Bir Bahadur · Manchikatla Venkat Rajam
Leela Sahijram · K.V. Krishnamurthy
Editors

Plant Biology and Biotechnology

Volume I: Plant Diversity, Organization, Function and Improvement

Springer

Plant Biology and Biotechnology

Bir Bahadur • Manchikatla Venkat Rajam
Leela Sahijram • K.V. Krishnamurthy
Editors

Plant Biology and Biotechnology

Volume I: Plant Diversity, Organization, Function and Improvement

Springer

Editors
Bir Bahadur
Sri Biotech Laboratories India Limited
Hyderabad, Telangana, India

Leela Sahijram
Division of Biotechnology
Indian Institute of Horticultural Research (IIHR)
Bangalore, Karnataka, India

Manchikatla Venkat Rajam
Department of Genetics
University of Delhi
New Delhi, India

K.V. Krishnamurthy
Center for Pharmaceutics, Pharmacognosy and Pharmacology, School of Life Sciences
Institute of Trans-Disciplinary Health Science and Technology (IHST)
Bangalore, Karnataka, India

ISBN 978-81-322-2285-9 ISBN 978-81-322-2286-6 (eBook)
DOI 10.1007/978-81-322-2286-6

Library of Congress Control Number: 2015941731

Springer New Delhi Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer (India) Pvt. Ltd. is part of Springer Science+Business Media (www.springer.com)

Foreword

Plants are essential to humanity for food, environmental intensification and personal fulfillment. Plants are also the foundations of healthy ecosystems ranging from the Arctic to the tropics. Plant biology is a living science dealing with the study of the structure and function of plants as living organisms, ranging from the cellular and molecular to the ecological stage.

It concerns the scientific study of plants as organisms and deals with the disciplines of cellular and molecular plant biology and the traditional areas of botany, e.g., anatomy, morphology, systematic physiology, mycology, phycology, ecology, as well as evolution.

The backbone of plant biology resides in its applications and spans from anatomy, plant physiology, and plant ecology to biochemistry, cell biology, and genetics.

Biotechnology is the use of living systems and organisms to develop or make useful products or "any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use." Depending on the tools and applications, it often overlaps with bioengineering and biomedical engineering.

For thousands of years, humankind has exploited biotechnology in agriculture, food production, and medicine. It is believed that the term *biotechnology* was coined in 1919 by Hungarian engineer Károly Ereky. During the twentieth and early twenty-first centuries, biotechnology was expanded to include diverse sciences such as genomics, recombinant gene technologies, applied immunology, and development of pharmaceutical therapies and diagnostic tests.

The past few years have witnessed the establishment of Departments or Institutes of *Plant Biology and Biotechnology* in different parts of the world. As the integration of the two subjects has expanded, undergraduate and postgraduate degrees have been instituted with distinct syllabi. Over the years, extraordinary developments have taken place, and significant advances have been made in biotechnology and plant biology. Unfortunately, there are not many texts on the confluence of the two subjects; hence, there is a dire need for texts that are pertinent for teaching courses and conducting research in this area. The present set of volumes is compiled to fill this gap and is edited by four eminent, talented, and knowledgeable professionals, Profs. Bir Bahadur, M. V. Rajam, Leela Sahijram, and K. V. Krishnamurthy. They have tried

to compile and cover major developmental processes to give the student a feel for scientific research.

Volume 1 contains 33 chapters, describes the past, present, and future of plant biology and the principles and strategies, and summarizes the landmark of research done on various aspects. The same authors have also compiled the first five chapters along with other colleagues to set the stage for the reader to comprehend the ensuing chapters. One chapter gives a comprehensive description of plant biodiversity; two chapters give an overview of plant–microbe interaction. Reproductive strategies of bryophytes, Cycads: an overview constitute the contents of two chapters. A single cohesive chapter on AM fungi describes them as potential tools in present-day technologies required for sustainable agriculture and to lessen the dependence on chemical fertilizers. The use of AM fungi as biofertilizers and bioprotectors to enhance crop production are well accepted, e.g., mining the nutrients, stimulating growth and yield, and providing resistance against water stress and pathogen challenge. The reproduction process by which organisms replicate themselves in a way represents one of the most important concepts in biology. Through this, the continuity of the existence of species is ensured. At the base level, reproduction is chemical replication and with progressive evolution, cells with complexity have arisen and in angiosperms involving complex organs and elaborate hormonal mechanism. Three chapters that exclusively deal with genetics of flower development, pre- and postfertilization growth, and development respectively are written in a masterly way. A single chapter on seed biology and technology should be of special interest to crop breeders and geneticists alike. The role of apomixis in crop improvement is most striking, and attract the attention of crop breeders wanting to secure pure lines.

Physiological aspects spanning from photosynthesis to mineral nutrition, which are important aspects of improving yield, have been reviewed pithily. Four chapters discuss details of induced mutations, polyploidy, and male sterility in major crops, and the potential of the utilization of these techniques is essential to shaping scientific minds. These have been discussed in depth.

Each chapter is compiled by a distinguished faculty who has taken seriously its commitment to satisfy the intellectual urge of lifelong learners. Areas of faculty research interest include cell and molecular biologists, geneticists, environmental biologists, organism biologists, developmental and regenerative biologists, and bioprocess technologists. Each chapter provides an authoritative account of the topic intended to be covered and has been compiled by one or more experts in the field. Each chapter concludes with carefully selected references that contain further information on the topics covered in that chapter. I am privileged to have known some of the authors both professionally and personally and am very excited to see their invaluable contributions.

For the students wishing to update themselves in the convergence of biology and biotechnology, the present volume not only furnishes the basics of the life sciences but provides plenty of hands-on functional experience, starting with plant diversity, organization, function, and improvement. Experienced life scientists, biologists, and biotechnologists have collaborated and pooled their talent and long experience in cross-disciplinary topics centered

on recent research focus areas. Interdisciplinary experts have combined their academic talent and strengths to further scientific discoveries in areas such as microbial diversity; divergent roles of microorganisms; overview of bryophytes, cycads, and angiosperms; etc. The strength of the volume lies in reproductive biology e.g., genetics of flower development, pre- and postfertilization reproductive growth, and development in angiosperms.

From finding better ways to deliver crop improvement, perk up the quality of produce, and exploit plant genomics and plant-based technologies to the myriad other ways, the life sciences touch our world, and there has never been a more exciting – or important – time to be a life scientist. If you want to learn more about what biology and biotechnology in plants can do for you, please pick up this volume and browse in depth.

This volume is intended for scientists, professionals, and postgraduate students interested in plant biology and biotechnology or life sciences. The volume will be indispensible for botanists, plant scientists, agronomists, plant breeders, geneticists, evolutionary biologists, and microbiologists.

Satish C. Maheshwari
Honorary Scientist of the Indian, National Science Academy, Biotechnology Laboratories
Centre for Converging Technologies
University of Rajasthan, Jaipur, India

Preface

Plant biology has been a fundamental area of biology for many centuries now, but during the last 30 years or so, it has undergone great transformation leading to a better and deeper understanding of many key fundamental processes in plants.

The idea of preparing these two volumes grew out of a need for a suitable book on plant biology and biotechnology for contemporary needs of students and researchers. The present volumes, to the best of our belief and knowledge, cover the most contemporary areas not adequately covered in most, if not all, books currently available on plant biology, plant biotechnology, plant tissue culture and plant molecular biology. Every effort has, therefore, been made to integrate classical knowledge with modern developments in these areas covering several new advances and technologies. This will definitely enable a better understanding of many aspects of plants: molecular biology of vegetative and reproductive development, genetically engineered plants for biotic and abiotic stress tolerance as well as other useful traits, use of molecular markers in breeding, all the '-omics' and various biotechnological aspects of benefit to mankind to meet challenges of the twenty-first century, to mention just a few.

These books have been designed to provide advanced course material for post-graduates in plant sciences and plant biotechnology, applied botany, agricultural sciences, horticulture and plant genetics and molecular biology. These also serve as a source of reference material to research scholars, teachers and others who need to constantly update their knowledge.

Volume 1 of the book provides an in-depth analysis on topical areas of plant biology, with focus on Plant Diversity, Organization, Function and Improvement, including mechanisms of growth, differentiation, development and morphogenesis at the morphological, cellular, biochemical, genetic, molecular and genomic levels.

Contributors to these volumes were selected from a wide range of institutions in order to introduce a diversity of authors, and at the same time, these authors were selected with vast expertise in their specific areas of research to match with the diversity of the topics. These authors not only have a deep understanding of the subject of their choice to write critical reviews by integrating available information from classical to modern sources but have also endured an unending series of editorial suggestions and revisions of their manuscripts. Needless to say, this is as much their book as ours.

We hope these books will help our fellow teachers and a generation of students to enter the fascinating world of plant biology with confidence, as perceived and planned by us.

Hyderabad, Telangana, India — Bir Bahadur
New Delhi, India — Manchikatla Venkat Rajam
Bangalore, Karnataka, India — Leela Sahijram
Bangalore, Karnataka, India — K.V. Krishnamurthy

Acknowledgements

First and foremost, we are immensely grateful to all the contributing authors for their positive response. We are also grateful to Prof. S.C. Maheshwari for kindly agreeing and writing a Foreword for this volume.

We wish to express our grateful thanks to a number of friends and colleagues for their invaluable help in many ways and for their suggestions from time to time during the evolution of the two volumes. We also thank research scholars of Prof. M.V. Rajam (University of Delhi South Campus) – Shipra Saxena, Meenakshi Tetorya, Mahak Sachdeva, Bhawna Israni, Mamta, Manish Pareek, Anjali Jaiwal, Jyotsna Naik, Sneha Yogindran and Ami Choubey for their help in formatting the chapters. We also thank Dr John Adams for his help in preparing the subject index.

We wish to express our appreciation for help rendered by Ms. Surabhi Shukla, Ms. Raman, N.S. Pandian and other staff of Springer for their cooperation and valuable suggestions. Above all, their professionalism, which made these books a reality, is greatly appreciated.

We wish to express our grateful thanks to our respective family members for their cooperation.

Editors
Bir Bahadur
Manchikatla Venkat Rajam
Leela Sahijram
K.V. Krishnamurthy

Contents

Contributors

Anjali Anand Division of Plant Physiology, Indian Agriculture Research Institute, New Delhi, India

Bir Bahadur Sri Biotech Laboratories India Limited, Hyderabad, Telangana, India

Rajesh Bajpai Lichenology Laboratory, Plant Diversity, Systematics and Herbarium Division, CSIR – National Botanical Research Institute, Lucknow, Uttar Pradesh, India

Pooja Bansal Plant Diversity, Systematics & Herbarium Division, CSIR – National Botanical Research Institute, Lucknow, Uttar Pradesh, India

Jaume Bastida Department of Natural Products, Plant Biology and Soil Science, The School of Pharmacy, University of Barcelona, Barcelona, Spain

S.G. Bhagwat Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, MS, India

K. Bhanuprakash Department of Seed Science and Technology, Indian Institute of Horticultural Research (IIHR) Hessaraghatta, Bangalore, Karnataka, India

Dinesh Narayan Bharadwaj Department of Genetics and Plant Breeding, C.S. Azad University of Agriculture & Technology, Kanpur, Uttar Pradesh, India

D. Dhanasekaran Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Tanmoy Ghosh Academy of Scientific and Innovative Research, New Delhi, India

Discipline of Salt & Marine Chemicals, CSIR-Central Salt and Marine Chemicals Research Institute, Bhavnagar, Gujarat, India

Anil K. Goel Botanic Garden, CSIR – National Botanical Research Institute, Lucknow, Uttar Pradesh, India

Anupama J. Hingane Pigeonpea Breeding, Grain Legumes, ICRISAT, Patancheru, India

S. John Adams Center for Pharmaceutics, Pharmacognosy and Pharmacology, School of Life Sciences, Institute of Trans-Disciplinary Health Science and Technology (IHST), Bangalore, Karnataka, India

M.S.L. Karuna Centre for Lipid Research, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad, Telangana, India

Sangeeta Khetarpal Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi, India

J.S. Khuraijam Botanic Garden, CSIR – National Botanical Research Institute, Lucknow, Uttar Pradesh, India

N. Kishore Department of Microbiology, Palamuru University, Mahabubnagar, Telangana, India

K.V. Krishnamurthy Center for Pharmaceutics, Pharmacognosy and Pharmacology, School of Life Sciences, Institute of Trans-Disciplinary Health Science and Technology (IHST), Bangalore, Karnataka, India

I.K. Kunwar Department of Botany, Osmania University, Hyderabad, Telangana, India

C. Manoharachary Department of Botany, Osmania University, Hyderabad, Telangana, India

Rahulkumar Maurya Academy of Scientific and Innovative Research, New Delhi, India

Discipline of Salt & Marine Chemicals, CSIR-Central Salt and Marine Chemicals Research Institute, Bhavnagar, Gujarat, India

S.J. Mirajkar Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, MS, India

Sandhya Mishra Academy of Scientific and Innovative Research, New Delhi, India

Discipline of Salt & Marine Chemicals, CSIR-Central Salt and Marine Chemicals Research Institute, Bhavnagar, Gujarat, India

Tavva S.S. Mohan Dev Botany Department, Andhra University, Visakhapatnam, Andhra Pradesh, India

M.H. Muhammad Ilyas PG & Research Department of Botany, Jamal Mohamed College, Tiruchirappalli, Tamil Nadu, India

G. Muralitharan Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Virendra Nath Plant Diversity, Systematics & Herbarium Division, CSIR – National Botanical Research Institute, Lucknow, Uttar Pradesh, India

S. Nayaka Lichenology Laboratory, Plant Diversity, Systematics and Herbarium Division, CSIR – National Botanical Research Institute, Lucknow, Uttar Pradesh, India

Chetan Paliwal Academy of Scientific and Innovative Research, New Delhi, India

Discipline of Salt & Marine Chemicals, CSIR-Central Salt and Marine Chemicals Research Institute, Bhavnagar, Gujarat, India

Renu Pandey Mineral Nutrition Laboratory, Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi, India

S.R. Pandravada National Bureau of Plant Genetic Resources, Regional Station, Hyderabad, Telangana, India

Pavan K. Pindi Department of Microbiology, Palamuru University, Mahabubnagar, Telangana, India

R.B.N. Prasad Centre for Lipid Research, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad, Telangana, India

T. Pullaiah Department of Botany, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India

S. Ram Reddy Department of Microbiology, Kakatiya University, Warangal, Telangana, India

S.M. Reddy Department of Botany and Microbiology, Kakatiya University, Warangal, Telangana, India

K.B. Saxena Pigeonpea Breeding, Grain Legumes, ICRISAT, Secunderabad, Telangana, India

K.R. Shivanna Conservation Biology, Ashoka Trust for Research in Ecology and the Environment, Royal Enclave, Bengalore, Karnataka, India

M.A. Singara Charya Department of Microbiology, Kakatiya University, Warangal, Telangana, India

M.V. Subba Rao Botany Department, Andhra University, Visakhapatnam, Andhra Pradesh, India

C. Sudhakar Reddy Forestry and Ecology Group, National Remote Sensing Centre, Balanagar, Hyderabad, Telangana, India

B. Sujatha Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India

N. Sunil Winter Nursery Centre, Indian Institute of Maize Research, Rajendranagar, Hyderabad, Telangana, India

P. Suprasanna Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, MS, India

N. Thajuddin Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Umesha Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences GKVK, Bangalore, Karnataka, India

D.K. Upreti Lichenology Laboratory, Plant Diversity, Systematics and Herbarium Division, CSIR – National Botanical Research Institute, Lucknow, Uttar Pradesh, India

Pathipati Usha Rani Biology and Biotechnology Division, CSIR, Indian Institute of Chemical Technology, Tarnaka, Hyderabad, Telangana, India

Padma Venkatasubramanian Center for Pharmaceutics, Pharmacognosy and Pharmacology, School of Life Sciences, Institute of Trans-Disciplinary Health Science and Technology (IHST), Bangalore, Karnataka, India

B. Venkateswara Rao Botany Department, Andhra University, Visakhapatnam, Andhra Pradesh, India

Y. Venkateswara Rao Botany Department, Andhra University, Visakhapatnam, Andhra Pradesh, India

Francesc Viladomat Department of Natural Products, Plant Biology and Soil Science, The School of Pharmacy, University of Barcelona, Barcelona, Spain

About the Editors

Prof. Bir Bahadur
FLS, C Biol, FI Biol (London)

Dr. Bir Bahadur, born 5 April 1938, studied at City College, Hyderabad, for 5 years, including an Intermediate Course (Osmania University), graduated from Nizam College, and postgraduated from University College, Osmania University, both in the first division. He obtained his Ph.D. in Plant Genetics from Osmania University. He was closely associated with late Prof. J.B.S. Haldane, F.R.S., a renowned British geneticist who encouraged him to study heterostyly and incompatibility in Indian plants, a subject first studied by Charles Darwin.

He made significant contributions in several areas, especially heterostyly, incompatibility, plant genetics, mutagenesis, plant tissue culture and biotechnology, morphogenesis, application of SEM in botanical research, plant asymmetry, plant morphology and anatomy and lately the biofuel plants Jatropha and castor.

He served as Lecturer and Reader at Osmania University, Hyderabad, and as Reader and Professor at Kakatiya University, Warangal. He also served as Head of Department; Chairman, Board of Studies; Dean, Faculty of Science; and Coordinating Officer/Dean, UGC Affairs at Kakatiya University. He has over 40 years of teaching and over 50 years of research experience. He has supervised 29 Ph.D. students and 3 M.Phil. students in both these universities

and has published about 250 research papers/reviews, which are well received and cited in national and international journals, textbooks and reference books.

He was a postdoctoral fellow at the Institute of Genetics, Hungarian Academy of Sciences, Budapest, and worked on mutagenesis and chromosome replication in *Rhizobium.* He is a recipient of the direct award from the Royal Society Bursar, London. He also worked at Birmingham University (UK). He was conferred with the title of Honorary Research Fellow by the Birmingham University. He studied species differentiation in wild and cultivated solanums using interspecific hybridization and the enzyme-etched seeds technique in combination with scanning electron microscopy to assess the relationship among various *Solanum* species. At the invitation of the Royal Society, he visited Oxford University, Leeds University, Reading University and London University, including the Royal Botanic Gardens, Kew, and various research labs. He was invited for international conferences by the US Science Foundation at the University of Missouri, St. Louis, and the University of Texas, Houston (USA), and at the SABRO international conference at Tsukuba, Japan. He has extensively visited most countries of Eastern and Western Europe as well as Tanzania and the Middle East.

He has authored/edited ten books. One of his important books is entitled *Jatropha, Challenges for a New Energy Crop*, Vol. 1 and 2, published by Springer, New York, USA, 2013, jointly edited with Dr. M. Sujatha and Dr. Nicolas Carels. These books are considered significant contributions to bioenergy in recent times. He was Chief Editor, Proceedings of Andhra Pradesh Akademi of Sciences, Hyderabad, and Executive Editor, *Journal of Palynology* (Lucknow).

He is the recipient of the Best Teacher Award by the Andhra Pradesh Government for mentoring thousands of students in his teaching career spanning over 40 years. He was honoured with the Prof. Vishwamber Puri Medal of the Indian Botanical Society for his original contributions in various aspects of plant sciences. He has been honoured with the Bharat Jyoti Award at New Delhi for outstanding achievements and sustained contributions in the fields of education and research. He has been listed as 1 of the 39 prominent alumni of City College, a premier institution with a long history of about 90 years as per the latest update on its website. He has been chosen for distinguished standing and has been conferred with an Honorary Appointment to the Research Board of Advisors by the Board of Directors, Governing Board of Editors and Publications Board of the American Biographical Institute, USA.

He is a fellow of over a dozen professional bodies in India and abroad, including the following: Fellow of the Linnean Society, London; Chartered Biologist and Fellow of the Institute of Biology, London. Presently, he is an Independent Board Director of Sri Biotech Laboratories India Ltd., Hyderabad, India.

Prof. Manchikatla Venkat Rajam
FNA, FNASc, FNAAS, FAPAS, FABAP

Dr. Manchikatla Venkat Rajam is currently Professor and Head, Department of Genetics, University of Delhi South Campus, New Delhi, India. He obtained his Ph.D. in Botany (1983) from Kakatiya University, Warangal, India. He was a postdoctoral fellow at the prestigious Yale University, New Haven (1984–1985), and also worked at BTI (Cornell University, Ithaca) for a couple of months as a visiting research associate. At Yale University, his work led to the discovery of a new method for the control of fungal plant infections through selective inhibition of fungal polyamine biosynthesis. This novel method has been adapted by several research groups globally for the control of a variety of fungal infections, and a large number of research articles have been published in this line of work. He returned to India to join as Pool Officer (CSIR) and worked for about 2 years (1986–1987) at Kakatiya University. Subsequently, he joined the University of Delhi South Campus, where he has been on the faculty since 1987. He had worked in ICGEB, New Delhi, for 6 months as a National Associate of DBT (1994). He made several short visits to various countries including France, Italy, China and Indonesia under collaborative projects supported by the Europeon Union and Indo-French organizations. He is a Fellow of the prestigious Indian National Science Academy (FNA), National Academy of Sciences, India (FNASc); National Academy of Agricultural Sciences (FNAAS); Andhra Pradesh Akademi of Sciences (FAPAS); and Association of Biotechnology and Pharmacy (FABAP) and is an elected member of the Plant Tissue Culture Association, India, since 1995. He was awarded the Rockefeller Foundation Biotech Career Fellowship in 1998 (but could not avail it); the 'Shiksha Rattan Puraskar' by the India International Friendship Society in 2011; Department of Biotechnology National Associateship in 1994; and National Scholarship for Study Abroad (Government of India) in 1984 and for Research in 1985 by the Rotary International Club of Hyderabad. He is serving as an Associate Editor and member of the editorial board of several reputed journals including *BMC Biotechnology* and the OMICS journal *Cell and Developmental Biology* and is a member of the advisory or other committees of some universities, institutions as well as other bodies. He has guided

28 Ph.D. students, 7 M.Phil. students and over 22 postdoctoral fellows and has published over 120 papers (80 research articles in peer-reviewed journals, 15 review articles, 20 book chapters and general articles). He has one Indian patent to his credit. He has vast experience in plant biotechnology and RNA interference and has handled over 22 major projects in these areas.

Dr. Leela Sahijram

Dr. Leela Sahijram is currently Principal Scientist, Division of Biotechnology, Indian Institute of Horticultural Research, Bangalore, India, and heading the Plant Tissue Culture Laboratory. She obtained her M.Sc. in Botany (Plant Physiology) with distinction from Osmania University, Hyderabad, India (1976), and her Ph.D. in Plant Physiology (1983) from the Indian Agricultural Research Institute, New Delhi, India. She was deputed under the USAID Program to the University of California at Davis, USA (1992), for plant transformation. She has also undergone training in bioinformatics at IISR, Calicut, India (2003). She has published several papers in national and international journals and has guided students for their master's and doctoral degree programmes. She was identified by the Department of Biotechnology (DBT), New Delhi, for training on 'Biotechnology and Intellectual Property Rights (IPR)' at the National Law School of India University (NLSIU), Bangalore (2003). She attended a residential course on 'Creative Writing in Agriculture' at the Indian Institute of Mass Communication (IIMC), New Delhi (2011).

Her team pioneered the micropropagation of banana (globally, the leading tissue culture–propagated fruit crop), which has spawned a multibillion-dollar industry worldwide. In 1990, she successfully demonstrated over 20 choice clones of banana from across India to be 'micropropagatable', including cultivars of the Cavendish Group. She was member of the Task Force for the rehabilitation of Nanjangud Rasabale (Pride of Karnataka) syn. Rasthali, 'Silk' group – a clone threatened with extinction. She has also worked extensively on micropropagation and 'specific-pathogen-free' (SPF) plantlet production through meristem culture/micrografting in crops like citrus, caladium, bougainvillea and chrysanthemum besides bananas and plantains. She specializes in hybrid embryo rescue in perennial horticultural crops

(intergeneric/interspecific/intervarietal crosses), particularly in fruit crops, namely, mango, seedless grapes/citrus, banana and papaya. In 2000–2001, she pioneered hybrid embryo culture and *ex vitro* grafting in controlled crosses of mango.

She was conferred with the Dr. Vikram Govind Prasad Award 1999–2000 for research on molecular diagnostics of viruses in micropropagated bananas. She was also honoured with the Horticultural Society of India Award 2006–2007 for research on hybrid embryo rescue in seedless grapes and with the Rashtriya Samman Award 2007 for developing biotechnologies for horticultural crops. She has been editing the *Journal of Horticultural Sciences*, an international journal, for the past 9 years as a Founder Editor. She has also edited a book entitled *Biotechnology in Horticultural and Plantation Crops*. She has several book chapters in national and international publications to her credit. She is the author of many technical and semi-technical popular articles and a laboratory manual besides having trained hundreds of personnel from development departments for setting up commercial plant tissue culture laboratories. She has travelled widely.

Dr. K.V. Krishnamurthy

Dr. K.V. Krishnamurthy is currently an Adjunct Professor at the Institute of Trans-Disciplinary Health Science and Technology (IHST), Bangalore, India, and offering consultancy services in Ayurvedic Pharmacognosy. He obtained his M.Sc. in Botany with University First Rank from Madras University, Chennai, in 1966 and his Ph.D. in Developmental Plant Anatomy from the same university in 1973. After a brief stint in government colleges in Tamil Nadu, he joined the present Bharathidasan University, Tiruchirappalli, in 1977 and became a Full Professor in 1989. He has an overall teaching and research experience of more than 47 years and has guided 32 Ph.D. scholars, more than 50 M.Phil. scholars and hundreds of master's degree holders. He has published more than 180 research papers and 25 books, including *Methods in Cell Wall Cytochemistry* (CRC Press, USA) and a textbook on biodiversity (Science Publishers, USA), *Bioresources of Eastern Ghats: Their Conservation and Management* (with Bishen Singh Mahendra Pal Singh, Dehradun). His major research areas include plant morphology and morphogenesis, biodiversity,

wood science, cytochemistry, plant reproductive biology and ecology, tissue culture, and herbal medicine and pharmacognosy. He has operated more than 15 major research projects so far. He has been a Fulbright Visiting Professor at the University of Colorado, Boulder, in 1993 and has visited and lectured in various universities in the UK in 1989. His outstanding awards and recognitions include the following: INSA Lecture Award 2011; Prof. A Gnanam Endowment Lecture Award 2010; President 2007, Indian Association for Angiosperm Taxonomy; Prof. V. Puri Award 2006 by the Indian Botanical Society; Rashtriya Gaurav Award 2004 by India International Friendship Society, New Delhi; Scientist of the Year Award 2001 by the National Environmental Science Academy, New Delhi; Tamil Nadu State Scientist Award 1997–1998 in the Field of Environmental Science; Dr. V.V. Sivarajan Gold Medal Award by the Indian Association for Angiosperm Taxonomy for Field Study in the year 1997–1998; Prof. Todla Ekambaram Endowment Lecture Award, Madras University, 1997; Prof. G.D. Arekal Endowment Lecture Award, Mysore University, 1997–1998; Prof. V.V. Sivarajan Endowment Lecture Award, Calicut University, 1997; Prof. Rev. Fr. Balam Memorial Lecture Award, 1997; the 1984 Prof. Hiralal Chakraborty Award instituted by the Indian Science Congress in recognition of the significant contributions made to the science of botany, 1960; Dr. Pulney Andy Gold Medal awarded by Madras University as University First in M.Sc. Botany, 1966; Dr. Todla Ekambaram Prize awarded by Madras University for standing first in M.Sc. Plant Physiology, 1966; The Maharaja of Vizianagaram Prize awarded by Presidency College, Madras, for outstanding postgraduate student in science, 1965–1966; and Prof. Fyson Prize awarded by Presidency College, Madras, for the best plant collection and herbarium, 1965–1966. He has been the following: Fellow of the National Academy of Sciences of India (FNASc); Fellow of the Linnean Society, London (FLS); Fellow of the Indian Association for Angiosperm Taxonomy (FIAT); Fellow of the International Association of Wood Anatomists, Leiden; Fellow of the Plant Tissue Culture Association of India; and Fellow of the Indian Botanical Society. He has been the Editor and editorial member of many journals in and outside India and has also been reviewer of research articles for many journals. He has also served in various committees, the major funding organizations of India and several universities of India. He has been the Registrar and Director, College and Curriculum Development Council; Member of Syndicate and Senate; Coordinator of the School of Life Sciences and Environmental Sciences; Head of the Department of Plant Sciences; and a Visiting Professor in the Department of Bioinformatics at Bharathidasan University, Tiruchirappalli, before assuming the present job after retirement.

Plant Biology: Past, Present and Future

1

Bir Bahadur and K.V. Krishnamurthy

Abstract

This chapter deals with a history of botanical science. Major advancements made in the ancient, medieval, Renaissance and modern periods in different subdisciplines are detailed. Particular emphasis has been provided to the importance of instruments and techniques that enabled these advancements. The importance of *Arabidopsis* as a model plant in contributing to modern botanical knowledge and in plant molecular biology is emphasized. The future of plant biology is briefly discussed. *Arabidopsis*-like researches must be extended to other plant taxa, especially those that are of economic value. Plants and ecosystems must be continued to be studied in order to save and sustain the earth in the context of population explosion not only of human but of animals as well.

Keywords

Ancient period • *Arabidopsis* • Future of plant biology • Medieval period • Model plant • Modern period • Plant biology • Renaissance period

1.1 Introduction

Botany, often also called *plant science(s)* or *plant biology,* may be defined as the science of plant life. This, along with zoology (science of animals), historically forms the core discipline of *biology* (*bios* = life; *logos* = discourse or science), a term coined by Lamarck. The history of biology is closely associated with *natural sciences* (or *natural history*) of chemistry, physics, mathematics and geology (Krishnamurthy 2005).

B. Bahadur (✉)
Sri Biotech Laboratories India Limited, Hyderabad, Telangana, India
e-mail: birbahadur5april@gmail.com

K.V. Krishnamurthy
Center for Pharmaceutics, Pharmacognosy and Pharmacology, School of Life Sciences, Institute of Trans-Disciplinary Health Science and Technology (IHST), Bangalore, Karnataka, India

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_1,

The term 'botany' is derived from the ancient Greek word '*βοτανη*' (= botane), which means 'pasture', 'grass' or 'fodder' (Morton 1981), and also from the medieval Latin word 'botanicus', which means a herb or plant. *Βοτανη*, in turn, is derived from 'βόσκιεν' (= *boskein*), which means 'to feed' or 'to graze'. The science of botany involves observing, recording and describing of plants and their morphological features; classifying them; analyzing their structure, development, physiology and function and reproduction; and exploiting them because of their economic uses and value. Now, botanists examine both the internal functions and processes within cell organelles, cells, tissues, organs, whole plants, plant populations, plant communities (made by different species and their populations), ecosystems (of which plants form a part), landscapes (made of many ecosystems) and the whole biome of the earth.

The above account naturally leads to the questions: What are plants? How to define a plant? Historically, plants represented all organisms other than animals. Hence, plants at one time included viruses, bacteria, fungi, lichens, algae, bryophytes, pteridophytes, gymnosperms and angiosperms. According to some researchers, the strictest definition of plants should include only 'land plants' or embryophytes. But today, viruses have been removed from the list of plants because they are acellular and bacteria have been removed as they are prokaryotic. Whittaker's (1969) five kingdom concept excluded fungi from plants and treated them as a separate kingdom based on their absorptive mode of nutrition as different from the photosynthetic mode of plants. However, detailed research have enabled us to fix the following characteristic/diagnostic features of plants: stationary habit, eukaryotic cells, presence of microfibrillar cell walls, presence of vacuoles, presence of plastids (particularly chloroplasts with chlorophyll), oxygenic photosynthesis (that releases oxygen through an oxygen-evolving complex), presence of photosystem I and photosystem II, invariable presence of starch as a principal reserve material, etc. (Krishnamurthy 2010). Embryophytic land plants share all these features. Algae share the most, if not all, of all the above characteristics. Lichens are autotrophic as they have a photosynthetic partner. Fungi are heterotrophic and non-photosynthetic, but yet they are included by many under plants as they possess eukaryotic cells, microfibrillar cell walls and vacuoles. Even today fungi and photosynthetic protists are usually covered in introductory botany courses, and researchers working on these taxa form the core botany faculty in many botany departments of universities throughout the world. Hence, botany is treated here as including fungi and photosynthetic protists.

Botany is subdivided into subdisciplines based on two important criteria. Either the subdivisions deal with the plant groups in question, such as algology (phycology), mycology, lichenology, bryology, pteridology, Gymnospermae and Angiospermae, or with the different basic aspects of study of plants, such as morphology, anatomy, palynology, taxonomy, physiology, ecology, genetics, cytology, etc. (Sachs 1890), and with the different applied aspects of study of plants, such as agriculture, horticulture, forestry, pharmacognosy, ethnobotany, etc. Since the origin of traditional and applied botany in the ancient period, there has been a progressive increase in the scope of the subject as technology has opened up newer techniques and areas/disciplines of study that increasingly required inter- and multidisciplinary inputs. This chapter examines the human efforts to study and understand plant life on earth by tracing the historical and chronological development of the discipline of botany. In tracing the history of any scientific discipline like botany, it is convenient to divide the past into the following periods (Krishnamurthy 2005): ancient period, medieval period, Renaissance period and modern period. This division has been followed in the present discussion also. Such a historically based study of plants is vital because the plants underpin almost all animal (including human) life on earth by generating a large amount of oxygen and food (through photosynthesis) that provide humans and other organisms with aerobic respiration and the necessary chemical energy which they need for their existence. Hence, a study of plants is crucial to the future of humanity globally.

1.2 Ancient Period

This period might be said to extend from the period of origin of modern man on the planet earth, estimated to be around 200,000 years ago in Olduvai Gorge in the Great Rift Valley of Tanzania, East Africa (excavated by Louis and Mary Leakey in the mid-1950s), until the fifth century CE. This is a very long period, much longer than any historic period. The modern man was nomadic and might have spread to other parts of the African continent, but his movement outside Africa happened only around 70,000 years ago. He moved to different parts of the world including Europe, West Asia, Middle East, Central Asia, India, China, East Indies and Australia. He was a hunter-gatherer, hunting animals and foraging plants for his food. During his hunter-gatherer phase, he was largely using stone implements for hunting and other purposes (and hence this period was called Stone Age). In the Old Stone Age (Palaeolithic period) which extended up to about 12,000 years ago, he gained great knowledge about the animals he hunted and the plants he collected/used for food, shelter, medicines, poisons, ceremonies and rituals, etc. Thus, the initial botanical science began with the empirically based plant lore passed from one generation to the next orally as writing was not invented by then. Botany particularly started with human effort to identify the useful plants and this use of plants might have also influenced the way in which the plants were named and 'classified' according to folk taxonomies that varied in different parts of the world and that were used in everyday communication between each other (Walters 1981). The efforts of the foragers were stated to be mainly focused on exploring the carbohydrate-containing and to some extent fat-containing plant foods such as tubers, fruits and oil seeds since they were able to get enough protein food from hunted animals (Crowe 2005). Today, we still have a few glimpses of how a hunter-gatherer society works from studies of indigenous people of Amazon, New Guinea, Andamans (India) and a few other places. These old-stone age societies are very much plant-based cultures, and it is amazing how many uses for plants they have developed (Prance 2005). Hence, it may be stated with conviction that basic botany started in close association with applied botany in the Palaeolithic Age itself and that knowledge about one was absolutely vital for the other to develop.

In the Neolithic period which started around 12,000 years ago, the nomadic hunter-gatherer lifestyle of humans got drastically changed into settled communities in many parts of the world, particularly in major river banks, although hunter-gatherer communities continued to persist in remote forest areas and islands. The reasons for this change from nomadic to settled life are debated, but most people agree that climate change and associated changes in vegetation at the end of the Pleistocene period about 12,000–13,000 years ago are the major factors responsible, at least for the initial transitions from foraging to farming (Harris 2005). These transitions involved three steps: (1) *cultivation*, which refers to the 'sowing and planting, tending, and harvesting of useful wild or domesticated plants, with or without tillage of the soil'; (2) *domestication*, which means that 'plant have been changed genetically and/or morphologically as a result of human selection (inadvertent or deliberate) and have become dependent on people for their long-term survival'; and (3) *agriculture*, which is defined as the 'growing of crops (i.e. domesticated plants) in systems of cultivation that normally involve systematic tillage of the soil' (Harris 2005). The earliest evidence for these transitions comes from three regions of the world: Southwest Asia, China and India. Legumes were domesticated in almost all the continents, while, among cereals, rice was domesticated in East and Southeast Asia, wheat and barley in the Middle East referred to as 'Golden Crescent', maize in Central and South America and millets mainly in Africa; vegetable and fruit crops were domesticated in many different parts of the world. Thus, 'the cultivated plants are mankind's most vital and precious heritage from remote antiquity' (Stearn 1965), particularly between 12,000 and 2,500 years ago, depending upon the region of the world.

With the invention of writing around 5,000–6,000 years ago, as evidenced from the writings in Babel Tower in Sumeria (South Mesopotamia) (see Krishnamurthy 2005), the passing of systematic knowledge, including those on plants, and culture from one generation to the next became easier, wider and faster (Morton 1981). Around 3500 BCE the first known illustrations of plants (Reed 1942) and descriptions of impressive gardens in Egypt (Morton 1981) could be got. Although protobotany that was brought to light by the first pre-scientific written record of plants in the late Neolithic period (i.e. from 3,000 years ago) was claimed to be not concerned with food plants but was born out of the medicinal literature of Middle East, Egypt, China and India (Reed 1942), it is not true, as it laid equal attention to basic botanical as well as applied botanical aspects such as agriculture, horticulture and medicinal plants. The claim that agriculture is an occupation of the poor and that medicine was the realm of socially influential priests, shamans, apothecaries, magicians and physicians, who were considered to be more likely to record their knowledge for posterity in the Neolithic period (Morton 1981), also appears to be not true, since both agriculture and medicine were practiced by poor as well as the influential people.

Very active and substantial contributions to botany were made in ancient India, particularly from N.W. India, Gangetic plain and the ancient Tamil country (now Tamil Nadu) in S. India. Extensive information has been obtained on plants through archaeological excavations (in the so-called Indus Valley regions and S. India), literary sources (Vedic Sanskrit, Pāli, Prakrut and Tamil literature), epigraphical data (including those on copper plates) and folklore. The Indus Valley Civilization extended from around 3500 to 1300 BCE and covered from Dasht valley of the Makran coast in the west to Meerut and Saharanpur in the upper Ganga-Yamuna Doab in the east and from Shortugai near the Oxus in North Afghanistan to upper Godavari in Maharashtra (Chakrabarti 2004). The literature of Vedic period include the four *Vedas* (*Rigveda*, *Yajurveda*, *Sāmaveda* and *Atharvaveda*), the *Samhitās*, the *Brahmanās* and the *Āranyakas-Upanishads*, and the post-Vedic ancient literature includes the works of Charaka, Sushruta and Vāghbhata; *Agnipurāna*; *Arthasāstra*; *Brhatasamhitā*; various *Vṛkshāyurveda* texts including those of Parasara (Chowdhury 1971a; Ghosh and Sen 1971); and the Tamil literary works including the *Tolkappiyam*, *Sangam* literature and the great Tamil Epics (Krishnamurthy 2006). Among cereals wheat, barley and rice were recorded in the Indus Valley. The wheat remains recovered from Mohenjodaro belonged to *Triticum vulgare* (*T. aestivum*) and *T. compactum* and that from Harappa to *T. compactum* and *T. sphaerocarpum*. Barley from both these regions belonged to *Hordeum vulgare* var. *nudum* and *H. vulgare* var. *hexastichum* (respectively, the two-rowed and six-rowed types). Rice belonged to *Oryza sativa.* Excavations made in South India reveal the *indica* group of the archaeological presence of *Eleusine coracana*, the rāgi millet (Chowdhury 1971a). Remnants of cotton cloth and strings were found in Indus Valley Civilization and this belonged to *Gossypium arboreum* (also frequently mentioned in very early Tamil literature). Indus Valley data also show the use of woods belonging to deodar (*Cedrus deodara*), rosewood (*Dalbergia latifolia*) (both were used in coffins), *Ziziphus mauritiana* (used in mortar) and species of *Acacia*, *Albizia*, *Tectona*, *Adina*, *Soymida*, *Dalbergia*, *Holarrhena*, *Shorea,* etc. (see details in Chowdhury 1971a; Ghosh and Sen 1971).

The Vedic period is believed to have extended from 1700 to 1000 BCE although some Vedic scholars put the date from around 3000 to 1000 BCE (Frawley 1991) and include the Indus Valley Civilization also under Vedic civilization. The Vedas have frequent references to the use of plough, sowing of seed, harvest seasons, harvesting of grains, agricultural produces like wheat, beans, *Sesamum*, sesamum oil, medicinal properties of plants, plant diseases and their treatment with herbals, classification of plants, etc. The *Rigveda* contains many references to at least 107 species of plants, while *Atharvaveda* refers to some plants not mentioned in *Rigveda*. The *Atharvaveda* in particular classified plants into eight classes: *sāsa* (herbs) *visakha* (plants with

spreading branches), *manjari* (plants with long clusters), *sthambini* (bushy plants), *prastanavati* (those which extend on the ground, i.e. creepers), *amsumati* (with many branches), *ekasringa* (with monopodial growth) and *kandini* (plants with knotty joints). Different parts of a plant body such as the root (*mūla*), shoot (*tūla*), stem (*kandā*) branch (*sākhā*), leaf (*parnā*), flower (*puspā*) and fruit (*phala*) are distinguished and clearly named in *Samhitās*, *Brahmanās* and *Upanishads*. The different colours of plants/plant parts are also mentioned. The stem had been shown to have a *valkala* (skin) or *tvac* (epidermis), an inner *sāra* (wood) and a *majja* (pith). The trees were called *vrksā,* herbs *osādhi* (or *ausādi*) and creepers *virudh*. The terms *vana* and *druma* were also used for trees. These indicate that in the Vedic period morphological terminologies were already in place to describe and classify the various plants (Ghosh and Sen 1971). It is also evident that the botany of this period was mainly on basic botany and agriculture.

In the post-Vedic period (800 BCE to 100 AD), not only aspects of plants related to medicine, agriculture and horticulture, but also basic aspects were paid attention to and a good body of information was obtained. Two thousand five hundred years ago, the University of Taxila, considered to be among the earliest universities in the world (now in Pakistan), flourished, followed later by one at *Nalanda* in Eastern India, now in Bihar, India. When Jivika completed his studies, his teacher Bhikshu Atreya asked him to collect, identify and describe properties of all the plants growing within 36 miles of the University for the doctoral degree (acharya). Medical treatises like those of Charaka, Sushruta, V*ā*ghbhata and others were produced in this period. Charaka unhesitatingly accepted that a pharmacologist is one who knows the uses and actions of herbs though he may not know their forms (morphology), but an expert physician is one who knows the herbs botanically, pharmacologically and in every other respect (Ghosh and Sen 1971). Although plant taxonomy of this period is based on *Udhvida* (botanical), *Virechanadi* (medicinal) or *Annapanadi* (food), Charaka interestingly classified plants into four categories based essentially on botanical characteristics, while into two major groups subdivided into 13 groups. *Prasastapada*, another medical practitioner, classified plants into seven categories, again based only on morphological features. Manusmriti, the law book of Hindus, classified plants into eight major categories (Ghosh and Sen 1971; Morton 1981).

The greatest contribution of the Vedic and post-Vedic period is the emergence of botany as a distinct science discipline, a fact that has not received the recognition that it rightly deserves. This discipline is called *Vṛkshāyurveda* which literally means the science of plants (Ghosh and Sen 1971; Roy 2008). The term first appears in Kautilya's *Arthasāstra* and subsequently Varāhamihira and *Agnipurāna* and *Brhatsamhitā* mention this term. In all these, a section dealing with *Vṛkshāyurveda* is found. This covers the complete process of the life of a plant, beginning from sowing of seed to the harvesting of crops; thus, it comprises all aspects of plant life—seed morphology, germination, morphology, physiology, ecology, taxonomy and reproduction of plants. Parāsara's *Vṛkshāyurveda* (*c* first century BCE to first century CE) text is the best known text and also the complete text covering many aspects of plant life. It is divided into six parts: *Vijotpattikānda*, *Vanaspatikānda*, *Vanaspatyakānda*, *Virudhavallikānda*, *Gulmakşupakānda* and *Cikitsitakānda*; the last part dealing with plant diseases is missing. The first part is further subdivided into eight chapters dealing, respectively, with plant morphology, nature/properties of soil, description/distribution of forests, more detailed morphology of plants, flowers and classification, fruits and description/discussion on root, stem, bark, heartwood, sap, excretion, oleogenous products and spines and prickles and seeds and seedlings. Parāsara's work recognized *ganas* (groupings) equivalent to modern angiosperm families. These included the following: *Samiganiya* (Fabaceae), *Puplikaganiya* (Rutaceae), *Swastikaganiya* (Cruciferae), *Tripuspaganiya* (Cucurbitaceae), *Kurcapuspaganiya* (Asteraceae), etc. (Majumdar 1982). *Vṛkshāyurveda* evidently formed the basis of botanical teaching preparatory to pharmaceutical studies in ancient India and perhaps also to

agricultural and horticultural studies. A further importance attached to this work is that it can help in the identification of plants mentioned in ancient medical and other botanical treatises. For detailed account on *Vṛkshāyurveda*, the reader may refer to Ghosh and Sen (1971a), Roy (2008) and Geetha et al. (2013). Roy's (2008) work may also be referred to for a detailed list of plants known in ancient India, both cultivated and wild, as well as grasses. A significant contribution to botanical sciences was done by the ancient Tamils who lived in South India and whose civilization in known as Dravidian civilization. Besides being the first to suggest the Ecosystem concept with emphasis from a cultural perspective (a perspective for whose introduction we now give credit to UNESCO) 2,000 years ago, the Tamils were also the first to record ecosystem degradation (Krishnamurthy 2006). They recognized four major ecosystems: *Kurinji* (hill ecosystem), *Mullai* (scrub forest ecosystem), *Marutham* (the agricultural ecosystem) and *Neithal* (the seashore ecosystem) each with its own primary components (*Mudalporul*), core components (*Karupporul*) and cultural components (*Uripporul*). The primary components are land (space) and time (temporal component, both seasonal and daily); the core components are animals, plants, gods, crops, used products, food, names of people, profession, etc. (totally 14 components all which differed between different ecosystems); and the cultural components include lifestyle, moral values and cultural values. The degradation of *Mullai* and *Kurinji* ecosystems results in *Paalai* ecosystem which also has its own components. The Tamils had a clear concept on plants which they called stationary organisms (*Thavara*). *Tolkappiyam* considered by Tamils as the oldest available literary work of about 2,000 years old calls a plant (irrespective of its habit) 'maram' (which in modern Tamil means only a tree); this is in agreement with ancient Sanskrit word for tree, *Vriksa* (hence study of plants was called *Vṛkshāyurveda*). *Tolkappiyam* mentions 53 species of plants, although it is a grammar text. The *Sangam* literature (200 BCE–250 CE) mentions about 260 plant species, while the post-Sangam literature (250–600 CE) mentions around 185 plant species (Krishnamurthy 2006). The Tamils had an elaborate list of terms to describe the characteristics of different plants, which comes roughly to around 150 terms; they had also used a large number of similes to describe a plant part (e.g. comparing the flower bud of Jasmine to teeth of women, the tepals of *Glorisa* to the fingers of women dyed with *Lawsonia* leaf extract or the leaf margin of neem to the cutting edge of a saw). These were used for not only identifying but also classifying plants. Tolkappiar was perhaps the first person to distinguish plants into two major groups, monocots (belonging to grass group) and dicots (woody plants), based respectively on the absence or presence of wood (*Akakkaaz*), and based on this feature, he correctly classified palmyrah and other palms and bamboos under monocots (grasses). Seven stages in flower development were recognized and each stage in the order of development was indicated by separate terms: floral primordium (*Nanai*), young floral bud (*Arumbu*), almost mature bud (*Mugai*), (often with scent), mature bud (*Podhu*), open flower (*Malar*), pollinated flower (*Alar*) and fertilized flower in which all parts other than ovary are about to fall (*Vee*). They were also aware of all the floral parts and importance of pollen and pollination (including pollinating insects) and nectar. They classified flowers based on their colour, texture, shapes and structure. The Tamils had a unique way of naming plants; the names were extremely short (mostly two to four lettered) and easy to pronounce. When new plants were introduced, they were called by adding prefixes or suffixes to existing names of plants to which they show similarity [e.g. *Nyctanthes arbor-tristis*, when introduced, was called *Pavala malli* (coral jasmine) because its flowers resembled *malli* (*Jasminum* species)] (for details, see Krishnamurthy 2006).

In ancient China, lists of plants and herbal concoctions for medicinal purposes were made. These date back to the time of the warring states (481–221 BCE). During the Han dynasty (202–220 BCE) important works of the *Huangdi Neijing* and the famous pharmacologist Zhang Zhongjing were made. The Chinese dictionary

cum encyclopaedia *Erh-ya* (*c* 300 BCE) described 334 plants and classified them either as trees or shrubs, with a name and illustration.

Ancient Greece has also made equally good contribution to botanical knowledge. Since ancient Athens of the sixth century BCE was a very busy trade centre at the confluence of Egyptian, Mesopotamian and Minoan cultures at the height of Greek colonization of the Mediterranean region, there was active generation of botanical knowledge in this part of the world. Empedocles (490–430 BCE) foreshadowed Darwinian evolutionary theory in a crude formulation of the mutability of species and natural selection (Morton 1981). It is claimed that at this time a genuine non-anthropocentric curiosity about plants emerged. The major works on plants extended beyond the description of their medicinal uses to the topics of plant geography, morphology, physiology, growth and reproduction (Morton 1981). The most important botanist of this period was Theophrastus of Eressus (*c* 317–287 BCE). He was the student of Aristotle (384–322 BCE) who is considered as the Father of Biology. Aristotle was heading the *Lyceum*, an educational institution comparable to a modern University, in Athens. Although Aristotle's special treatise on plants is now lost, he at *Lyceum* not only questioned the superstitious medical practices, in which plants were also employed as medicines, called *rhizotomi*, but also promoted systematic medical use of plants (Morton 1981). Aristotle established the *Lyceum* botanic garden (Singer 1923; Reed 1942). Theophrastus is frequently and rightly referred to as the 'father of botany'. Of the 20 treatises believed to have been written by him, only two remained without being lost. These were *Historia Plantarum* (*Enquiry into Plants*) and *Cause Plantarum* (*Causes of Plants*) and they formed his lecture notes for the *Lyceum* (Morton 1981). The first work is nine books of 'applied' botany and deals with the forms and classification of plants, economic botany, agricultural methodologies, horticulture, etc. About 500 plants are described in detail by him and some botanical names such as *Crataegus*, *Daucus* and *Asparagus* used by him are in use even now. His second work deals with plant growth and reproduction (Reed 1942). In this work, he classified plants into trees, shrubs and herbs. He also stated that plants could be annuals, biennials or perennials. He distinguished determinate and indeterminate growths and made accurate descriptions of flowers (Thanos 2005; Morton 1981). His works thus included a clear exposition of the rudiments of plant morphology and anatomy, physiology and ecology (Harvey-Gibson 1919) and were believed (wrongly) by many, mainly in the western world, to form the starting point for modern botany.

The other famous work of Greek region is the five volume *Materia Medica*, a complete synthesis of ancient Greek pharmacology that appeared around 60 CE, by Pedanius Dioscorides (*c* 40–90 CE). It is a very important text on medicinal herbs obtained both from occidental and oriental regions. It contained a description of the medicinal information of about 600 herbs, but had only limited information on the botany of these herbs (Morton 1981). Hence, it remained the most important work for a very long time on medicinal aspects of plants (Singer 1923).

In ancient Rome, not much was contributed to basic botanical science but a good contribution was made to agriculture. In works titled *De Re Rustica*, four Roman workers, Cato (234–149 BCE), Varro (116–27 BCE), Columella (04–70 CE) and Palladius (fourth century CE), contributed to a collective work called *Scriptores Rei Rusticae*. This set the principles and practice of agriculture. Roman encyclopaedic author, Pliny the Elder (23–79 CE), dealt with plants in books 12–26 of his 37-volume *Naturalis Historia*. He often quoted Theophrastus and drew a distinction between true botany and applied botany (including agriculture and medicine) (Morton 1981).

1.3 Medieval Period

The medieval period or the *Middle Ages* is said to have started with the fall of Roman Empire in the fifth century CE up to the rise of the Italian Renaissance in the fourteenth century CE (a period of about 1,000 years). There is, however, a growing tendency to restrict the term 'medieval'

to the 400 years between the 'dark ages' (from ninth century CE) up to the Renaissance (Krishnamurthy 2005). It was a dark age for European science including botany and was a period of disorganized feudalism and indifference to learning and doing science. It was Greek science that was still followed in Europe without anything new being contributed by European scientists. For example, Theophrastus' botany remained the sole source of botanical knowledge for Europeans during this period. However, the Middle Ages were a golden period for science in India, China and the Arab world. In these places it may be called a period of herbals. Many new works on medicinal plants were produced in China and these included encyclopaedic accounts and treatises compiled for the Chinese Imperial Court. The most important characteristic of these works was that these were free of superstition and myth and with carefully researched plant descriptions and nomenclature; these also contained data on cultivation and economic and medicinal uses. Also produced during this period in China were elaborate monographs on ornamental taxa. However, there were no experimental studies in these works; neither were there detailed analyses of sexual reproduction, plant nutrition or anatomy in these works (Morton 1981).

The medieval period, particularly the period between the ninth and thirteenth centuries CE (400 years), was an age of Islamic Renaissance, when Islamic culture and science were at its best (Krishnamurthy 2005; Raju 2009). It is believed that Graeco-Roman texts were preserved, copied, rewritten in Persian and Arabic languages and extended (but strongly refuted by Raju that they are really not Graeco-Roman texts). The new texts prepared mainly emphasized the medicinal importance of plants. Kurdish biologist Dawud Dīnawarī (812–896 CE) is known to be the founder father of Arabic botany. His *Kitāb al-Nabat* (*Book of Plants*) described 639 plant species and discussed plant development from germination to their death (Fahd 1996). The Mutazilite philosopher and physician Ibn Sina (popularly known as Avicenna) (*c* 980–1037 CE) was very well known for his book *The Canon of Medicine*, which was a landmark book in the history of medicine. This book was very popular till the Renaissance period (Morton 1981). In the early thirteenth century, the Andalusian-Arabian biologist Abu al-Abbas al-Nabati developed an early scientific method for botanical study and introduced experimental as well as empirical techniques for testing, describing and identifying numerous materia medica and separating unverified reports from those supported by actual tests and observations (Huff 2003). His disciple Ibn al-Baitar (*c* 1188–1248) *wrote Kitab al-Jami fi al-Adwiya al-Mufrada*, a pharmacopoeia that described 1,400 species of which 300 were discovered by him. This was translated into Latin in 1758. This book effectively summed up centuries of Arab medical botany. For further information by early Islamic world, the reader may refer to Watson (1983).

The Indian contribution to botany in the medieval period is very substantial. This happened independently in North India and the Dravidian Tamil country in South India. While Chowdhury (1971b) has summarized the advancements made in North India, Krishnamurthy (2006) did it for South India. The medieval North Indian works on plants included *Prithviniraparyam* of Udayana, *Nyayavindutika* of Dharmottara, *Saddarsana-samuccaya* of Gunaratna and *Upasakara* of Sankara Misra as well as writings of Amir Khusrau. The South Indian works include a large number of Tamil literary works. Lot of information has also been obtained from several epigraphs (Swamy 1973, 1976, 1978). All these sources provide information on natural flora of India, the main agriculture crops cultivated, plants that were introduced into India through trade and other means from various parts of the world (particularly from West Asia, Central Asia, Africa, Europe, China and Southeast Asia), botanic gardens including *Nandavanas* (temple gardens), sacred groves and temple plants (*Sthala-Vṛiksās*) and plants that were used for medicine and purposes other than food. More than 600 wild plants in various vegetation types have been listed in the Tamil country alone (see list given in Krishnamurthy 2006). According to Chowdhury (1971b) and Krishnamurthy (2006), the main

agricultural crops cultivated in India in the medieval period include wheat, barley, rice, sesame, banana, sugar cane, cotton, millets, pulses and legumes, dye plants, plantation crops, a number of fruit trees (as plantations), palmyrah, coconut, oil crops, fibre plants, etc. Many varieties have been recognized in all the above. For instance, the Tamil Pallu literature mentions more than 150 rice varieties in the Tamil country alone (Krishnamurthy 2006). The most important plants introduced during this period are betel nut, betel leaf, coconut, pomegranate, castor, ground nut, chilli, cashew nut, cloves, mace, cardamom, camphor, some millets (from Africa), rose (in the Mughal period), almond, saffron, tamarind (the word being derived from Arabic *tamar-e-hind*), pear, apple, grape, water melon, strawberry, *Ocimum*, etc. Kitchen gardens became very popular during the medieval period in India. Most of these were growing vegetables and spices needed for food preparation at home. The most important plants recorded in such gardens (Chowdhury 1971b) included, among others, the following: brinjal, cucumber, bitter gourd, melons, turmeric, cumin, fenugreek, bottle gourd, ridge gourd, snake gourd, etc.

Although not much is known about botanic gardens in North India, the Mughal gardens established by Mughal kings in Delhi and other places like Agra are well known. Although these gardens contained exotic and native herbaceous and shrubby taxa, they lacked or are poor in trees. However, the Tamil country promoted the establishment of parks, gardens and *Nandavanas*, as evident from literary and epigraphic evidences (Krishnamurthy 2006). There were public gardens also. They provided three kinds of services: they provided the venues for people to spend their spare time; they provided plants required for the social ceremonies/rituals, worships, food and medicines of people and public institutions like temples; and they served as a means of plant conservation. Special bodies/officers were given the tasks of managing these gardens. Special mention must be made of *Nandavanas* (Krishnamurthy 2000). Temple Nandavanas served two purposes: they provided plants and plant parts offered (flowers and leaves) to the presiding deity and provided medicinal plants required for the local people since the temple priest was invariably the local physician also in medieval Tamil Nadu. The sacred groves attached to many temples of India were remnants of native forest vegetation and were the excellent means of conserving vegetation and plants (also animals) from a cultural perspective (Krishnamurthy 2004). Detailed account on sacred groves of the medieval period was not only brought to light, but also their role in conservation is provided by Krishnamurthy (2004). Dendrolatry (tree worship), another method of conservation of plants, was introduced as a specific method of conservation in medieval South India. Each temple was assigned (for various reasons) a specific plant (invariably a tree) as the sthala-vṛksum (=temple plant) which, in turn, is protected by people. Detailed account on temple plants is given in Krishnamurthy (2006) and Swamy (1978).

1.4 Renaissance Period

This period extends from fourteenth to eighteenth centuries CE. Also known as *Period of Enlightenment,* this period includes the so-called *Age of Confidence* or *Age of Reasoning* (Krishnamurthy 2005). The Renaissance period was first evident in Italy, followed by France, Germany and then Western Europe. During this period, the scientific method was perfected through the works of Gilbert, Descartes, Francis Bacon and others. Till the renaissance period the lives of people in Europe were mainly based in agriculture. But with the arrival of the printing press with movable type and woodcut illustrations, works on medicinal plants with descriptions of their useful virtues were published. The first of these medicinal books, called *Herbals*, showed that botany was still a part of medicine, particularly in Europe and the Arab world (but not in India), as it had been for most of ancient history (Morton 1981). Most authors of these herbals were curators of university gardens (Sachs 1890) and most of these herbals were refined compilations made out of classical texts, particularly of *De Materia Medica*. Otto Brunfel's

(1464–1546 CE) *Herbarium Vivae Icones* (1530), however, contained descriptions of about 47 species new to science accompanied by accurate illustrations. Another German botanist Hieronymus Bock's (also called Hieronymus Tragus) (1498–1554) *Kreutterbuck* of 1539 described plants that he found in nearby woods and fields using his own system of plant classification; these plants were illustrated in the 1546 edition of the work (Reed 1942). These two botanists along with Leonhart Fuchs (1501–1566) were called the 'three German fathers of botany'. However, it was Valerius Cordus (1515–1544) who pioneered the formal botanical-pharmacological description that was concerned with flowers and fruits, some on pollen, and also distinguished inflorescence types (Reed 1942). His 50-volume *Historia Plantarum* was published about 18 years after his early death at the age of 29. He also published a pharmacopoeia of great importance called *Dispensorium* in 1546. The term 'morphology' was coined by the German philosopher/biologist Johann Goethe (1749–1830). His very famous book 'Die Metamorphose der Pflanzen' (1790) linked comparative morphology with phylogeny through the word *metamorphosis;* by that time Charles Darwin's concept on evolution was not yet proposed.

In Holland, Rembert Dodoens (1517–1585), in his *Stirpium Historiae* (1583), included descriptions of many new species of plants from the Netherlands (Reed 1942). In England William Turner (1515–1568) in his *Libellus de Re Herbaria Novus* (1538) published names, descriptions and localities of many native British plants (Arber 1986). The major contribution of herbals to botany was to train people (especially those interested in medicinal plants) of the science of description, classification and botanical illustration. Even up to the end of the eighteenth century, medicine and botany were one and the same, but these works on herbals started to emphasize only medicinal aspects, thus eventually omitting the plant lore, and became the modern pharmacopoeias. Those works that omitted medicinal aspects became more botanical and evolved into the floras, which are modern compilations of plant descriptions. These floras were often backed by specimens deposited in a herbarium, a collection of dried plants that verified and authenticated the plant descriptions given in the floras (Oliver 1913).

The church, feudal aristocracy and an increasingly affluent merchant community supported science and art, particularly in Europe. Trade between different parts of the world started in right earnest and sea voyages were undertaken along with exploration of land resources, including plants. The voyages to distant places coupled with the eighteenth century enlightenment values of reason and science, thus, instigated another phase of encyclopaedic plant collection, identification, nomenclature, description and illustrations. More new lands were opening up to European colonial powers and the botanical riches collected and looted from these new lands returned to European botanists for description. This was a romantic era of botanical explorers, intrepid plant hunters and gardener-botanists. Significant collections came from West Indies (Hansloane 1660–1753); China (James Cunningham); East Indies, especially Moluccas (George Rumphius 1627–1702); Mozambique (João de Loureiro 1717–1791); West Africa (Michel Adanson 1727–1806); Canada, Hebrides, Iceland and New Zealand by Captain Cook's chief botanist, Joseph Banks (1743–1820); etc. (Reed 1942). These land and sea explorations brought botanical treasures to the public, private and newly established botanic gardens and introduced to a very eager population novel plants and crops, drugs, spices and condiments. Thus, plant trophies from distant lands decorated the gardens of Europe's powerful and wealthy in a period of great interest in botany ('botaniophilia') (Williams 2001). Bray's (1986) book agriculture, science and civilization in China provides detailed information as also of Astill and Langdon (1997), and Stone (2005) provides a glimpse of medieval farming and agriculture technology and decision-making in northwest Europe.

The medieval botanic gardens, usually attached to academic institutions, were mainly physic gardens which were often used for teaching medicine. The gardens were managed by

directors who were physicians. Botanic gardens of the modern type were established in North Italy, the first being at Pisa (1544) by Luca Ghini (1490–1556) who was the first chairman in botany, i.e. Materia Medica, at the University of Padua. Ghini, who was earlier in the University of Bologna, initiated collections of pressed and dried specimens, called *hortus siccus* (= garden of dry plants), and the first accumulation of plants in this way (Sachs 1890; Morton 1981). Special buildings called Herbaria were used for housing these specimens mounted on cards with descriptive labels. They were stored in cupboards in a systematic order for posterity or for easy transfer or exchange with other institutions. By the eighteenth century these physic gardens had changed into 'order beds' that demonstrated the classification systems that were being devised by botanists of the day. They also had to accommodate the influx of curious, beautiful and new plants pouring in from voyages of exploration associated with European colonial expansion.

The number of scientific publications started increasing during the Renaissance period. These publications and communications were facilitated by learned societies like Royal Society of London, founded in 1660. There were also support and activities of botanical institutions like the Jardin du Roi in Paris; Chelsea Physic Garden; and Royal Botanical Gardens at Kew, Oxford, Cambridge and Birmingham, as well as the influential and renowned private gardens and wealthy entrepreneurial nursery men (Henray 1975).

The seventeenth century marked the beginning of experimental botany and application of a rigorous scientific method, while improvements in the compound microscope (first discovered in 1590 by Jensen) initiated by Leeuwenhoek launched the new disciplines of plant anatomy. The latter's foundations were laid by the careful observations by Englishman Nehemiah Grew (1628–1711) of the Royal Society, London, who wrote his books *The Anatomy of Plants Begun* (1671) and *Anatomy of Plants* (1682) (Arber 1913), and Italian Marcello Malpighi (1628–1694) (Morton 1981), who was in the University of Bologna. Malpighi wrote *Anatome Plantarum* (1675). These two botanists made careful observations, descriptions and illustrations of the transitions from seed to mature plant, recorded the formation of stem and wood and discovered and named parenchyma and stomata (Reed 1942).

By the middle of eighteenth century, the collections of plants obtained through exploration needed to be systematically catalogued. This became the task of taxonomists. The word 'taxonomy' was coined by de Candolle in 1813. The basis for this taxonomic effort was laid through works carried out in the sixteenth and seventeenth centuries. Konrad Gesner (1516–1544) discovered many new plants and these were defined by similar flowers and fruits. Carolus Clusius (1526–1609) journeyed throughout most of Western Europe. He was the first to divide plants into classes. Italian physician Andrea Cesalpino (1519–1603) of the University of Pisa and the director of botanic garden of Pisa (1554–1558) wrote a 16-volume book *De Plantis* (1583). This book described 1,500 species of plants and his herbarium details of 260 pages and 768 mounted specimens are still preserved. He proposed classes of plants based largely on the detailed structure of flowers and fruits (Meyer 1854–1857); he also applied the concept of genus (Woodland 1991). He was the first to try and propose the principles of a natural classification reflecting the overall similarities between plants, and hence, his classification scheme was well in advance of his days (Morton 1981). Gaspard Bauhin (1560–1624) proposed two influential publications: *Prodromus Theatrici Botanici* (1620) and *Pinax* (1623). These publications brought order to the 6000 species described till then. In *Pinax* he used binomials and synonyms that may well have influenced Linnaeus's thinking. He was perhaps the first person to have insisted that taxonomy should be based on natural relationships between plants (Morton 1981). Joachim Jung (1587–1657) compiled a much-needed botanical terminology, till then used, to aid taxonomy. Based on Jung's work, John Ray (1623–1705) of England established the most elaborate and insightful classificatory system of the day (Reed 1942). His earlier work *Catalogues*

Stirpium Circa Cantabrigiam Nascentium (1680) was later expanded into *Synopium Methodica Stirpium Britannicarum* (a British Flora). This was followed by his *Historia Plantarum* (1682, 1688, 1704), a step towards world flora with addition of more and more plants that arrived into Britain from colonized countries and from explorations in distant lands. His classification was an extension of Cesalpino's system. He emphasized all parts of plants are important in classification. Ray's system included families, and this system was extended by Pierre Magnol (1638–1715) and Joseph de Tournefort (16765–1708) (Woodland 1991). Meanwhile, the term 'angiosperm' was coined in 1690 by Paul Hermann.

Carl Linnaeus (1707–1778) of Uppsala Botanic gardens in Sweden is considered as the most important taxonomic botanist. He proposed the *sexual system of classification,* based on stamen and pistil characteristics, although *artificial*. He also standardized the binomial nomenclature whereby each plant is known by a generic name and a specific epithet. His important works include the following: *Systema Naturae* (1735), *Genera Plantarum* (1737), *Philosophia Botanica* (1751) and *Species Plantarum* (1753), which is the most important among all his works. He described around 6,000 species of plants. The Linnaeus' system was later elaborated by Bernard de Jussieu (1699–1777) and then by Antoine-Laurent de Jussieu (1748–1836) by including about 100 orders (i.e. present-day families). Frenchman Michel Adanson (1727–1806) wrote his work entitled *Familles des Plantes* (1763–1764), in which he extended the then current system of plant names; he emphasized that a natural system of classification must be based on all characters and that an equal importance must be given to all characters (Morton 1981).

During the Renaissance period, knowledge on plant physiology also started to emerge. Jan Helmont (1577–1644), through his experiments and calculations, noted that the weight increase of a growing plant cannot be derived purely from the nutrient inputs of the soil and that it must be related to water uptake (Reed 1942). Stephen Hales of England (1677–1761) established through quantitative experiments that there is an uptake of water by plants by roots and a loss of the same by transpiration; both these processes are influenced by environmental conditions. He distinguished 'root pressure', 'leaf suction' and 'imbibition'. He also recorded that the major direction of sap flow in woody tissue is upward. His results were published in *Vegetable Staticks* (1727). It was during the Renaissance period that it was established that air makes a very considerable percentage of the substance of vegetables (Morton 1981). Joseph Priestley (1733–1804) discovered oxygen and its production by plants. Jan Ingenhousz (1730–1804) mentioned that only in sunlight the green parts of plants absorb air and release oxygen, while at night the air (CO_2) is released from all parts. He published these results in his work *Experiments upon Vegetables* (1779) (Reed 1942).

Rudolf Jacob Camerarius (1665–1721) was the first to establish the sexuality of plants conclusively by experiments. He wrote a detailed paper dated 1694 titled *De Sexu Plantarum Epistola* in which he stated 'no ovule of the plant could ever develop into a seed from the female style and ovary without first being prepared by the pollen from the stamens, the male sexual organs of the plant' (Reed 1942). More information on the subject can be found in Sturtevant's *A History of Genetics* written in 1966. The German naturalist Joseph Gottlieb Kolreuter (1733–1806) extended this work by recording the function of nectar in attracting pollinating insects and the role of wind in pollination. He also produced deliberate hybrids, observed pollen grains under the microscope and demonstrated how the transfer of 'matter' from the pollen to the ovary induces the formation of the embryo (Reed 1942). In 1793, Christian Konrad Sprengel (1750–1816) from Germany did considerable research on the pollination of plants and the interaction between flowers and their insect visitors later called as pollination syndrome. His monumental work *Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen* (Berlin 1793) made him as one of the founders of pollination ecology. He was also the first to describe the role of nectar guides in pollination and the adaptive floral mechanisms for pollinations.

1.5 Modern Period

This period extends from nineteenth century till date. This is also considered as the golden period of science, when science became a forefront of human activity. Much advancement took place in botanical science during this period. Emphasis was given (and continues to be given) to technology that developed on scientific principles. Great changes took place in the way science was done and practiced. Theoretical approaches, intuition, reasoning, experimentation, mathematical applications and inter-, trans- and multidisciplinary approaches, either individually or in coordination, were followed to solve intricate problems (Krishnamurthy 2005). People who did science for the sake of science in their homes and garages also disappeared. Science became a full-time profession and scientists started specializing in specific disciplines or subdisciplines. Scientists also began to specialize between theorists and experimentalists, since the expertise for both roles did not occur in the same person. Science also became big involving large funds, huge laboratories, sophisticated instruments and techniques and involved great teamwork (Krishnamurthy 2005). Science also became highly institutionalized.

In the nineteenth century, Botany was greatly stimulated by the appearance of the first 'modern' textbook by Matthias Schleiden entitled *Grundzüge der Wissenschaftlichen Botanik* (Principles of Scientific Botany) published in English in 1849 (Morton 1981). The method of botanical communication changed very drastically. Scientific journals started publishing results of botanical investigations (Reynolds Green 1909). Botanical research papers were initially published in general botany journals such as Annals of Botany, American Journal of Botany, Botanical Journal of the Linnaean Society, New Physiologist, Botanical Review, etc., but because of increasing specialization several specialized journals relating to specific groups of plants such as algae, fungi, lichens, bryophytes, pteridophytes and seed plants as well as those related to specific areas of plant sciences such as molecular biology, genetics, cytology, cytogenetics, plant morphology, taxonomy, wood science (and technology), anatomy, palynology, ecology, physiology, plant reproduction, etc. More importance is being given to clearly exciting, emerging and rapidly advancing areas of research. There was also an evolution of journals in terms of their contents: journals with original research papers, with short communications and/or reports, with review articles alone, with abstracts of articles only or with contents of journals only. Several e-journals have also come into existence with the advent of the twenty-first century. Botanical information and data were increasingly being obtainable through rapid advancements in information technology. Several databases are now available.

One of the very important and refreshing trends in botanical research that got initiated in the 1970s was the use/selection of *model plants* system on which research would be concentrated upon. This research would place emphasis on the molecular basis of all developmental/physiological/genetical events. The foremost among these model plants was *Arabidopsis thaliana* (Brassicaceae) because of its several advantages, especially its short life cycle. The *Arabidopsis* genome project was initiated on a grand scale in 1990 as a multinational project and this 10-year effort ended in 2000 with great success. An outcome of this project is the finding that there are about 25,500 genes in this taxon (Somerville and Dangl 2000; Slater et al. 2003). However, only about 1,000 of these genes have been assigned a function by direct experimental evidence. About 70 % of the 25,500 predicted genes have been assigned role, a function based on their sequence similarity to proteins of known function in other organisms. Incidentally most of the genes are involved in plant metabolism and defence. The 30 % genes were not assigned a function since they have a high degree of similarity to genes of unknown function from other organisms.

Encouraged by the success of this project, a new 10-year project of comparable import was started in 2000 entitled 'Plant Biology in 2010' by the US National Science Foundation (the NSF 2010 project) with the ambitious goal of knowing

the functions of all plant genes by the year 2010 either by inactivation or overexpression followed by analysis of the resulting phenotypes. This will enable us to understand the genetic basis of basic biology of plants within 2010. This knowledge will also facilitate the development of a virtual plant—a computer model that will use information about each gene product to simulate the growth and development of a plant under many environmental conditions. The goals of this 10-year project were as follows:

1. Plant artificial chromosomes (PAC).
2. Identify *cis*-regulatory sequences of all the genes.
3. Identify regulatory circuits controlled by each transcription factor.
4. Determine biochemical function for every protein.

   Determine and describe 3-D structures of members of every plant-specific protein family.
5. Undertake systems analysis of the uptake, transport and storage of ions and metabolites.
6. Describe globally protein-protein, protein-nucleic acid and protein-other interactions at organ, cellular and subcellular levels under various environmental conditions.
7. Survey genomic sequencing and deep EST sampling from phylogenetic node species.
8. Define a predictive basis for conservation versus diversification of gene functions.
9. Compare genomic sequences within species (comparative genomics).
10. Develop bioinformatics, visualization, and modelling tools that will facilitate access to all biological information about a representative virtual molecular plant.

1.6 Taxonomy and Systematics

Because of the already laid strong foundation in the previous periods of botanical history, the nineteenth century systems of plant identification were developed that were comparable to dichotomous keys for all taxa like family (or natural order), genus and species. The choice and sequence of the characters may be artificial in keys designated purely for identification (*diagnostic keys*) or more closely related to the natural or phyletic order of taxa (*synoptic keys*). Augustin de Candolle (1778–1841) succeeded de Jussieu in managing the plant taxonomic project *Prodromus Systematis Naturalis Regni Vegetabilis* (1824–1841); this project involved 35 authors. It contained all the dicots known in his time, around 58,000 in 161 families, and he doubled the number of recognizable plant families. This project was completed by his son Alphonse (1806–1893) between 1841 and 1873 (Morton 1981).

The next major work in plant systematics was that of Bentham and Hooker; their work *Genera Plantarum* (1862–1883) covered 202 families and this remained influential until the end of nineteenth century (still followed and influential in India and its Herbaria). This is a natural system of classification, based on relative similarities and difference between taxa. But, Darwin's theory of evolution introduced modifications in plant classificatory systems. Hence, the subsequent systems of botanical classifications reflected and discovered evolutionary and phylogenetic relationship between plants. The more important phylogenetic systems that emerged subsequently are those of Eichler, Engler and Prantl, Hutchinson, Takhtajan, Cronquist, Oswald Tippo and others. All these systems involved the characters and characteristics (mostly morphological) that were considered, on consensus by most botanists, as either primitive or advanced. Subsequently, molecular phylogenetic systems of classification emerged, which ignored morphological characters; these relied mainly on DNA sequence data, although anatomical, embryological, serological, ecological, cytological, biochemical and other characters have also been exploited by many botanists (see, e.g. Soltis et al. 2005). These data enabled the Angiosperm Phylogeny Group (APG) to publish in 1998 (with subsequent updates) a phylogeny of flowering plants giving explanations to many questions about relationships between angiosperm families, genera and species. Angiosperms were the first major group of organisms, whose

classification was overhauled as the result of molecular studies. The theoretical possibility of a practical method for identification of plant species and commercial plant varieties by DNA barcoding and fingerprinting is the subject of active current research. DNA barcoding is of particular interest in the correct authentication of substitute and adulterant plant raw drugs.

1.7 Cytology and Anatomy

Charles Mirbel (1776–1854) published in 1802 his work entitled *Traite d'Anatomie et de Physiologie Vegetable*, while Johann Moldenhawer (1766–1827) published in 1812 his book on *Beytrage zür Anatomie der Pflanzen*. These two books summarized all the available information on the anatomy of plants till that time. The second book described the method of separating plant cells from one another as well as vascular and parenchymatous tissues, vascular bundles, cambium, tree rings, stomata with guard cells, etc. (Morton 1981). All these led Matthias Jakob Schleiden, Theodore Schwann (1810–1882) and Rudolf Virchow (1821–1902) to propose the *cell theory* in 1838–1839. Robert Brown first discovered the nucleus in 1831. Carl Wilhelm von Nägeli (1817–1891) of Switzerland published a paper in 1842 accurately describing cell division; the thread-like bodies that he called 'transitory cytoblasts' were later identified as chromosomes. From 1870 to 1880 it became very clear that the nucleus of the cell is never formed a new, but that it always is derived from another pre-existing nucleus. In 1882, Walther Flemming (1843–1905) observed the longitudinal splitting of chromosomes in the dividing nucleus and concluded that each daughter nucleus received half of each of the chromosomes of the mother nucleus. In early twentieth century it was found that the number of chromosomes in a given species is constant. Carl Wilhelm von Nägeli (1817–1891) was also one of the first to differentiate the plant cell wall from cytoplasm and nucleus; in 1846 German botanist Hugo von Mohl called the latter two together as protoplasm, although many attribute to Purkinje the coining of the word protoplasm in 1858. Leydig (1857) was the first to describe and define that a plant cell is made of two parts, the first part being nucleus that is embedded in the cytoplasm which is the other part. Hugo von Mohl (1805–1872) summarized the work on plant anatomy up to 1850 in his book *Die Vegetabilische Zelle*. Julius von Sachs established during the later part of the nineteenth century the continuity of protoplasm between plant cells through cell walls. Golgi bodies were discovered by Camillo Golgi in 1898 and were named after him.

Nägeli was also the first to use the botanical term 'meristem' to refer to the undifferentiated, actively dividing apical cell at the growing point of plants. In 1858, he showed how important the sequence of events in cell division was in determining the form of plant parts. The *histogen theory* of meristem organization was proposed by Johannes Ludwig Emil Robert von Hanstein (1822–1880) in 1868 and then perfected in 1870. He was also the first to use the word 'hypophysis' to the initial root meristem at the radicular pole of the embryo. Heinrich Anton de Bary in 1877 published his encyclopaedic work on comparative plant anatomy. The *tunica-corpus* organization of the shoot apical meristem organization was first brought to light by Ernst Johannes Schmidt (1877–1933) in 1924. That the shoot apices of many gymnosperms did not have a tunica-corpus organization prompted Foster to describe the *cytohistological zonation* concept for this group of plants in 1938. Subsequently, this concept was extended to angiosperms as well. *The quiescent centre (QC) concept* of root apical meristem organization was first brought to light by F.A.L. Clowes in 1957. The fact that a quiescent zone is an integral part of shoot, root and lateral meristems like vascular cambium was emphasized by B.G.L. Swamy and Krishnamurthy in 1978 and that the quiescent zones of shoot and root apical meristems were respectively derived from epiphysis and hypophysis of the embryo was also brought to our attention first by same two authors in 1975 and 1977. The non-procambial origin of vascular cambium was demonstrated by B.G.L. Swamy and Krishnamurthy in 1981. Structure of nodes and the three types of

nodes (unilacunar, trilacunar and multilacunar) were first described by Sinnott and Bailey in 1914. Detailed information have been contributed on the development and structure of the difference of plant organs, tissues and cell types and the detailed history of all these has been summarized in Esau (1965), Larson (1994) and Evert (2006).

1.8 Plant Physiology and Biochemistry

Studies on plant nutrition were initiated with the work of Théodore de Saussure's (1767–1845) entitled *Recherches Chimiques Sur la Végétation*. He demonstrated the similarity of respiration of both plants and animals and the fixation of CO_2 and water. He also showed that just minute amounts of salts and nutrients (after analysis of plant ash) have a powerful influence on plant growth (Morton 1981). In 1847, Mayer suggested the capture of solar energy by plants. In 1771, Joseph Priestley implicated oxygen (at that time it has not known by this name) in photosynthesis. In 1782, Jean Senebier implicated CO_2 in photosynthesis and in O_2 production during photosynthesis. In 1804, N.T. de Saussure implicated water in photosynthesis. By 1850, the chemical structure of many plant constituents was taken up for serious research (Morton 1981). The connection between *chlorophyll* (the name was first given in 1818) and starch production in the presence of sunlight only was found in 1864 (Reed 1942). In 1862, Sachs observed that starch was formed by green cells only. These discoveries led to the understanding of *C3 photosynthesis*. In 1903, chlorophyll *a* and *b* were first separated by thin layer chromatography (TLC). The production of carbohydrate during photosynthesis was shown by van Sachs in 1884. In the early 1930s, C. B. van Niel showed the similarity in the photosynthetic process between the different photosynthetic organisms. In the late 1930s, Robin Hill and R. Scarisbrick showed that isolated chloroplasts could release O_2 in light. Hill was also known for deciphering the reactions during light reaction of photosynthesis, which is also called Hill reaction, after his name. The most important process during light reaction is the *photolytic splitting* of water to release O_2 (which for a long time was believed to come from CO_2); this aspect came into light in 1941 through the work of Samuel Ruben and Martin Kamen. *Photosynthetic phosphorylation* was discovered by Daniel Arnon in 1954. In the 1950s, Robert Emerson's researchers resulted in the discovery of *Emerson effect*, which in turn paved the way for the discovery of photosystems I and II and oxygen-evolving complex (OEC). Mervin Calvin, Andrew A. Benson and James A. Bassham between 1946 and 1953 explained the details of *Calvin cycle* (C_3 cycle) and the details of fixation of carbon (from CO_2). This phase of photosynthesis was called Dark reaction or *Blackman's reaction*. The C_4 photosynthetic cycle was discovered due to the researches of H.P. Kortschak, C.E. Hartt, G.O. Burr, M.D. Hatch and C.R. Slack. It was also shown that C_4 plants have *Kranz anatomy* which is characterized by the presence of special bundle sheath parenchyma cells around the vascular tissue of the leaf. *Crassulacean acid metabolism* (CAM) was subsequently discovered and found to be unique to succulent plants. The process of *photorespiration* was elucidated through the researches of Otto Warburg of Germany, who in 1920 discovered Warburg effect, where photosynthesis is inhibited by O_2.

Jagadish Chandra Bose's (1858–1937) contributions in the field of biophysics led to the demonstration of the electrical nature of the conduction of various stimuli like wounds in plants, which were subsequently proved. He was also the first to study the action of microwaves in plant tissues and corresponding changes in the cell membrane potential. His work was on the mechanism of the seasonal effect on plants, the effect of chemical inhibitors on plant stimuli and the effect of temperature. From the analysis of the variation of the cell membrane potential of plants under different circumstances, he hypothesised that plants can 'feel pain and understand affection'.

The discovery of respiratory pathway between 1912 and 1935 involving *glycolysis* (the term was first introduced in 1909) by Carl and Greti Cori) and *Krebs cycle* [discovered by Hans Krebs

(1900–1981) in the 1920s and 1930s]. The term citric acid cycle was proposed by Hans Adolf Krebs in 1937. The *pentose phosphate pathway* (PPP) was elucidated soon. The discovery of ATP as the energy house of the cell and its location in mitochondria threw light on the energy turnover in plants. In 1968, David E. Atkinson explained many reasons as why ATP, ADP and AMP should be the master controllers of respiration.

Hugo von Mohl (1805–1872) explored solute transport and the theory of water uptake by the roots using the concepts of cohesion, transpirational pull, capillarity and root pressure (Morton 1981). The discovery and naming of *Osmosis* was done by Dutrochet, René-Joachim-Henri (1776–1847). In 1855, Adolf Fick published his famous Fick's law of diffusion of molecules, which enabled the calculation of the rates of molecular diffusion in biological systems, including the diffusion of water and gases. J. Willard Gibbs in the 1880s developed a thermodynamic measure that allows us to link the energy available to do work as it passes across the boundary between a system and its surrounding. This measure is called Gibbs free energy. In 1887, J.H. van't Hoff discovered an empirical relationship that allows the calculation of an approximate osmotic potential from the molal concentration of a solution. The actual measurement of osmotic potential of plant cells was made by P. Pfeiffer in 1877. Henry H. Dixon, in 1914, proposed the cohesion *theory of ascent of sap*, but he started working on this concept as early as 1884 with the help of John Joly. Although efforts to understand transport of photosynthates and other organic substances inside the plant may be said to have started as early as 1675 by the Italian botanist Marcello Malpighi through his girdling experiments on trees, it was Stephen Hale, in 1727, who repeated these experiments and indicated the probable transport of these substances. E. Munch of Germany suggested the pressure-flow (or mass-flow) hypothesis of phloem transport in 1926. Phloem loading of photosynthates was suggested in 1949 by Brunhild Roeckl, but was proved conclusively by Donald R. Geiger and his co-workers in 1973 using autoradiography techniques. Particularly since the 1960s, there have been rapid advances in the understanding of processes such as transpiration and transport of water and sap within plant tissues, the temperature dependence of rates of water evaporation from the leaf surface and the molecular diffusion of water vapour and CO_2 through stomata. Precise description of rates of gas exchange between plants and the atmosphere was also made. Movement of chemicals through extracellular matrix was investigated. The proton pump hypothesis was first suggested by Peter Mitchell in 1961, while work on plant channel proteins began only around the mid-1980s.

A textbook on plant physiology *Vorlesungen Über Pflanzenphysiologie* was published in the year 1882 by the German botanist Sachs, which summarized the available knowledge on plant physiology till that time. The discovery of enzymes paved the way to explain many biochemical reactions in plants. Enzymes are proteins or glycoproteins that serve as organic catalysts. The mechanism of enzyme action was first explained by the *lock-and-key hypothesis* in the year 1884 by Emil Fischer. However, the present understanding of enzyme action is based on the *induced fit hypothesis*, proposed by Daniel E. Koshland, Jr. in 1973. According to this hypothesis the active site of enzyme action can change. Another important aspect of the mechanisms of enzyme action is the *Michaelis-Menten constant* or the K_m value which refers to the substrate concentration required to cause half the maximal enzyme reaction rate.

Significant discoveries relating to nitrogen metabolism, including ammonification, nitrification and nitrogen fixation, had to wait for advances in biochemistry in the late nineteenth century and this was followed by the elucidation of protein synthesis mechanisms in the twentieth century.

The concept of plant growth regulators or hormones first emerged in the latter half of the nineteenth century when Julius von Sachs (1832–1897), in 1878, first suggested the possible presence of specific organ-forming substances. Charles Darwin experimented with shoot movement towards light and root movement in response to gravity. Further work on growth

implicating growth regulators was done by Boysen Jensen in 1919 and Paal in 1919. The *auxins* (from the Greek word 'auxein', which means to grow) were first named in 1926 and their role in controlling growth was first outlined in 1934 by the Dutch biologist Frits Warmolt Went (1903–1990) whose 1928 experiment demonstrated the existence of auxin in plants. He was also perhaps the first to devise the *Avena* coleoptiles assay for auxins. The first known auxin IAA was first isolated from plants about 50 years later, although it was first identified in urine in 1930, it was Kenneth Vivian Thimann (1904–1997) an English-American plant physiologist and microbiologist known for his studies of plant hormones, which were widely influential in agriculture and horticulture. He first isolated and determined the structure of auxin, the first known plant hormone. In 1948, the role of auxins was greatly highlighted, through external application experiments on intact plant parts, as well as on plant explants grown under in vitro conditions as attempted first by *Frederick Campion Steward* (1904–1993), a British botanist and plant physiologist in the 1950s. The synthetic auxin 2, 4-D was the first commercial selective herbicide; it formed an ingredient of Agent Orange that was used as a defoliant and herbicide by the US Army during the Vietnam War. The other synthetic auxins were NAA, MCPP, picloram, etc. *Cytokinins* were discovered in 1964, although their presence was suggested by *Gottlieb Haberlandt* (1854–1945) as early as 1913. Its presence as a component of coconut milk was suggested by van Overbeek in the 1940s, while Skoog and his coworkers named kinetin as a cytokinin in the 1950s. Gibberellic acids (GAs) were first discovered by Yabuta and others from the fungus *Gibberella fujikuroi* in 1934, although their presence was first inferred in the 1880s with the study of bakanae disease (foolish seedling disease of rice). From the 1950s, several GAs were discovered and more than 120 GAs are known from different groups of plants. Ethylene was suggested as a plant growth regulator by the Russian Dimitry N. Neljubow in 1901 but was shown to be decisively so only in 1934. Brassinosteroids were known as plant hormones first in the pollen of rape seed plant by 1979. ABA as a growth regulator was first demonstrated by Addicott and his co-workers as well as by Wareing independently in 1963. Turgorins as the growth regulators in leaf movements (sleep and touch movements) were demonstrated in 1983 by Schildknecht. We now know how the concentration of hormones and receptors can regulate cell, tissue, organ and even whole plant responses and how the levels of non-receptors, but interacting proteins, can modify the effectiveness of receptors. We also now know that there are complex interacting components of hormones, signals, receptors and attendant proteins, linked to the transduction events required to instruct the plant genomes (nuclear, chloroplast and mitochondria).

Gravitropism was first recognized by Thomas Knight, and the role of root cap in this process was suggested by Ciesielski in 1872; he found statoliths in them. The statolith theory of gravitropism was proposed almost simultaneously by Bohumil Némec in 1901 and Gottlieb Haberlandt in 1902. *Circumnutation* was discovered by the Darwins in 1880. Circumnutation refers to the oscillatory movement of the shoot tip while growing. To explain this, Johnsson first proposed the *gravitropic overshoot theory* in 1971. The Cholodny-Went model for phototropism was proposed following discoveries made on phototropism. Blaauw in 1909 discovered the role of light in phototropism, which led to the concept of photomorphogenesis (control of morphogenesis by light) and discovery of phytochromes as photoreceptors. Research leading to phytochrome detection and isolation was accomplished at the US Department of Agricultural Research Station at Beltsville, Maryland, between 1945 and 1960. The P_r and P_{fr} pigments, homodimers of two identical polypeptides, each with a molecular weight of about 120 KDa which respectively absorb red and far red wavelengths of light, were discovered. This was followed by cryptochrome, the blue/ UV-A photoreceptor, discovery.

In the 1920s, the work of Rose Stoppel of Germany suggested the first idea on *biological clock*. The idea was further refined by Erwin Bünning (1906–1990) and Stern in 1930; their

work added strongly to the concept of *circadian rhythm*. WW Garner and HA Allard of USDA, Beltsville, Maryland, discovered *photoperiodism* in 1920 and their work led to the discovery of long day (LD), short day (SD) and day neutral (ND) plants. How light/dark duration affect flowering was explained in their work and the works of others who followed them. Mikhail Chailakhyan suggested, in 1937, the presence of the hypothetical *florigen*, which is synthesized in leaves under inductive day length and transported to the shoot apex, where it induces flowering. Hans Mohor, in 1983, proposed that photomorphogenesis has two important stages: (1) pattern specification in which cells and tissues develop and become competent to react to light and (2) pattern realization when light-dependent process occurs. Recent work based on genetical and biochemical data suggest that florigen is a protein encoded by the gene, *FLOWERING LOCUS T* (*FT*). The structural and biochemical features of the florigen protein complex and molecular basis for the multifunctionality of FT proteins is now known but further details are beyond the scope of this chapter. The importance of temperature in flowering, a phenomenon known as *thermoperiodism* [a word introduced by Frits Warmolt Went (1903–1990) in 1957], was realized. The role of cold treatment or *vernalization* was first discussed by Trofim Lysenko, a Russian botanist; it was he who first introduced this word. Georg Melchers of Germany suggested the existence of a hypothetical flowering hormone *vernalin*, which is believed to control vernalization.

1.9 Reproduction

Much was learnt about reproductive mechanisms of bryophytes (both liverworts and mosses) and algae even by the middle of the nineteenth century. In his book *Vergleichende Untersuchungen* written in 1851, Wilhelm Hofmeister (1824–1877) demonstrated that the process of sexual reproduction entails an 'alternation of generations' between sporophytes and gametophytes in ferns and bryophytes (Reed 1942). Most initial information on the life cycle patterns and alternation of generation were brought out through the combined work of William Farlow (1848–1919), Nathanael Pringsheim (1833–1894), Frederick Orpen Bower (1855–1948), Eduard Adolf Strasburger (1844–1912) and others (Reed 1942). Strasburger found that the nuclei of reproductive cells have a reduction division (halving of chromosomes, now called meiosis).

Detailed studies on angiosperm flower may be said to have started with the German poet Johann Wolfgang von Goethe (1749–1832) who published the very influential essay *Versuch die Metamorphose der Pflanzen erklären* in 1790. The flower, accordingly, is a metamorphosed vegetative shoot and the floral organs are modified basic generalized organ 'blatt', and leaves are just one of the forms that *blatt* could adopt. This is often called *classical concept of flower* (and floral organs). In line with classical concept of flower, the sepals and petals are quite similar to the leaves in form, but stamens and carpels are not so. However, Robert Brown (1773–1858), a Scottish botanist and palaeobotanist, independently got evidence to show that all floral organs share leaf-like properties. For carpels, his interpretation is that leaf-like carpels joined along their edges and that the ovules arise along these edges. [He also made important contributions to botany largely through his pioneering use of the microscope. His contributions include one of the earliest detailed descriptions of the cell nucleus and cytoplasmic streaming and the observation of Brownian motion.] In contrast to this classical concept of carpel, the conduplicate carpel concept was proposed by Bailey and Swamy in 1948 after detailed and critical analysis of the primitive angiosperms. According to the conduplicate carpel concept, the ancestral leaf-like carpel folds conduplicately and not involutedly and that the fusion does not take place on the margins but typically submarginal. The carpels of all ancient primitive angiosperms show these features. Further modifications from this ancestral carpel resulted in the different forms of carpels evident in more advanced taxa. Comparative studies on the development of flowers reached their zenith in the later part of the twentieth century. For example, SEM studies of flower development by

Sattler and his students in Canada enabled us to understand precisely the order of initiation of floral organs. Such comparative studies provided insights into the relative rates and possible directions of evolution of floral form. For instance, Endress in 1994 highlighted three aspects of flower structure with different underlying rates of evolutionary change: organization, construction and mode. Essentially, these correspond with first, the floral blueprint or bauplan that defines the number and position of floral organs (including the degree of their fusion); second, the basic three-dimensional structure of the flower (*gestalt*); and third, the later elaboration of specialized characteristics. The blueprint is fairly stable in evolutionary terms, growth patterns that decide the size and shape of the flower less stable and elaboration such as colour and aroma relatively unstable. Genetic control of floral initiation, floral meristem identity, floral organ identity, floral symmetry, microsporogenesis and microgametogenesis, megasporogenesis and megagametogenesis, events leading to double fertilization, embryogenesis, endosperm development, seed and fruit development (including ripening) and seed germination has been extensively investigated in the last four to five decades. The information relating to these have been obtained not only through mutants but also through the MADS box genes in model plants like *Arabidopsis*, *Antirrhinum*, cereals like rice and maize, etc. The *MADS box* is a conserved sequence motif found in genes which comprise the MADS-box genes family. The MADS box encodes the DNA-binding MADS domain. MADS-box genes in a number of plants have now been worked out as also their evolution.

The subject of pollination biology was extensively detailed in Paul Knuth's (1906) *Handbook of Flower Pollination* and also by Charles Darwin in 1868, 1876 and 1877 and several others. The impact of these studies led to the discovery of *heterosis* (hybrid vigour) in 1914 by George Harrison Shull (1874–1954), an eminent American plant geneticist who revolutionized plant breeding and agriculture crops. There was revival of research on pollination biology/pollination ecology, reproductive biology during the mid-1950s to date by H.G. Baker, K.S. Bawa, S.C.H. Barrett Real (1982) and their associates in the USA and Canada and in India by R. P. Kapil, K.R. Shivanaa, Bir Bahadur, C. Subba Reddy, A.J.S. Raju and others. The impact of these studies led to better understanding of various pollination mechanisms in crop plants, the role of bees in pollination, evolution of various breeding systems, the genetic basis of incompatibility, pollen flow, plant-insect interactions, nectar chemistry and the role of nectaries in agriculture.

With reference to details of angiosperm reproductive development, the work of Amici in 1947 on *Orchis* is the starting point. He reported that a germinal vesicle is present in the ovule before the entry of pollen tube and that the vesicle, in due course, developed into the embryo. William Hofmeister in 1862 demonstrated the formation of embryo from the egg within the embryo sac in flowering plants. This was followed by similar observations on embryo development by Hanstein in 1868 and 1870 and by Treub in 1879. In 1878, Strasburger (1849–1912) noticed the fusion of sperm nucleus with egg nucleus. The classification of embryo sac types was standardized by Panchanan Maheshwari (1904–1966), noted for his extensive work on classical embryology and for his invention of the technique of test-tube fertilization of angiosperms in 1950. This work enabled later researchers for the creation of new plant hybrids that could not previously be obtained. The dual origin of anther tapetum (i.e. partly from the parietal wall tissue and partly from the connective) was first demonstrated by K. Periasamy and BGL Swamy in 1960.

The classification of fertilization (syngamy) into pre-mitotic, intermediate and post-mitotic types was done by Gerassimova-Navashina. Work on intra-ovarian pollination and test-tube fertilization was first carried out by Kusum Kanta and P Maheshwari in 1962. In vitro fertilization of isolated sperm and egg was subsequently effected. The importance of chalaza in the pre-fertilization ovule development was indicated by Netolitsky in the early years of the twentieth century, while its importance in seed development was brought to light by E. J. H. Corner of Cambridge University in 1960 and by

K. Periasamy in 1962. However, ultrastructural details of testa of various families using SEM were made by Heywood (1971), Behnke and Barthlott (1983), Barthlott (1984), Bahadur et al. (1989) and several other researchers in India. Bir Bahadur (1983, 1984, 1989) made SEM studies on seeds and on pollen by Bahadur et al. (2013), Murthy and Bahadur (1995) and Murthy et al. (2005). Vast contributions by Delhi group especially Prof. B.M. Johri, H.Y. Mohan Ram, I.K. Vasil, R.N. Kapil and N. Bhandari, A. K. Pandey and others were made to embryology. A detailed analysis and classification of helobial endosperm and the report of its restriction to monocots were done by B.G.L. Swamy (1918–1980) and Parameswaran in 1959. Details of ruminate endosperm and its development and classification were done by K. Periasamy in 1962. Detailed accounts on embryo development in dicots were given by R. Souèges, P. Créte, D.A. Johansen and P. Maheshwari and in monocots by Barbara Haccius and B.G.L. Swamy (see Krishnamurthy 2015).

Although vegetative reproduction in plants is known from historic days, non-sexual reproduction involving the ovary/ovule was not known for a long time; seedless fruits, however, were known to people. *Apomixis*, the term applied to non-sexual reproduction (in contrast to *amphimixis* which means sexual reproduction), was coined by Winkler in 1908. Apomixis results in 'seeds' without fertilization. The term parthenocarpy was introduced by Noll in 1902 to indicate 'fruits' that develop without fertilization and even without pollination; such 'fruits' do not contain true seeds, but often shrivelled seed-like structures. True polyembryony was reported in gymnosperms and orchids among angiosperms, but other forms of polyembryony were also reported.

1.10 Genetics and Heredity

The cytological basis of chromosome-gene theory of heredity may be said to have originated with the hybridization experiments done on garden pea plant by the Austrian monk Gregor Johann Mendel (1822–1884). This work resulted in the establishment of the laws of inheritance, often called Mendelian laws. August Weismann (1834–1914) identified chromosomes as the hereditary-material carrier and proved that inheritance takes place only through gametes or germ cells. William Bateson (1861–1926) first introduced the word 'genetics'. The 'factors' of Mendel that controlled characters were actually proved to be genes and the word 'gene' was introduced by Wilhelm Johansen in 1909. In 1911, Thomas Hunt Morgan (1866–1945), an American evolutionary biologist and geneticist proposed that genes are arranged linearly in the chromosome and that they are all linked. By 1926, he was able to outline a theory of gene and its structure and function. Morgan, Sturtevant, Muller and Bridge wrote in 1915 the book 'The Mechanism of Mendelian Heredity' and in 1926 the book 'The Theory of Gene'. These discoveries enabled to construct genetic maps of organisms.

Molecular genetics may be said to have started with the discovery of nucleic acids in the nucleus in 1869 by Johannes Friedrich Miescher (1844–1895). Phoebus Levene (1869–1940) discovered that nucleic acids are made up of the building blocks called nucleotides. In 1941, George Wells Beadle (1903–1989) and Edward Lawrie Tatum (1909–1975) proposed the one gene-one enzyme (protein) hypothesis, paving the way for the fact that genes express themselves through proteins. Thus, the genomics-proteomics relationship was initiated. In 1944, Oswald Avery, Colin MacLeod and Maclyn McCarty showed that the DNA carries the genetic information. It was in this year that DNA was extracted for the first time. With George Palade's discovery in 1950, through electron microscopic studies, of microsomes and in 1956 of ribosomes rich in RNA, studies on the genetic control of all plant processes through its protein products were intensified. In 1956, Mahlon Bush Hoagland (1921–2009) discovered different species of RNA in the cytoplasm and that these are bound to specific amino acids. These were called transfer RNAs (tRNAs). Each tRNA molecule has three nucleotides that form the anticodons. Based on the sequence in which these three nucleotides are present in the

tRNA molecule, the sequence in which the three nucleotides that are present in the codons of the messenger RNA (mRNA), with which they pair, is decided; this process is called *translation*. J.L. Monad (1910–1976) and F.J. Jacob (1976) discovered *transcription* (i.e. passing on information from DNA to mRNA, which in turn determines the sequence of amino acids on the ribosome surface). The genetic code involved in transcription was discovered in the 1960s and 1970s through the original researches of Severo Ochoa, Arthur Kornberg, Warren Nirenberg, Har Gobind Khorana (1922–2011) and Robert Holley. These researches enabled the artificial synthesis of genes in later years.

In 1948, Barbara McClintock (1902–1992) proved that genes (discrete DNA segments) are not fixed in their location and that they can 'jump' from one place to another in the chromosome or to another chromosome. Hence, they were termed 'transposable elements', 'transposons' or 'jumping genes', exactly 60 years ago. James Dewey Watson (1928) and Francis Harry Compton Crick (1916–2004) in 1953 established the three-dimensional, double-helical right-handed model of DNA based on inputs from John Griffith, Erwin Chargaff, Maurice Wilkins and Rosalind Franklin. This was immediately followed by the work of Matthew Meselson and Franklin Stahl in 1958 which conclusively demonstrated semi-conservative method of DNA replication during cell division using cesium chloride density sedimentation.

Genetic engineering and molecular hybridization began in the 1970s with the invention of recombinant DNA (rDNA) technology, followed by its commercial applications to crops in the 1990s. However, the background researches for this might be said to have started with Joshua and Esther Lederberg, who convincingly showed the presence of sex in bacteria, $F^{+\,(male)}$, $F^{-\,(female)}$, Hfr (high-frequency recombinant cells) and hence bacterial conjugation in 1952, followed by the discovery of the phage plasmid, the Ti (tumor-inducing) plasmid of *Agrobacterium tumefaciens*. The fact that the phage plasmid is also a type of DNA was established by William Hayes in 1953. Werner Arber discovered in 1968 the restriction enzymes, which can fragment (break) the phage DNA into small bits that have sticky ends, which will enable the bits to rejoin with one another, in the absence of these enzymes. He was able to obtain bits or fragments of DNA are of variable length (random fragment length, RFL) and these fragments can be used as important molecular markers for taxonomic purposes which was emphasized by Tansley and his colleagues in 1989 (RFLP or Random fragments length polymorphism). Jonathan Beckwith and his colleagues first isolated a bacterial gene in 1969. This was followed by the discovery of many independent restriction enzymes that can specifically cut the DNA strand at the desired places by Daniel Nathans and Hamilton Smith in 1970. Hence, DNA bits with desired lengths could be obtained. In 1973, Stanley H. Cohen and Herbert W. Boyer used restriction enzymes to isolate the desired DNA (genes) and then inserted the thus isolated DNA bits into plasmids and then showed that these plasmids could make copies (cloning) of the inserted gene. This led to the emergence of the transgene technology whereby desired genes from any organism, mostly prokaryotes, can be isolated through restriction enzymes and then inserted into the DNA strand of any other organism and allowed it to express in the new organism called transgenic organism or GMO. This technique led to the emergence of the era of genetically modified crops or GM or biotech crops. In GM crops by and large, only useful trait/s, such as resistance to certain pests, diseases or environmental stresses, or resistance to chemical treatments (e.g. resistance to herbicides, etc.), or improving the nutrient profile of the crop, their shelf life, etc, which do(es) not occur naturally in the plant species are introduced. Examples of non-food traits introduced include pharmaceutical products, edible vaccines, insulin, biofuels, bioplastics and other industrially useful products. GM technology has also applications in environmental biotechnology, especially in bioremediation.

During the last 15 years or so, farmers of 25 countries have widely adopted GM technology; the total area of land cultivated with GM crops

had increased substantially (4,200,000–395,000,000 acres). Ten per cent of the world's crop lands were planted with GM crops in 2010. As of 2012, 11 different transgenic crops (maize, rice, soybean, papaya, cotton, canola, sugar beet, tomato, squash and sweet pepper) were grown commercially on 395 million acres. The world's population by 2050 is expected to be 9.2 billion. Global demand for cereal is expected to double and biotech crops will fill the gap to feed the world despite Green Revolution heralded by the Nobel Laureate for Peace Norman E. Borlaug. It is gratifying to note that over 3,000 high-yielding mutant cultivars of number of crops are released globally for use as food by IAEA, Rome, mostly by Chinese, Indian and Japanese mutation breeding researchers.

Once DNA structure was known, the sequencing of this molecule started. Two methods for sequencing DNA were published in 1977. Walter Gilbert and Allan Maxam of Harvard University, USA, independently developed the Maxam-Gilbert method of rapid DNA sequencing technique, while Sanger and co-workers developed an enzymatic method, known as didoxy sequencing in the UK. This was followed by efforts to make analysis of the genome of different organisms, including plants. *Arabidopsis thaliana* (thale cress) became the first plant to have its genome sequenced, through the *Arabidopsis* genome project in 2000. This plant remains the most important model plant even today; it has 120,000 kbp DNA and is one of the smallest genomes (115 megabases) among plants. The sequence of some other relatively small genomes like rice (450,000 kbp) and *Brachypodium distachyum* has also been determined; these plants are also used as model plants to understand the molecular mechanisms of photosynthesis. The molecular genetics of *Chlamydomonas reinhardtii* and of *Cyanidioschyzon merolae* (both are algae) has been used to study some basic chloroplast functions. Genomes of mitochondria and chloroplasts have been worked out in a number of plants and it is now possible to transfer at will suitable genes from these organelles for developing better plants with better functions and higher (*metabolomics)* yield, etc.

With advances in molecular genetical techniques and knowledge, *epigenetics* also became an intense area of research. It is the study of mitotically and/or meiotically heritable changes in gene function that cannot be examined by changes in the underlying DNA sequence, but cause the organism's genes to express themselves differently. One example of epigenetic change is the marking of the genes by DNA methylation, which determines whether they will be expressed or not. Gene expression can also be controlled by repressor proteins that attach to silencer regions of the DNA and prevent that region of DNA code from being expressed.

The subdiscipline of *functional genomics* was born in the 1990s. It meant that large quantities of high-quality genetical data become available. These genetical data were generated by the various genome projects (*Arabidopsis* was one among them).

1.11 Evolution

Although very early Indian philosophers like Uddalaka and Yajnavalkya wrote about evolution more than 2,000 years ago (Chowdhury 1971a), until the 1860s, it was believed that a species remained unchanged through time. This was called 'fixity of species', a concept derived from the Aristotelian idea of the 'Great Chain of Being' or the 'ladder of life'. This idea emphasized that each species remains as it was first created (by God) and that each species with increasing complexity of form and function occupied different rungs of the ladder from base upwards. Thus, the *creation theory* or the *theory of intelligent design* was widely prevalent till around the 1860s. The explanation for the perpetuation of organisms was offered by the spontaneous origin or *abiogenesis* concept, supported by Buffen (1707–1788), John Needham (1713–1781) and van Helmont. However, detailed and carefully designed experiments by Franceso Redi (1626–1698), Spallanzani (1729–1799) and Louis Pasteur (1822–1895) disproved the spontaneous origin of living organisms. Only life begets life. The presence of fossils and other

palaeobotanical evidences had enabled Georges Curvier in the early eighteenth century to propose the *catastrophism theory*, which stated that living organisms were subjected to alternate periods of sudden and often mass extinctions and creations (by God). Almost at the same time, Lamarck (1744–1829), a colleague of Cuvier in the French Natural History Museum, proposed the *theory of use and disuse* (often also called the theory of *inheritance of acquired characters*). According to this theory, organisms acquire, through constant use or disuse of their parts, new characters in their lifetime, which are passed on to their offsprings. Charles Darwin (1809–1882) proposed in 1859, through his famous book shortly known as 'Origin of Species', the organic theory of evolution, which revolutionized the biological science. Darwin based his theory on his experiences during the voyage to different parts of the world in the ship H.M.S. Beagle, especially his experiences with finches (often called Darwin's finches) in the Galapagos Islands. He was also influenced by Lyell's books of geology and by Thomas Robert Malthus's (1776–1834) concept on population growth (i.e. population increases geometrically, while the means to support the population increases only arithmetically). Darwin's theory also included most, if not all, ideas of an essay written by Alfred Russell Wallace in 1858 that was sent to him by Wallace. Darwin's theory stated that organisms undergo slow and gradual changes because of competition between organisms for various requirements and that the organisms with best adapted changes succeed in this competition and are selected by nature (natural selection) and those that do not (that lack fitness) get eliminated; these gradual changes result in a new species which is different from the one from which it evolved.

Subsequently *neo-Darwinism* concept evolved as a result of not only combining the aspects of Darwinism with the tenets of population genetics but also combining Darwin and Mendel. The main proponent of this was Theodosius Dobzhansky (1900–1975), the Ukrainian-American evolutionary biologist who published his book 'Genetics and the Origin of Species' in 1937. This book emphasized that in evolution nature, instead of selecting the fittest species as emphasized by Darwin, selects the fittest gene and rejects those which are unfit. Later Sir Julian Sorell Huxley (1887–1975), a British evolutionary biologist and eugenicist, a proponent of natural selection and a leading figure in the mid-twentieth century, advocated for an evolutionary synthesis and mixing of Darwinian evolution with Mendelian genetics. This is called the 'modern synthesis', the basis of neo-Darwinism. His book *Evolution: The Modern Synthesis* was published in 1942.

Hugo de Vries in 1901 proposed *in his book Die Mutationstheorie* the *mutation theory* of evolution, which stated that more than the small and incremental changes as proposed by Darwin, sudden and large changes in a species are more important in evolution. Although what de Vries proposed was really the result of chromosomal changes (especially chromosome number), mutation theory persisted with the discovery of point mutations (gene mutations) in the DNA strand and the obtaining of several hundreds of gene mutants in several organisms, including, for example, *Arabidopsis*. These mutants enabled us to understand the genetic basis of several characters of organisms. In 1972, Niles Eldredge and Stephen Jay Gould proposed the *theory of punctuated evolution*, according to which fossils and fossiliferous rocks are not in support of a gradual evolution, but that evolution happed only due to intermittent and sudden changes. Mass extinctions that have happened so far on this earth are cited as evidences for this theory. In 1980, Walter Alvarez proposed the *asteroid theory,* which was supported by D. R. Raup and J. J. Sepkowski. According to this theory, mass extinctions happened due to asteroid collision with earth. In a way, this theory provides a basis for explaining punctuated evolution.

1.12 Plant Breeding

Plant breeding may be defined as an act of crossing two different plants and producing a hybrid between them due to human intervention. Breeding of plants has been a very old practice

and evidences are there for its practice of hand pollinations of female inflorescence with pollen from male inflorescences in date palms by the Assyrian and Babylonian civilizations and also the Arabs. Plant breeding may be said to have started with artificial pollination, i.e. dusting the pollen of a desired plant on the stigma of the flower of the plant with which it has to be crossed; this was soon accompanied by removing or isolating the stamens of the artificially pollinated flower through selective bagging. These plant breeding experiments have been undertaken for various purposes such as increasing the yield, producing resistance against biotic and abiotic stresses, shortening the lifetime of crops, etc. Artificial pollination resulted in two types of problems. In many cases, particularly involving unrelated species, either the pollen did not germinate or even if germinated, the pollen tube is arrested. Thus, the phenomenon of incompatibility in angiosperms and several related aspects was brought to our attention in great detail, particularly by D. de Nettancourt (1977). Hence, a critical and extensive study of stigma was undertaken by Harrison, Heslop-Harrison and Shivanna (1977) as also the interaction between the pollen and stigma in deciding compatibility or otherwise of the hybridization effort. Their study brought to light the presence of *wet stigma* with wet stigmatic exudates and *dry stigma* with the stigmatic papillae covered by proteins in different angiosperms; pollen proteins released onto the stigma decide the compatibility or otherwise (see also Krishnamurthy 2015). In some cases, pollen germination and pollen tube formation are not affected in the stigma but pollen tube growth is affected in the style through the transmitting tissue present in the style (details summarized in Krishnamurthy 2015). In view of incompatibility operating at either the stigma or stylar levels, some efforts were made to cut the stigma and put the pollen grains on the cut surface of the style (stub pollination) or both stigma and style are avoided and the pollen is directly placed on the placental tissue of the ovary (placental/intra-ovarian pollination). In some instances, in vitro fertilization was tried with success using isolated egg and sperm cells in culture vials.

In spite of all these efforts, the male gamete may fail to fuse with the egg and/or secondary nucleus or polar nuclei, leading to abortion of the egg and secondary nucleus, single fertilization or *hemigamy* (*semigamy*) (independent development of male and female nuclei in the egg cytoplasm to result in a chimeral embryo, first discovered in *Rudbeckia* by Battaglia in 1945). Even if a zygote is formed, it may not develop further or abort at various stages of development because of intrinsic reasons, lack of endosperm development or due to overgrowth of somatic tissues like integuments or nucellus crushing the zygote/proembryo (a phenomenon called *somatoplastic sterility*) (see details in Krishnamurthy 2015). Hence, *embryo rescue technique* was developed in which the hybrid zygote/proembryo is excised from the ovule and grown in artificial nutrient medium.

Molecular hybridization might be said to have started with Joshua and Esther Lederberg, who studied bacterial conjugation in 1952, followed by the discovery of the phage plasmid; the fact that the phage plasmid is also a type of DNA was established by William Hayes in 1953. The early work of Marmur and Doty in 1962 and Britten and Kohne in 1968 established denaturation (breaking of base pair bonds of DNA) and renaturation (re-forming of base pair bonds) formed the basis of rapid and simple nucleic acid hybridization. To make many copies of the isolated desired gene, the polymerase chain reaction (PCR) technique was discovered by Kary Mullis in 1983. Ti plasmid was the first to be used as a vector for transferring the N_2 fixation (Nif) genes by Schell and Montagu in 1977. Today, genetic modification of the Ti plasmid is one of the main techniques for the introduction of foreign genes into plants and the creation of genetically modified (GM) crops already mentioned above. Apart from *Agrobacterium*-mediated gene transfer, electroporation, particle bombardment (biolistic), DNA uptake into protoplast and silicon carbide fibres technology have been developed and standardized for gene transfer. The use of reporter genes to study expression of genes was also developed. Methods to verify whether the introduced transgene expresses itself in the new

organism were then developed. The common reporter genes used included chloramphenicol acetyltransferase (CAT), neomycin phosphotransferase (NPT), green fluorescent proteins (GFP), and glucuronidase (GUS). To check whether a selective duplication of a DNA sequence (gene amplification) has taken place or not, Southern blotting technique was perfected. Here the DNA sequences restricted with an appropriate restriction endonuclease are size-fractionated by electrophoresis in *agarose acrylamide* gel stained with ethidium bromide and the banding pattern observed under UV light. The Western blotting for DNA expression studies at protein level was developed by Burnette in 1981 and almost simultaneously Thomas (1980) developed the Northern Blotting technique enabhng transfer of RNA to nitrocellulose was a dramatic improvement over the previous ones. These genes which code for proteins can be assayed readily.

1.13 Ecology and Biogeography

At the beginning of the nineteenth century, there was an increased interest in the connection between climate and plant distribution. Carl Willdenow (1756–1812) studied the connection between seed dispersal and plant distribution and the nature of plant associations. He noted the similarities between the floras of North America and North Asia, the Cape and Australia. He also examined the ideas of 'centres of diversity' and 'centres of origin'. Robert Brown (1773–1852) noted the similarities between the floras of South Africa, India and Australia. Joakim Schouw (1789–1852) studied the influence of environmental factors on plant distribution (Morton 1981). Joseph Hooker (1817–1911), expanded the boundaries of floristic studies with his work on Antarctica, India and the Middle East with special attention to endemism or restricted distribution of plants. August Grisebach (1814–1879) wrote a book 'Die Vegetation der Erde' in 1872, in which he examined the physiognomy in relation to climate.

The discipline of ecology (which is a science of functional relationship between plants and their environment), in reality, refers to physiological biogeography. It emerged out of floristic biogeography in the late nineteenth century, as environmental influences on plants received greater attention and recognition. The early work by Danish ecologist Eugenius Warming (1841–1924), through his book *Plantesamfund* (*Ecology of Plants*) initiated the beginning of modern plant ecology. He included the new idea that plants form their own communities, and he made analysis on the structure of these communities, their adaptations and the effects of environment on them. He was followed by Andreas Schimper (1856–1901) who made a very grand synthesis of all plant geographical aspects in his book *Pflanzengeographie auf Physiologischer Grundlage*, published in 1878. It was an excellent book combining plant ecology and plant geography; hence, it was published in English in 1903 under the title *Plant Geography upon a Physiological Basis* (a translation done by W.R. Fischer) (Reed 1942). Raunkiaer later proposed the life-form concept in, which is in use even today to analyse the vegetation of any area. The publication of Alfred Wegener's (1880–1930) *theory of continental drift* (1912) initiated the field of historical biogeography and past distribution patterns of plants. Nikolai Vavilov (1887–1943) gave detailed accounts on biogeography, centres of origin and evolutionary history of economic plants.

The *ecological succession concept* was developed by Henry Chandler Cowles, Arthur Tansley and Frederic Clements. Clements also created the concept of *climax vegetation*. Subsequently, Clements and Weaver produced the classical book on *Plant Ecology*. The concepts of *synecology* (study of plant communities) and *autecology* (study of the ecology of individual plants) emerged immediately. The concept of energy flow, food chains, food webs, energy pyramids, food pyramids, etc., slowly emerged in the 1930s. Tansley's ecosystem concept also became stabilized taking into account the above and integrating them in the ecosystem functioning in the 1940s and 1950s. Because of this, ecology matured into an independent discipline, particularly through the works of Eugene Odum (1913–2012).

A separate subdiscipline of *interaction ecology* developed when the concepts of ecosystem and food chain crystallized. People realized that no plant (animal or microbe) can live in isolation without having any kind of interaction with one or more of other kinds of organisms. These interactions may be mutual/symbiotic (when both organisms are benefitted by the interaction), antagonistic (when one benefitted and the other(s) was affected; the relationship may be predatory or parasitic) or commensalic. Meanwhile, the effects of abiotic environmental factors on plants as well as other living organisms were increasingly appreciated. Detailed research on land, water and air pollution was initiated. The climax of such studies was the realization of *global warming* due to greenhouse gases like CO_2, methane, etc.

1.14 Plant Cell, Tissue and Organ Culture

The first approach to culture cells and tissues in artificial culture media was made by Rechinger in 1893, who experimented with isolated buds, roots slices and stem bits. Gottlieb Haberlandt (1854–1945), an Austrian botanist, first formulated in 1902 the principles of plant cell/tissue culture and indicated the theoretical possibility of producing whole plants from single cells. Then the nutritional requirements for the cells were determined through the development of correct nutrient media. Media developed by Hoagland, White, Gautheret, Murashige and Skoog (MS medium) and others were adopted through several modifications made on original Knop's medium. The mineral sources (in the form of inorganic salts), carbon sources (in the form of various sugars and sugar derivatives) and growth regulators were all standardized through several trials and errors. This is how coconut milk was first tried as a constituent of growth media. Agar was standardized as the base of the medium. F.C. Steward in 1957 finally obtained a whole carrot plant from cultured isolated phloem parenchyma cell, thus proving the totipotency of parenchyma cells. Production of whole plants from isolated cells/tissues involved an intermediate *callus* stage from which shoot buds and roots were then produced or an *embryoid* (an embryo-like structure) from which a plantlet is produced. Callus production and micropropagation protocols were then exploited for the large-scale production of threatened and endangered species, elite and virus-free genotypes as well as secondary metabolites, medicinal chemicals, nutraceuticals and other useful products. In vitro production of androgenic haploids through anther/pollen/ microspore culture was first obtained by Sipra Guha-Mukherjee and S.C. Maheshwari in 1964 in *Datura innoxia*. Now the technique for obtaining androgenic haploids have been standardized in several plants like rice, *Nicotiana* and *Brassica*, and these haploids are used for crop improvement, often after producing dihaploids (through colchicine treatment) out of them. Haploids, in plant breeding programme, are useful as means of reducing the time required to complete backcrossing and as a means of helping to retain the character/s under transfer and also to stabilize the genetic material in the homozygous condition. Dihaploids are currently used in various breeding programmes in China, India and other countries. It is gratifying to note that the number of species amenable to doubled haploidy has reached a staggering 250 in just a few decades. A related development is the production of protoplasts from living cells by Edward Cocking of University of Reading. Protoplasts were initially isolated from cells through mechanical removal of cell walls or digestion of cell walls by cellulase enzyme. The latter method has been perfected for the production of somatic hybrids between species closely or distantly related. Protoplasts of different species are fused to produce somatic hybrids (*parasexual hybridization*). Protoplasts are also used in genetic engineering. Novel gene transfer through electroporation, biolistic methods, PEG or by using electric fields and electrofusion is generally preferred for higher yield, and then cultured in vitro, to produce either interspecific and intergeneric hybrids and even transgenic plants via callus-regeneration mode. In addition, tissue culture technique finds application in embryo

rescue, somaclonal variation, micropropagation, somatic embryogenesis, synseeds, cryopreservation, in vitro flowering and production of useful chemicals like Taxol, etc. There is immense potential in this area in the years ahead.

1.15 Instruments and Techniques

The botanical advances made in this modern period were largely due to advances in physics, chemistry, biochemistry, mathematics, computer/information technology and instrumentation. This was the period when methods, protocols, techniques and instrumental methods of analysis reached very great advancements taking botanical research to its zenith. It will be difficult to describe here all advancements made with reference to the instruments designed/fabricated and the methods, techniques and protocols developed, and only the most important are highlighted.

The optical microscope discovered and perfected in the earlier two centuries became highly sophisticated with greater precision and perfection in light sources, lens, methods of specimen preparations and image analysis. Along with advancements in light microscopy, specimen preparation methods also underwent several sophistications. Cryomicrotomes were discovered to take ultrathin sections of fresh materials. Chemical fixation methods initiated by the middle of the nineteenth century gradually became more and more refined with the discovery of newer fixatives. Plastic and other embedding media gradually replaced the paraffin wax to obtain very thin sections. Great advances were made in staining the sections for light microscopy. Many orthochromatic and metachromatic dyes were introduced. This gradually led to the emergence of the new field of histochemistry/cytochemistry that enabled the in situ localization of several cellular chemicals using specific dyes and other reagents. Since the intensity of staining is proportional to the amount of the cellular chemical localized, quantitative microscopy developed using the principles of Beer-Lambert laws. Pollen/stigma prints or live specimens of any tissue can be studied for their cytochemistry including enzymes, etc. By altering the quality and quantity of light used, other forms of light microscopy emerged: phase-contrast, dark-field, polarization, fluorescence and interference microscopies. In the last two decades, confocal microscopy developed that enabled the examination of specimens at different depths so as to get a three-dimensional perspective of the specimen. Such confocal studies helped in understanding the structure and functions of Golgi bodies, actin filaments, microtubules and other cell organelles and widely used in plant molecular biological studies.

Optical microscopy has its own limitations because the specimens cannot be magnified beyond a particular resolution limit as it employs wavelengths of visible and near-visible ranges. Hence, transmission electron microscopy (TEM) was developed in the middle of the twentieth century. This employs electrons instead of light. TEM helped us to know the detailed ultrastructure of the cell and its various organelles. The construction of TEM was soon followed by scanning electron microscopes (SEM), scanning tunnelling microscope (STM), atomic force microscope (AFM), laser scanning confocal microscope (LSCM), etc., that enabled us to get three-dimensional images of the specimen, their molecular structure, etc. Sophistication in microscopy along with instrumentation and techniques has made it possible to get images of living cells. Meanwhile developments in immunology resulted in the production of monoclonal and polyclonal antibodies against specific cellular antigenic chemicals; these developments lead to the developments in radioimmunoassay techniques. Radioisotope techniques resulted not only in *autoradiography* that helped in understanding cell cycle events but also in tracing metabolic pathways such as the key steps involved in photosynthesis and phloem loading/unloading.

Developments in the field of electromagnetic radiation physics resulted in advancements in the field of colorimetry and spectroscopy. Spectroscopic methods were beginning to be increasingly employed to identity chemicals. UV-visible, atomic absorption, nuclear magnetic

resonance, electron spin resonance and mass spectroscopic techniques were developed, and all these helped in the identification or quantification of the various chemical constituents of diverse species of plants. Separation techniques such as chromatography, and its various brands (column, paper, chromatography, gas chromatography, i.e. GC, HPLC, HPTLC, etc), electrophoresis and blotting also got developed very rapidly and enabled us to separate the various plant chemicals one from the other. Chromatography refers to any of the several techniques for separating or analyzing mixtures of liquids or gases by selective absorption. Electrophoresis is a technique used for the separation of charged molecules such as proteins or DNA, developed by Tiselius in 1937. Charged molecules in solution will migrate in the presence of an electric field, according to their charge; the size and shape of the molecule also affect the rate of migration. The technique, commonly performed by using an inert porous medium such as starch (for generally enzymic proteins), silica gel, polyacrylamide gel, agarose gel, etc., separates proteins, DNA, etc. Non-pigmented molecules are detected in the gel by staining or by differential optical absorption. Southern blotting technique, a widely used method in gene manipulation and invented by Edward Southern in 1975, employs an agarose gel containing denatured DNA fragments that are placed between buffer-saturated filter paper and a cellulose nitrate filter. DNA is eluted from the gel onto the cellulose nitrate filter, to which it binds strongly. This filter can then be used for complementary nucleic acid hybridization, which reveals the identity of the sequences in the blotted filter and thus in the original agarose gel. Other similar blotting tests are the 'Northern' and 'Western' blot gels. In situ hybridization techniques were developed to reveal the chromosomal location of DNA sequences involved in the hybridization process.

Biometrical and biostatistical methods/techniques were developed for biological data analysis, for estimating mutation frequencies and evolutionary studies in populations. Their contributions laid much emphasis on innovations in statistical analysis made by stalwarts like Sir Ronald Fisher, Sewall Wright, J. B. S. Haldane (founding fathers of population genetics) and Frank Yates. Because of the complexities and time involved in such analysis due to the enormity of data, computerized software of statistical packages like UPGMA were soon developed. Packages were also developed to construct and analyse cladistic, phenetic and phylogenetic dendrograms, parsimony methods, calculation of genetic distances and microarray data analyses. Software were also made for mathematical modelling of botanical characteristics such as architecture or physiological phenomena such as enzyme kinetics and water/organic substance conduction and transport. Such models were also developed to understand stochastic and deterministic environmental ecological events that control plants and their population growth. New models such as the use of *process calculi* to model biological process that include stochastic π-calculus, BioAmbients, Beta-Binders, Bio-PEPA and Brane Calculus, and constraint-based modelling. There are also efforts to develop syntactically and semantically sound ways of representation of biological models.

Another major advance made in the last five to six decades was the emergence of the field of bioinformatics. Bioinformatics tools were developed to analyse raw data, to derive information from data, to gain knowledge from information and to attain wisdom from knowledge. Several thousands of online databases and metadatabases and repositories have been created related to several aspects of plant sciences and technology for sharing data and models. The whole area of *data mining* (text mining) or *information extractions* got initiated and advanced within a very short time. It is now possible to get data/information on any aspect of botany at our table top within seconds. There are also approaches to database integration and software interoperability via loose coupling of software, websites and databases or commercial suits.

The most recent technology to develop relates to nanobotany, which forms the interface between botany and nanotechnology. It serves as a blanket for various related technologies including biotechnology. It describes the use of various

nanotools to manipulate molecular processes in living cells and biomolecules. It also discusses the employment of nanoparticles, nanophoton, array sensor, imaging via AFM/optical tweezers, X-ray diffraction and various computational approaches.

Developments in *systems biology* (*systeomics*), first launched as a distinct discipline by Minjalo Mesarovic in 1966, and *cybernetics* have emerged in the last five decades and have contributed greatly to the advancement of plant biology and biotechnology. Around the year 2000, Institutes for Systems Biology were established in Seattle and Tokyo. Systems biology is an interdisciplinary field holistically dealing with complex interactions within biological systems. The main aim of this field is to model and discover emergent properties and interactions between cells, tissues and whole organism/plant functioning as a system, i.e. it integrates, instead of being reductionistic, and then models a phenomenon in a very refined manner. Since the objective is a model of the interactions in a system, those that are system-wide are most suited to systems biology to make the model as complete as possible. Hence, phenomics, genomics, epigenomics, transcriptomics, interferomics, metabolomics, proteomics, glycomics, lipidomics, biomics (analysis of biomes) and other high-throughput techniques are used to collect data for the construction and validation of these models. The main conceptual difference between systems biology and bioinformatics is the focus made on the dynamics of the studied systems in the former. This is exemplified by the application of dynamical systems theory to molecular biology.

The systems biology approach often involved cybernetics. Cybernetics (or control theory), the science of coordination, regulation, control and communication in the biological systems and machines, has been gaining increasing importance in plant biology, especially in systems biology, in the last few decades. Cybernetics applies the theory of machines, particularly relating to behavioural and functional aspects, to biological systems. Cybernetics, for example, provides mechanistic interpretations for the morphogenesis of specific geometric forms of plants and its constituent organs. It is a study of systems that are open to energy but closed to information and control. There are two peculiar scientific virtues of cybernetics: (1) it provides a single vocabulary and a single set of concepts suitable for representing the most diverse types of biological system and (2) it offers a method for the scientific treatment of the system in which complexity is outstanding and too important to be ignored. A number of studies have already been undertaken to apply concepts of systems biology and cybernetics to explain and model plant phenomenon and functions from plant molecular biology to morphogenesis to ecosystem analysis. Max Planck Institute at Tubingen, Stuttgart, Germany, is among the few pioneers in this field.

1.16 Future of Plant Biology

Predicting the future is always risky, especially to predict the future of any scientific discipline like botany. The present status of botany grew out of the solid foundations laid by the breadth of vision and detailed experimental observations of the botanist of the previous centuries made possible through the power of scientific methods and sophisticated instrumentation and techniques. Most of the basic questions concerning the structure and functions of plants at all levels of their organization, from molecules to global biome, in principle, have been resolved to a fair amount of success and satisfaction. A vastly increased research work force has now been obtained and is also rapidly expanding. A growing awareness of the unity of biological structure and function at all the hierarchical levels of organization has been established. Consequently, now, a clear distinction between pure and applied aspects of botany has become blurred.

However, plants continue to remain crucial to the future welfare and sustenance of human society globally as they provide food, fodder, fuel, oxygen, medicine and other useful products including green plastics on which our daily life depends, as well as do several ecosystem services including the creation and preservation of soil, photosynthesis, gas turnover, biogeochemical

cycles, etc. Among the very vital botanical questions of the twenty-first century are the roles of plants as primary producers in the global cycling of life's basic ingredients—energy, carbon, oxygen, nitrogen and water—and the ways in which the plants can address the global environmental issues of resource managements and conservation, human food security, biologically invasive organisms, carbon sequestration, climate change and overall sustainability. Hence, botany should continue to be studied and researched for the very survival of humans in the future. Our historically accumulated botanical wisdom is absolutely needed to improve human custodianship of the planet earth.

Hence, attention should be particularly focused in the future on monitoring and conserving our ecosystems, particularly the tropical ecosystems (see Bawa et al. 2004) since they support a diversity of species and ecological processes that are unparalleled anywhere else in the world. Botanists must, therefore, provide critical knowledge on three areas: (1) structure and functioning of ecosystems, (2) the nature and magnitude of human interference on ecosystems and (3) socioeconomic drives of these anthropogenic effects. In the immediate future effective strategies for conservation restorations and sustainable management of the various ecosystems must be developed to integrate scientific perspectives with social perspectives and at the same time to maintain the ecological services that they provide. Three principles for guiding ecosystem research are suggested (as done for tropical ecosystems by Bawa et al. 2004): (1) broadening the set of concerns, (2) integrating botanical/ biological knowledge with social science and traditional knowledge and (3) linking botanical science to policy and action (for details see Bawa et al. 2004).

The botanical advancements made through *Arabidopsis* (the first model plant) projects should also be obtained in as many other plants as possible in the immediate future so as to test whether molecular basis of development and functioning known for *Arabidopsis* are applicable to other plant taxa and to draw general and universal statements on the genetic bases of structure and function. All the goals mentioned for *Arabidopsis* on a previous page of this article should also be the focus points of future research on other taxa.

One of the major problems that we face now pertains to the fact that the number of good, young botanists/plant scientists is rapidly declining in India, the UK and many other countries. Hence, recruitment of good botanists is becoming more and more difficult. Students do not opt for programme in botany in the colleges and universities, both at the undergraduate and postgraduate levels. Also, investment of industry and research funding organizations in botany is also rapidly dropping in many countries. Career development prospects are also gradually becoming dimmer. There is also a strong bias among the few students who opt for botanical research in choosing the disciplines for their research work. Subjects like morphology, anatomy, embryology, taxonomy, etc., are largely neglected, while genetics, molecular biology, biotechnology, microbiology, etc., are often preferred. This greatly reduces the inter- and multidisciplinary approaches (while is very much needed and need of the hour) in solving the problems in their own chosen fields and as much as at national/global interest.

References

Arber A (1986) Herbals, their origin and evolution, a chapter in the history of botany 1470–1670. Cambridge University Press, Cambridge

Astill G, Langdon J (eds) (1997) Medieval farming and technology – the impact of agricultural change in northwest Europe. Brill, Leiden

Bahadur B, Farooqui SM (1984) Seed and seed coat characters of Australian *Nicotiana* under light and scanning electron microscope. In: Arcy WGD (ed) Proceedings of 2nd international symposium on biology and systematics *Solanaceae*. Columbia University Press, St. Louis, pp 114–137

Bahadur B, Farooqui SM, Bhaskar KV (1983) Light and scanning electron microscopic study of seeds in *Nigella* (Ranunculaceae). Proc Indian Acad Sci 92:429–435

Bahadur B, Venkateswarlu K, Ramaswamy N (1989) SEM studies of seeds in nine species of *Petunia* Juss. (Solanaceae). Phytomorphology 39:121–128

Bahadur B, Murthy GVS, Sujatha M (2013) Pollen of *Jatropha* L. taxonomic and phylogenetic considerations. In: Bahadur B, Sujatha M, Carels N (eds) *Jatropha*, challenges for a new energy crop. Springer Publishers, New York, pp 45–74

Barthlott W (1984) Microstructural features of seed surfaces. In: Hey wood VH, Moore DM (eds) Current concepts in plant taxonomy. Academic Press, London, pp 95–106

Bawa KS, John KW, Nadkarni Nalini M, Lele S (2004) Beyond paradise-meeting the challenges in tropical botany in 21st century. Biotropica 36:4337–4448

Behnke HD, Barthlott W (1983) New evidence for the ultrastructural and micromorphological fields in angiosperm classification. Nord J Bot 3:43–66

Bray F (1986) Agriculture science and civilization in China. Cambridge University Press, Cambridge

Chakrabarti DK (ed) (2004) Indus civilization sites in India, new discoveries. Marg Publications, Mumbai

Chowdhury KA (1971a) Botany: pre-historic period. In: Bose DM, Sen SN, Subbarayappa BV (eds) A concise history of science in India. Indian National Science Academy, New Delhi, pp 371–375

Chowdhury KA (1971b) Botany in the medieval period from Arabic and Persian sources. In: Bose DM, Sen SN, Subbarayappa BV (eds) A concise history of science in India. Indian National Science Academy, New Delhi, pp 392–400

Crowe I (2005) The hunter-gatherers. In: Prance G, Nesbitt M (eds) The cultural history of plants. Routledge, New York, pp 3–11

Darwin C (1868) The variation of domestication of animals and plants under domestication. Aplleton, New York, p 473

Darwin C (1876) The effects of cross and self pollination in the vegetable kingdom. John Murray, London

Darwin C (1877) The different forms of flowers on plants of the same species. John Murray, London

de Nettancout D (1977) Incompatibility in angiosperms. Springer, New York

de Vries H (1901) Die mutationstheorie, vol 1. Veit, Leipzig

Esau K (1965) Plant anatomy. (2nd Edition). John Wiley & Sons, New York

Evert RF (2006) Esau's plant anatomy. John Wiley, New York.

Fahd T (1996) Botany and agriculture. In: Morelon R, Rashed R (eds) Encyclopedia of the history of Arabic science. Routledge, London

Frawley D (1991) Gods, sages and kings. Vedic secrets of ancient civilization. Motilal Banarsidass Publishers Pvt Ltd, Delhi

Geetha S, Haridasan K, Krishnamurthy KV (2013) Relevance of Vṛksāyurveda and other traditional methods for organic production of nursery seedlings of usage plants. Anc Sci Life 33:68–78

Ghosh AK, Sen SN (1971) The vedic and post-vedic period. In: Bose DM, Sen SN, Subbarayappa BV (eds) A concise history of science in India. Indian National Science Academy, New Delhi, pp 375–391

Harris DR (2005) Origins and spread of agriculture. In: Prance G, Nesbitt M (eds) The cultural history of plants. Routledge, New York, pp 13–26

Harvey-Gibson RJ (1919) Outlines of the history of botany. A. and C Black, London

Henray B (1975) British botanical and horticultural literature before 1800, vol 1–3. Oxford Univ. Press, Oxford

Heslop-Harrison Y, Shivanna KR (1977) The receptive surface of the angiosperm stigma. Ann Bot 41:1233–1258

Heywood VH (1971) The characteristics of scanning electron microscope and their importance in biological studies. In: Scanning electron microscopy. Academic, London, pp 1–16

Huff T (2003) The rise of early modern science: Islam, China and the West. Cambridge University Press, Cambridge

Krishnamurthy KV (2000) Nandavanas (sacred groves) in Medieval South India: epigraphical data. In: Krishna N, Amirthalingam (eds) Sacred groves. CPR Environmental Education, Chennai

Krishnamurthy KV (2004) Textbook of biodiversity. Science Publishers, Plymouth

Krishnamurthy KV (2005) History of science. Bharathidasan University, Tiruchirappalli

Krishnamurthy KV (2006) The Tamils and plants (in Tamil). Bharathidasan University, Tiruchirappalli

Krishnamurthy KV (2010) Origin and evolution of plants a brief review. J Swamy Bot Cl 27:1–12

Krishnamurthy KV (2015) Growth and development in plants. Scientific Publishers, Jodhpur

Larson PR (1994) The vascular cambium: development and structure. Springer-Verlag, Berlin

Majumdar GP (1982) Studies in history of science in India. In: Chattopadhyaya D (ed) The history of botany and allied sciences in India (c. 2000 B.C. to 100 A.D.). Asha Jyoti, Editorial Enterprise, New Delhi

Meyer EHF (1854–1857) Geschichte der Botanik. Verlag de Gebrüder Borntäger, Königsberg

Morton AG (1981) History of botanical science: an account of the development of botany from ancient times to the present day. Academic, London

Murthy GVS, Bahadur (1995) Pollen morphology of caprifoliaceae. In: Bir Bahadur (ed) Vistas in palynology: perspectives and prospects. J Palynol 31:79–129

Murthy GVS, Nair PKK, Bahadur B (2005) Pollen morphology of Indian campanulaceae. In: Bir B, Reddy PR, Varma YNR, Ramakrishna H (eds) Gleaning in botanical research. Dattsons Publishers, Nagpur, pp 11–33

Oliver FW (ed) (1913) Makers of British botany. Cambridge University Press, Cambridge

Prance G (2005) Introduction. In: Prance G, Nesbitt M (eds) The cultural history of plants. Routledge, New York, pp 1–2

Raju CK (2009) Is science western in origin? Multiversity & Citizens International, Penang

Real L (1982) Pollination biology. Academic, New York

Reed HS (1942) A short history of the plant sciences. Ronald Press, New York
Reynolds GJ (1909) History of botany 1860–1900. Clarendon Press, Oxford
Reyond Green J (1909) History of botany 1860–1900. Clarendon Press, Oxford
Roy BP (2008) Vrksāyurveda in ancient India. In: Gopal L, Srivastava VC (eds) History of science, philosophy and culture in Indian civilization, vol V, Part. 1. History of Agriculture in India (up to *c.* 1200 AD). Centre of studies in Civilization, New Delhi, pp 550–596
Sachs JV (1890) History of botany (1530–1860) (trans: Garnsey HEF and Balfour IB). Clarendon Press, Oxford
Singer C (1923) Herbals. Edinb Rev 237:95–112
Slater A, Scott N, Fowler M (2003) Plant biotechnology. Oxford University Press, Oxford
Soltis DE, Soltis PS, Chase MW, Endress PK (2005) Phylogeny, evolution, and classification of flowering plants. Sinauer, Sunderland
Stearn WT (1965) The origin and later development of cultivated plants. J R Hortic Soc 90:279–291, 322–341
Stone D (2005) Decision-making in medieval agriculture. Oxford University Press, Oxford
Sturtevant AH (1966) A history of genetics. Harper and Row, New York
Swamy BGL (1973) Sources for a history of plant science in India. I. Epigraphy. Indian J Hist Sci 8:61–98
Swamy BGL (1976) Sources for a history of plant science in India. III. The maritime trade of ancient Tamils in plant products A critique. Indian J Hist Sci 11:33–48
Swamy BGL (1978) Sources for a history of plant sciences in India. IV "temple plants" (*Sthala-vrksas*) a reconnaissance. Trans Arch Soc S India
Thanos CA (2005) The geography of Theophrastus' life and of his botanical writings. In: Karamanos AJ, Thanos CA (eds) Biodiversity and natural heritage in the Aegean. Proc Conf 'Theophrastus 2000' (Eressos – Sigri, Lesbos, July 6–8, 2000). Fragoudis, Athens, pp 23–45
UN World Assessment Report. http://www.unesco.org/waterwwap/wwdr
Walters SM (1981) The shaping of Cambridge botany. Cambridge University Press, Cambridge
Watson AM (1983) Agricultural innovation in the early Islamic world. Cambridge University Press, Cambridge
Whittaker RH (1969) New concepts of kingdoms of organisms. Science 163:150–160
Williams RL (2001) Botanophilia in eighteenth-century France: the spirit of the enlightenment. Springer, Amsterdam
Woodland DW (1991) Contemporary plant systematics. Prentice Hall, Englewood Cliffs

Organization at the Cellular Level

2

K.V. Krishnamurthy and Bir Bahadur

Abstract

This chapter deals with plant organization at the cellular level which is the fundamental level of biological integration also. Since cells are often considered as the basic structural and functional unit of any biological organism, organization at the cellular level forms the basis for understanding organization at the higher levels. This chapter deals with the structural and functional aspects of cytoplasm and its various endomembrane system, organelles, storage and other cellular chemicals, nucleus and cell wall.

Keywords

Cell wall • Cytoskeleton • Endomembrane system • Endoplasmic reticulum • Golgi bodies • Levels of biological organization and integration • Mitochondria • Plastids • Peroxisomes • Plasma membrane • Ribosomes • RNA • Vacuoles

2.1 Levels of Biological Organization and Integration

The living components of the earth together make up the *biosphere*, which can be considered as the highest *level of biological organization*. Starting from this highest, largest and the most complicated level, there are several lower hierarchical levels of biological organization that ultimately end in the lowest, smallest and the least complicated level of biomolecules; all these levels are interconnected (Fig. 2.1). The best analogy is an automobile antenna that is made up of several cylinders that are tucked into one another, in

K.V. Krishnamurthy (✉)
Center for Pharmaceutics, Pharmacognosy and Pharmacology, School of Life Sciences, Institute of Trans-Disciplinary Health Science and Technology (IHST), Bangalore, Karnataka, India
e-mail: kvkbdu@yahoo.co.in

B. Bahadur
Sri Biotech Laboratories India Limited, Hyderabad, Telangana, India

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_2,

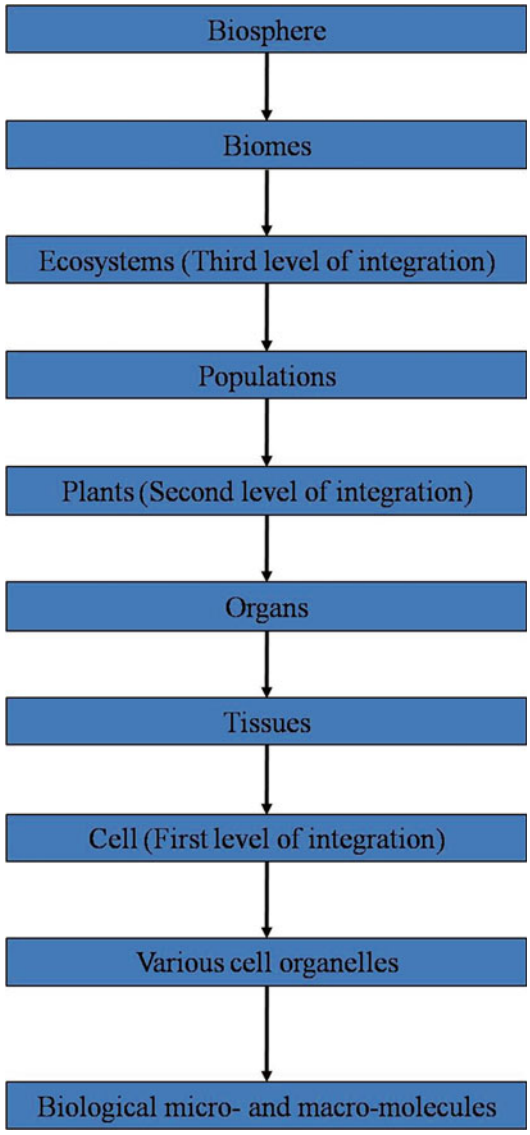


Fig. 2.1 Levels of biological organization and integration (*top down*) (Based on Krishnamurthy 2015)

which the narrowest and innermost represents the lowest level of biological organization and the broadest and outermost represents the highest level of biological organization, separated by cylinders of intermediate hierarchical levels of biological organization (Krishnamurthy 2015). The biosphere is constituted of several *biomes* which may be defined as follows: particular region or set of regions that has characteristic physical conditions (including climatic) and supports plants, animals and microbes that show adaptations to these conditions; for example, the sea, tropical forests or temperate forests may be considered as biomes. The biomes are made of *ecosystems* which are dynamic but complex natural units of living and nonliving components interacting not only among themselves but also between themselves. For example, a tropical forest biome may have tropical evergreen forest, tropical deciduous forest, tropical savanna and a tropical scrub jungle ecosystem. Ecosystems in turn possess *populations* of specific plants, animals and microbes that share a common descent and are often considered as the units of genetic diversity of a species. The individual *organisms* (a plant, animal or microbe) form the next lower hierarchical level of biological organization. Each organism is made up of several *organs* which in turn are made of different *tissues* that come together in a location-specific manner to form the organ. Tissues are made of *cells* (of the same type or of different types). Cells are made of *organelles*, which in turn are made of *biomolecules*, both micro and macro. At each increasing hierarchical level of organization, there is not only an increasing complexity in form, structure and function but also in size and volume. In this hierarchy of biological organization, we may recognize three levels of *biological integration*: cell (which integrates several different biomolecules and organelles to form a unit of structure and function; see next section of this chapter), *organism* (a plant or animal) (where cells, tissues and organs are integrated to form a whole individual) and ecosystem (where all abiotic and biotic components are integrated to form a dynamic but stable system or ecological unit).

2.2 Cell as a Structural and Functional Unit

The term ‘cell’, meaning a ‘little room’, was first introduced by Robert Hooke in the seventeenth century. They are considered as the smallest structural, organizational and functional units of life (Sitte 1992). Plants (defined in the way in Chap. 1 of this book) are either single celled

(as in some algae and fungi) or complexes of cells which vary in size, form, structure and function. Despite this great diversity, cells are strikingly similar in their physical organization and biochemical attributes. Cells of fungi are called *hyphae*.

There are two different theories that discuss the importance of cells as the structural and functional units of plants and animals. The first one is the *cell theory* proposed independently by Schleiden and Schwann in 1838 and 1839, respectively. As per this theory, all living organisms are made of one or more cells. The cell theory in its modern form states the following: (1) All organisms are made of one or more cells. (2) Chemical reactions, including energy-related processes, occur within cells. (3) Cells arise from pre-existing cells through cell divisions, and cell divisions are considered as important in plant growth, development and organ formation (Evered and Marsh 1989). The second theory, called *organismal theory*, was proposed in the later part of the nineteenth century as an alternative to cell theory. According to this theory, the whole organism is not merely a group of independent units (i.e. cells), but rather is a living unit that is subdivided into cells, which are connected and coordinated into a harmonious whole entity. Further, this theory emphasizes that cell division is merely a 'marker' of plant growth and development and that it does not influence plant growth and development (Kaplan and Hagemann 1991). Cells do not pinch off during cell division and that cells cannot be easily be separated from one another, because of plasmodesmal connections, particularly in plants. Hence, plants are supracellular organisms (Lucas et al. 1993). According to the organismal theory, cell enlargement, differentiation and formation of tissues and organs are essentially independent of cell division in plants and that cell division is simply a necessary consequence of their execution. In view of the above discussion, there is a paradox with cell division being viewed as both important and unimportant for plant development (Meyerowitz 1996). The arguments on this topic have tended to be clouded by these two polarized views (Fleming 2006), encapsulated in the aphorisms attributed to de Bary (1879) and Barlow (1982), '*Die pflanze bilder zellen*, *nicht the zelle bildet pflanzen*' ('It is the plant that forms cells, and not the cells that form plants' – translation by Sitte 1992), supported by many (Kaplan and Hagemann 1991; Cooke and Lu 1992; Kaplan 1992), and the other to Meijer and Murray (2001), 'The plant makes cells and the cells make the plant'. It is emphasized by the followers of cell theory that the cell theory is based on actual observations and analyses of ontogeny of various plant organs and that cell divisions should not simply be disregarded as a secondary consequence of growth (Meijer and Murray 2001). However, it should also be emphasized that both theories are not mutually exclusive.

2.3 Organization of the Plant Cell

The contents of the living cell together constitute the *protoplasm*. It consists of two main parts: *cytoplasm* and *nucleus*. The nucleus was discovered by Robert Brown in 1831, and the cytoplasm was named by Albert von Kolliker in 1862. The term *protoplast*, used to denote the unit of protoplasm inside the cell, was introduced by Hanstein (1880).

2.3.1 Cytoplasm

Cytoplasm refers to the protoplasmic material that surrounds the nucleus. *Cytosol* now refers to the cytoplasm minus all the organelles and endomembrane systems. Plant biologists refer to the cytoplasmic matrix through terms such as *cytoplasmic ground substance* and *hyaloplasm*. A few biologists equate the cytosol with cytoplasm. The cytoplasm of a living cell is always in motion along with its organelles, and this movement is called *cytoplasmic streaming* or *cyclosis*. It is the result of an interaction between *actin filament* bundles and the so-called motor protein, *myosin*. This protein molecule has an ATPase-containing 'head' that is activated by actin (Baskin 2000; Reichelt and Kendrick-Jones 2000) (see more

details on a later section of this chapter). Cyclosis is metabolically a very costly process, but it is a necessary evil as it facilitates movements of materials within the cell and between it and the environment. The cytoplasm is limited by the plasma membrane.

2.3.1.1 Plasma Membrane

It is also known as *plasmalemma*. It is a unit membrane that isolates the cytoplasm from the environment. It is about 0.01 μm thick. Its functions include mediation of transport of substances into and out of protoplasts, coordination of the synthesis and assembly of cell wall cellulose and callose and transduction of hormonal and environmental signals involved in cell growth and differentiation. Plasma membrane is a lipid bilayer that provides impermeability. Most membranes have 40–50 % lipids. Embedded in this lipid bilayer are globular proteins called *transmembrane proteins*. Many of these proteins extend across the lipid bilayer and protrude on either side of the membrane (Fig. 2.2). These are hydrophobic proteins whose protruding portions alone are hydrophilic. The inner and outer surfaces of the plasma membrane greatly differ in their chemical composition, with reference to the more abundant phospholipids and sterols, particularly stigmasterol. The protruding portions of the proteins also have different amino acid compositions and tertiary structures. The plasma membrane also has other membrane-associated proteins: (1) *peripheral proteins*, which lack hydrophobic sequences and which do not penetrate into the lipid bilayer, and (2) *integral proteins*, which are transmembrane proteins and other lipid-bound proteins. There are 50–60 % proteins in the plasma membrane.

The membrane proteins are responsible for most of the functions of the plasma membrane. The amount and type of protein in a plasma membrane reflects its function. Some membrane proteins are enzymes that are involved in the catalysis of membrane-associated reactions. Some proteins are transport proteins that are involved in the transport of materials in and out of the cells, yet others are receptor proteins that receive signals meant for the cell. Some of the plasma membrane proteins are anchored on the membrane, forming various patterns or mosaics that vary temporally and/or spatially. In view of this, the name *fluid mosaic model* is given for the membrane structure (Singer and Nicolson 1972; Jacobson et al. 1995). In addition to lipids and proteins, oligosaccharides, which are short-chain carbohydrates, are also attached to the protruding membrane proteins forming glycoproteins. These are believed to help in cell-to-cell adhesion processes and in the 'recognition' of molecules such as hormones, viruses and pathogen-related chemicals.

Particular mention should be made here on transport proteins (Logan et al. 1997; Delrot et al. 2001). These are of two types: *carrier proteins* and *channel proteins*. The carrier proteins bind to specific solutes that need to be transported across

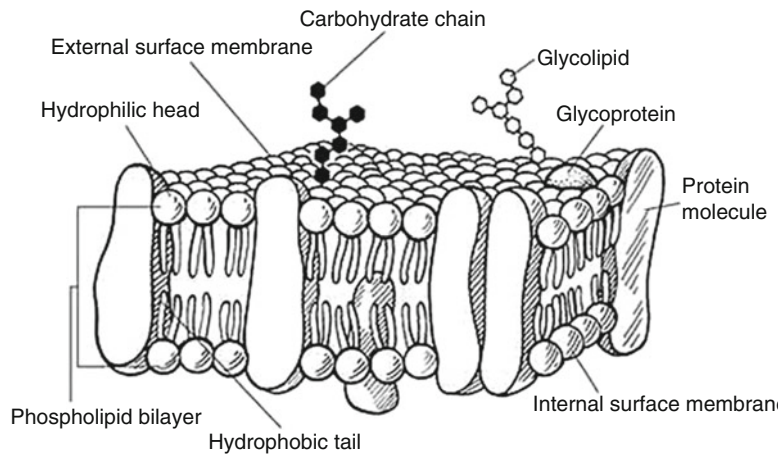


Fig. 2.2 Fluid mosaic model of cell membrane (Source: http://www.getting-in.com/guide/a-level-biology-plasma-membranes-and-cell-structure/)

the membrane and undergo a series of conformational changes in order to transport the same. The channel proteins form water-filled 'pores' that extend across the width of the plasma membrane and, when open, show specific solutes like K^+, Na^+, Ca^{2+} and Cl^- to pass through them. The channels are not open continuously but are 'gates' that open briefly and then close again; this process is referred to as *gating*. Special water-transporting channel proteins called *aquaporins* are also present that facilitate water transport and control water entry and exit (Schaffner 1998). Both transport across a membrane only down the substance's electrochemical gradient and serve as passive transporters. The carrier proteins belong to two categories: *uniporters*, which transport only one solute from one side of the membrane to the other side, and *cotransporters*, which are involved in a transfer process where transport of one solute depends on the simultaneous or sequential transport of a second solute, either on the same direction (=*symporter*) or in the opposite direction (=*antiporters*). There is also transport of substance against their electrochemical gradient and this requires energy input. This type of transport is called *active transport*. This energy is provided by an ATP-powered proton pump and a membrane-bound H^+-ATPase (Palmgren 2001) or in some species by H^+- pyrophosphate (H^+-PPase).

Many macromolecules like proteins, polysaccharides lipids, etc., cannot be transported across membranes by transport proteins. These are transported by means of vesicles, a process called *vesicle-mediated transport*. The vesicles that contain these macromolecules fuse with the plasma membrane and/or bud off it (Battey et al. 1999). If this happens into the cell from the plasma membrane, the process is called *endocytosis*, and if it is from the cell into the outside, the process is *exocytosis*. Both these processes involve portions of the membrane called *coated pits*, which are depressions in the membrane containing specific receptors to which the macromolecules to be transported across the membrane must first bind and then get coated on their surface with *clathrin*, a protein (Fig. 2.3). Invaginations of coated pits pinch off to form

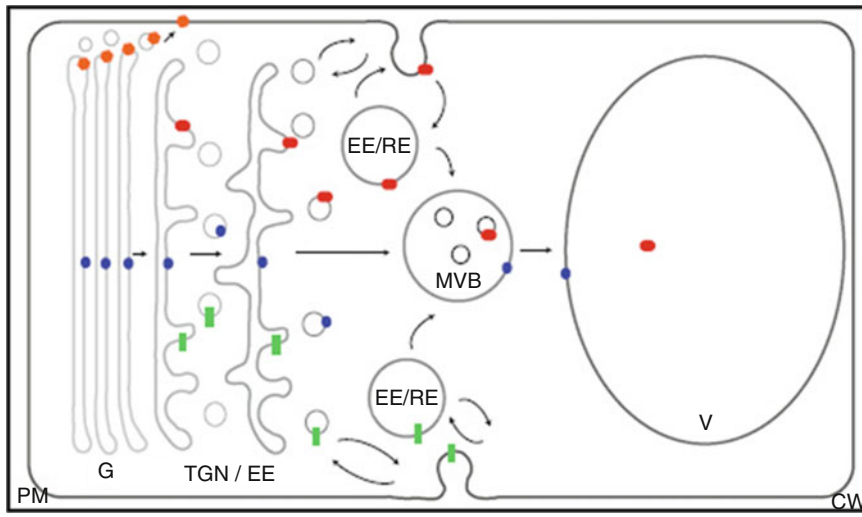


Fig. 2.3 Overview of trafficking pathway of proteins through endomembrane system that is from ER to Golgi to TGN to PM. *Orange polygons* show Golgi to PM traffic. Blue circles show Golgi to tonoplast traffic. *Red ovals* show a PM protein that is undergoing turnover in the vacuole. *Green rectangles* show a protein that is constitutively recycled. *CW* cell wall, *EE* early endosome, *G* Golgi, *MVB* multivesicular body (prevacuolar compartment), *PM* plasma membrane, *RE* recycling endosome, *TGN* trans-Golgi network, *V* vacuole. The nucleus, mitochondria, plastids, actin filaments, microtubules and ER have been omitted for clarity. *Arrows* indicate the direction of movement (Peer 2011)

coated vesicles. The vesicles shed off their coats and bind to Golgi bodies or small vacuoles inside the cell. In some fungi, multivesicular bodies are seen to be implicated in the transport of macromolecules, and these are called *lomasomes* and *plasmalemmasomes*. The former are membranous vesicular material embedded in the wall external to plasmalemma, while the latter are similar vesicular material projecting into the cytoplasm internal to the plasmalemma. However, there are gradations between these two kinds of structures.

2.3.1.2 Plastids

Plastids are semi-autonomous organelles, believed to have evolved from free-living cyanobacteria through *endosymbiosis* (Margulis and Sagan 2002). They are characteristic of plant cell and are covered by a double membrane. The inner membrane is the main barrier for permeability between the cytosol and plastid matrix. The outer, though also forms a barrier to cytosolic proteins, was earlier believed to be permeable to low molecular weight (<600 Da) solutes only. It is likely to be a wrong belief. The homogenous matrix of the plastid called the *stroma* and a system of stacked membranes called *thylakoids* are enclosed by the double membrane. Stroma-filled tubules called *stromules* have been found to emanate from the surface of some plastids and to interconnect different plastids (Kwok and Hanson 2004). They are likely to help in the exchange of water but are also believed to enhance specific metabolic activities. The plastids also contain *nucleoids*, which are regions having circular DNA (like bacteria); these are not associated with histones. The genomes of plastids are quite small when compared to the nuclear genome. Plastids also have 70S ribosomes. Plastids reproduce by binary fission. Plastids are derived from colourless *proplastids* (Fig. 2.4), which are immature plastids, found in undifferentiated parts of the plant body and also in the zygote derived from an egg cell.

Plastids are classified on the basis of presence or absence of pigments and the colour of the pigments, if present, into a few categories (Fig. 2.4). The *chloroplasts* contain the chlorophyll and

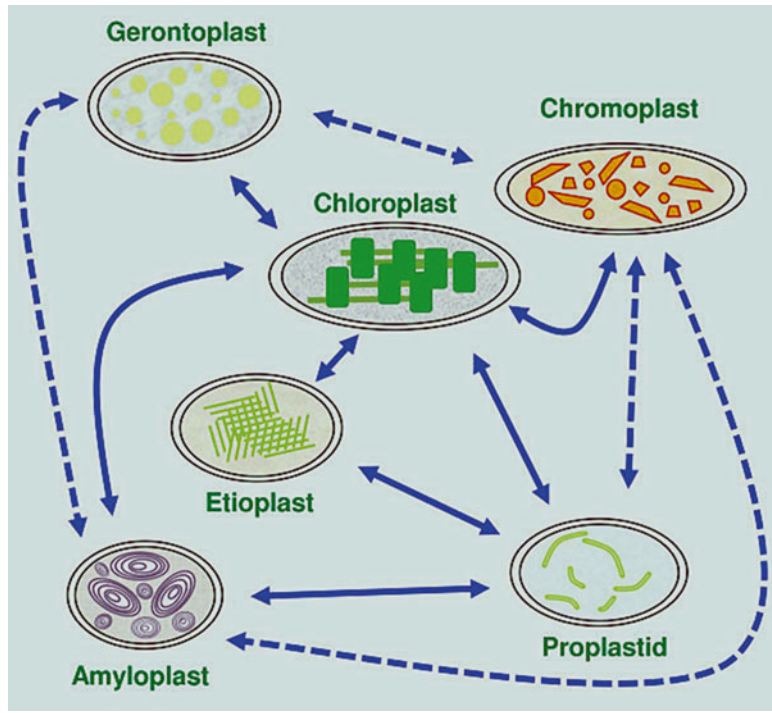


Fig. 2.4 Different types of plastids and their interrelationship (Thomas et al. 2009)

carotenoid pigments and give green colour to the organs (like leaves) containing them. Chloroplasts are usually discoid in shape, 2–6 μm thick by 5–10 μm diameter. Chloroplasts can reorient themselves inside the cells depending upon the light and hence can optimize the utilization of light; under high light intensity, they orient themselves parallel to the wall perpendicular to the leaf surface. Blue-UV spectrum is the most effective in chloroplast reorientation, and this movement probably involves actin-myosin system of the concerned cell. The stroma of chloroplasts is traversed by an elaborate system of thylakoids consisting of *grana*, which are stacks of discoid thylakoids, like a stack of coins. The stromal thylakoids that traverse the chloroplast matrix are interconnected to granal stacks. The thylakoids are not physically connected to the chloroplast membrane but are totally embedded in the stroma. The pigments, along with chloroplast proteins, are embedded in the thylakoid membranes in discrete units of organization called *photosystems*. Chloroplasts may contain starch (as a temporary storage product), phytoferritin and lipids (in the form of plastoglobuli). There are many copies of circular plastid DNA molecule, which encode for around 100 proteins, although most proteins involved in chloroplast function are coded by the nucleus.

The *chromoplasts* contain only carotenoid pigments and do not contain any chlorophyll. The pigments may be yellow, orange or red. Chromoplasts occur in old leaves, flowers, fruits and in some roots. Chromoplasts are of four types: (1) *globular chromoplasts*, which have many carotenoid-bearing plastoglobuli and remnants of thylakoids; (2) *membranous chromoplasts*, which are characterized by a set of up to 20 concentric (double), carotenoid-containing membranes; (3) *tubular chromoplasts*, in which carotenoids are incorporated into filamentous lipoprotein tubules; and (4) *crystalline chromoplasts*, which contain crystalline inclusions of pure carotene; these are called *pigment bodies*. Chromoplasts generally arise from chloroplasts with the loss of chlorophyll and thylakoid membranes; disappearance of ribosomes and rRNAs, but not of DNA; and with the accumulation of carotenoids. The precise functions of carotenoids are not well understood, but more likely, they attract insects for pollination and animals for fruit/seed dispersal. Carotenoids are antioxidant and prevent photooxidative damage to chlorophyll molecules (Niyogi 2000).

The colourless plastids are called *leucoplasts* and are nonpigmented. They have a uniform granular stroma, several nucleoids and 70S ribosomes but lack thylakoids; they often store substances like starch (*amyloplasts*), proteins (*proteinoplasts*) or oil (*elaioplasts*). The etioplasts that are leucoplasts of etiolated plant contain *prolamellar bodies*, which are quasicrystalline and with tubular membranes.

Plastids that are on the verge of senescence and degeneration are called *gerontoplasts*.

2.3.1.3 Mitochondria

These are double-membraned cell organelles of 0.5–1.0 μm long by 1–4 μm broad. The inner membrane is inwardly convoluted into folds known as *cristae*, which increase the surface area available to enzymes and to their chemical reactions (Fig. 2.5). The two membranes surround the mitochondrial matrix, which contains enzymes, coenzymes, water, phosphatases and other respiratory molecules. Mitochondria are the sites of cellular respiration and the release of energy from organic molecules in the form ATP. The outer mitochondrial membrane is relatively permeable to most smaller molecules, but the inner is relatively impermeable and permits only certain molecules such as pyruvate and ATP.

Mitochondria are in constant motion inside the cell, and their movement is probably controlled by actin–myosin-based system. Since mitochondria are semi-autonomous, they are able to synthesize some of their own proteins. Each mitochondrion contains one or more DNA-containing nucleosomes, and the DNA is without histones. It also has many 70S ribosomes. The mitochondrial genome is much smaller in size and is 200–2,400 kb. The DNA molecule is linear, circular or more complex depending on the taxon and is able to encode for about 30 proteins. Mitochondria are believed to have evolved from α-proteobacteria through endosymbiosis, and

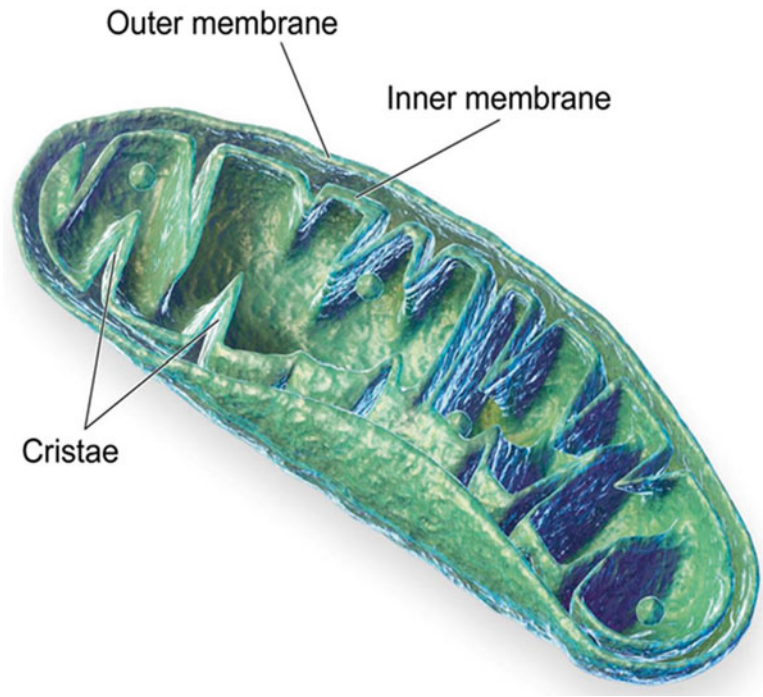


Fig. 2.5 Diagrammatic representation of a vertically split mitochondrion (Source: http://en.wikipedia.org/wiki/Mitochondrion)

that during further evolution, like plastids, mitochondria have passed on many of their DNA to the host cell's nuclear DNA. There are also evidences for the transfer of some genetic information from plastids to mitochondria and from nucleus to mitochondria.

Mitochondria are involved in the control of cytoplasmic sterility. They are involved in PCD (apoptosis) of animal cells through the release of cytochrome C, but their role in plant PCD is questionable and surely no cytochrome C is released during PCD.

2.3.1.4 Peroxisomes

These were earlier called *microbodies*. They are invariably spherical and are single-membrane bodies (Olsen 1998). Neither DNA nor ribosomes are present in them, and hence, all their proteins are nuclear coded. They range in size from 0.5 to 1.5 μm diameter. Although they can self-replicate, they may also be generated de novo, according to some. Their granular interior sometimes contains amorphous or crystalline protein bodies. Biochemically, they are characterized by at least one hydrogen peroxidase, and hence, they are called peroxisomes. They, however, show metabolic plasticity, since their enzyme content can vary very greatly. Because of this, peroxisomes do a wide array of metabolic activities/functions (Hu et al. 2002).

Two very different peroxisomes have been studied in great detail in plants. The first one is found in green leaves and is involved in glycolic acid metabolism that is associated with *photorespiration*, a process in which oxygen is consumed and CO_2 is released, and in cooperation with mitochondria and chloroplasts. The other is present in endosperm or cotyledons of germinating seeds and is essentially needed for converting fat into carbohydrates; hence, these are called *glyoxysomes*. Both these types of peroxisomes are interconvertible. Peroxisomes are motile organelles and their movement involves the actin–myosin complex.

2.3.1.5 Ribosomes and RNA

Ribosomes are small cellular particles of about 15–25 nm, but they are larger than prokaryotic ribosomes. They contain proteins and RNA. The number of protein molecules in ribosomes greatly exceeds the number of RNA molecules. RNA constitutes about 60 % of the total mass of a

ribosome. Ribosomes form the sites where amino acids are linked together into polypeptides/proteins. Each ribosome has two structural units, a small and a large one, with specific ribosomal RNA and proteins molecules. The ribosomes may occur freely in the cytosol or attached to the surface of endoplasmic reticulum (ER) and outer surface of nuclear envelope. They are also found in nuclei, plastids and mitochondria.

Ribosomes that are involved actively in protein synthesis from amino acids and that occur in clusters are called *polysomes* or *polyribosomes* and are united by mRNA molecules that carry genetic information from the nucleus. The amino acids needed for protein synthesis are brought to the polysomes by the tRNAs of cytoplasm. The proteins are synthesized by tRNAs of cytoplasm, and proteins are synthesized by *translation*. The energy needed for this process is provided by the hydrolysis of guanine triphosphate (GTP).

Special mention must be made here on microRNAs (=miRNAs). These are ~21 nucleotide noncoding RNAs that regulate the expression of target genes by binding to the complementary sequences located in their transcripts. Such a binding can lead to translational attenuation or target the mRNAs for cleavage and degradation (Kidner and Martienssen 2005; Carraro et al. 2006). Several hundred miRNAs have been identified from a diverse group of plant species, and, of them, many putative target genes have been predicted. The functions of a number of miRNAs have been demonstrated by overexpression or knockout studies and by characterization of their target genes. One of the best characterized miRNAs in plants is miR165/166 which is shown to be localized in shoot apical meristems (SAM), leaf primordia and vascular tissues in *Arabidopsis*. This is important in the regulation of *HD-ZIP III* genes involved in SAM activity, floral development, auxin signalling and vascular development. The other target genes include members of *TCP*, *NAC*, *ARP*, *AP2* or *MYB* families. Thus, miRNAs may provide an important level of regulation during plant development. MiR159 targets *DUO1* gene required for cell division in *Arabidopsis* male gametophyte.

2.3.1.6 Endomembrane System

All cellular membranes other than those of mitochondria, plastids and peroxisomes constitute the *endomembrane system* (Fig. 2.6). Thus, this continuous, interconnected membrane system is

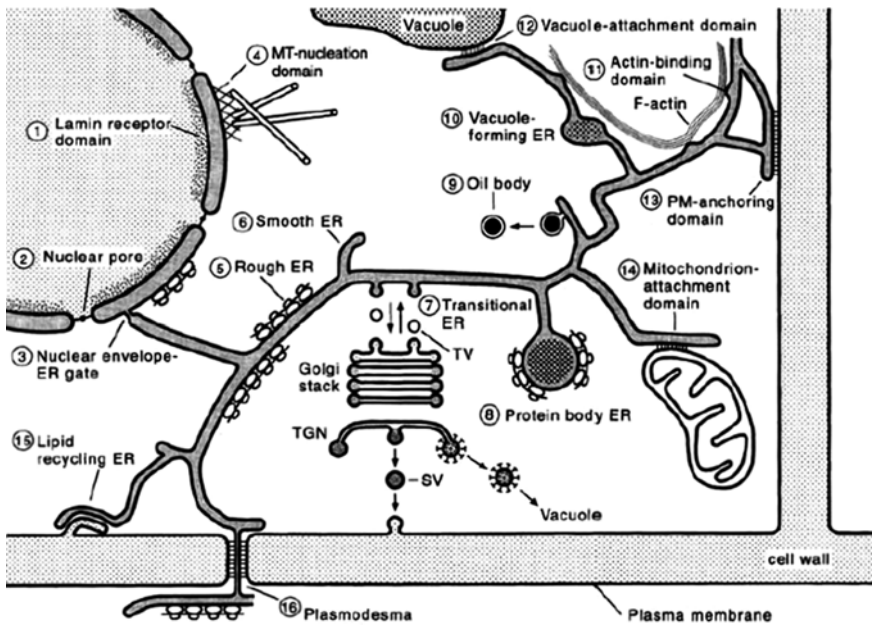


Fig. 2.6 A diagrammatic representation of the endomembrane system depicting the 16 types of ER domains (Staehelin 1997) © Blackwell Publishing

formed by the membranes of plasmalemma, nuclear envelope, ER, Golgi bodies, tonoplasts and various kinds of vesicles of the cell. The ER forms the initial source of the endomembrane system. Transition vesicles originating from the ER transport new membrane materials to the Golgi bodies, while the secretory vesicles derived from the Golgi contribute membrane materials to the plasma membrane. Thus, the ER and Golgi bodies constitute the most important functional unit in which the latter serve as the main vehicle for the transformation of ER-like membranes into plasma membrane-like membranes (Staehelin 1997). Membrane deformation, as a process of vesicle trafficking, budding and fusion, in endomembrane system, is regulated by the interplay between lipids and proteins, which act like wedges in the membrane. Changes in lipid composition via, for example, phospholipid acylation and/or deacylation, is also a potential mechanism of membrane deformation (Kim et al. 2011).

2.3.1.6.1 Endoplasmic Reticulum (ER)

The ER forms a continuous, three-dimensional endomembrane system that permeates the entire cytosol. The ER is made of two parallel membranes with a narrow space between them and should not be confused with a single unit membrane since each of the two parallel ER membranes is itself a unit membrane. Each membrane is about 7.5 nm thick. The cisternae with two membranes vary in thickness. The abundance and form of ER varies quite considerably from cell to cell with the extent of metabolic activity and with the stage of its development. Cells involved in secretion or in storage of proteins have an abundant quantity of *rough ER*, which are made of flattened sacs or *cisternae* with numerous ribosomes located on their outer surface. However, cells that are very actively involved in the production of lipids in large amounts have extensive systems of tubular *smooth ER*, which lack ribosomes on their outer surface. Both types of ER can occur in the same cell (Fig. 2.6) and are often physically continuous.

The ER is involved in several functions of the cell. Staehelin (1997) had recognized 16 types of functional ER domains or subregions (Fig. 2.6) to which two more domains were added in 2001. The most important among these 18 domains are the following: nuclear pores, ER-Golgi transitional domain near the latter, nuclear envelope–ER connection gates, rough ER domain acting as the port of entry proteins into the secretory pathway, smooth ER domain involved in lipid synthesis (including isoprenoids and flavonoids), protein body-forming domain, oil body-forming domain, vacuole-forming domain, plasmodesmal domain, ricinosome-forming domain (where *ricinosomes* of senescing endosperm of germinating castor seeds are formed by the budding off of ER to initiate PCD in order to deliver huge quantities of cysteine endopeptidase to the cytosol) and the 'nodal ER' domain, which is unique to root cap columella cells that perceive gravitropic signals. An extensive two-dimensional network of ER (*cortical ER*) made up of interconnected cisternae and tubules is found just inside the plasmalemma in the peripheral or cortical cytoplasm. Its membranes are continuous with those of the ER lying deeper in the cytoplasm including those of the transvacuolar strands of greatly vacuolated cells. Similarly, the outer nuclear membrane is also continuous with the rough and smooth cytosolic ER, and this membrane continuum encloses a single lumen and pervades the entire cytosol. The cortical ER functions as a structural element that stabilizes/anchors the cytoskeleton of the cell (Lichtscheidl and Hepler 1996). It also regulates Ca^{2+}, thus playing an important role in a number of developmental and biochemical processes. Studies made on living cells and cells stained with vital fluorescent dyes have shown that ER membranes are in constant motion with concomitant change in their shape and distribution. This motion is more active in deep-located ER than in cortical ER, although the latter are constantly restructured. This slow motion of cortical ER is presumed to be due to its anchorage at plasmodesmata and adhesion to the plasmalemma.

2.3.1.6.2 Golgi Bodies

Golgi bodies are highly polarized membrane systems involved in cell secretion. They are also called *Golgi stacks* or *dictyosomes*; some consider Golgi bodies as being made of dictyosomes (Salisbury and Ross 2004). The term 'Golgi body' refers collectively to all the Golgi body–*trans*-Golgi network. Golgi bodies are 0.5–2.0 μm in diameter, and their membranes are 7.5 nm in thickness. Each Golgi body consists of five to eight stacks of flattened cisternae, which invariably have bulbous and fenestrated margins (Fig. 2.6). The two opposite poles of a stack are referred to as *cis*- and *trans*-faces. Three morphologically distinct cisternae may be recognized across a Golgi stack: *cis*-, medial, and *trans-cisternae*, each of which differs from one another both in structure and in biochemistry (Driouich and Staehelin 1997). The *trans-Golgi network* (*TGN*), a tubular network with clathrin-coated and non-coated budding vesicles, is associated with the *trans*-face of the stack, and each Golgi-TGN complex is embedded in and surrounded by a ribosome-free zone, often called *Golgi matrix*. Unlike mammalian Golgi bodies, those of plant cells consist of several separate stacks that remain functionally active during cell division (Andreeva et al. 1998; Dupree and Sherrier 1998). Just before mitosis, the number of Golgi stacks doubles by cisternal fission, and this doubling happens along the *cis*-to-*trans* direction. The Golgi stacks undergo stop-and-go movements and rapidly oscillate between directed movement and random 'wiggling'. This movement is considered to be regulated by the 'stop signals' produced by ER export sites and locally expanding cell wall domains in order to optimize the ER to Golgi and Golgi to cell wall trafficking of materials (Nebenführ et al. 2000).

In the majority of plant cells, Golgi bodies serve the following two main functions: (1) synthesis of noncellulosic cell wall polysaccharides such as hemicelluloses (like xyloglucans) and pectins (like polygalacturonic acid–rhamnogalacturonan I, PGA-RGI) and (2) glycosylation of proteins to form glycoproteins such as hydroxyproline-rich proteins (or extensins). Most of the work concerning these two functions have been done at professor Staehelin's lab at the University of Colorado at Boulder using both monoclonal and polyclonal antibodies raised against these polysaccharide and glycoprotein antigens. This work has shown that the different steps in polysaccharide synthesis occur in the different cisternae of the Golgi body. The, thus, produced wall polysaccharides are packaged in secretory vesicles, which then migrate to and fuse with the plasma membrane and then involving exocytosis. The vesicles subsequently discharge their contents, and the released polysaccharides become part of the cell wall (Moore et al. 1991; Zhang and Stahelin 1992; Driouich et al. 1993). The initial stages of protein glycosylation occur in the rough ER, and subsequently, the glycoproteins transferred to the *cis-face* of the Golgi body via transition vesicles. Then, these glycoproteins proceed stepwise across the Golgi stack to the *trans*-face and then are sorted in the TGN for delivery to the vacuole or for secretion at the cell surface. Both polysaccharides and glycoproteins can be simultaneously processed in the same Golgi body. Glycoproteins and complex noncellulosic polysaccharides meant for cell wall are packaged in non-clathrin-coated smooth vesicles, while hydrolytic and storage proteins destined for vacuoles are package at the TGN into clathrin-coated vesicles and smooth, electron-dense vesicles, respectively.

2.3.1.6.3 Vacuoles

Vacuoles are unique to plant cells, where they are almost about 95 % of the cell volume (Fig. 2.7). The vacuole is bound by a single membrane, the *tonoplast* or *vacuolar membrane* of about 7.5 nm thick. Vacuoles are multifunctional organelles and vary in form, size, content and dynamics (Wink 1993; Marty 1999). The same cell may contain more than one type of vacuole. Some vacuoles function mainly in storage; some others serve primarily as lytic compartments. The storage and lytic types of vacuoles often are characterized

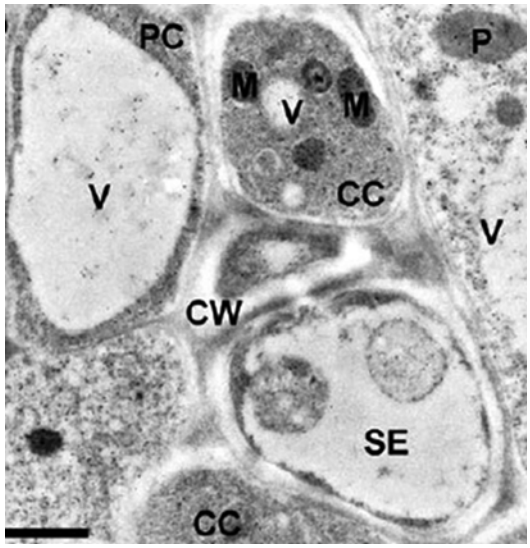


Fig. 2.7 TEM of a T.S. of a portion of leaf vascular bundle of *Arabidopsis* showing vacuoles (*V*) in parenchyma cells (*PC*). Also seen are companion cells (*CC*), sieve elements (*SE*). *CW* cell wall, *M* mitochondria, *P* plastids (Zechmann and Müller 2010)

by the presence of specific *tonoplast integral/intrinsic proteins* (*TIPs*), with α-TIPs associated with the tonoplast of protein-storage vacuoles and γ-TIPs with lytic vacuoles. Smaller vacuoles of both storage and lytic types can merge individually to form larger vacuoles of the two types during cell expansion. Many meristematic cells and their immediate derivatives contain numerous extremely small vacuoles, and as they enlarge, their vacuoles also enlarge and often fuse with one another. In fact, cell enlargement itself is often due to increase in vacuole number and size. Increase in vacuole size increases the surface area of the cytoplasm. The tonoplast is involved in the regulation of osmotic phenomena and cell turgidity changes. New vacuoles can arise from the dilation of specialized regions of smooth ER or Golgi vesicles.

Storage vacuoles store different types of storage components. The principal component of nonprotein-storing vacuoles is water, along with

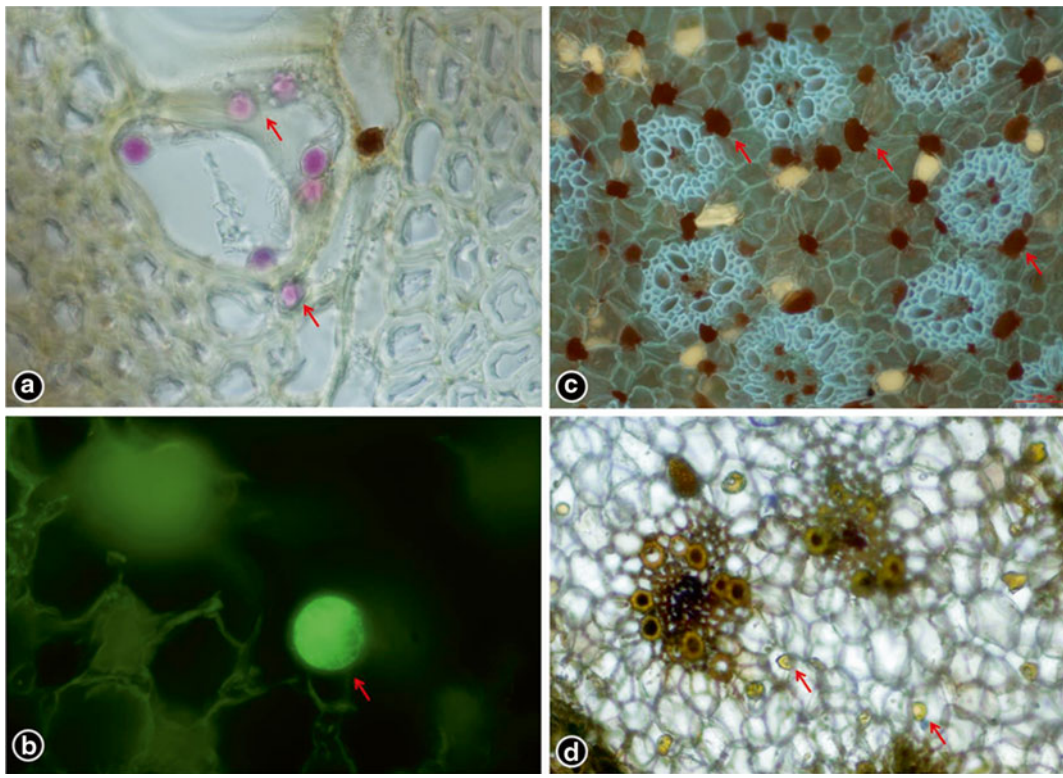


Fig. 2.8 (**a**) T.S. of wood of *Aquilaria* stained for terpenoid using vanillin perchloric acid. Terpenoids stained to a pink colour. (**b**) T.S. of rhizome of *Cyperus scariosus* showing terpenoid deposit (*arrow*) as examined under fluorescence. (**c**) T.S. of rhizome of *Cyperus scariosus* showing brownish red tannin deposits (viewed under fluorescence microscope). (**d**) T.S. of rhizome of *Cyperus rotundus* showing alkaloid deposits (*arrows*) (Courtesy of S. John Adams)

inorganic ions such Ca^{2+}, Cl^-, K^+, Na^+, NO_3^- and PO_4^{2-}, in aqueous solution called *cell sap*. Cell sap plays an important role in cell shape and turgidity. If the concentration of any one of the solutes in a vacuole is sufficiently high for it, it forms crystals, as for example, calcium oxalate, calcium carbonate, silica, etc. Vacuoles are also important in the storage of several primary and secondary metabolic chemicals such as various sugars, organic acids, proteins, lipids, etc. Such a storage is either temporary or for a long term. Vacuoles are also known to sequester toxic secondary metabolites such as alkaloids, tannins, phenolic compounds, terpenoids, etc. (Fig. 2.8), thus helping in detoxification. However, most, if not, all of them, are recycled by the plant. Hence, many vacuolar chemicals which were earlier considered as waste products or *ergastic substances* are now known to be recyclable and reusable. These secondary metabolites help the plants in self-defence against pathogens, predators and herbivorous animals. Many vacuoles are also sites of pigment deposition, and these pigments include anthocyanins (frequently in epidermal tissue of aging leaves, fruits, petals, etc.), imparting different colours.

The lytic vacuoles serve as lytic compartments where macromolecules are broken down and recycled. Entire cytoplasm with organelles of cells undergoing senescence/PCD may be engulfed by the lytic compartments, and a number of hydrolytic enzymes, called *lysozymes* (comparable to the *lysosomes* of animal cells), break down these components (Krishnamurthy et al. 2000). This phenomenon is called *vacuolar autophagy*.

2.3.1.7 Cytoskeleton

The cytoskeleton forms a dynamic, three-dimensional network of filamentous proteins that extends throughout the cytosol. It is involved in many processes that include cell division, cell expansin, cell differentiation, cell-to-cell communication and movement of cell organelles and other cytoplasmic components from one location to another within the cell (Seagull 1989; Derksen et al.1990; Goddard et al. 1994; Brown and Lemmon 2001; Kost and Chua 2002; Sheahan et al. 2004).

Two types of cytoskeleton protein filaments are recognized: *microtubules* (*MTs*) (Fig. 2.9a) and *actin filaments* (*AFs*) (Fig. 2.9b). The MTs are cylindrical and about 24 nm in diameter with a central core of 12 nm and of varying lengths. Each MT is made of two different types of protein molecules (a dimer): *α-tubulin* and *β-tubulin*. Both come together to form soluble dimmers, which then self-assemble into insoluble tubules. The subunits are arranged in a helix to form 13 rows or 'protofilaments' around the core of lightly contrastable material. The tubulin proteins have a molecular weight of 110 KDa. MTs are polarized structures with + and – poles, with the former pole growing faster than the latter. The

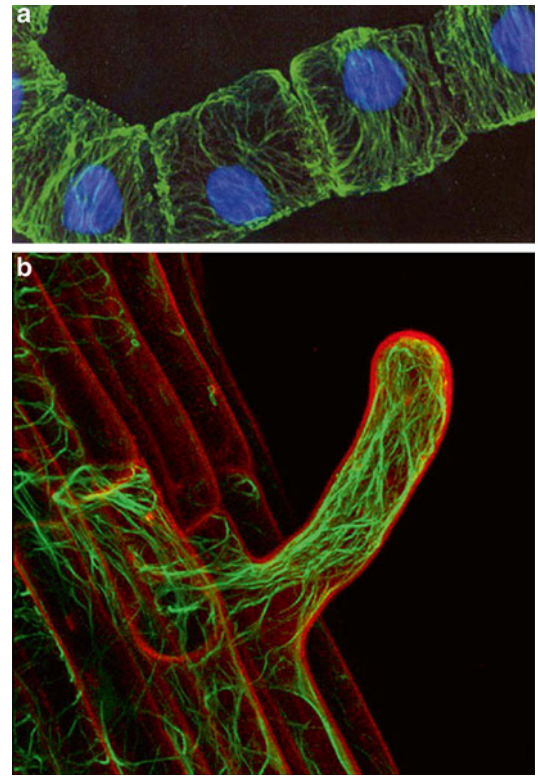


Fig. 2.9 (**a**) Cortical microtubules and nuclei in tobacco BY-2 cells (Courtesy of Dr. Seiichiro Hasezawa) (Source: http://hasezawa.ib.k.u-tokyo.ac.jp/zp/hlab/78147a765ba469828981-1/theme-of-study). (**b**) Root hair of an *Arabidopsis thaliana* seedling expressing GFP fused to the actin-binding domain of talin (GFP-mTalin). The root was counter stained with propidium iodide to reveal the outline of the cell walls (*red*). Image was taken with a Bio-Rad 1024 ES confocal microscope (Courtesy of The Samuel Roberts Noble Foundation, Inc)

poles of MTs can alternate between growing and shrinking states, and this behaviour is known as dynamic instability or '*treadmilling*'. MTs also undergo regular sequences of breakdown, reformation and rearrangement into new MT configurations or arrays at specific phases of the cell cycle and during cell differentiation (Azim-Zadeh et al. 2001). The most prominent cell cycle configurations of MTs are the interphase *cortical array*, the *preprophase band*, the *mitotic spindle* and *phragmoplast* (Kumagai and Hasezawa 2001). The growth and breakdown of MTs depend on a number of factors such as the availability and concentration of tubulin, presence of Ca^{2+} and Mg^{2+} (breakdown favoured by high concentration of Ca^{2+}), temperature (low temperature favours breakdown), high hydraulic pressure (favours breakdown), presence of chemicals like colchicine (arrests MTs during cell division), heavy water (promotes MT formation), etc. The MTs are shown to have many functions (Wasteneys 2004): control the alignment of cellulose microfibrils in growing and differentiating cells, the direction of cell enlargement, chromosome movement during nuclear division, phragmoplast and cell plate formation, etc. During cell cycle interphase, MTs radiate from all over the nuclear surface, which is the primary *nucleating site* or *microtubule organizing centre* (MTOC). Secondary MTOCs, whose materials are supplied by primary MTOCs, are localized in the plasmalemma, where they organize arrays of cortical MTs, which are essential for ordered cell wall synthesis.

The second category of cytoskeletal protein filaments, the AFs, is also called *microfilaments* (*MFs*) or *filamentous actins* (*F actins*). AFs are 5–7 nm thick and consist of two linear chains of actin molecules in the form of a helix. Like the MTs, the AFs are also polar structures with + and − poles. The AFs are made of actin monomers, which self-assemble into filaments that resemble a double-stranded helix (Staiger 2000). AFs may occur either singly or commonly in bundles and can assemble and function independently of MTs, for example, in driving cytoplasmic components, establishment of cell polarity, orientation of cell division planes, cell signalling, tip growths of pollen tubes and root hairs, control of plasmodesmal transport, thigmotropism/thigmonasty, etc.

2.3.1.8 Stored Substances

Cells store an array of metabolites which are products of primary and secondary metabolism. As already indicated, some of these products were originally considered as waste or ergastic substances. The most important organic storage substances are starch, inulin, proteins, lipid bodies and essential oils.

2.3.1.8.1 Starch

Starch is found as starch grains in plastids called amyloplasts. It is the most common storage polysaccharide found in green plants and is a primary metabolic product of photosynthesis. It is broken down to sugar and recycled in times of need. Starch grains may be simple or compound, vary greatly in size and shape (depending on the taxon) and commonly show layering (concentric or eccentric) around the *hilum* (Fig. 2.10). All starch grains are made of unbranched amylose chains and branched amylopectin, the two in alternate layers. Amylose is the predominant component of the starch grains of the leaves of sorghum and maize, while those of the seeds of the same plants have around 7–90 % amylopectin; potato starch grains also have a dominance of amylopectin. Storage starch occurs in different parts of the plant body in the parenchyma cells but more abundantly in endosperm, underground tuberous parts, rhizomes, corms and in fruits (especially mature unripe fruits), etc.

2.3.1.8.2 Protein Bodies

Protein bodies (Fig. 2.11a) are also important in storage and are formed in different ways. The salt-souble protein bodies are called *globulins*, which are the major storage proteins of many legume seeds. Globulins are present in the storage vacuoles to which place they are transported from rough ER through Golgi bodies, as mentioned earlier in this chapter. The alcohol-soluble proteins are called *prolamines*; these form the major storage proteins of most cereals. In wheat, these accumulate directly within the rough ER as

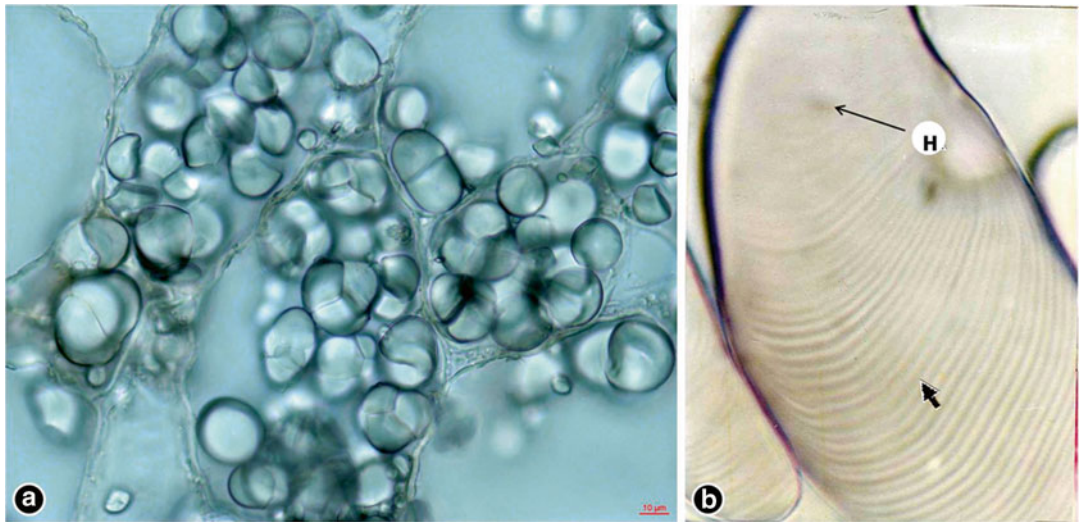


Fig. 2.10 (**a**) Cells of *Aconitum* fully filled with starch grains (Courtesy of S. John Adams). (**b**) A single starch grain of banana unripe fruit enlarged to show the amylose and pectin in alternate eccentric layers (*arrow*) around a hilum (H) (Courtesy of Dr. C. Santhakumari)

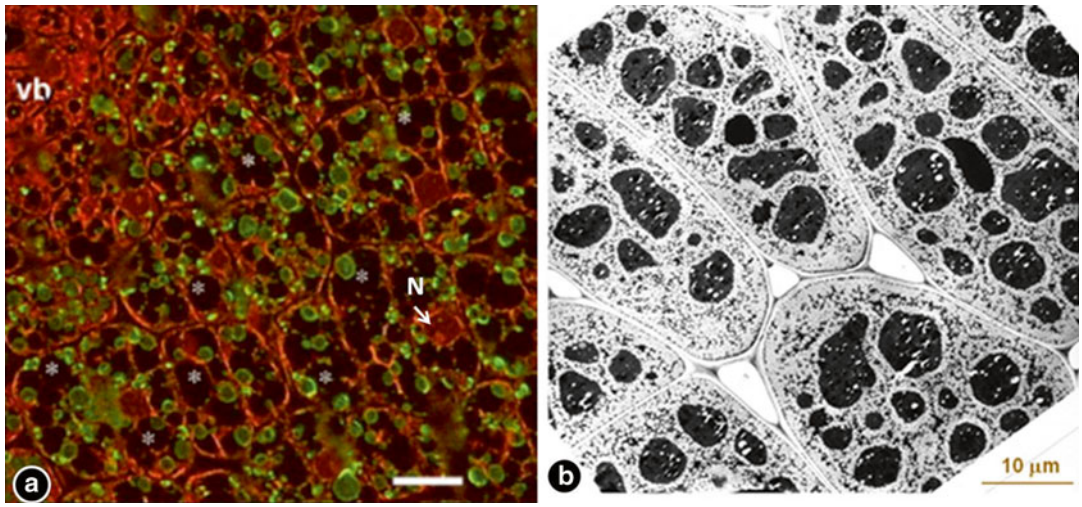


Fig. 2.11 (**a**) Light microscopy immuno-detection: the anti-vicilin protein antibodies are visualized in the multivacuolar compartment (*) with Alexa 488 green fluorescence and counterstained with the red fluorescent propidium iodide to stain nucleus (N). *vb* vascular bundle (Courtesy of Dr. Abirached-Darmency Mona). (**b**) TEM image of oil bodies seen in *Brassica* seed (Courtesy of Dr. Anthony H.C. Huang)

protein bodies and then are transported in distinct vesicles to vacuoles without involving Golgi bodies, while in other cereals, the similarly formed protein bodies are not transported to vacuoles but remain within the rough ER and bounded by ER membranes.

The structurally simplest of protein bodies consist of an amorphous proteinaceous matrix covered by a bounding membrane. The more complex protein bodies may have one or more nonproteinaceous globoids and one or more proteinaceous crystalloids, in addition to the

proteinaceous matrix. Complex protein bodies also contain a number of enzymes and a good amount of phytic acid, a cation salt of myoinositol hexaphosphoric acid that is usually stored in the globoids. Phytic acid is an important source of phosphorus during seed germination and seedling development. Some protein bodies of Apiaceae members may have calcium oxalate crystals. Proteins are also found in the form of crystalloids in the cytosol, as for example in parenchyma cells of potato tubers, among starch grains in banana fruits and in the parenchyma of the capsicum fruits. Proteinaceous crystalloids are also known in the nuclei of some taxa.

2.3.1.8.3 Oil Bodies and Essential Oils

Oil bodies (Fig. 2.11b), also known *spherosomes* and *oleosomes*, are spherical reserve bodies formed as buds of smooth ER membranes by an oleosin-mediated process. Each oil body is bounded by a phospholipid monolayer on which is embedded the oleosin. The oleosin and phospholipid layer stabilize the oil body and prevent it from coalescing. Oil bodies impart a granular appearance to the cytoplasm under the light microscope, while under electron microscope, they have an amorphous appearance. Oil bodies are fairly widely distributed, although they are the most abundant in fruits and seeds of some taxa. They arise by the accumulation of triacylglycerol molecules at ER 9 domain areas in the interior of the ER lipid bilayer (Ohbrogge and Browse 1995). These accumulation sites are defined by the presence of oleosins (integral membrane proteins) which are thumbtack-like molecules and which cause the oil bodies to bud into the cytosol (Huang 1996). Oil bodies usually occur in a liquid state, although crystalline forms of oil bodies are rarely present as in the endosperm of oil palm.

Essential oils, also called *ethereal oils*, are volatile chemicals that contribute to the essence or odour of the plants possessing them. They are synthesized by certain special cells and are excreted into intercellular spaces/cavities.

2.3.1.8.4 Tannins

Tannins (Fig. 2.8c) are heterogeneous group of polyphenolic substances that typically occur in vacuoles, although they may also get deposited on the cell wall facing the cytoplasm. They have an astringent taste. Two types of tannins are found among plants: *hydrolysable tannins*, which are hydrolysable with hot dilute acids to form glucose and phenolic acids, and *condensed tannins*, which cannot be hydrolyzed. The latter appear, under light microscope, as coarsely or finely granular material or as bodies of various sizes and are coloured yellow, red or brown. Many cells possess tannins, particularly in infected plants and galls. In some plants, there are special *tannin idioblasts* or *tannin sacs* or tubular tannin cells. Tannins apparently originate in ER. The primary function of tannins is protection as they repel predators, pests and invading microbes. In some allelopathic plants, they are excreted through roots. The cell wall tannin deposits arise from phenolics that initially get accumulated in the vacuoles, then appear in a solubilized form in the cytosol and then get impregnated on the walls.

2.3.1.8.5 Crystals

Crystals are formed when the concentrations of certain ions like Ca^{2+} or silica exceed a certain limit in the cell in which they are present. The most common crystals present in plants are calcium oxalate crystals, followed by calcium carbonate and silica. The first develop in vacuoles but are also present in the cell wall and cuticle in some taxa. Calcium oxalate is found in either mono- or di-hydrate forms (Prychid and Rudall 1999), of which the first type is more common and more stable. Calcium oxalate crystals are (1) *prismatic* (Fig. 2.12a) that are variously shaped prisms, usually one per cell; (2) *raphides* (Fig. 2.12b) that are needle shaped, usually in bundles (four types of raphides have been recognized so far) (Horner and Wagner 1995); (3) *druses* (Fig. 2.12c), which are spherical aggregates of several prismatic crystals; (4) *styloids* (Fig. 2.12e), which are elongated crystals with pointed or ridged ends, one or two in a cell; and (5) *crystal sands* (Fig. 2.12d), which are very small and often crystalline masses. Crystal-containing cell is either special idioblasts or may occur in series as *crystal strands*.

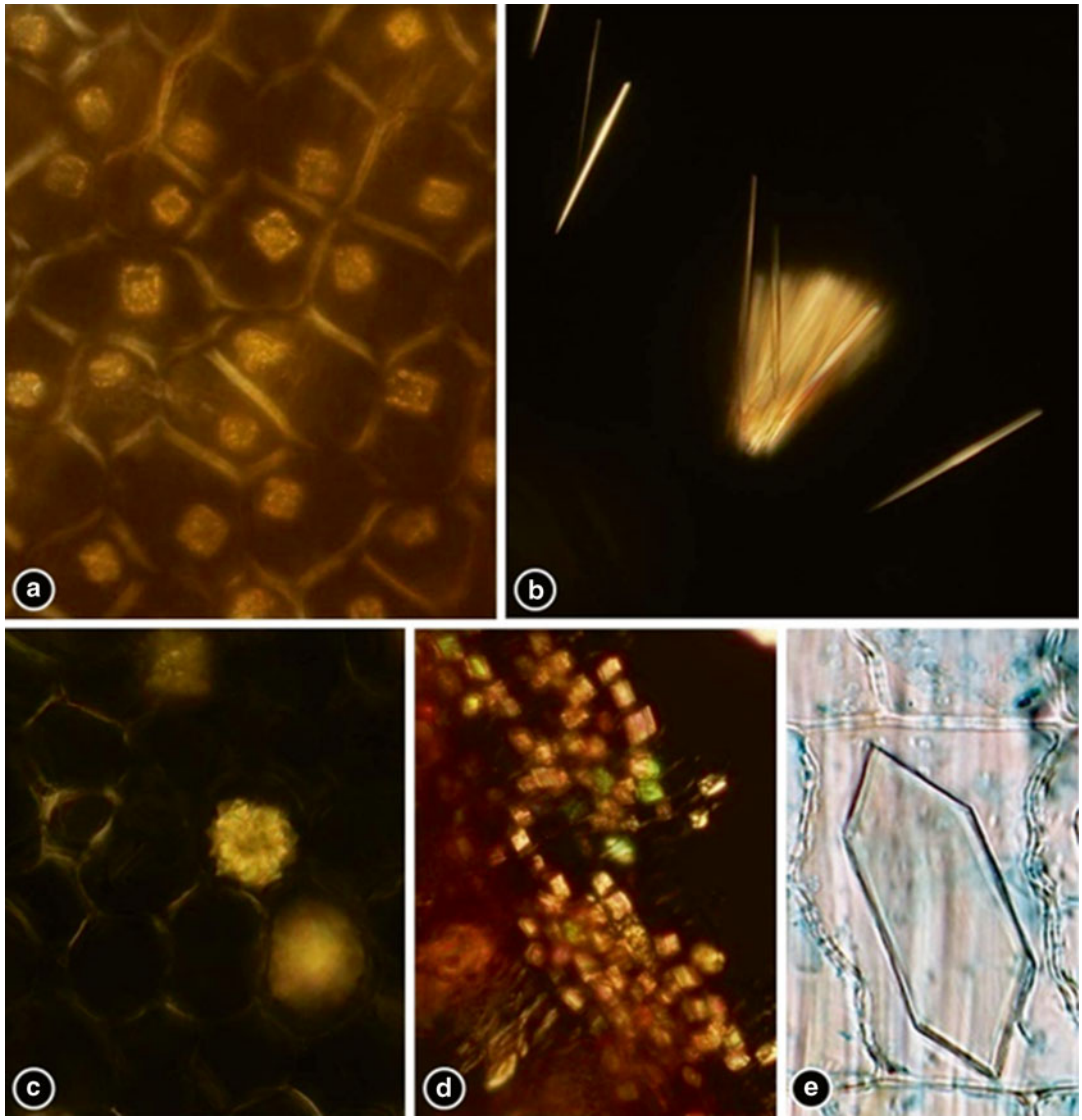


Fig. 2.12 Types of calcium oxalate crystals. (**a**) Prismatic crystals on the epidermal cells of *Vanilla* sp. (**b**) Raphides crystal bundle of *Cryptocoryne spiralis*. (**c**) Druse crystals of *Jatropha* sp. (**d**) Highly enlarged image of crystal sands in the stem of *Berberis lycium*. All polarized images (Courtesy of S. John Adams). (**e**) Styloid crystal of *Glycydendron amazonicum* (Source: Ritchter and Dallwitz 2000; http://delta-intkey.com/wood/en/www/euglyam.htm)

Calcium oxalate crystals usually develop in vacuoles. Crystal formation is commonly preceded by the formation of some type of membrane system, which, in some taxa, becomes complex crystal chambers (Webb 1999), through the deposition of cell walls, often made up of callose. Thus, the crystal is totally isolated from the protoplast of the concerned cell. Horner and Wagner (1995) have recognized two general systems of vacuolar crystal formation. The first system is characterized by the presence of vacuolar membrane complexes and of organic paracrystalline bodies that display subunits with large periodicity. To this system belong the druses of *Capsicum* and *Vitis*, raphides of *Psychotria*, crystal sand of *Beta* (all dicots), etc. The second system

is characterized by the absence of tonoplast complexes and the presence of paracrystalline bodies with closely spaced subunits. To this second system belong raphides of *Typha*, *Vanilla*, *Yucca* and *Dracaena* (all monocots). Calcium oxalate crystals can also be deposited on cell walls and cuticles; this type of deposition is uncommon in flowering plants (present in *Casuarina*, some Aizoaceae in cuticles; between epidermal cell wall and cuticle in *Dracaena* and between primary and secondary walls of astrosclereids of *Nymphaea* (Fig. 2.13) and *Nuphar* but more frequent in conifers).

Calcium oxalate crystals deposition is often reversible and, thus, is recyclable, when there is a requirement for calcium. Hence, this recycling depends on the Ca^{2+} in the cells at their immediate vicinity; if there is excess, then crystal formation is triggered. Thus, crystal calcium formation and dissolution are highly controlled and regulated processes. In *Pistia stratiotes*, the raphide-bundle-containing cells are greatly enriched with *calreticulin*, a calcium-binding protein present in the ER. This protein is involved in keeping cytosolic calcium activity low and allowing for a rapid accumulation of calcium used for crystal formation.

Calcium oxalate crystals are believed to be involved in the following functions:

1. Removal of oxalic acid (oxalate) which is not easily metabolized by the plant.
2. Protect plants against herbivory and pests.
3. As stated above, serve as a storage source of calcium.
4. Detoxification of heavy metals.
5. Provide mechanical strength and weight to the tissue containing them.

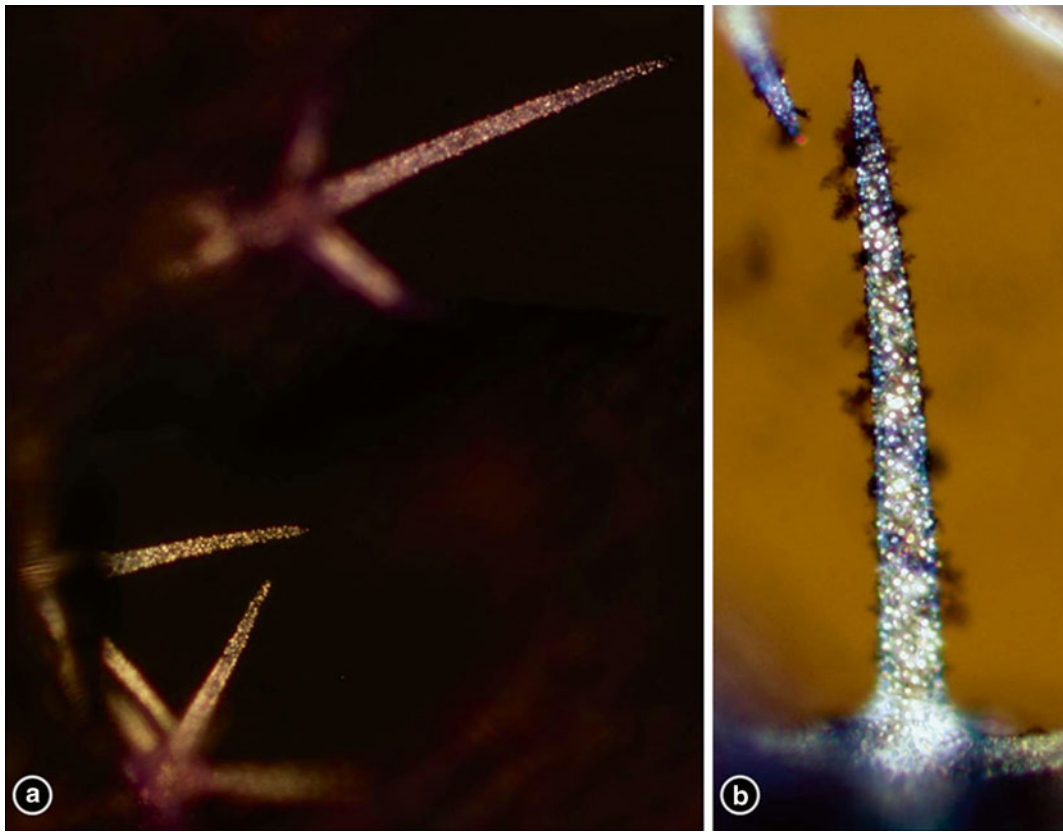


Fig. 2.13 Astrosclereids of *Nymphaea* petiole (**a**) showing crystals of calcium oxalate in the arms of the sclereids (**b**) as viewed under polarization microscope (Courtesy of S. John Adams)

6. The raphide cells provide defence to the plant as they forcibly eject their 'needles' and an irritative protease enzyme, which causes swelling and soreness, or an irritative 26 kDa protein, probably a cysteine protease.

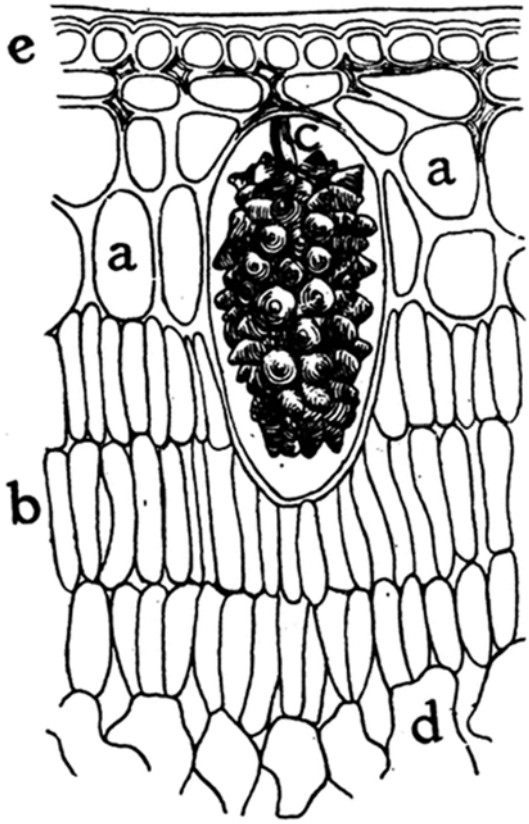


Fig. 2.14 Cross section of a portion of leaf of *Ficus elastica* showing the multiple epidermis from *e* to *a* with a cystolith (*c*,), (*b*) palisade parenchyma, (*d*) spongy parenchyma (Stevens 1911)

Calcium carbonate crystals are not very common in seed plants. The best known calcium carbonate crystals are the *cystoliths*, which are formed in special, enlarged cells called *lithocysts*; the lithocysts may occur in epidermis as in species of *Ficus* or in parenchyma cells of various plant organs as in members of Cucurbitaceae and Acanthaceae, in the latter family often as double cystoliths. In *Ficus*, cystoliths arise outside the plasmalemma in association with the cell walls of the lithocysts and then protrude into the lumen of the cell as grape-bunch-like structures (Fig. 2.14). Many cell wall materials like cellulose, silica, pectic polysaccharides and mainly callose form components of cystoliths, particularly of the wall materials protruding from the outer tangential wall of the lithocyst into the cell lumen and bearing the crystal bunch.

Silica is commonly deposited in cell walls of many grasses, sedges, *Equisetum* and a few other taxa. In grasses, it contributes 5–20 % to shoot's dry weight. It may be impregnated on the cell wall or may form *silica bodies* or *phytoliths* (Fig. 2.15) within the cell lumen. Silica crystals/deposits add strength to the plant body as well as protect the plants from herbivory.

2.3.2 Nucleus

The most prominent component of eukaryotic cells is the nucleus (Fig. 2.16). It generally ranges

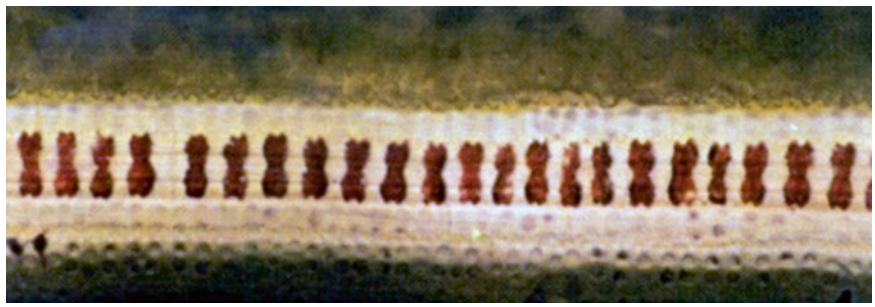


Fig. 2.15 Silica crystals in the epidermal cells of a rice variety stained with silver amine chromate (SAC) (Courtesy of Prof. P. Dayanandan)

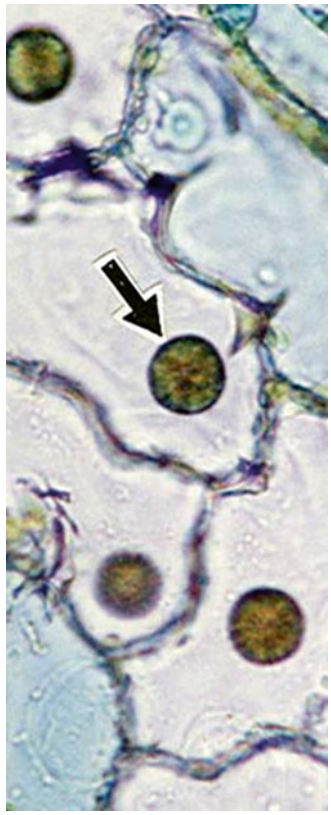


Fig. 2.16 Surface view of epidermal cells of *Basella alba* showing the nucleus (*arrow*) (Courtesy of Dr. V. Nandagopalan)

in diameter from 5 to 12 μm, but some cells have a much larger nucleus. Generally, only one nucleus is present in a cell, but in a number of fungi, some algae and a few cell types of angiosperms have more than one nucleus; this latter condition is called *coenocytic*. In basidiomycetous fungi, each hyphal cell contains two genetically distinct haploid nuclei, a condition known as *dikaryotic state*. The nucleus has two fundamental functions: (1) it controls cell's activities by determining which RNA and protein molecules are to be produced by the cell and when they should be produced, and (2) nucleus is a repository of most of cell's genetic information and passes on this information to the daughter cells in the course of cell division. The total genetic information found in a nucleus is called *nuclear genome*.

Each nucleus is bound by a pair of membranes called *nuclear envelope* of about 25–60 nm thickness, with a *perinuclear space* between them (Fig. 2.6) (Rose et al. 2004). The outer membrane is continuous with the ER in many places, and hence the perinuclear space is also continuous with the ER lumen. Hence, it is often considered as a locally specialized ER. The nuclear envelop has many cylindrical *nuclear pores* that provide contact between the nucleus, especially the *nucleoplasm*, and the cytosol (Fig. 2.6). Both the nuclear membranes join around the nuclear pores to form the border for the pore. The nuclear pore is structurally very complicated. The pore complex is more or less wheel-shaped consisting of a cylindrical central channel or the hub from which eight spoke-like structures extend outwardly to an interlocking collar that is associated with the nuclear membrane lining the pore. The structurally complicated pore complexes span the entire nuclear envelope and each measure about 9 nm diameter (Talcolt and Moore 1999; Lee et al. 2000). The pore complex allows certain ions and small molecules to pass through it. However, the proteins and other macromolecules that pass it greatly exceed its diameter. Hence, their passage through the pore is facilitated by a highly selective energy-dependent, active transport mechanism, thus making its functional diameter extended up to about 26 nm.

The nucleoplasm forms the matrix of the nucleus; it is granular and fibrillar. This texture is due to the presence of *chromatin*, which is made of *DNA* combined with a large amount of proteins called *histones*. During nuclear division, the chromatin gradually becomes more and more condensed to form *chromosomes*. Chromatin of nondividing or interphase nuclei is attached to the inner nuclear membrane at one or more places. Invariably, their bulk of chromatin is diffuse and lightly staining. In this uncondensed condition, the chromatin is called *euchromatin*, which is genetically active and associated with high rates of RNA synthesis. The remaining, genetically inactive, condensed chromatin is called *heterochromatin*; it does not take part in RNA synthesis (Franklin and Cande 1999). Before DNA replication (which precedes actual nuclear division), each chromosome is composed of a single, long DNA molecule. Only a very small percentage of total chromosomal DNA is involved in encoding essential proteins or RNAs, and thus, there is a substantial amount of surplus

DNA in the genome of plants. The chromosome number varies from four to several hundreds depending upon the plant. The nucleus may be *haploid* (n), as in gametes, spores and gametophytic tissues; *diploid* (2n), as in zygote and sporophytic tissues; or *polyploidal* (3n or more).

The nucleus, in its nucleoplasm, possesses one or more spherical bodies called *nucleoli* (singular: *nucleolus*). They are about 3–5 nm in diameter and contain a large amount of RNA and proteins.

2.3.3 Cell Wall

The cells of plants are bound externally by a cell wall and thus distinguish a plant cell from an animal cell. The cell wall forms the basis for several of the characteristic features of plants. Some people use the term *extracellular matrix* (*ECM*) (Staehelin 1991; Bolwell 1993; Roberts 1994) instead of cell wall or as an alternate for it. But many botanists object to the use of this term based on many compelling reasons.

2.3.3.1 Macromolecualr Composition

The plant cell wall has a vast array of chemical components. Of these, *cellulose* is the principal component in most plant cells. It is a homopolysaccharide that is made of linear chains of (1➔4) β-linked, D-glucan molecules that tend to form *microfibrils* through hydrogen bonding. Each microfibril is about 4–10 nm in diameter on the average, although it may be as small as 1–2 nm to as large as 25 nm. Microfibrils together form *macrofibrils* of about 0.5 μm diameter and 7 μm in length (visible in light microscope). The cellulose molecules have a tensile strength approximately equal to that of steel (i.e. 50–160 Kg/mm^2). Cellulose constitutes about 20–30 % of the dry weight of primary walls and about 40–60 % of the dry weight of secondary walls, depending on the taxon. Cellulose is a crystalline macromolecule (with monoclinic unit cells) and has a molecular weight of *ca* 1,000,000; hence, the wall containing cellulose is *anisotropic* and doubly refractive (*birefringent*) when viewed under polarized light (Fig. 2.17). Cellulose forms the *framework* or *skeletal component* of the cell wall,

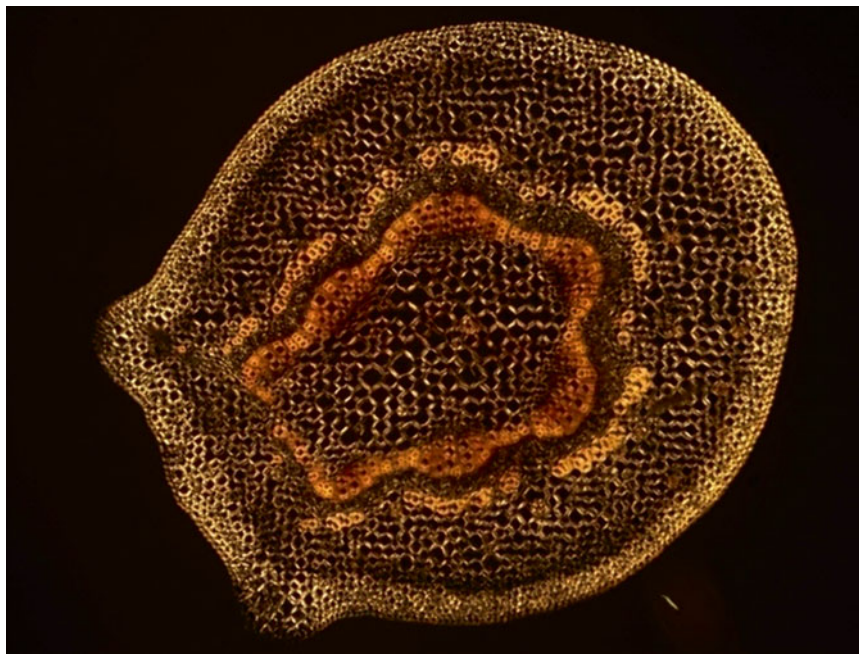


Fig. 2.17 T.S. of *Ricinus* petiole examined under polarization microscope showing birefringent cellulosic cell walls (Courtesy of S. John Adams)

since it is responsible for the mechanical properties of the cell wall and since it resists hot water, dilute alkali and acid treatments. Its microfibrils are embedded in a ground or matrix of noncellulosic polysaccharides, which can be extracted by hot water and dilute alkali or acids or both. The cellulose of some fungal cell walls is called *fungal cellulose*. Cellulose with *glycogen* is present in Acrasiales, and cellulose with *glucan* is present in Oomycetes. A number of fungi and a few algae (such as *Cladophora*, *Pithophora*) have *chitin*, instead of cellulose, a carbohydrate (polyglycan), as the framework material of their cell walls. These have spirally oriented fibrils of chitin, forming complexes with proteins. Chitin is a linear polymer of N-acetyl glucosamine (Fig. 2.18). Crystalline chitin has orthorhombic unit cells. Chitin occurs along with *chitosan* in Chytridiomycetes, Ascomycetes, Basidiomycetes and many Deuteromycetes, along with *mannan* in Sporobolomycetaceae and Rhizotorulaceae and along with cellulose in Hypochytridiomycetes. Some algae and fungi have other polysaccharides as the framework material of the cell wall: mannans-glucan in *Saccharomyces* and Cryptococcaceae, *polygalacturosamine-galactan* in Trichomycetes (Webster 1993).

The cell wall matrix is made of *hemicelluloses*, *pectins* and *structural glycoproteins*, of which the first ones are the most dominant. The commonest hemicelluloses is *xyloglucan*, which makes up 20–25 % of the dry weight of primary walls of dicots and 2 % of monocots. It is a linear chain of (1➔4) β, D-glucan as in cellulose with short side chains having *xylose*, *galactose* and often a terminal *fucose* (Carpita and McCann 2000). Xyloglucan is tightly hydrogen bonded to cellulose microfibrils, thus potentially limiting the extensibility of cell walls. Thus, xyloglucans play a very important role in controlling cell enlargement (Cosgrove 1997, 1999). Xyloglucan also functions as a storage polysaccharide. Primary cell walls with xyloglucan as the principal hemicelluloses are called *Type I walls* (Darley et al. 2001). The other hemicelluloses of cell walls are *glucuronoxylans*; *glucuronoarabinoxylans* (*Type II walls*, according to Darley et al. 2001); *xylans*, especially in secondary walls (Bacic et al. 1988); *glucomannans*, especially in the secondary walls of gymnosperms (Brett and Waldron 1990); etc. Pectins are chemically diverse acidic polysaccharides (Bacic et al. 1988; Willats et al. 2001) that are characteristic of the primary walls and middle lametta of eudicots but to a smaller extent of monocots. They account for 30–50 % of the dry weight of eudicots and 2–3 % of monocots; only traces are recorded in grasses (Fry 1988). Pectins may be totally lacking in secondary walls. The principal types of pectins are *polygalacturonic acid* (*PGA*)–*rhamnogalacturonan I* (*RG I*) and *RG II*. Pectins often form a gel matrix in which the cellulose-hemicellulose network is embedded. Because of their great hydrophilic nature, pectins provide a plastic property to the cell wall and modulate its stretching. Cell walls of meristematic cells are particularly low in Ca^{2+}, but its amount increases in their derivative cells. PGA has two major chemical forms: *nonesterifed pectin* in which the galactosyluronic acid residues contain carboxyl group at C-6 position and *esterified pectin* in which there is methylesterification at C-6 position. Pectins have zero birefringence, show little UV absorption and are isotropic. Pectins largely determine the porosity of the cell walls, the pore size ranging from 4.0 to 6.8 nm; these pores help

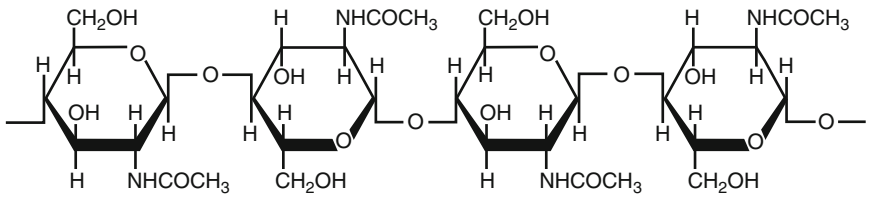


Fig. 2.18 Chemical structure of chitin

in the transport of salts, sugars, amino acids and phytohormones (Baron-Epel et al. 1988) but do not allow pathogens. Pectins play an important role in cell wall hydration, adhesion of adjacent cells and wall plasticity during cell elongation; they are also involved in fruit ripening (see Krishnamurthy 1999). Oligosaccharides derived from pectins are important in cell signalling events.

Alginic acid is an important cell wall component of brown seaweeds (Persival 1979). It is a mucilaginous, carboxylated polysaccharide that prevents desiccation of the thallus. It is a linear polyuronate compound of D-mannopyranosyluronic acid and L-glucopyranosyluronic acid residues, the proportion of the two varying in different taxa. Alginic acid is likely to be crystalline in nature. *Sulphated polysaccharides* are also common in the cell walls of algae. *Fucoidins* (including *sargassan*, *ascophyllan*, *glucaranoxylofucan*, etc.), *carrageenan*, *agar* and green algal sulphated polysaccharides are the well-known sulphated polysaccharides. Fucoidins are a family of polydisperse heteromolecules containing, in addition to fucose, varying amounts of galactose, mannose, xylose, uronic acid and glucuronic acid. These are extremely complex and highly branched molecules. Carrageenan has an alternating sequence of (1➔3)-linked β-D-galactopyranosyl and (1➔4)-linked β-D-galactopyranosyl residues (Rees 1969), containing various degrees and sites of sulphation. Agar contains a neutral gelling component *agarose* and an anionic fraction containing sulphate groups, the former containing chains of alternating (1➔3) and (1➔4) linkages; the predominant monosaccharide of carrageenan and agar is galactose, although xylose and mannose may also be present (McCandless 1981). The green algal sulphated polysaccharides are classified into three categories: glucuronoxylomannans, glucuronoxylorhamnogalactans, and xyloarabinogalactans (Persival 1979). The sulphated polysaccharides have the following functions: (1) their capacity to imbibe water helps the thallus against the physical buffeting caused by sea wave action; (2) their capacity to protect the thallus against desiccation; and (3) their anionic nature helps to serve as a sort of ion exchange material and might help to sequester certain ions (McCandless 1981).

Glycoproteins of cell walls are often structural in function (Cassab 1998). Structural glycoproteins constitute 10 % of the dry weight of primary walls. The most important among them are *lectins*; *hydroxyproline-rich glycoproteins* (*HRGPs*), also known as extensins; *proline-rich glycoproteins* (*PRPs*); *glycine-rich glycoproteins* (*GRPs*); *threonine-rich proteins* (*TRPs*); *thionins*; etc. (Krishnamurthy 1999; Jothi et al. 2010). HRGPs are known in the cell walls of many cell types such as tracheary elements, phloem, sclerenchyma and vascular cambium. Two types HRGPs are known, extensin 1 and extensin 2 (see full literature in Krishnamurthy 1999). HRGPs strengthen the cell walls through the formation of cross-linked insoluble cell wall matrix, help in protection, limit cell wall extension and protect the cell from biotic and abiotic stresses. Structural proteins are implicated in the strengthening of cell walls and have been reported in developing secondary walls of sclerenchyma and xylem cells as being important in lignification (Hariharan and Krishnamurthy 1995). *Arabinogalactan-rich proteins* (*AGPs*) are also glycoproteins that are found in cell walls, although they do not have any apparent structural role. Since they are soluble and diffusible, they act as messengers in cell-to-cell interaction particularly in incompatibility reactions between pollen/pollen tube and stigma/style, somatic embryogenesis, root epidermal cell expansion, pollen tube growth, etc. Another important cell wall protein is *expansin*, which is vital in cell wall extension (Li et al. 2002). Lectins are specific sugar-binding glycoproteins. They are also known as *agglutinins*, *phytoagglutinins*, *phytohaemagglutinins* or *prolectins*. Lectins are found in the cell walls of almost every plant tissue but are particularly abundant in seed cell walls. They are reported in all groups of plants. The most common lectins are *concanavalin A* (*con A*), *ricin*, *wheat germ agglutinin* and *peanut agglutinin*. Lectins are important in a variety of cell-to-cell recognition phenomena, facilitated by their specific sugar-binding

properties. Enzymic proteins are also known in cell walls. Numerous enzymes are associated with primary wall and some with secondary wall formation. These are known from several plant structures including pollen and pollen tubes. The most important enzymes known in cell walls are peroxidase, polyamine oxidase, phosphatases, proteases, prolyl hydroxylase, glycosyl hydrolases, transferases, esterases, etc.

Callose is one of the widely distributed cell wall polysaccharides, known right from algae to angiosperms. It is a linear chain of (1➔3) β-glucans. It is often laid between the plasma membrane and primary cellulosic wall as an adcrusting substance. It is associated with the developing sieve pores and fully lines them in the phloem sieve elements. It is reported in pollen tubes, micro- and megaspore mother cells, endothecial cells of anther developing fibres of cotton, cell plates of dividing cells, etc. Callose is also laid in cells immediately around a wound, infection site and sites subjected to other biotic and abiotic stresses. A polysaccharide called *laminarin*, which is similar to callose, is known in some marine seaweeds like *Laminaria* (Persival 1979). Another polysaccharide similar to callose, called *mycolaminarin*, is found in the cell walls of yeasts and some fungi. In these fungi, the (1➔3) β-glucan backbone is either substituted at intervals by (1➔6)-linked-β-glucosyl residues and the molecules are water-soluble or multiple-branched with varying proportions of (1➔3): (1➔6) linkages and the molecules are insoluble (Stone 1984; Blaschek et al. 1989). In all cases, callose is involved in insulating and isolating the concerned cells from the effects of adjacent cells as well as stress factors associated with biotic and abiotic stresses; callose is known to be impermeable to many chemical substances (see details in Krishnamurthy 2015).

Lignins form the most predominant incrusting secondary cell wall macromolecular chemical of vascular land plants, have a molecular weight of *ca* 11,000 and occur in supporting tissues like sclerenchyma and water-conducting xylem cells (Fig. 2.8c, d). Lignin is formed from polymerization of three principal units, the monolignols *P*-coumaryl, coniferyl and sinapyl alcohols (Ros Barcelo 1997). Based on the predominance of one or more of these monolignols in the fully polymerized lignin, lignins are classified into *guaiacyl* (formed mainly of coniferyl alcohol), *syringyl–guaiacyl* (formed mainly of synaphy and coniferyl alcohols) and *hydroxyphenyl–syringyl–guaiacyl* (formed of all three alcohols). These three types are, respectively, abundant in gymnosperms, woody angiosperms and grasses. However, lignins of different tissues in the same plant may belong to different categories mentioned above. For example, the protoxylem, secondary xylem and sclereids of the same plant may show different types of lignin. Lignin is apparently covalently linked to the cell wall polysaccharides. Lignification is an irreversible process and typically proceeded by the deposition of cellulose and noncellulosic matrix components, mentioned earlier (Grunwald et al. 2002; Kaliamoorthy and Krishnamurthy 1998). Lignin deposition waterproofs the secondary walls and hence limits lateral diffusion of water and promotes its longitudinal diffusion. Lignin deposition in water-conducting cells also provides the necessary mechanical rigidity to withstand the negative pressure generated by transpiration and to prevent collapse of the cell. Lignin is generally resistant to microbial attack, especially in a living plant; it is also resistant to environmental stresses, and hence lignified tissue is often preserved as fossils. Lignin is also deposited in non-lignified cells in response to wounding or attack by pests and parasites. Lignin strongly absorbs UV light, is strongly autofluorescent and has zero birefringence.

The cell wall often has phenolic acids as regular constituents. The most important among them are ferulic acid, diferulic acid, *p*-Coumaric acid, truxillic acid and *p*-hydrobenzoic acid. They are bonded to cell wall polysaccharides by ester linkage (Harris and Hartley 1980). Ferulic acid has been reported in many cell types of diverse tissues (barring perhaps phloem), including cutinized and suberized cells. Phenolic acids can also occur in secondary cell walls, covalently linked to lignin or may form bridges between polysaccharides and lignin (Iiyama et al. 1990). Wall-bound fungistatic phenolic amides

(=phytoalexins) have been reported in walls of infected host cells; these are non-specific toxins that act in plant defence.

Cutin and *suberin* are insoluble lipid polymers, often containing additional chemicals such as phenolics. Cutin and in some cases suberin also form a matrix in which *waxes* (long-chain lipid compounds) are embedded. Cutin and suberin together form barrier layers that prevent water loss as well as loss of many other molecules (Kolattukudy 1980). Cutin embedded with waxes forms the *cuticle*, which covers the aerial parts of vascular land plants. Cuticle is of variable thickness and has different amounts of cutin, waxes and cellulose; it is often multilayered. Suberin is a major component of cell walls of *cork* or *phellem*, of *endodermal cells*, of *exodermal cells* of roots (Fig. 2.19) and of bundle sheath cells of many Cyperaceae, Juncaceae and Poaceae. Suberin, in addition to preventing water loss, forms a barrier to microbial attack. Suberin has a *polyphenolic domain*, which is incorporated within the primary wall and is covalently linked to the *polyaliphatic domain*, which is deposited on the inner surface of the primary wall. The polyaliphatic domain in electron microscope looks polylamellar or layered with alternating light (aliphatic) and dark (phenolic) bands. Very long-chain fatty acid and waxes are found intercalated between aliphatic domains (Bernards 2002).

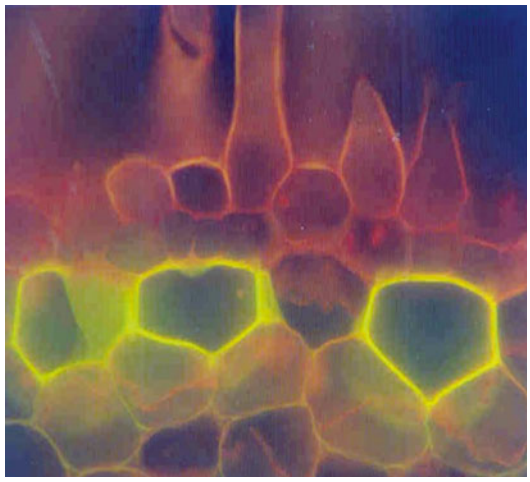


Fig. 2.19 T.S. of the peripheral region of the root of *Spathoglottis plicata* showing suberized exodermal cells with yellow fluorescence (Courtesy of Dr. Senthil Kumar)

Sporopollenin is a major cell wall material of pollen exine and spore coats and found nowhere else. It has highly polymeric lipidal esters consisting of carotenoid subunits and hence is extremely resistant to microbial and chemical degradation. An exact chemical analysis of sporopollenin is difficult, but the probable chemical formula of the sporopollenin of rye pollen is $C_{90}H_{134}0_{31}$ and of pine pollen is $C_{90}H_{158}O_{44}$. *Pollenkitt* is a hydrophobic material that covers the pollen exine of a number of taxa and is made up of lipoidal materials, carotenoids, and degenerated products of tapetal proteins (Heslop-Harrison 1975). Its probable functions include (1) insect attraction, (2) pollen dispersal (3) protecting pollen from UV radiation, (4) controlling sporophytic incompatibility and (5) controlling water loss from pollen grains. *Tryphine* is a complex mixture of hydrophilic substances including some proteins, all being derived from tapetal breakdown. Its functions are not yet clear.

A few *mineral substances* are present in the cell wall either in the mineral state or as complexes with organic chemicals. In the former instance, mineral occurs in cell walls either as incrustations or as impregnations. Silica occurs in a number of plants, especially in grasses, sedges, Commelinaceae, Equisetum, Urticaceae, Moraceae (Fig. 2.20) and diatoms (algae). Silica has a role in transpiration and prevents grazing by animals; silica-less diatoms do not survive. *Calcium* occurs forming complexes with pectin (*calcium pectate*), alginates (*calcium alginate*) or as in organic crystals (*calcium oxalate*, *calcium carbonate*) in cell walls; boron, magnesium, sodium and iodine are also reported in some cell walls.

2.3.3.2 Cell Wall Layers

The cell wall varies very greatly in thickness depending on the functions of the cell, age of the cell, plant organ and taxon on which the cells are located, etc. Young cells generally have growing thinner cell walls, while in cells which have stopped their growth, the cell wall stops its thickness growth. Wall formation by the protoplasts

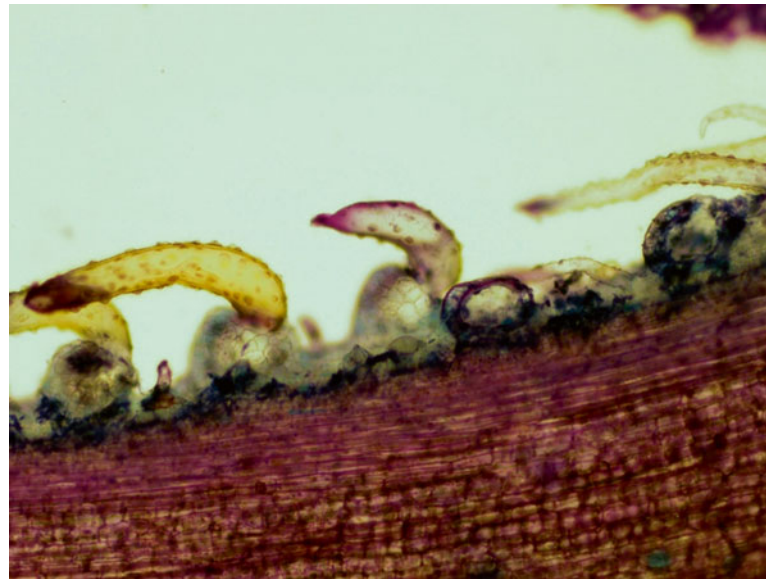


Fig. 2.20 L.S. of shoot bud region of *Ficus* sp. showing trichomes impregnated with silica (Courtesy of S. John Adams)

proceeds from the outside towards the inside, and therefore, the most recently formed and youngest wall layer adjoins the plasma membrane. The first-formed wall by a plant cell is the *primary wall*, and the region where the primary walls of two adjacent cells unite is called the *middle lamella* or *intercellular layer*; it is largely made of pectic polysaccharides and is isotropic. Many cells produce additional wall layers inside the primary wall, and these together constitute the *secondary wall*. Very often it is very difficult to distinguish, under a light microscope, the middle lamella from the primary wall, particularly in cells that have also produced a secondary wall. But the middle lamella could be easily distinguished under the electron microscope. Middle lamella may get lignified in cells with secondary walls. The term *compound middle lamella* is applied to the structure made up of the outer layers of primary walls of adjacent cells plus the middle lamella.

The primary wall is the wall produced by the protoplast before and during cell growth. It is weakly birefringent. It is usually thin but may attain thickness as in collenchymas, endosperm cells and cotton hairs. Such thick walls often show a polylamellar texture caused by the variations in the orientations of cellulose microfibrils or by the alternation of cellulose-rich and pectin-rich layers as in collenchymas cells. There are currently two models that explain the architectural construction of the primary walls (Cosgrove 1999), which have a network of cellulose microfibrils intertwined with hemicelluloses like xyloglucans and with both embedded in a pectin gel. As per the first model, xyloglucan-like hemicelluloses coat the surface of cellulose microfibrils to which they are non-covalently bonded and form cross-links or tethers that bind the cellulose microfibrils together. As per this model, the xyloglucans have three domains or fractions: (1) one that forms cross-links between cellulose microfibrils, (2) another that is closely associated with the surface of cellulose microfibrils, and (3) one that is entrapped within or between cellulose microfibrils. As per the second model, there is no direct link between cellulose microfibrils. The hemicelluloses that are tightly bound to the microfibrils are sheathed in a layer of less tightly bound hemicelluloses, which in turn are embedded in the pectin gel matrix that fills the spaces between the microfibrils (see Fig. 2.6 in Evert 2006).

The secondary wall is generally deposited inside the primary wall after growth in surface area of the primary wall has ceased. However, in many cases, its deposition starts even when the primary wall is growing in surface area. This wall

is generally developed in special cells that have a mechanical function and/or water conduction, and their protoplast die invariably after the secondary wall is deposited (see more details in chapter 3 of this volume). Cellulose is more abundant in this wall than the primary walls; some structural proteins and enzymes have been reported in developing secondary walls. Lignin is the most common material in secondary walls. The cells of the wood have three secondary wall layers, S_1, S_2 and S_3 (outer, middle and inner), which differ in the orientation of their cellulose microfibrils. Of these, S_2 is the thickest and S_3 the thinnest or even absent. In S_1, the microfibrils run along crossed helices which make a large angle with the long axis of the cell, and hence this layer shows birefringence. In S_2, the angle is smaller and the slope of the crossed helices steeper, and hence this layer does not show birefringence under polarized light. In S_3, the microfibril is as in S_1.

In algae and other thallophytes, a distinction between primary and secondary walls cannot be made, and one may speak of only growing and nongrowing cell walls (Preston 1974).

2.3.3.3 Primary Pit Fields, Pits and Plasmodesmata

The primary walls have *primary pits* which are very thin-walled areas in the wall, and these pits are located either singly or in groups in special areas of cell walls called *primary pit fields*. Most often *plasmodesmata* (singular: *plasmodesma*) are common in the primary pit fields, but are not restricted to them. Secondary walls possess pits, which are cavities in the wall. Invariably, pits of one cell lie opposite to pits of the adjacent cell to form *pit pairs*, in which the middle lamella and primary wall of both the cells constitute the *pit membrane*. Pits of secondary walls arise as areas where secondary wall is not deposited.

Pits vary considerably in size, distribution, abundance and detailed structure. However, there are two broad categories of pits: *simple pits* and *bordered pits* (Fig. 2.21). Simple pits do not have any border formed by the overarching of the secondary wall over the *pit cavity*. The bordered pit has an overarching secondary wall over the pit

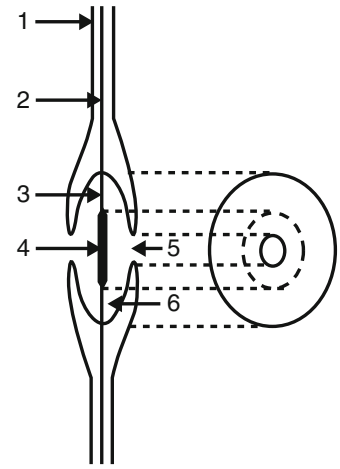


Fig. 2.21 Structure of bordered pit in sectional and surface views: (*1*) cell wall, (*2*) middle lamella, (*3*) pit membrane, (*4*) torus, (*5*) pit aperture, (*6*) pit chamber (Krishnamurthy 2015)

cavity and narrows down its opening to the lumen of the cell; the overarching secondary wall constitutes the *border*. That part of the cavity enclosed by the border is the *pit chamber*, and the opening in the border is *pit aperture*. There are simple pit pairs, bordered pit pairs, half-bordered pit pairs (where a bordered pit of one cell has a simple pit opposite to that of the adjacent cell), *blind pits* (where a pit of one cell has no counterpart in the adjacent cell or where a pit of a cell faces an intercellular space) and *unilaterally compound pits* (where a pit of one cell faces more than one pit on the adjacent cell). If the secondary wall is very thick, as in certain brachysclereids, the pit, instead of being shallow, forms a pit canal (Fig. 2.22). When these pits coalesce, they form branched or ramiform pits. In such cases, pits have an inner aperture (facing cell lumen) and an outer aperture (opening into the pit chamber).

Plasmodesmata are narrow cytoplasmic strands that connect the protoplasts of two adjacent cells, and these are often located in primary pit fields. They, thus, help in the movement of substances from one cell to another (vanBel and van Kestersen 1999; Haywood et al. 2002). The plasmodesmata may be classified as *primary* or *secondary* according to their origin (Evert 2006). Primary plasmodesmata arise during cytokinesis as strands of tubular ER that are entrapped within

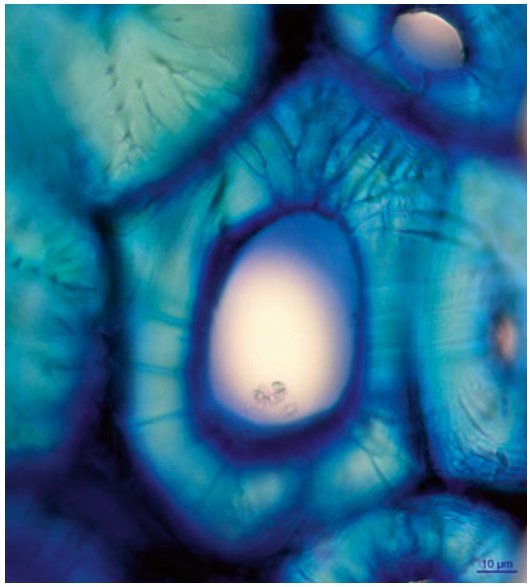


Fig. 2.22 A group of sclereids of *Gmelina arborea* root showing ramified pit canals extending from the lumen to the periphery of the wall (Courtesy of S. John Adams)

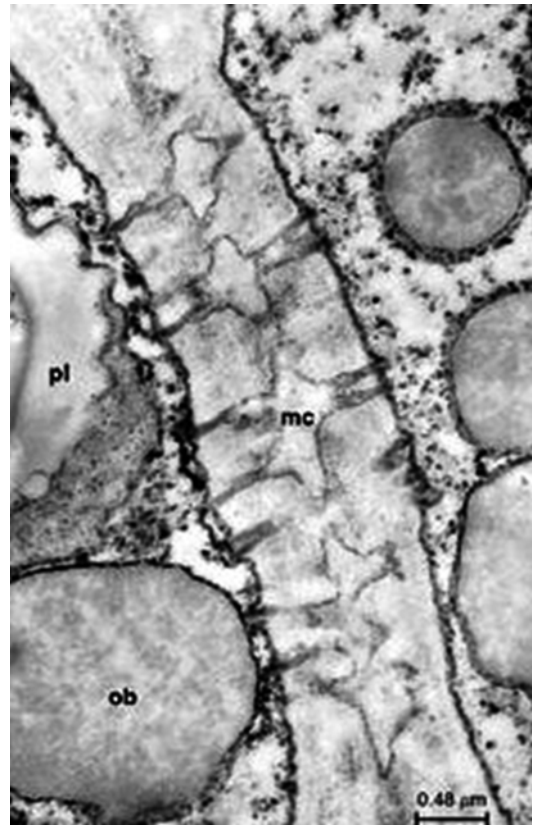


Fig. 2.23 Branched plasmodesmata in radial walls of ray parenchyma cells in secondary phloem of *Pinus strobus*. *mc* median cavities in the middle lamella, *ob* oil bodies, *pl* plastid (Murmanis and Evert 1967)

the developing cell plate. Secondary plasmodesmata arise de novo across the already existing primary cell walls that may belong to ontogenetically unrelated cells (Ding and Lucas 1996). Secondary plasmodesmata (Fig. 2.23) arise, according to some, due to localized enzymatic degradation (mediated by plasmalemma) of cell wall chemicals that enables cytoplasmic strands to penetrate the otherwise intact cell wall. According to others, the secondary plasmodesmata arise from the fusion of 'opposite secondary half-plasmodesmata formed simultaneously' by adjacent cells. At such sites, ER segments get attached to the plasma membrane on both sides of the extremely narrow cell wall followed immediately by the removal of wall material at this site. Secondary plasmodesmata are often branched, and most of them are characterized by the occurrence of a *median cavity* in the region of middle lamella. Primary plasmodesmata may also become branched either through lateral fusion of adjacent plasmodesmata in the region of middle lamella or through branched ER to which the original unbranched ER of plasmodesma gets connected (Ehlers and Kollmann 2001). Plasmodesmata contain two categories of membranes, the plasmalemma and *desmotubule*. The former lines the channel of the plasmodesmata, while the latter is the tubular strand of tightly constricted ER that transverses the channel. The desmotubule of most plasmodesmata does not resemble the very adjacent ER, although it itself is a segment of ER and is of much smaller diameter and contains a central, rod-like structure (Figs. 2.24 and 2.25). The desmotubule lacks a lumen or opening (unlike ER), and hence substances must move through plasmodesmata only between the desmotubule and the plasma membrane. This region is called *cytoplasmic sleeve*, and it is subdivided into 2.5 nm diameter microchannels by globular particles which are embedded in both the plasma membrane and desmotubule and interconnected by cycle spoke-like extensions. In some

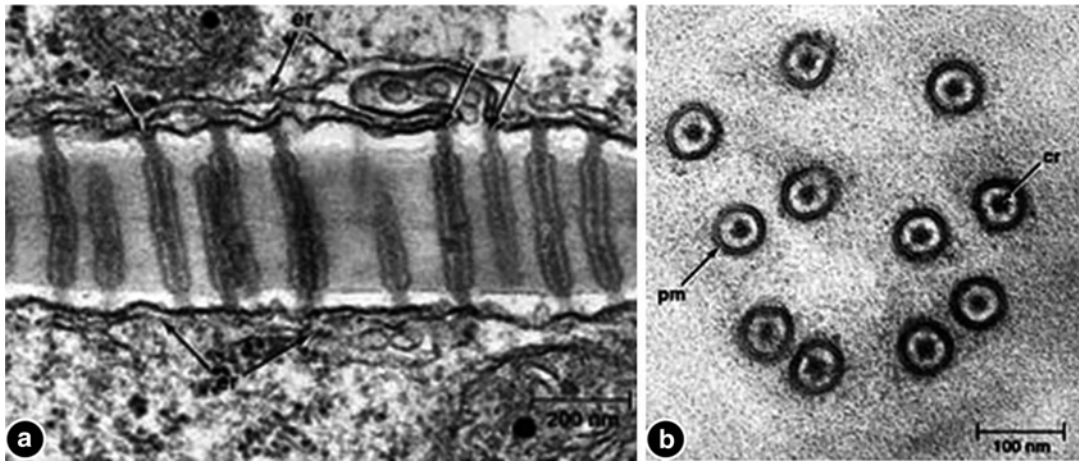


Fig. 2.24 Plasmodesmatas in cell walls of sugarcane leaf in L.S. (**a**) and T.S. (**b**) views; *er* endoplasmic reticulum, *pm* plasma membrane (Robinson-Beers and Evert 1991)

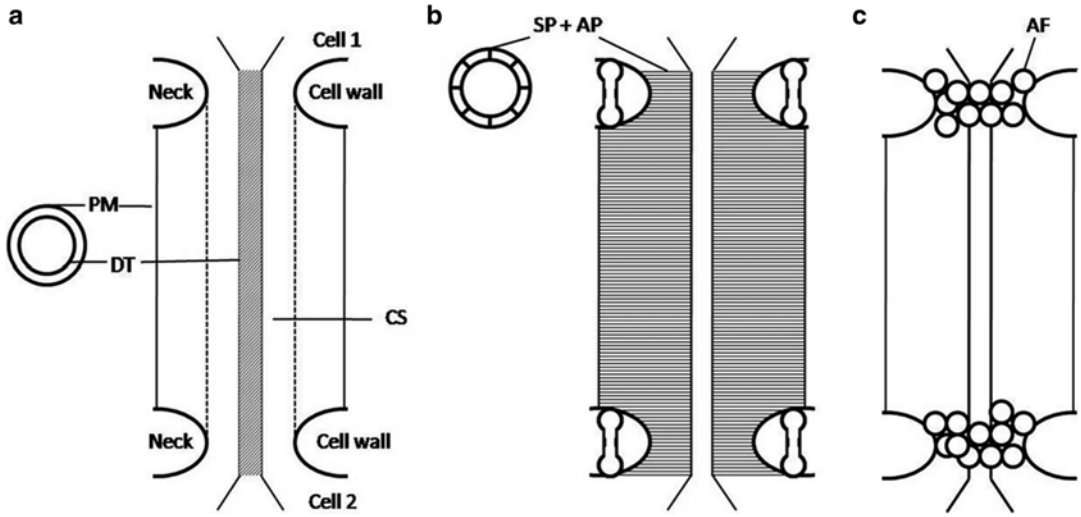


Fig. 2.25 Models to explain the structure and function of plasmodesmata (all shown in diagrammatic L.S.). (**a**) Skeleton model where the membranes delimiting the plasmodesmata are important in their functioning. *CS* cytoplamic sleeve, *DT* tube of appressed ER, *PM* plasma membrane. (**b**) Transmission electron microscopic model of plasmodesmata. The spokes (*SP*) and associated proteins (*AP*) in the neck region are involved in opening the plasmodesmata for movement of cytoplasmic material. An extracellular sphincter (*S*), associated with the neck region, is also important in the functioning of plasmodesmata. (**c**) An actin-based model for plasmodesmata function. Actins are shown in as *small circles*. These regulate the movement of molecules (Based on Gail McLean et al. 1997)

plasmodesmata, their two ends or orifices are conspicuous by narrow constrictions. In some plasmodesmata, the desmotubules are open (i.e. with a lumen) and are constricted only at the neck region (Fig. 2.25) or are fully open. More details on plasmodemata are given in Chap. 3 of this volume.

2.3.3.4 Origin of Cell Wall During Cytokinesis and in Isolated Protoplasts

Cytokinesis is the division of a cell into two by a wall, and it follows karyokinesis, the division of the nucleus. Cytokinesis is initiated along the cell division plane. The future division plane and the

site of future cell plate are predicted by the preprophase band. If the cell about to divide is highly vacuolated, a layer of cytoplasm called *phragmosome* appears across the future division plane, and the nucleus becomes located in this layer (Venverloo and Libbenger 1987). The phragmosome contains both MT and AF, and hence both are likely to be involved in its development. In addition, most dividing vegetative cells show a *preprophase band*, which is a cortical belt of MTs and AFs, that predicts the plane of future cell plate (Gunning and Wick 1985). The site of preprophase band is marked by tubules of ER that form a dense ringlike structure, as revealed by confocal laser scanning microscopy and immunolabelling techniques employed on dividing root tip cells of *Pinus brutia* (Zachariadis et al. 2001). The development of 'ER preprophase band' closely resembled that of the 'MT preprophase band'. This preprophase band disappears after the initiation of the mitotic spindle and breakdown of the nuclear envelope long before the initiation of the cell plate; yet, the cell plate fuses with the mother cell wall precisely at the zone marked earlier by the preprophase band. During late anaphase of mitosis, there is the formation a *phragmoplast*, which is a barrel-shaped structure made of MTs and remnants of mitotic spindle, between the two sets of daughter chromosomes. The phragmoplast is made of two opposing and overlapping sets of MTs that are formed on either side of division plane. Besides MTs, AFs also form prominent components of phragmoplasts and get aligned perpendicular to the division plane; AFs, although organized into two opposite sets like MTs, do not overlap. The phragmoplasts serve as the framework for the assembly of the *cell plate*, the initial partition between the two daughter cells. The cell plate is formed by the fusion of Golgi-derived vesicles, which apparently are directed towards the cell division plane by the phragmoplast MTs, probably with the help of *myosin*, the motor proteins; the role of AFs in this process is not very clear. The cell plate is formed in the centre of the division plane and extends centrifugally towards the lateral walls of the mother cell, but the MTs of phragmoplast do not extend to the lateral walls of dividing cell, but get depolymerized in the centre and are successively repolymerized at the margins of the cell plate. Thus, the MTs, AFs, Golgi vesicles and ER are implicated with the developing cell plate as being part of the phragmoplast (Smith 1999; Staehelin and Hepler 1996).

The actual events in cell plate formation are rather complicated and take place in several steps (Staehelin and Hepler 1996; Nebenführ et al. 2000; Verma 2001): (1) Arrival of vesicles of Golgi origin to the division plane: KNOLLE protein, a syntaxin-related protein of *Arabidopsis* (Lucowitz et al. 1996) apparently serves as a docking receptor for vesicles that are transported by the phragmoplast, and in its absence, these vesicles fail to fuse (Lauber et al. 1997). (2) Formation of 20 nm *fusion tubes* that grow out of these vesicles and their fusion with others to result in a continuous, netted, *tubular–vesicular network*: this network then gets a dense fibrous coat; among the many proteins implicated in cell plate formation (Smith 1999; Otegui and Stahelin 2000; Assaad 2001), *phragmoplastin* is a dynamic protein; this is believed to be involved in the formation of the fusion tubes and vesicle fusion at the cell plate. Its overexpression causes excessive accumulation of callose at the cell plate which in turn arrests cytokinesis. (3) Transformation of this network into a tubular network and subsequently into a *fenestrated plate-like* structure: during this transformation, the dense coat, as well as the associated phragmoplast MTs, get disassembled. (4) Formation of numerous fingerlike projections at the margins of the cell plate: these then fuse with the lateral plasma membranes of the mother cell. (5) Maturation of the cell plate into a new primary wall, closing of the fenestrae, entrapping of segments of tubular ER and formation of plasmodesmata in the new wall (Reichelt et al. 1999): simultaneously AF bundles get attached to the plasmodesmata.

During the cell division of BY-2 cells, callose begins to accumulate in the lumen of the developing cell plate in the tubular–vesicular network stage and becomes more abundant during transition to fenestrated plate stage. Callose is believed to exert a spreading force on the membranes and to help in conversion into a platelike structure (Samuels et al. 1995). Cellulose synthesis, to a

significant extent, begins when the cell plate has reached the fenestrated sheet stage. However, in the root meristem cells of *Phaseolus vulgaris*, by contrast, cellulose, hemicelluloses and pectins are deposited almost at the same time all along the cell plate (Matar and Catesson 1998). One should not confuse the cell plate with the middle lamella. The pectic middle lamella does not begin to develop until the cell plate makes contact with the lateral walls of the mother cell. The middle lamella then extends centripetally within the cell plate from the junction region of cell plate and the mother cell wall (Matar and Catesson 1998).

The protoplasts are isolated from cells after a very good experimental system to study cell wall formation. Under proper in vitro conditions, the protoplast will regenerate a new cell wall after getting over the initial shock and stress conditions. The comparison of the regenerated wall of the protoplasts with the original wall of the source cell shows that irrespective of the source cell, the regenerated wall has an identical chemical composition. The regenerated wall is laid in two phases: in phase 1, the newly regenerated wall around protoplasts was deposited in patches here and there; soon it extends around the entire protoplast. It contains initially callose, esterified pectins, extensins, xyloglucans, cellulose and some phenolics. Later it contains, in addition, the two hydrolytic enzymes, chitinase and 1, 3-β-glucanase. Cellulose was not laid in its usual crystalline form in the beginning. The initially laid wall, therefore, is more of a makeshift wall to ward off stress than serving as a regular wall. In phase 2, phenols, callose, extensin 2, 1, 3-β-glucanase and chitinase were slowly eliminated. The cellulose laid is more and more crystalline and comparable to that of normal cell walls. The wall also becomes more uniformly thick. Nonesterification of pectins also takes place slowly (Krishnamurthy and Nandagopalan, Unpublished data on protoplast regeneration in *Basella alba*).

2.3.3.5 Growth of Cell Wall

Once the cell plate formation is completed, additional wall materials are deposited on either side of it to contribute to the primary wall and its thickness. According to Matar and Catesson (1998), in meristematic cells, this deposition takes place in a mosaic fashion, as a consequence of which the new walls are characterized by a heterogeneous distribution of cell wall polysaccharides. As already mentioned, cellulose is synthesized by the plasma membrane, while matrix polysaccharides and glycoproteins are synthesized by Golgi bodies and delivered to the wall through Golgi-derived vesicles. Production of cellulose is facilitated by the ring or rosette-like cellulose synthase enzyme complexes present on the plasma membrane (Delmer 1999; Kimura et al. 1999). Each rosette synthesizes cellulose from UDP-glucose and exudes the cellulose microfibrils on the outer surface of the plasma membrane via Golgi vesicles.

There are a few hypotheses to explain the deposition of cellulose microfibrils, particularly their orientation. The most widely accepted hypothesis is called by Baskin (2001) the *alignment hypothesis*. According to it, the orientation of the nascent cellulose microfibrils is decided by the underlying MTs (Fisher and Cyr 1998). This hypothesis may explain microfibril deposition in elongating cells but is not adequate to explain this process in non-elongating cells, where cortical MTs are not parallel to the nascent microfibrils (Baskin 2001). Moreover, disordering or complete absence of MT in the *mor1-1* mutant of *Arabidopsis* did not affect parallel alignment of microfibrils in growing root cells (Sugiomoto et al. 2003). The second hypothesis is the *liquid-crystalline self-assembly hypothesis* (Bouligand 1976). According to this hypothesis, because of the similarity of helicoidal cell walls, whose cellulose microfibrils do not match the cortical MTs, to the cholesteric liquid crystals, the helicoidal wall structure could arise from a liquid-crystalline self-organizing principle. The third hypothesis, called *template incorporation hypothesis*, was proposed by Baskin (2001). According to this hypothesis, the nascent cellulose microfibrils can be oriented by MFs or become incorporated into cell wall by binding to a scaffold built and oriented around either already incorporated MFs or membrane proteins or both. The cortical MFs serve to bind and orient components of the scaffold at the plasmalemma. Hence, MTs are neither required for cellulose synthesis nor for the pro-

duction of cellulose microfibrils. The fourth hypothesis is called the *geometrical model*. This has been proposed based on detailed observations on the helicoid secondary cell wall architecture of *Equisetum hyemale* root hairs by Emons and his group (see Emons and Mulder 2001). In this mathematical model, the angle of deposition of cellulose microfibrils with reference to the long axis of the cell is quantitatively related to the following: (1) the density of active cellulose synthesis in the plasmalemma, (2) the distance between different microfibrils within a wall lamella and (3) the geometry of the concerned cell.

When we talk about the mechanism of cell wall growth, it is important for us to differentiate between wall growth in surface (i.e. wall expansion) and growth in thickness. Wall growth in surface takes place when cells enlarge in size. Growth in wall thickness takes place both in primary and secondary walls, particularly in the latter. As per classical ideas, increase in cell wall thickness takes place either through *apposition* or through *intussusceptions* (Fig. 2.26). In the former, additional wall materials are deposited on top of the already existing materials, while in the latter, new wall materials are inserted between the already existing materials. Lignin or cutin deposition follows intussusception, and hemicelluloses and cellulose as well as some secondary wall materials may be deposited by apposition.

2.3.3.6 Evolution of Cell Walls

The stages in the evolution of cell walls of plants have been postulated by Bartnicki-Garcia (1984). The earliest cells (prokaryotic) in this world were wall-less and bounded only by a unit cell membrane since the environment was hypertonic or because they operated under low turgor. The origin of the cell wall was suggested to go back to the time when some of these primitive prokaryotes had their plasmalemma strengthened by polymers such as polysaccharides, glycoproteins and proteins which remained anchored to the cell surface after being discharged by the cell. This enabled these cells to function at a greater turgor and to have faster growth, giving them a selective advantage over their naked counterparts. Prokaryotic cell walls, as we know them today, gradually evolved from these coated cell membranes probably through natural selection and/or mutation in order to finally result in *murein* or *peptidoglycan* walls. From the details provided earlier in this chapter, it is clear that the eukaryotic cell wall is with microfibrillar nature and that it is totally different from that of the peptidoglycan-containing prokaryotic cell wall. The prokaryotic cell wall was probably discarded during the evolution of eukaryotic organization that has greater complexity with elaborate endomembrane systems. The prevalence of microfibrillar walls from the lowest to highest members of the plant kingdom (including fungi) is *prima facie* evidence for the superiority of this wall over prokaryotic wall. However, we do not know yet whether the prokaryotic wall gradually evolved into a microfibrillar wall or if the eukaryotic wall was invented de novo on the surface of some ancestral, wall-less eukaryote. According to Bartnicki-Garcia (1984), the obstacle that had to be surmounted in order to construct a microfibrillar wall was not the production of the appropriate enzymes but the spatial organization of such enzymes. More studies are needed to know the actual process of evolution of eukaryotic cell walls.

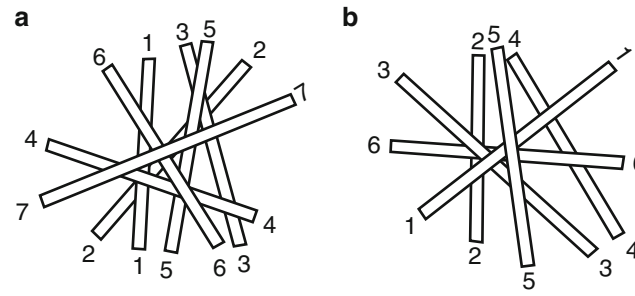


Fig. 2.26 Diagrammatic representations of growth in cell wall thickness by apposition (**a**) and intussusception. (**b**) Only microfibrils are shown and numbered (Krishnamurthy 2015)

2.3.3.7 Functions of Cell Wall

The cell wall has a number of functions. The following are among the known functions:

1. Since the cell wall is rigid, it helps to limit the size of the protoplast enclosed by it.
2. It prevents the rupture of plasma membrane following the uptake of water and when the cell becomes turgid.
3. It determines the size and shape of the cell. The protoplast without a cell wall (when removed) becomes spherical.
4. It determines the texture of the cell.
5. Since different cell types are often identified by the structure of their walls, a close relationship exists between the cell wall structure and cell function.
6. It plays an important role in absorption, transport and secretion of various chemical substances.
7. Once regarded as dead, the cell wall is now recognized as a metabolically dynamic component (Bolwell 1993; Carpita and McCann 2000). The primary cell wall is now considered as a 'vital extension of the cytoplasm'. It has been reported to contain several enzymes and hence is involved in a number of metabolic activities.
8. It is involved in cell-to-cell signalling.
9. The cell wall is involved in cellular differentiation.
10. It plays a direct role in defence against both biotic and abiotic stresses and in pathogenesis. It produces a number of chemicals in defence including phenolics, phytoalexins, lignins, suberins, callose, pathogenesis-related proteins, oligosaccharides (from xyloglucans and pectins), etc.
11. It is also involved in storage, especially many polysaccharides (e.g. in endosperm cells).

2.3.4 Flagella and Cilia

Flagella (singular: flagellum) and *cilia* (singular: cilium) characterize a number of algae, fungi and the sperms of lower vascular plants and in some gymnosperms (Cycads and *Ginkgo*). These are hair-like structures or *axonemes* projecting from the cell surfaces. They are around 0.2 μm thick and 2–150 μm long. The longer ones are flagella, while the short ones are cilia, but otherwise there is no sharp dividing line between them. They form the sources of locomotion for cells possessing them, and they are capable of beating back and forth at high speeds. Both flagella and cilia have a similar structure (Fig. 2.27a). Each one of them possesses nine pairs of MTs plus two additional MTs (it should be mentioned here that bacterial flagella/cilia lack MTs). Both structures grow out of the roughly cylindrical *basal bodies* or *kinetosomes* or *blepharoplasts* located in the cytoplasm. The basal bodies have a structure similar to that of the flagellum and cilium, except that there are nine triplets instead of pairs of MTs

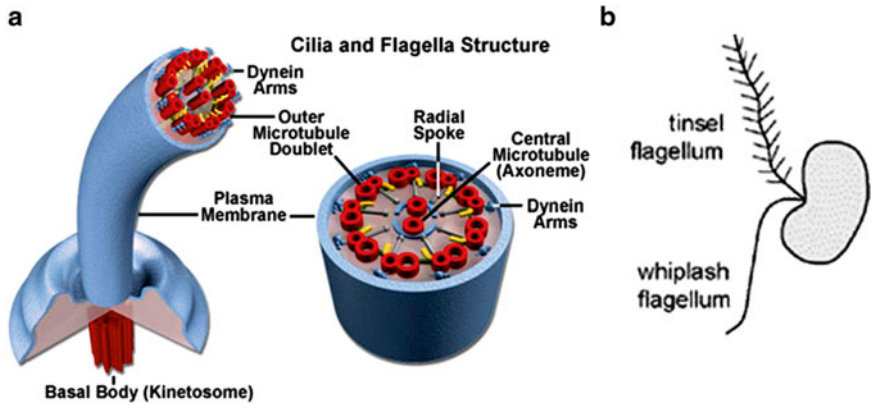


Fig. 2.27 (**a**) Structure of flagellum/cilium (Source: Dr. Michael W. Davidson and The Florida State University). (**b**) A zoospore with whiplash and tinsel flagella

and with no MTs in the centre. The basal body is shown to be derived from the larger of the two *centrioles* formed during nuclear division. When cells possessing flagella/cilia divide, they are cast off, and new ones are regenerated in the daughter cells from the basal bodies. The number of flagella may vary considerably depending on the taxon: one, two or many. Flagella may be inserted on the anterior, posterior or lateral sides of the cells or in some multiflagellate/ciliate cases; they may be all around the cell. Flagella may be of the *whiplash* type or of the *tinsel* type (Fig. 2.27b). In biflagellate, laterally inserted, taxa, one flagellum is of the whiplash type and the other is of the tinsel type (this condition is known as *heterokont*). The surface of the whiplash flagellum is invariably smooth (some show hairs under very high magnification), while that of the tinsel type bears a series of fine *mastigonemes/flimmer hairs* along its entire length. The mastigonemes consist of a wider basal shaft for about two-thirds of their length and end in a terminal part that is about one-fifth of the diameter of the basal part. The wider basal part has transverse or spiral bands of alternating light and dark material.

References

Andreeva AV, Kutuizov MA, Evans DE, Hawes CR (1998) The structure and function of the Golgi apparatus: a hundred years of questions. J Exp Bot 49:1281–1291

Assaad FF (2001) Plant cytokinesis: exploring the links. Plant Physiol 126:509–516

Azim-Zadeh J, Traas J, Pastuglia M (2001) Molecular aspects of microtubule dynamics in plants. Curr Opin Plant Biol 4:513–519

Bacic A, Harris PJ, Stone BA (1988) Structure and function of plant cell walls. In: Preiss J (ed) The biochemistry of plants, vol 14, Carbohydrates. Academic, New York, pp 297–371

Barlow PW (1982) 'The plant forms cells, not cells the plant': the origin of de Bary's aphorism. Ann Bot 49:269–271

Baron-Epel O, Gharyal PK, Schindler M (1988) Pectins as mediators of wall porosity in soybean cells. Planta 175:389–395

Bartnicki-Garcia S (1984) Kingdoms with walls. In: Dugger WM, Bartnicki-Garcia S (eds) Structure, function, and biosynthesis of plant cell walls. University of California, Riverside, pp 1–18, Proc 7th Annu Symp Bot

Baskin TI (2000) The cytoskeleton. In: Buchanan BB, Gruissem W, Jones RL (eds) Biochemistry and molecular biology of plants. Amer Soc Plant Physiol, Rockville, pp 202–258

Baskin TI (2001) On the alignment of cellulose microfibrils by cortical microtubules: a review and a model. Protoplasma 215:150–171

Battey NH, James NC, Greenland AJ, Brownlee C (1999) Exocytosis and endocytosis. Plant Cell 11:643–659

Bernards MA (2002) Demystifying suberin. Can J Bot 80:227–240

Blaschek W, Kasbauer J, Flanz G (1989) Analysis of cell-wall polysaccharides from *Pythium aphanidermatum*. In: Fry SC, Brett CT, Reid JSG (eds) V cell wall meeting. Edinburgh. p 28

Bolwell GP (1993) Dynamic aspects of the plant extracellular matrix. Int Rev Cytol 146:261–324

Bouligand Y (1976) Les analogues bioliques des cristaux liquids. La Rech 7:474–476

Brett C, Waldron K (1990) Physiology and biochemistry of plant cell walls. Unwin Hyman, London

Brown RC, Lemmon BE (2001) The cytoskeleton and spatial control of cytokinesis in the plant life cycle. Protoplasma 215:35–49

Carpita N, McCann M (2000) The cell wall. In: Buchanan B, Gruissem W, Jones R (eds) Biochemistry and molecular biology of plants. Amer Soc Plant Physiol, Rockville, pp 52–108

Carraro N, Peaucelle A, Laufs P, Traas J (2006) Cell differentiation and organ initiation at the shoot apical meristem. Plant Mol Biol 60:811–826

Cassab GI (1998) Plant cell wall proteins. Annu Rev Plant Physiol Plant Mol Biol 49:281–309

Cooke TJ, Lu B (1992) The independence of cell shape and overall form in multicellular algae and land plants: cells do not act as building blocks for constructing plant organs. Int J Plant Sci 153:S7–S27

Cosgrove DJ (1997) Relaxation in a high-stress environment: the molecular bases of extensible cell walls and cell enlargement. Plant Cell 9:1031

Cosgrove DJ (1999) Enzymes and other agents that enhance cell wall extensibility. Annu Rev Plant Physiol Plant Mol Biol 50:391–417

Darley CP, Forrester AM, McQueen-Mason SJ (2001) The molecular basis of plant cell wall extension. Plant Mol Biol 47:179–195

deBary A (1879) Lehrbuch der Botanik für mittlere und höhere Lehranstalten. Bot Ztg 37:221–223

Delmer DP (1999) Cellulose biosynthesis: exciting times for a difficult field of study. Annu Rev Plant Physiol Plant Mol Biol 50:245–276

Delrot S, Atanassova R, Gomés E, Coutos-Thévenot P (2001) Plasma membrane transporters: a machinery for uptake of organic solutes and stress resistance. Plant Sci 161:391–404

Derksen J, Wilms FHA, Pierson ES (1990) The plant cytoskeleton: its significance in plant development. Acta Bot Neerlandica 39:1–18

Ding B, Lucas WJ (1996) Secondary plasmodesmata: biogenesis, special functions and evolutions. In:

Smallwood M, Knoz JP, Bowles DJ (eds) Membranes: specialized functions in plants. BIOS Scientific, Oxford, pp 489–506

Driouich A, Staehelin LA (1997) The plant Golgi apparatus: structural organization and functional properties. In: Berger EG, Roth J (eds) The Golgi apparatus. Birkhäuser Verlag, Basel, pp 275–301

Driouich A, Faye L, Staehelin LA (1993) The plant Golgi apparatus: a factory for complex polysaccharides and glycoproteins. Trends Biochem Sci 18:210–214

Dupree P, Sherrier DJ (1998) The plant Golgi apparatus. Biochim Biophys Acta, Mol Cell Res 1404:259–270

Ehlers K, Kollmann R (2001) Primary and secondary plasmodesmata: structure origin, and functioning. Protoplasma 216:1–30

Emons AMC, Mulder BM (2001) Microfibrils build architecture. A geometrical model. In: Morohoshi N, Komamine A (eds) Molecular breeding of woody plants. Elsevier Science BV, Amsterdam, pp 111–119

Evered D, Marsh J (1989) The cellular basis of morphogenesis. Wiley, Chichester

Evert RF (2006) Esau's plant anatomy. Wiley, New York

Fisher DD, Cyr R-J (1998) Extending the microtubule/microfibril paradigm. Cellulose synthesis is required for normal cortical microtubule alignment in elongating cells. Plant Physiol 116:1043–1051

Fleming AJ (2006) The coordination of cell division, differentiation and morphogenesis in shoot apical meristem: a perspective. J Exp Bot 57:25–32

Franklin AE, Cande WZ (1999) Nuclear organization and chromosome segregation. Plant Cell 11:523–534

Fry SC (1988) The growing plant cell wall: chemical and metabolic analysis. Longman Scientific, Burnt Mill

Gail McLean B, Hempel FD, Zambryski C (1997) Plant intercellular communication via plasmodesmata. Plant Cell 9:1043–1054

Goddard RH, Wick SM, Silflow CD, Snustad DP (1994) Microtubule components of the plant cytoskeleton. Plant Physiol 104:1–6

Grunwald C, Ruel K, Kim YS, Schmitt U (2002) On the cytochemistry of cell wall formation in poplar trees. Plant Biol 4:13–21

Gunning BES, Wick SM (1985) Preprophase bands, phragmoplasts, and spatial control of cytokinesis. J Cell Sci Suppl 2:157–179

Hanstein J (1880) Einige Züge aus der Biologie des Protoplasmas. Bot. Abhandl. Aus dem Gebiet der Morphologie und Physiologie Band 4, Heft 2. Marcus, Bonn

Hariharan Y, Krishnamurthy KV (1995) A cytochemical study of cambium and its xylary derivatives on the normal and tension wood sides of the stems of *Prosopis juliflora* (SW) DC. Beitr Biol Pflanzenbau 69:459–492

Harris PJ, Hartley RD (1980) Phenolic constituents of the cell walls of monocotyledons. Biochem Syst Ecol 8:153–160

Haywood V, Kragler F, Lucas WJ (2002) Plasmodesmata: pathways for proteins and ribonucleoprotein signaling. Plant Cell 14(Suppl):S303–S325

Heslop-Harrison J (1975) The cronian lecture (1974). The physiology of the pollen grain surface. Proc R Soc Lond Ser B 190:275–299

Horner HT, Wagner BL (1995) Calcium oxalate formation in higher plants. In: Khan SR (ed) Calcium oxalate in biological system. CRC Press, Boca Raton, pp 53–72

Hu J, Aquirre M, PetoC AJ, Ecker J, Chory J (2002) A role for peroxisomes in photomorphogenesis and development of *Arabidopsis*. Science 297:405–409

Huang AHC (1996) Oleoresins and oil bodies in seeds and other organs. Plant Physiol 110:1055–1061

Iiyama K, Lam TBT, Stone BA (1990) Phenolic acid bridges between polysaccharides and lignin in wheat internodes. Phytochemistry 29:733–737

Jacobson K, Sheets ED, Simson R (1995) Revisiting the fluid mosaic model of membranes. Science 268:1441–1442

Jothi R, Parthasarathy S, Krishnamurthy KV (2010) Computational internal sequence repeats analysis of accelerated evolution and the role of extensins under abiotic and biotic stresses. Open Access Bioinformatics 2:157–168

Kaliamoorthy S, Krishnamurthy KV (1998) Secondary wall deposition in tracheary elements of cucumber grown in vitro. Biol Plant 41:515–522

Kaplan DR (1992) The relationship of cells to organisms in plants: problem and implications of an organismal perspective. Int J Plant Sci 153:S28–S37

Kaplan DR, Hagemann W (1991) The relationship of cell and organism in vascular plants. Bioscience 41:693–703

Kidner CA, Martienssen RA (2005) The developmental role of microRNA in plants. Curr Opin Plant Biol 8:38–44

Kim YJ, Zheng B, Yu Y, Won SY, Ma B, Chen X (2011) The role of mediator in small and long noncoding RNA production in Arabidopsis thaliana. EMBO J 30:814–822

Kimura S, Laosinchai W, Itoh T, Cui X, Linder CR, Brown RM Jr (1999) Immunogold labeling of rosette terminal cellulose-synthesizing complexes in the vascular plant *Vigna angnment* of cellulose microfibrils by cortical microtubules: a review and a model. Protoplasma 215:150–171

Kolattukudy PE (1980) Biopolyester membranes of plants: cutin and suberin. Science 208:990–1000

Kost B, Chua N-H (2002) The plant cytoskeleton: vacuoles and cell walls make the difference. Cell 108:9–12

Krishnamurthy KV (1999) Methods in cell wall cytochemistry. CRC Press, Boca Raton

Krishnamurthy KV (2015) Growth and development in plants. Scientific Publishers, Jodhpur

Krishnamurthy KV, Krishnaraj R, Chozhavendan R, Samuel Christopher F (2000) The program of cell death in plants and animals–a comparison. Curr Sci 79:1169–1181

Kumagai F, Hasezawa S (2001) Dynamic organization of microtubules and microfilaments during cell cycle progression in higher plant cells. Plant Biol 3:4–16

Kwok EY, Hanson MR (2004) Stromules and the dynamic nature of plastid morphology. J Microsc 214:124–137
Lauber MH, Waizennegger I, Steinmann T, Schwarz H, Mayer U, Hwang I, Lukowitz W, Jurgens D (1997) The *Arabidopsis* KNOLLE protein is a cytokinesis-specific syntaxin. J Cell Biol 139:1485–1493
Lee J-Y, Yoo B-C, Lucas EJ (2000) Parallels between nuclear pore and plasmodesmal trafficking of information molecules. Planta 210:177–187
Li Y, Darley CP, Ongaro V, Fleming A, Schipper O, Baldauf SL, McQueen-Masan SJ (2002) Plant expansions are a complex multigene family with an ancient evolutionary origin. Plant Physiol 128:854–864
Lichtscheidl IK, Hepler PK (1996) Endoplasmic reticulum in the cortex of plant cells. In: Smallwood M, Knox JP, Bowles DJ (eds) Membranes: specialized functions in plants. BIOS Scientific, Oxford, pp 383–402
Logan H, Basset M, Very A-A, Sentenac H (1997) Plasma membrane transport systems in higher plants: from black boxes to molecular physiology. Physiol Plant 100:1–15
Lucas WJ, Ding B, van der Schoot C (1993) Plasmodesmata and the supracellular nature of plants. New Phytol 125:435–476
Lucowitz W, Mayer U, Jürgens G (1996) Cytokinesis in the *Arabidopsis* embryo involves the syntaxin-related KNOLLE gene product. Cell 84:61–71
Margulis L, Sagan D (2002) Acquiring genomes: a theory of the origins of species. Perseus Books Group, New York
Marty F (1999) Plant vacuoles. Plant Cell 11:587–599
Matar D, Catesson AM (1998) Cell plate development and delayed formation of the pectic middle lamella in root meristems. Protoplasma 146:10–17
McCandless EL (1981) Polysaccharides of the seaweeds. In: Lobban CS, Wynne MJ (eds) The biology of the seaweeds. Blackwell Scientific Publications, Oxford, pp 559–588
Meijer M, Murray JAH (2001) Cell cycle controls and the development of plant form. Curr Opin Plant Biol 4:44–49
Meyerowitz EM (1996) Plant development: local control and global patterning. Curr Opin Genet Dev 6:475–479
Moore PJ, Swords KM, Lynch MA, Staehelin LA (1991) Spatial organization of the assembly pathways of glycoproteins and complex polysaccharides in the Golgi apparatus of plants. J Cell Biol 112:589–602
Murmanis L, Evert RF (1967) Parenchyma cells of secondary phloem in *Pinus strobus*. Planta 73:301–318
Nebenführ A, Frohlick JA, Staehelin LA (2000) Redistribution of Golgi stacks and other organelles during mitosis and cytokinesis in plant cells. Plant Physiol 124:135–151
Niyogi KK (2000) Safety valves for photosynthesis. Curr Opin Plant Biol 3:455–460
Ohbrogge J, Browse J (1995) Lipid biosynthesis. Plant Cell 7:957–970
Olsen LJ (1998) The surprising complexity of peroxisome biogenesis. Plant Mol Biol 38:163–189
Otegui M, Stahelin LA (2000) Cytokinesis in flowering plants: more than one way to divide a cell. Curr Opin Plant Biol 3:493–502
Palmgren MG (2001) Plant plasma membrane H+-ATPases: powerhouses for nutrient uptake. Annu Rev Plant Physiol Plant Mol Biol 52:817–845
Peer WA (2011) Plasma membrane protein trafficking. In: Murphy AS et al (eds) The plant plasma membrane, plant cell monographs, vol 19. Springer-Verlag, Berlin
Persival E (1979) The polysaccharides of green, red, and brown seed weeds: their basic structure, biosynthesis and function. Br Phycol J 14:103–117
Preston RD (1974) Plant cell walls. In: Robards RW (ed) Dynamic aspects of plant ultrastructure. McGraw-Hill, New York
Prychid CJ, Rudall PJ (1999) Calcium oxalate crystals in monocotyledons: a review of their structure and systematic. Ann Bot 84:725–739
Rees DA (1969) Structure, conformation and mechanism in the formation of polysaccharide gels and networks. Adv Carbohydr Chem Biochem 24:267–332
Reichelt S, Kendrick-Jones J (2000) Myosins. In: Staiger CJ, Baluska F, Volkmann D, Barlow PW (eds) A dynamic framework for multiple plant cell function. Kluwer Academic, Dordrecht
Reichelt S, Knight AE, Hodge TP, Baluska F, Samaj J, Volkmann D, Kenrick-Jones J (1999) Characterization of the unconventional myosin VIII in plant cells and its localization at the post-cytokinetic cell wall. Plant J 19:555–567
Ritchter HG, Dallwitz MJ (2000) Commercial timbers: descriptions, illustrations, identification and information retrieval. version 18. http://delta-intkey.com/wood/en/www/euglyam.htm
Roberts K (1994) The plant extracellular matrix: in a new expansive mood. Curr Opin Cell Biol 6:688–694
Robinson-Beers K, Evert RF (1991) Fine structure of plasmodesmata in mature leaves of sugarcane. Planta 184:307–318
Ros Barcelo A (1997) Lignification in plant cell walls. Int Rev Cytol 176:87–132
Rose A, Patel S, Meizer I (2004) The plant nuclear envelope. Planta 218:327–336
Salisbury FB, Ross CW (2004) Plant physiology. Thomson Asia Private Ltd, Singapore
Samuels AL, Giddings TH Jr, Staehelin LA (1995) Cytokinesis in tobacco BY-2 and root tip cells: a new model for cell plate formation in higher plants. J Cell Biol 130:1345–1357
Schäffner AR (1998) Aquaporin function, structure, and expression: are there more surprises to surface in water relations? Plant 204:131–139
Seagull RW (1989) The plant cytoskeleton. Crit Rev Plant Sci 8:131–167
Sheahan MB, Rose RJ, McCurdy DM (2004) Organelle inheritance in plant cell division: the actin cytoskeleton is required for unbiased inheritance of chloroplasts,

mitochondria and endoplasmic reticulum in dividing protoplasts. Plant J 37:379–390
Singer SJ, Nicolson GL (1972) The fluid mosaic model of the structure of the cell membranes. Science 175:720–731
Sitte P (1992) A modern concept of the "cell theory". A perspective on competing hypotheses of structure. Int J Plant Sci 153:S1–S6
Smith LG (1999) Divide and conquer: cytokinesis in plant cells. Curr Opin Plant Biol 2:447–453
Staehelin A (1991) What is a plant cell? A response. Plant Cell 3:553
Staehelin LA (1997) The plant ER: a dynamic organelle composed of a large number of discrete functional domains. Plant J 11:1151–1165
Staehelin LA, Hepler PK (1996) Cytokinesis in higher plants. Cell 84:821–824
Staiger CJ (2000) Signaling to the actin cytoskeleton in plants. Annu Rev Plant Physiol Plant Mol Biol 51:257–288
Stevens WC (1911) Plant anatomy from the standpoint of the development and functions of the tissues, and handbook of micro-technic. P Blakiston's Son & Co, Philadelphia
Stone BA (1984) Non-cellulosic β-glucans in cell walls. In: Dugger DM, Bartnicki-Garcia S (eds) Structure, function and biosynthesis of plant cell walls. American Society of Plant Physiologists, Rockville, pp 52–74
Sugiomoto K, Himmelspach R, Williamson RE, Wasteneys GO (2003) Mutation or drug-dependent microtubule disruption causes radial swelling without altering parallel cellulose microfibril deposition in *Arabidopsis* root cells. Plant Cell 15:1414–1429
Talcolt B, Moore MS (1999) Getting across the nuclear pore complex. Trends Cell Biol 9:312–318
Thomas H, Huang L, Young M, Ougham H (2009) Evolution of plant senescence. BMC Evol Biol 9:163
vanBel AJE, van Kestersen WJP (eds) (1999) Plasmodesmata: structure, function, role in cell communication. Springer-Verlag, Berlin
Venverloo CJ, Libbenger KR (1987) Regulation of the plane of cell division in vacuolated cells. I. The function of nuclear positioning and phragmosome formation. J Plant Physiol 131:267–284
Verma DPS (2001) Cytokinesis and building of the cell plate in plants. Annu Rev Plant Physiol Plant Mol Biol 52:751–784
Wasteneys GO (2004) Progress in understanding the role of microtubules and microfilaments during cell cycle progression in higher plant cells. Curr Opin Plant Biol 7:651–660
Webb MA (1999) Cell-mediated crystallization of calcium oxalate in plants. Plant Cell 11:751–761
Webster J (1993) Introduction to fungi, 2nd edn. Cambridge University Press, Cambridge
Willats WGT, McCartrey L, Mackie M, Knox JP (2001) Pectin: cell biology and properties for functional analysis. Plant Mol Biol 47:9–27
Wink M (1993) The plant vacuole: a multifunctional compartment. J Exp Bot 44(suppl):231–246
Zacchariadis M, Quader H, Galatis B, Apostolakos P (2001) Endoplasmic reticulum preprophase band in dividing root-tip cells of *Pinus brutia*. Plants 213: 824–827
Zechmann B, Müller M (2010) Subcellular compartmentation of glutathione in dicotyledonous plants. Protoplasma 246:15–24
Zhang GF, Stahelin LA (1992) Functional compartmentation of the Golgi apparatus of plant cells. Plant Physiol 99:1070–1083

Development and Organization of Cell Types and Tissues

3

K.V. Krishnamurthy, Bir Bahadur, S. John Adams, and Padma Venkatasubramanian

Abstract

This chapter deals with the development and organization of different cell types and tissues of plants. Development includes processes such as cell division (including cell cycle), cell enlargement, differentiation, pattern formation and morphogenesis. A detailed account on cell cycle and its hormonal and genetic control has been provided. Differentiation of cells happens by modification of cell cycle events at specific control points. The laws of governing cell division, planes of cell division and asymmetric cell division are discussed in relation to specific morphogenesis of cell and tissue types. The relative importance of cell division and cell enlargement in overall morphogenesis, organ size and organ shape is highlighted. Differentiation, dedifferentiation, redifferentiation and transdifferentiation are distinguished and their importance in cell type production is emphasized. The importance of diffusion reaction of theory and positional information theory on morphogenesis of cells, tissues, organs and organisms is explained. Finally, the differentiation of various cell types is described.

Keywords

Apoplast • Cell cycle • Cell division • Cell enlargement • Dedifferentiation • Development • Differentiation • Growth • Morphogenesis • Pattern formation • Programmed cell death • Redifferentiation • Symplast • Transdifferentiation

K.V. Krishnamurthy (✉) • S. John Adams
P. Venkatasubramanian
Center for Pharmaceutics, Pharmacognosy and Pharmacology, School of Life Sciences, Institute of Trans-Disciplinary Health Science and Technology (IHST), Bangalore, Karnataka, India
e-mail: kvkbdu@yahoo.co.in

B. Bahadur
Sri Biotech Laboratories India Limited, Hyderabad, Telangana, India

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_3,

3.1 Development

Development is a series of events by which a multicellular plant, plant organ, tissue or cell type arises from a single progenitor cell. This single cell may be a zygote, a spore or a meristematic initial cell. During development there is a continuous synthesis of a large number of biomolecules that, along with externally supplied materials like minerals and water, form the raw materials for development. The developmental events, in a real sense, are invariably not cascaded, but overlapping. In other words, development is not an *ultimate* end product but a *cumulative* end product. It should, however, be emphasized that development is a *progressive* event from a fairly simple starting point to a highly complex entity with progressive addition of complexity at every step. This gradual developmental strategy is called *epigenesis*, a term borrowed from animal developmental biology (Twyman 2003).

Development, according to some (Twyman 2003), involves the following five major but overlapping processes: *cell division*, *cell enlargement*, *differentiation*, *pattern formation* and *morphogenesis*. The authors of this chapter follow this approach. Some consider development as synonymous with growth, while others include cell division and cell enlargement alone under growth. Yet others consider cell division, cell enlargement, differentiation, pattern formation and development under morphogenesis (Wardlaw 1968). There are also others who consider the above five events as independent and that certain types of development may not and need not involve one or more of these five.

The formal methods of representing development are based on two main concepts (Barlow 1994): (1) Development, within limits, is *invariant*, and its manifestation depends upon the correct set of permissive conditions. If development only requires yes or no type 'decisions', as, for example, whether a seed should germinate or not depending on whether conditions promoting dormancy do not exist or exist. (2) Developmental process is regarded as essentially *self-perpetuating*; here too, decisions are taken as to which new developmental state is to be followed, but the information relating to this change of developmental state is inherent to the system itself and permissive environmental conditions are regarded as axiomatic (Krishnamurthy 2015). In the example of seed germination mentioned above, the outcome is a seedling, as it is the only state prefigured in the previous state. The first concept is basically physiological and considers the processes as the basic driving force of development, while the second concept is essentially structural and considers structural patterns as vital. It should, however, be stressed here that the above two concepts of development are not mutually exclusive.

3.1.1 Controls in Development

Development is controlled by genes and various gene products, by environment and by an interaction of all these. Gene products like enzymes and other proteins, growth regulators and other signalling molecules and other molecules control the chemistry of cells and account for development. Barring a very few exceptions, each cell in a developing plant contains the same DNA and hence the developmental diversification of plant body depends on the expression of the different genes in its various cells. *Gene expression* generally involves two major processes: *transcription* and *translation*. The former involves transcription of DNA into RNA and the latter translation of this RNA into proteins. The information carried in DNA (gene) specifies the amino acids to be incorporated into proteins as well as the sequence of their incorporation during protein synthesis. The gene is also flanked by further information in the form of *cis*-acting *regulatory elements*, which control where and when the gene is to be transcribed, as well as the rate of transcription. Because of this, the amount of RNA produced and its spatial and temporal patterns of expression are controlled. The flanking regions may also have *response elements*, which allow external signals to regulate gene expression resulting in linking *signal transduction* to gene expression. In view of this, the transcriptional regulation is often the most important level of

control in gene expression. Further, post-transcriptional regulation can take place to effectively control the amount of functional proteins formed in a cell. The transcribed RNA must be processed and exported from the nucleus, translated and then broken down. All these processes are also regulated. Moreover, all these proteins need processing in various ways before they can effectively function; these processes include folding, chemical modification, removing of some amino acids, conjugation to other molecules, formation of multiproteins, etc. These proteins are also to be transported to different loci of the cell (especially for incorporation into various organelles) as well as for secretion extracellularly. In the end, these proteins also need to be broken down and recycled. At all these levels also, gene expression needs to be regulated (Krishnamurthy 2015). The patterns of gene expression in the developing embryo and in various plant organs determine where, when and in what quantity particular proteins are to be made, thus governing the properties of each and every cell, tissue and organ. Our knowledge on the role of genes in plant development has, mostly, been brought to light through an analysis of developmental mutants, which provides clues regarding the function of specific genes.

As stated earlier, plant development is also controlled by environmental factors. Since plants, unlike animals, are stationary, a necessity for fine-tuning of their development is there in order to adapt themselves to the prevailing environmental conditions such as temperature (thermonasty and thermoperiodism), light (photomorphogenesis), water availability, etc.

3.2 Growth

The whole plant develops from a single cell (zygote or spore) through the involvement of growth (and development). Growth essentially involves an increase in size and volume, total biomass and, more importantly, complexity. Hence, growth is essentially measured by measuring any one of the above parameters. At the cellular level, growth involves two events: *cell division* and *cell enlargement*.

3.2.1 Cell Division

Cell division results in an increase in the number of cells, thus contributing to growth. Growth through cell division is called *hyperplasia* (or *hyperplasy*), although this word is more commonly used to designate an extreme form of cell division. Apparently at least, increase in cell number happens early in the development of plants (e.g. during embryo development) in the absence of visible cell enlargement; it also happens in meristematic cells and their immediate derivatives. The immobility of both plants and their constituent cells makes cell divisions the main means to achieve the correct/right size and shape of their organs and the whole body. For any given cell division, a new cell wall is deposited, and the orientation and position of the new cells are locked in the correct and appropriate place (Dupuy et al. 2010).

It is a *prerequisite* that the nucleus should divide before a mother cell divides into two daughter cells. Nuclear division is termed as *karyokinesis*, while cytoplasmic division is called *cytokinesis*. Nuclear divisions are of two types: (1) *mitosis*, during which a nucleus gives rise to two daughter nuclei which are morphologically and genetically alike to each other as well as to the parent nucleus from which they are derived. Mitosis, in essence, involves the duplication and separation of chromosomes; and (2) *meiosis*, in which the parent nucleus undergoes two successive divisions, the first of which is a *reduction division* (or *heterotypic division*), while the second is a normal mitosis (or *homotypic division*) (Swamy and Krishnamurthy 1975). Chromosome replication occurs only once. Meiosis is vital in gamete formation.

3.2.2 Cell Cycle

The cells that actively divide mitotically pass through a series of regular events, which together are referred to as *cell cycle* (Fig. 3.1). The cell cycle is divided into *interphase* and *mitosis* (Strange 1992). The interphase precedes mitosis. Mitosis and cytokinesis together are referred to as *M* phase of the cell cycle. The interphase is

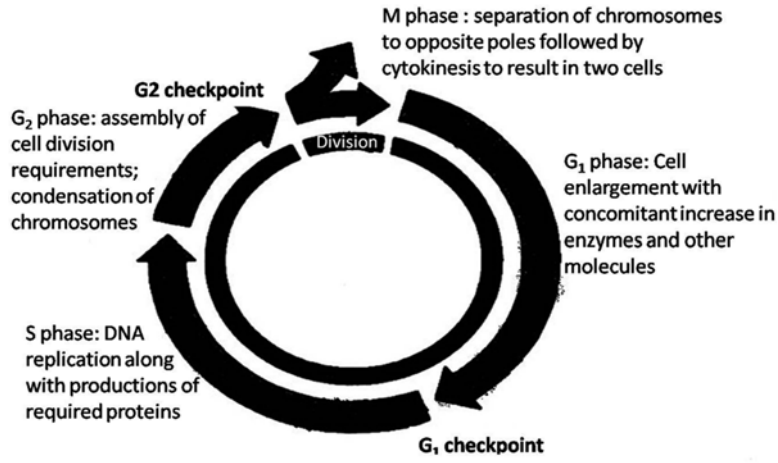


Fig. 3.1 The cell cycle (Krishnamurthy 2015)

divided into three phases, respectively, designated as *G1*, *S* and *G2* phases, where *G* refers to a gap and *S* to synthesis. The *G1* phase, the interval between *M* and *S* phase, occurs after mitosis, and during this phase there is an intense biochemical activity and increase in cell size, number of many cell organelles, endomembranes and other components of the cytoplasm. The *S* phase is a period when the DNA and chromosomes replicate. When initiation of DNA replication takes place, the diploid nucleus of the about-to-divide cell has 2C DNA value, but at the completion of the *S* phase, the DNA content becomes 4C. During *S* phase, histones are also synthesized and repair of damaged DNA, if any, carried out. Then the cell enters the *G2* phase, which is the interval between *S* and *M* phases. The microtubules of preprophase band (see Chap. 2 in this volume) also develop during *G2* phase. During *M* phase, the DNA synthesized in the *S* phase is equally appropriated to the two daughter nuclei, thus restoring the original 2C value in them.

The control and regulation of cell cycle are very vital in deciding the further fate of the daughter cells derived after cell division. The method of this control and regulation is basically similar in all eukaryotic organisms (including plants) and hence is conserved across eukaryotes. Cell cycle mechanisms involve as core regulators *cyclins*, *cyclin-dependent kinases* (*CDKs*), *CDK inhibitors* and *retinoblastoma*-related (*RBR*) substances (De Veylder et al. 2003; Dewitte and Murray 2003). Stress and redox status are also involved as regulators. These affect the expression and/or activities of these and other cell cycle regulators and thus lead to cell cycle arrest. This control and regulation can happen in response to both internal and external environment. In a typical cell cycle, the continuance of the cycle is basically controlled/regulated at two crucial transition points, called *checkpoints*: one at *G1-S* transition and the other at *G2-M* transition (Boniotti and Griffith 2002). The first checkpoint will decide whether or not a cell should enter *S* phase, and the second whether mitosis should be initiated or not. There is often a third checkpoint also, and this is known as *metaphase checkpoint*, which delays anaphase and/or arrests nuclear division. The first checkpoint arrest of cell cycle is noticed during the early stage of root nodule development in the root cortical cells in legumes (arrested in *G0*/*G1*) and in the cells of an embryo before its germination into a seedling (arrested in *G1*). The second checkpoint arrest is found in lateral root primordia derived from pericycle cell (arrested in *G2*). However, these pericycle cells can go to *M* phase upon auxin treatment and then continue to divide and produce lateral root primordium (den Boer and Murray 2000).

As already mentioned, progression through cell cycle depends on the successful formation, activation and subsequent deactivation of cyclins (CYCs) and cyclin-dependent protein kinases (CDKs) at the first two checkpoints mentioned

above; these are the crucial plant cell cycle regulators. Significant progress has been made on the identification and characterization of cyclins and protein kinases. The kinases have a catalytic (enzyme) subunit and an activating cyclin subunit (Hemerly et al. 1995; Huntley and Murray 1999; Mirnov et al. 1999). They belong to a common class of heterodimeric serine or threonine protein kinases. Many types of CDK-like proteins have been identified in diverse plant species based on their amino acid sequences: *A-type* (with PSTAIRE as cyclin-binding motif), *B-types* (PPTALRE and PPTTLRE as cyclin-binding motifs) and three non-classified types (respectively with NFTALRE, PITAIRE and SPTAIRE as cyclin-binding motifs). Cyclins are classified at least under eight groups: A1, A2, B1, B2, D1, D2, D3 and D4. In plants, A-type cyclins are involved in *S* phase progression. But we do not yet know very clearly as to how many of these kinases are actually involved in the plant cell cycle. Data available on plants support the relevance for cell division control only for CDC2aA1, CDC2b A1, pCYCLINB1;1(CYCB1;1) and CDKB1 and CDKB2 and by extrapolation, their orthologues from other species. Donnelly et al. (1999) analyzed the expression pattern of a *CYCB1*; *1*:: GUS fusion gene (with a destruction box inside the *GUS* coding region for β-glucoronidase). The expression of this gene is a specific marker of *G2*/*M* phase of the cell cycle; the same is true for CDKB1 and CDKB2. There is no evidence for cell cycle functions of PITAIRE and SPTAIRE CDKs. CDK and CYCs have been suggested as putative target genes of *PLETHORA* (*PLT2*) which is an auxin-inducible gene. A1 CDK inhibitor that regulates cell cycles representing ICK1 has been known from *Arabidopsis*. It is known to interact with both a CDK and a cyclin. An ICK2 has also been subsequently reported.

RBR protein is a master negative regulator of cell cycle progression. Reduced RBR activity promoted cell division, while increased activity promoted premature differentiation and exit from cell cycle. KRYPTONITE 2 (KPR2), a histone H3 methyltransferase, inhibits cyclin D/Kinase; the latter inhibits RBR which in turn inhibits cell cycle-promoting transcription factors (E2F), thus modulating cell differentiation (Jackson et al. 2002).

Stress and redox controls (den Boer and Murray 2000) include downregulation of *G1* D-type cyclins, inhibition of the ubiquitin pathway by reducing the activities of proteosome or the enzymes involved in the ubiquitination process, upregulation of CKIs and suppression of retinoblastoma (RB) phosphorylation. Under oxidative stress, the expression of two A-type cyclins is downregulated in BY-2 tobacco cells and results in cell cycle arrest at *G1-S*. These cells in *G1* are more sensitive to oxidative stress than in *S* phase.

Specific inhibitors of gamma-glutamyl cysteine synthetase (the first enzyme in glutathione biosynthesis) can block cell cycle (Potters et al. 2004, 2009). Ascorbic acid increases the rate of cell proliferation, but the oxidized form of the same, DHA, delays cell cycle progression (Liso et al. 2004; Potter et al. 2004).

Plant cell cycle is also known to be regulated by both auxin and cytokinins (den Boer and Murray 2000) but it is not clear whether they influence cell division directly or indirectly and at what point of the cell cycle these two growth regulators act (Kende and Zeevaart 1997). Auxins alone increase the level of a CDK protein, for example, in the cultured tobacco cells and stem pith explants, but addition of a cytokinin is required for the activation of this kinase. Auxin and cytokinins are associated with progression through the *G1-S* and *G2-M* control points. As indicated earlier, auxin stimulates radish pericycle cells arrested in the *G2* phase of the cell cycle to enter into mitosis. Cytokinin-depleted cells accumulate inactive CDK-cyclin complexes. Cytokinin inhibitor lovastatin blocks cells in *G2*; cytokinins are also known to regulate the *G1-S* transition. Ascorbic acid is also shown to be important for *G1-S* transition in root apical cells (Liso et al. 1984, 1988). Houssa et al. (1994) observed that exogenously supplied cytokinins reduced the size of DNA replication units in the shoot apical meristem (SAM) of *Sinapis alba* and *Lolium temulentum* and in the ovules of tomato; on the basis of these results, these authors suggested

that the activation of latent replication origin is a universal effect of cytokinins in the promotion of cell division. Cell cycle length change is one of the major effects of some of the growth regulators and other chemicals, and *G1* phase is the most responsive to such signals. Artificial overexpression of CYCLIN D3, a key regulator of CDK activity, can bypass the requirement for cytokinin in SAM. Sucrose promotes cell division through the induction of cyclin D2 and cyclin D3, probably through protein phosphatases. In *Dactylis* exposed to elevated levels of CO_2, SAM cells increased their rate of cell division, resulting in faster growth, and this happens partly through the shortening of mitotic cycle, particularly the *G1* phase (den Boer and Murray 2000). When the cells are in *G1* phase, in the presence of sufficient stimulus, the cells may proceed further in the cell cycle to *S* phase or they may remain there in response to unfavourable environmental cues (i.e. become dormant); this dormant state is often called the *G0* phase (*G-zero*) phase. The cells may undergo differentiation into a distinct cell type without losing their protoplasts, or may undergo terminal differentiation through programmed cell death (PCD) as in sclerenchyma or tracheary elements (Fig. 3.2).

The *M* phase of the cell cycle is also often under developmental control. This depends on the ubiquitin ligase (E3) activity of the anaphase-promoting complex/*cyclosome* (APC/C) (Peters 2006). The APC/C in *Arabidopsis* contains at least 11 subunits (Capron et al. 2003a, b; Lima et al. 2010). Without APC/C, cells cannot separate their sister chromatids during anaphase, cannot exit from mitosis, and to divide into two daughter cells, and cannot do DNA replication in *S* phase. The APC/C was found to be important in targeted proteolysis of A-and B-type cyclins, thus facilitating exit from mitosis. The apc/c mutants have increased CYCB1;1 transcript levels. Thus, in addition to its role in degrading CYCB1;1, APC/C leads to reduced CYCB1;1 transcription. APC2 and APC6 are involved in cell cycle.

Several modifications of the cell cycle are known and all these play a very important role in growth and development. Some cells show only DNA replication and gap phases but no nuclear division. This process is *endoreduplication* (D'Amato 1998; Larkins et al. 2001). Such a DNA replication cycle, often called an *endocycle*, occurs within the nuclear envelope itself and without spindle formation; the nucleus becomes polyploidal by a process referred to as *endopolyploidy* or *endoploidy*. In endocycles of some cells, structural changes similar to those that occur in normal mitosis are seen, and the replicated DNA strands become separate chromosomes; this type of cycle is called *endomitotic cycle* and results in *polytene* chromosomes with numerous strands of DNA lying adjacent to one another. Growth involving endocycles has significant advantages as it provides a mechanism to

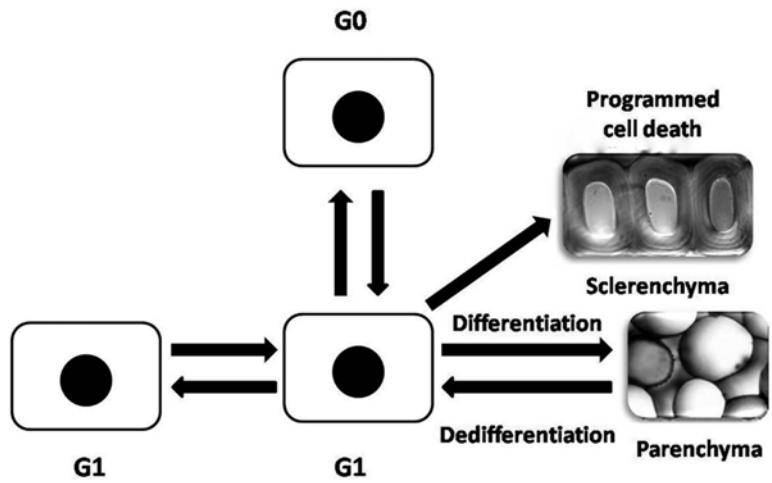


Fig. 3.2 Options for *G*1 cells in plants (After Krishnamurthy 2015)

increase the amount of gene expression (Nagl 1981; Larkins et al. 2001). Contrary to mitotically dividing cells, RNA and protein synthesis are not interrupted during an endocycle so as to enable the cells with endocycle to grow in size faster, to assume maturity more rapidly and to have high physiological activity. Polyploidal nuclei might be required for the formation of large cells in plants. The absolute necessity of endoreduplication of DNA as a prerequisite for cytodifferentiation in some root cells (Rost 1994), endosperm haustorial cells, chalazal chamber of helobial endosperm, bacterioid-containing root nodule cells and embryo suspensor cells has been emphasized by some investigators, although some others consider it as simply a step in a differentiation sequence and not as a prerequisite (Dyer 1976). A reduction in CDK activity is a primary feature of cells that enter the endocycle. *HIGH PLOIDY2* (*HYP2*) prevents endoreduplication (Ishida et al. 2009).

3.2.2.1 Laws of Cell Division

Ever since the proposal of cell theory in the second half of the nineteenth century, many investigators started to emphasize the importance of cell divisions in plant growth and development. Hofmeister, Sachs, Errera, Schwendener, Berthold and D'Arcy Thompson have established/discussed a series of empirical rules to describe the behaviour of dividing plant cells. Hofmeister (1863) noticed that in a tissue that grows in different directions, cell divisions, in general, tend to be perpendicular to what was previously the principal direction of the fastest growth. As an example, we can mention the predominantly transverse divisions in the vertical files of a rib meristem. Sachs (1878) proposed the rule of rectangular section (often called Sach's law), which states that in all tissues, a new cell wall meets the side wall of the parent cell at a right angle. Both Hofmeister's and Sach's laws were concerned with the positions of new walls (after division) from a biological standpoint rather than their dependence on physical factors. Berthold (1886), on the contrary, adopted physical features such as the principle of minimal areas and compared the forms of cells and the disposition of partition walls with those which would be assumed by a system of weightless films under the influence of surface tension. Errera (1888) definitely ascribed the properties of a semi-liquid film to the newly formed cell wall and deduced that this wall must be subjected to ordinary physical laws and must, accordingly, assume the form that would be got under the same conditions by a weightless liquid film. Accordingly, Errera's law states that new cell walls follow the shortest path that will divide the parent cell, 'as if the nascent wall transiently possessed the surface minimization properties of a fluid' (Dupuy et al. 2010). Another important rule that was proposed by Errera is that, 'the incipient partition wall of a dividing cell tends to be such that its area is the least possible by which the given space-content can be enclosed'. All these laws emphasized that many of the properties of dividing plant cells are decided by physical factors, as was also emphasized by D' Arcy Thompson (1942) in his book *On Growth and Form*. These laws/rules which govern cell division in plant (Smith 2001) were deduced from observations on the final arrangement of cells subsequent to growth. D'Arcy Thompson (1942) made a very detailed analysis of surface tension as a factor in deciding cell division pattern in a growing tissue mass, like that of a developing embryo. He emphasized that a living cell is a complex energy field in which the energy is distributed internally through cytoplasm and externally over the surface. He further emphasized that any change in the ratio of these two energy components will disturb the equilibrium of the system. Cell division, by keeping constant the ratio of surface to mass, will, therefore, tend to maintain this equilibrium. Hence, there will be a tendency to maximally reduce the extent of surface created by cell division forcing the cell to divide by walls of minimal area.

Errera's laws on cell division, although were opposed initially, steadily gained importance and support, especially from embryologists. The segmentation pattern during embryogenesis was believed to be purely physical in nature and referable to molecular physics (Krishnamurthy 1994, 2015). Critics of Errera's laws mainly indicated

on the inoperability of the laws in the division of fusiform initials of a vascular cambium in which the division wall is not of minimal area. But, according to Wardlaw (1968), Errera's laws should be accepted in its general aspect and that in different circumstances other factors will inevitably become incident and may affect the pattern of wall formation to a greater or lesser extent; then the value of the law, far from being diminished by noting exceptions, is enhanced by them.

3.2.2.2 Planes of Cell Division

Planes of cell division are also important in plant growth and development. According to Dupuy et al. (2010), the plane or orientation of cell division is correlated with cell shape and size. Cells that are longer in radial dimension generally undergo *anticlinal* divisions, while those longer in the tangential direction undergo *periclinal* divisions. Early plant biologists described the empirical relationship between cell shape, mechanical force and direction of growth that determines the axis of cell division (Théry and Bornens 2006); however, the processes that control the positioning of nascent cell walls remain still poorly understood (Lloyd and Buschmann 2007).

There are four types of cell divisions based on their planes: anticlinal, periclinal, transverse and diffuse. In anticlinal division the cell divides by a wall laid at right angles to the surface of the concerned plant/organ (Fig. 3.3). If anticlinal divisions occur parallel to the radius of a cylindrical organ like the stem or root, they are referred to as *radial*, *radial anticlinal* or *radial longitudinal* division. Anticlinal division is very vital for maintaining the integrity as well as the specific identity of the surface layer of both vegetative and floral organs, the *tunica* in the shoot apical meristem and the *epidermis* in all other parts of the plants; they are also very important to maintain the identity of the *fusiform initials* of vascular cambium. The vascular cambium, a lateral meristem, forms a continuous cylindrical sheath in stems and roots of dicots and gymnosperms and cuts off secondary xylem (or wood) towards inside and secondary phloem (bast) towards outside. As more and more wood is formed on the inside, the cambial cylinder has to keep pace with it by expanding in circumference with a corresponding increase in the number of fusiform and ray initials. These two requirements are satisfied by the radial anticlinal divisions of the fusiform initials. In *storeyed cambium* the anticlinal division is through a straightforward radial longitudinal wall (Fig. 3.4a). This results in the grouping of fusiform initials in horizontal rows, provided that differential elongation of daughter cells does not occur (and it does not occur, avoids crowding past one another of initials and maintains the stratified arrangement). If differential elongation of daughter cells occurs, the average length of initials would increase considerably with each anticlinal division. In *non-storeyed cambium*, anticlinal division of fusiform initials is through a more or less horizontal division wall, often called *pseudotransverse division* (Fig. 3.4b) with a subsequent increase in length of daughter cells. In

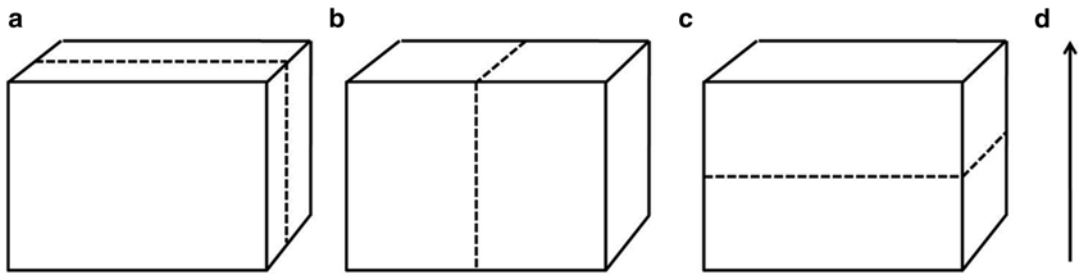


Fig. 3.3 Diagrams illustrating planes of cell division. (**a**) Periclinal division, (**b**) radial anticlinal division and (**c**) transverse division. Division wall is shown by *broken lines*, while the *arrow* (for **a** and **b**) indicates the direction towards the periphery and (for **c**) indicates the longitudinal axis of the plant organ in whose cells these divisions take place (Krishnamurthy 2015)

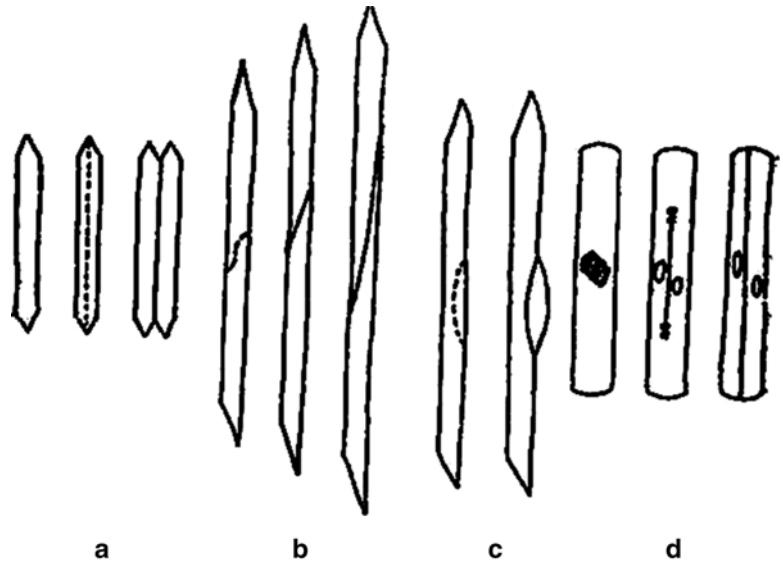


Fig. 3.4 Types of cell divisions in fusiform initials of vascular cambium. (**a**–**c**) Types of anticlinal divisions. (**a**) Radial division in stratified cambium; (**b**) pseudotransverse division in non-stratified cambium; (**c**) lateral division in non-stratified cambium; (**d**) periclinal division as seen in radial longitudinal sections (Krishnamurthy 2015)

pseudotransverse division, the dividing wall is usually laid down near the centre of the cell. The length and pitch of the wall are very variable, ranging from very short and transverse (or nearly so) to very oblique and up to half the length of the dividing cell in conifers and considerably more in dicots. The length of the dividing wall in coniferous taxa is related to the length of the fusiform initial, with longer initials tending to have longer partitions; the slope of the dividing walls tends to be the same in the neighbouring dividing fusiform initials. After varying lengths of time, reversal in the direction of slope of the dividing walls of groups of fusiform initials takes place. The continued occurrence of anticlinal divisions with a particular direction of slope results in the development of *spiral grain* in the wood derived from this cambium. The term *domain* is often used to designate that sector of the vascular cambium where the anticlinal partitions are tilted in one direction. In vascular cambial cylinder that increases in circumference, another type of anticlinal division takes place. This was called originally (*longitudinal*) *division off the side*, but this is now called *lateral division*. The dividing wall is laid down longitudinally and to one side of the fusiform initial and often looks curved; this results in a small lateral cell that often looks spindle shaped (Fig. 3.4c). Such cells function as the progenitors of ray initials after undergoing a few transverse divisions, as seen in TLS. There needs to be a constant ratio between pseudotransverse and lateral divisions in the fusiform initials during the increase in cambial circumference in order to constantly maintain the proportion of fusiform and ray initials.

If a cell divides by a wall laid parallel to the surface of the concerned plant part/organ, the division is known as periclinal (Fig. 3.3a). In cylindrical organs like stems and roots, such periclinal divisions are called *tangential* or *tangential longitudinal divisions*. Periclinal divisions in cells are important in the following instances: (1) on the flanks of shoot apical meristems (other than in tunica) to initiate the leaf primordia, (2) help to differentiate the mantle from core region in the floral meristem, (3) distinguish the parietal tissue from the archesporium in the young anther and ovule primordia, (4) help to produce all vertically aligned cell types of wood and secondary phloem from fusiform initials (Fig. 3.4d) of the vascular cambium and (5) help to form the cork cells (phellem) and secondary cortex cells (phelloderm) from the cork cambium (phellogen). In vascular cambium the fusiform initials are long cells and so the process of cell-plate formation is greatly extended in time; the cell plate has to extend to the entire length of the cell (Fig. 3.4d).

Once the division is over, one of the daughter cells becomes a xylem or phloem mother cell, while the other remains as the initial.

When a new cell wall of a dividing cell is laid down at right angles to the longitudinal axis of a cylindrical organ, the division is *transverse*. Such a division is important in megaspore mother cell, zygote, rib meristem in the young internode, etc. *Diffuse divisions* refer to divisions that take place in cells without any specific direction/orientation and in diverse planes. Such divisions are vital in the development of pith and take place in the cortex of roots and stems, mesophyll tissue of leaves and pericarp of fleshy fruits. Diffuse growth is related to volume increase.

3.2.2.3 Asymmetric Cell Divisions

The symmetry of cell division is very important in development. Cell division is symmetrical when the two resultant daughter cells are almost equal in size, at least immediately after the division is over. It is asymmetrical if the two resultant daughter cells are of strikingly different sizes from the beginning. *Asymmetric divisions* are very important in plant development as the two daughter cells have different fates and with totally different pathways of differentiation (Gallagher and Smith 2000). Some investigators conceptually include under asymmetric divisions those divisions where the resultant daughter cells are equal in size but are destined to have different fates from the beginning.

Asymmetric cell divisions are very strong reflections of the existence of polarity in the parent cell before cell division (Stebbins and Jain 1960). Such a strong polarity has been noticed in spores of lower vascular plants, zygotes, root hair mother cells, stomatal mother cells, microspores, phloem sieve element mother cells and the innermost root cortical cells. The division of the spore of *Onoclea sensibilis* is preceded by migration of its nucleus from the centre of the spore to one end. This is followed by an asymmetric division of the spore; the longer daughter cell forms the protonema, while the smaller cell becomes the rhizoid. Zygotes of brown algae like *Fucus* and *Pelvetia* (Fowler and Quatrano 1997) were the first to be investigated in detail for asymmetric division. In these taxa, the zygote is initially spherical and apolar but polarity is established once it is subjected to illumination. A rhizodal pole and body pole are organized before asymmetric division sets in to form a rhizoidal cell and a thallus cell, each with a different type of cell wall. The rhizoidal cell contains a wall made of sulphated polysaccharides (fucoidins), while the thallus has a different wall chemical (carboxylated polysaccharides). The asymmetries fixed in the cell wall are stated to signal positional information to the cytoplasm that is very crucial for subsequent development (Fowler and Quatrano 1997). The zygotes of angiosperms, in contrast to those of *Fucus* or *Pelvetia*, are asymmetric from the beginning and inherit the asymmetry from the polarized egg, which in turn is inherited from the polarized embryo sac. The asymmetric division of the zygote results in a large micropylar *basal cell* and a small chalazal *apical cell*, respectively, organizing the embryo suspensor and embryo proper. This asymmetric division is under strong genetic control; for example, *gnom* and *emb30* mutants of *Arabidopsis* have defective zygotic division, which appears near-symmetric (Mayer et al. 1993).

Root hair of some plants is formed by an asymmetric division from dermal cells which give rise to a larger epidermal cell and a smaller cell called *trichoblast*. The nucleus of the trichoblast mother cell moves towards that pole where the trichoblast will be cut off (Sinnott 1960). The specification of the mother cell is initiated before the emergence of the root hair. Following laser ablation of hairless cells of the root epidermal layer of *Arabidopsis*, root hairs were not formed by these cells, emphasizing the importance of the asymmetrically derived cells in developing into root hairs. The GL2 promoter-β-glucuronidase reporter gene fusion construct directs the expression preferentially in future ordinary epidermal cells (atrichoblasts) located over cortical cells, while the trichoblasts do not show the expression and are located over the junction walls of cortical cells. Cytochemical studies have shown that the developing trichoblast of *Phleum pratense* and *Hydrocharis morsus-ranae* has greater activities of enzymes such as acid phosphatase,

peroxidase, succinic dehydrogenase and cytochrome oxidase than its sister cell and other epidermal cells (Cutter and Feldman 1970a; Avers and Grimm 1959). The trichoblasts also contain more proteins and RNA and organelle-rich cytoplasm. The trichoblasts of some taxa also have endoreduplicated nuclei (Cutter and Feldman 1970b).

Stomatal development also involves asymmetric division. The *meristemoid mother cell* (*MMC*) of the protoderm cuts off a smaller cell called *meristemoid,* which develops into the stoma, and a larger cell, which continues to remain as an epidermal cell. The meristemoid may directly generate two *guard cells* by a symmetric division or may undergo additional divisions before forming guard cells (Sylvester et al. 1996). There are many taxa where stomata have *subsidiary cells* which are derived from *subsidiary mother cells* (*SMC*s), either through symmetric or asymmetric divisions, depending on the type of stoma. A conserved mechanism to segregate cell fate determinants is operating during stomatal asymmetric divisions. Studies on stomatal development in *Tradescantia* (Croxdale et al. 1992; Cleary 1995; Kennard and Cleary 1997) have shown that the first sign of asymmetry in the SMC is a cytoplasmic aggregation at a particular cortical site nearer to the *guard cell mother cell* (*GMC*), and this is followed by a migration of the nucleus towards this locus. Subsequently microfilaments also accumulate at this cortical site between the nucleus of SMC and the GMC and simultaneously in the adjacent cortex of the GMC. The nucleus gets connected to the newly accumulated cortical microfilaments by new actin filaments. The SMC division takes place with the asymmetrically shifted microtubules and microfilaments and preprophase bands appearing at the SMC cortical site that predicts the future division plane. A similar set of changes occur in the asymmetric divisions that produces the GMC. It is suggested that pressure application to SMC walls leads to cytoplasmic and nuclear migrations to the locus of pressure and consequent stretch-activated ion channels and ion fluxes may serve as intermediates in the signalling events associated with asymmetric divisions. It is further suggested that an extracellular *cue* originating from the GMC is interpreted by the SMC to orient both the nuclear migration and the local accumulation of cortical microfilaments. Either of these asymmetric events could serve to reinforce the GMC-originating spatial *cue*, thus establishing a more permanent polarity, which might in turn influence, extracellularly, the microfilaments and cytoskeleton of the adjacent GMC and, intracellularly, the SMC cytokinetic apparatus (Fowler and Quatrano 1997).

The division of the haploid microspore to form pollen grains is also asymmetric. This asymmetric division is due to the polarity of mitotic spindle and results in two cells different in both size and subsequent fate. The large cell is the *vegetative cell* (*VC*) and the smaller cell is the *generative cell* (*GC*). Immunofluorescence studies have indicated that the bulk of the microtubules appear as radial arrays surrounding the centrally located microspore nucleus. The microtubules then get concentrated at the distal surface of the microspores between the plasma membrane and the nuclear envelope, and this is the first indication of polarity. This perhaps facilitates the nuclear migration towards this region just before mitosis. During nuclear division the microtubules disappear and the locus marks the pole of the mitotic spindle. There is no change in distribution pattern of microfilaments during premitotic stages, but during division they get co-aligned with the spindle microtubules (Brown and Lemmon 1991, 1992, 1994). In tobacco, the *lat52* promoter is sufficient to direct VC-specific transcription of a nuclear-targeted β-glucuronidase (GUS) fusion, providing a useful marker for VC identity (Twell 1992). Thus, the symmetric division is necessary to establish the GC fate, and a microtubule structure is *involved* in anchoring asymmetric factors influencing the division and GC fate.

In the angiosperm phloem tissue, the *sieve tube element* and its associated *companion cell* are derived from the sieve tube element mother cell through an asymmetric tangential longitudinal division. The larger daughter forms the sieve tube element and the smaller the companion cell.

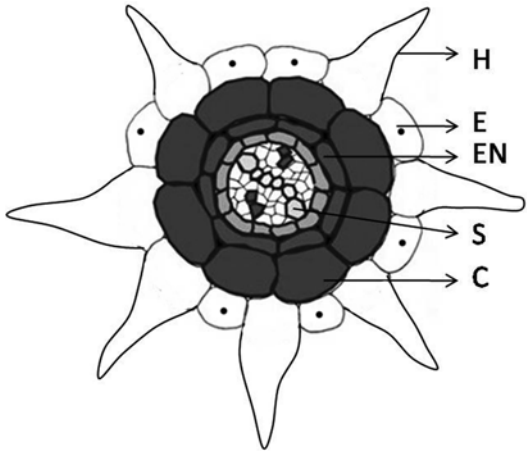


Fig. 3.5 Diagrammatic T.S. of *Arabidopsis* root showing the distribution of different tissue types. The cells of the epidermis (*E*) in between two cortical cells (*C*) elongate as root hairs (*H*), while those (atrichoblast) located over cortical cells show the preferential expression of the *GL2* promoter-β-glucuronidase reporter gene fusion construct (shown with a *black dot*). The stele (*S*) is bound externally by pericycle and endodermis (*EN*) (Krishnamurthy 2015)

In *Arabidopsis* root apical region asymmetric cell divisions occur in the initial cells to give rise to the inner, smaller endodermal cells and to the outer, larger cortical cells (Fig. 3.5) (Scheres et al. 1994). The occurrence of the asymmetric divisions is supported by histological and clonal evidences (Dolan et al. 1993). The *Arabidopsis* mutant for the *SCARECROW* gene has only one layer instead of the normal two (one cortical and one endodermal) present in the wild type (Di Laurenzio et al. 1996). This single layer exhibits cell type markers of both cell layers (Scheres et al. 1995). The *Arabidopsis short-root* (*shr*) mutation also affects the asymmetric division that results in endodermis formation (Benfey et al. 1993), and the single layer expresses both endodermis and cortex cell fates.

3.2.3 Cell Enlargement

Cell enlargement refers to the increase in the size of the cell after the cell is derived from a meristematic initial. In many cases, the size of the cell increases before entering into another division. This prompted investigators like Dupuy et al. (2010) to propose that a minimum cell volume is required to trigger mitosis; this is also consistent with the requirement for a minimum cell volume during progression through the cell cycle. Korn (1984) recognized three basic categories of growing cells in plants: (1) cells that grow in all three dimensions and that too equally in all directions; this results in uniform increase along all its radii. The cells finally become isodiametric; this is seen in some parenchyma cells; (2) cells that grow in one plane only resulting in an increase of length or width of the cell. Such an increase may be through unipolar or bipolar growth in one plane. Growth in one plane is seen in fibres, laticiferous cells, tracheary elements, phloem sieve elements, pollen tubes, root hairs, fungal hyphae, cotton hairs, etc.; and (3) cells that grow only in two dimensions, as in epidermal cells. In addition to these three categories, one more category can be added: cells in which growth is restricted to certain sectors alone along the perimeter to result in armed or stellate or irregularly outlined cells. The stellate parenchyma in the diaphragms that prevent collapse of air canals in water plants, pith of *Juncus*, leaf spongy mesophyll cells, astrosclereids, etc. are good examples of this category (Fig. 3.6).

3.2.3.1 Intercellular Adjustments During Cell Enlargement

The interplay of forces between cells can constrain as well as promote cell expansion in a population of cells. In a population of cells, all cells cannot increase at the same rate, and this unequal increase of adjacently located cells is very important in tissue differentiation. In other words, in a growing tissue some cells may continue to divide with no or little enlargement, some enlarge slightly and some enlarge considerably; the direction of this enlargement and the type of enlargement may also vary between adjacent cells. For instance, developing fibres elongate considerably when compared to adjacently lying tracheary elements or sieve elements which elongate only to some extent; parenchyma cells undergo the least elongation or may keep pace with other cells through cell divisions. The same is true for an increase in width of the cells. The

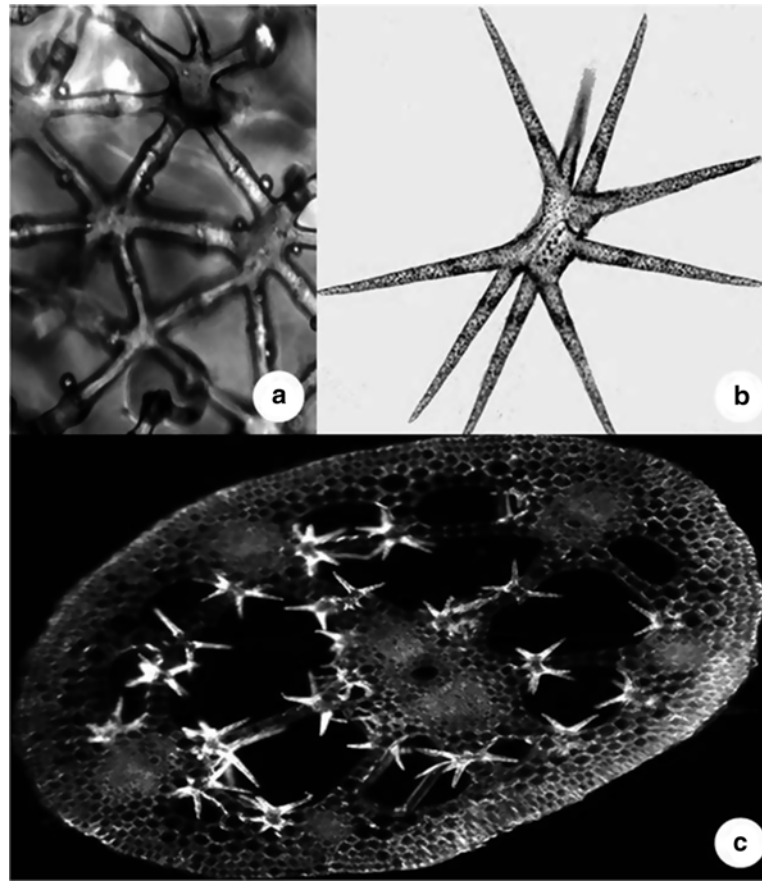


Fig. 3.6 (**a**) T.S. of portion of the diaphragm of *Juncus* stalk showing star-shaped aerenchyma cells. (**b**) An isolated astrosclereid of *Gnetum* leaf. (**c**) T.S. of petiole of lotus leaf showing astrosclereids (Krishnamurthy 2015)

differential behaviour of the different cells of a population of cells is called *intercellular adjustment*. Intercellular adjustments (Fig. 3.7) during tissue differentiation involve one or more of the following (Krishnamurthy 2015): (1) *coordinated* or *symplastic growth*, which involves adjustments in the growth of cell walls of participating cells; the contiguous growing cell wall layers of two adjacent cells do not separate but expand together as the cells elongate (Fig. 3.7). In this type of growth, it is possible that parts of the common wall between the two cells may expand and but nor another part; (2) *Intrusive* or *interpositional growth*, which also involves adjustments in the growth of cell walls of participating cells; the contiguous growing cell wall layers of two adjacent cells separate and the growing cell *intrudes* into the just-formed space. In this case, of the two adjacent cells, one grows in size, while the other does not. This type of growth is common in elongating cambial initials, fibres, tracheids, laticifers and some types of sclereids (Fig. 3.7b). Invariably, the elongating fibres or tracheids show only *apical intrusive growth*, i.e. growth is seen only at one tip or at both tips. The intercellular pectin substances through which the elongating fibres grow are probably hydrolyzed in advance, thus facilitating their easy intrusion. Intrusive growth concept has replaced the earlier *gliding* (or *sliding*) *growth* (Fig. 3.7a), according to which a growing cell was supposed to separate from and glide over the wall of adjacent cell. Some investigators still believe in the existence of gliding growth (Rao and Krishnamurthy 1976).

3.2.3.2 Unipolar Growth

Unipolar growth is a strong manifestation of polarity. This type of growth is shown by pollen tubes and root hairs. Pollen tubes have a distinct

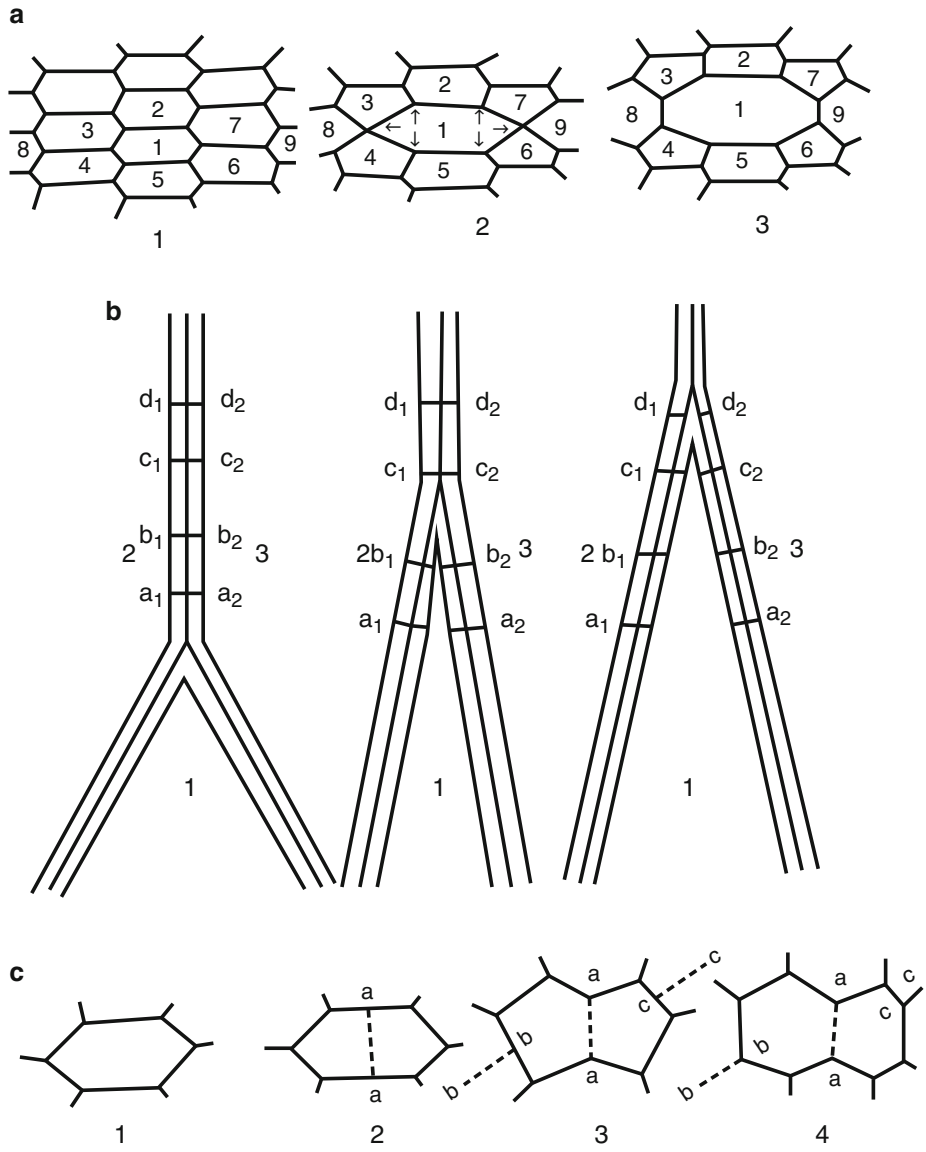


Fig. 3.7 Diagrammatic sketches to show gliding (sliding) (**a**), apical intrusive (**b**) and symplastic growth (**c**). In (**a**) cell 1, which previously was not in contact with cells 8 and 9, comes to have contacts with them during gliding growth. In (**b**) cell 1 with apical intrusive growth grows through cells 2 and 3 that face each other across a common middle lamella. In (**c**) there is an adjustment of cell shape and cell position after each division (*dotted lines*) (Krishnamurthy 2015)

apical growth zone, besides a nuclear zone, a vacuolar zone and a callose plug zone; these are not rigidly demarcated regions in terms of their extent of occupation but invariably overlap. The growth of the tube is restricted to the tip region of about 4–7 μm (Fig. 3.8). This region exhibits characteristic cytological features (Cai et al. 2005). The plasma membrane is connected to the tubular and smooth ER. Mitochondria amyloplasts, secretory Golgi bodies, Golgi vesicles, several enzymes, cytoskeletal elements, etc. are in abundance; the Golgi vesicles are of two types, one of 0.1–0.3 μm in diameter, bound by a unit membrane and rich in polysaccharides required for pollen wall synthesis by the tube tip, and the other of 0.01–0.05 μm in diameter and rich in

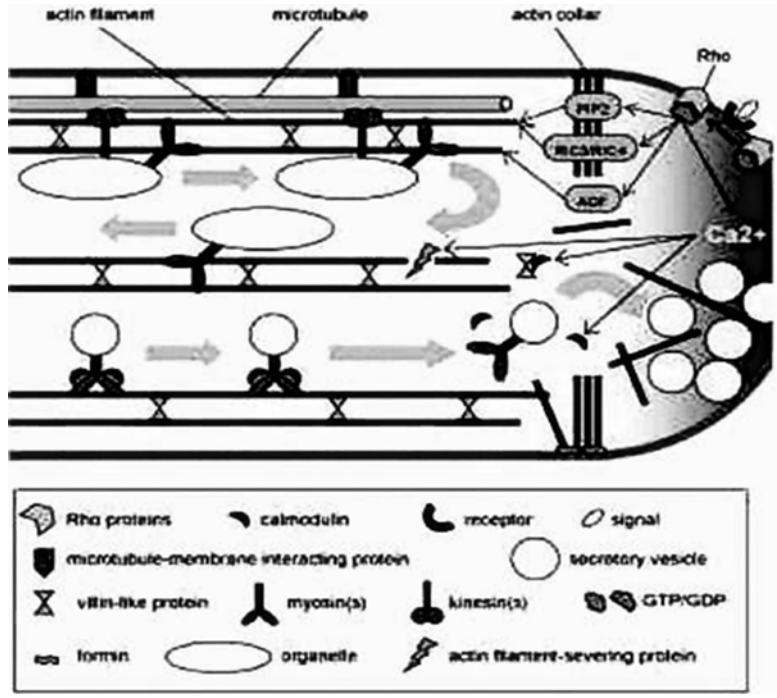


Fig. 3.8 Detailed composite structure showing the putative interactions between the Ca^{2+} gradient, regulatory proteins and the cytoskeleton in the pollen tube tip (Cai et al. 2005)

RNA, and both are transported to the tube tip through cytoplasmic streaming (described as *reverse foundation streaming*). The unipolar growth of pollen tubes is associated with polar electric currents and polar distribution of calcium ions (revealed by proton probe analysis, fluorescence cytochemistry and by ^{45}Ca autoradiography). The rate of pollen tube tip growth is very high and may be up to 600 μm × h^{-1}.

The root hairs range from 80 to 1,500 μm in length (Krishnamurthy 2015) and, like pollen tube, show tip growth. Root hair tip also shows a polarized distribution of almost all cytoplasmic constituents that are recorded above for pollen tube tip. Ca^{2+} influx at its tip is believed to regulate the secretory process through its effect on actin (Baluska et al. 2000). The manner in which actin is present in the tip region is debated, some describing the presence of an actin cap (a three-dimensional network of filaments) (Fig. 2.9b) and others describing a fewer filament or even its absence. There is cytoplasmic streaming. On completion of growth, the cytoplasmic polarity seen all along is lost, with the position of the nucleus becoming either random or basal. Unlike the pollen tube, there is no callose plug in root hairs, as it will hinder absorption and movement of water and sap. Schiefelbein et al. (1993) have described a mutant in *Arabidopsis* that shows defects in both pollen tube and root hair growth; compound to wild-type tubes and root hairs, growth of the mutant tubes and root hairs is tardy and stymied.

3.2.3.3 Mechanism of Cell Expansion

The mechanism of cell expansion is central to the regulation of cell shape (Dupuy et al. 2010). Cell expansion may involve two mechanisms: (1) continued production of new cytoplasm with new wall materials being laid only in the region of growth of the cell, this is seen in pollen tube and root hair growth, as described above, and (2) expansion happening mainly due to stretching of the already existing cell with concomitant changes in the cell wall and deposition of new wall materials. In this section, attention would be focused only on the second mechanism.

Growth in volume of a cell is mainly caused by an uptake of water, and this process necessarily stretches the cell wall as well as the plasma

membrane inside the cell wall. Consequently, new cell wall and membrane materials need to be synthesized. The latter are needed not only to avoid the cell wall and plasma membrane from becoming thinner due to stretching but also to avoid their rupture since both these cell entities cannot stretch beyond a reasonable limit. Thus, growth of cells causes an increase in surface area of cell wall and plasma membrane. When cells enlarge in size due to water uptake, the water taken inside the cell would dilute the solute of the cell and thus lower the internal solute potential. Then, solutes are to be absorbed by this cell from the surrounding cells or are to be newly synthesized in the tissue of which the cell is a part; this, in fact, happens after cell growth. Water entry into a cell causes changes in cell turgor and so the cell wall has to be strong enough to withstand the high physical stresses generated by the cell's turgor pressure (*P*). When *P* of growing cells is typically in the range of 0.3 to ±9.0 MPs and that wall stress is more or less Px cell radius/wall thickness, it can be calculated that wall stresses are in the order of 10–100 MPs (Cosgrove 1997). The inevitable consequence of this physical stress, i.e. the critical physical event required for cells to expand, is *cell wall relaxation*. Cell wall relaxation is the reduction in wall stress at constant cell wall dimensions; it is the means by which growing cells simultaneously loosen their walls and reduce their turgor wall potential, thereby enabling them to imbibe water and to expand physically. Thus, wall expansion is a very complex physical process and requires loosening of wall structure resulting in relaxation of wall stress, reduction of turgor pressure, synthesis of polysaccharides and proteins and active respiration in the cell (McQueen-Mason 1995).

Heyn (1931) first introduced the concept of *plasticity* and *elasticity* of cell walls, with reference to expanding cells. Plastic wall stretching is achieved as the cell wall is loosened (this is also called *wall yielding*), so as to enable cellulose microfibrils to slide past each other more easily. This sliding of cellulose microfibrils is called *shear*, which actually involves the breaking of bonds between adjacent microfibrils. *Creep* refers to a time-dependent, irreversible extension, typically due to slippage of the wall polymers relative to one another in growing cells which exhibit a steady long-term extension. The relative growth rate of a cell is proportional to the extent that turgor pressure (*P*) exceeds a value called the *yield threshold* or *yield potential,* and this proportionality factor is the wall *extensibility*. Wall extensibility and yield threshold are shown to be modulated by hormones such as auxin and gibberellins, while ABA and ethylene decrease wall extension.

The mechanisms as to how loosening, wall relaxation and cell wall expansion happen in an enlarging cell have attracted the attention of several investigators. One mechanism proposed is the *acid growth hypothesis* (see Brett and Waldron 1990). According to this hypothesis, specific genes activated by auxin influence the synthesis and delivery of new cell wall polymers needed for wall extensibility and simultaneously lead to wall expansion. However, it fails to explain how the secretion of wall polymers may lead to wall stress relaxation that is important for water imbibition and expansion of the turgid cell. The acid-induced creep or acid growth, as it is called, is characteristic of the walls of growing cells. The change to an acidic pH appears to convert the growing wall from a viscoelastic solid to a viscoelastic liquid due to weakening of one or more key bonds linking wall polymers together. There is no experimental proof for this explanation. The more commonly accepted explanation for this is that auxins activate a proton-pumping ATPase in the plasma membrane. The protons are pumped from the cytoplasm into the cell wall resulting in pH alteration, which in turn is believed to cause a loosening of cell wall structure and the subsequent turgor-driven extension.

The second explanation is that wall-yielding results from a biochemical loosening of the wall to allow turgor-driven extension of the wall polymer network (Fig. 3.9) (Cosgrove 1997). It is evident from this figure that the cell wall contains plastic and elastic elements which are believed to be in series with each other. When the plastic elements relax, they stretch, the elastic elements shrink and shorten, and this can happen only if wall stress and turgor are reduced. If water enters

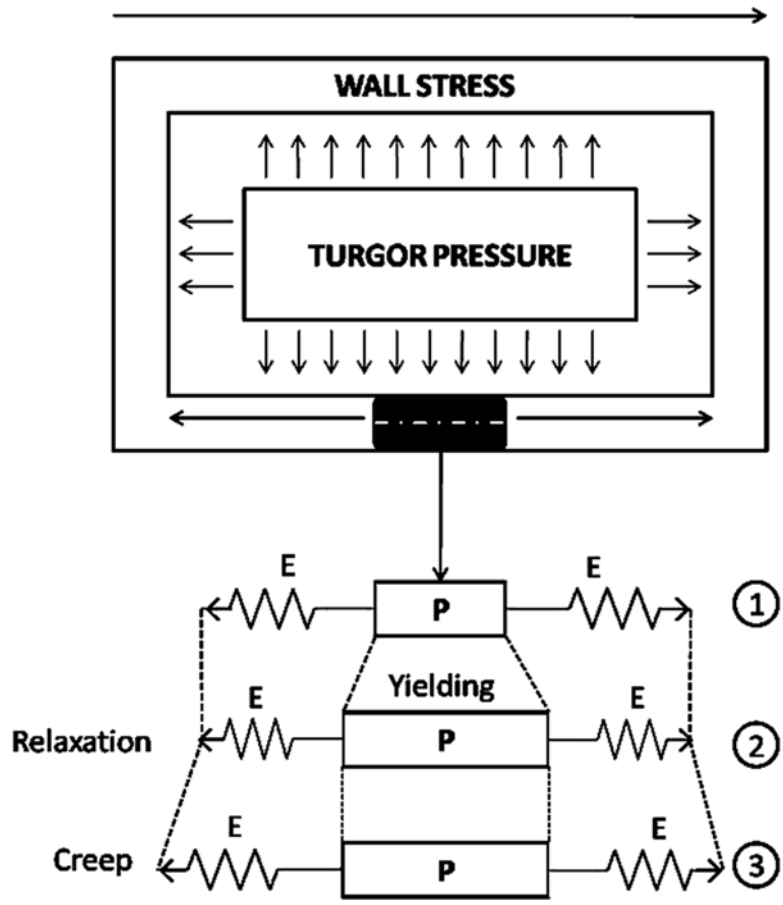


Fig. 3.9 A mechanistic model for the growing primary cell wall as per Cosgrove (1987). Turgor pressure (*small arrows*) exerts a force against the primary cell wall and causes a tensile stress in the plane of the wall. (*1*) Shows both elastic (*E*) and plastic (*P*) elements, the latter bearing the wall stress. First the plastic elements yield and relax but the elastic elements shrink (*2*) and hence there is no net increase in cell wall length. (*3*) Shows the maintenance of wall stress through the expansion of E but not of P (creep), resulting in increase of wall length (Diagrams based on Cosgrove 1987)

almost simultaneously as pressure decreases in response to relaxation of the plastic elements, the elastic elements might shorten only infinitesimally as pressure decreases only infinitesimally. In this steady-state growth process called creep, wall stress and turgor remain constant. Although this explanation is supported by many, it does not take into consideration the long-term need for integration of new cell wall polymers into the expanding wall.

Many researchers have suggested the involvement of wall enzymes like (1→4)β-glucanases or xyloglucan endotransglycosylase (XET) in cell wall loosing since cell wall matrix glucans show an enhanced turnover upon cell wall growth stimulation by auxins. The wall polymers hydrolyzed by the enzymes are xyloglucans and (1→3),(1→4) β-glucans in dicot and grass cell walls, respectively. However, in vitro experiments failed to detect cell wall loosening or extension of the cell wall by XET. The xyloglucans that anchor cellulose microfibrils to the cell wall matrix may be cut by XET that transfers the newly formed reducing end to water (i.e. hydrolysis) or to non-reducing end of another hemicelluloses (i.e. transglycosylation). Alternatively, wall loosening agents may weaken the non-covalent bonds that bind matrix polymers to cellulose microfibrils or to other matrix polymers (Cosgrove 1997). Further biochemical analysis of extending walls led to the discovery of a novel family of cell wall proteins called *expansins* that mediate the rheological behaviour of growing cell walls. Wall growth is lost if the wall is treated with either proteases or protein-denaturing agents, thus proving the role of expansins in wall extensibility. However, not all plant cell walls are susceptible to the action of expansins, particularly cell

walls of cells that are located in nongrowing plant parts and of mature cells. Although the molecular basis for the action of expansin on wall extensibility is still not clear, Cosgrove (1997) came up with a tentative model. The primary wall is initially assembled in a form that is mechanically tough, but it also has 'hot spots' where expansin can weaken cellulose microfibril-matrix polymer bonding. This expansin activity induces stress relaxation and polymer creep required for wall elongation and water inhibition by the cell. Enzymes like glucanases, other cell wall hydrolases and XEt alter the viscosity of the matrix and control the amount of wall extension that results from expansin activity. These enzymes may also control the yield threshold by modulating the size of the polysaccharide cluster that is dragged along by expansin-induced creep. Xylosidases and other enzymes remove the side chains from hemicelluloses and promote their binding to cellulose microfibrils. Potential cross-linking enzymes finally lock up the wall during cell maturation, preventing further creep. Generally, cell wall does not grow in surface area after cell maturation, accompanied by a mechanical stiffening or rigidification. After cell expansion is completed, the cell wall matures; wall maturation involves (1) reduction in wall loosening, (2) an increase in cross-linking of cell wall polymers, (3) change in the chemical composition of the cell wall and (4) a loss in expansin expression.

3.2.4 Role of Genome on Cell Size and Cell Division

There is considerable debate over the relative contribution of physical and genetic processes to the coordination of cell division and cell expansion during plant development (Schopfer 2009). At one extreme, geneticist believe that both cell division and enlargement are controlled by DNA and that this control regulates plant growth and development, while at the other extreme biophysicists argue that cell division and cell enlargement, and consequently growth and development of plants, are controlled by physical factors (Green 1980; Lintilhac and Vesecky 1984; Lynch and Lintilhac 1987). A number of studies indicate a positive correlation between DNA amount (=genome size) on the one hand and carbon content per cell, cell size, pollen volume, log seed weight, seed mass and mean leaf dimension on the other (Bennett 1987; Bennett et al. 1981, 1983). The presence of direct relationship between DNA amount and cell size (and metabolism) is the basis of the *nucleotype theory*, proposed by Bennett (1971, 1972). He coined this term to describe 'that condition of the nucleus (most notably DNA content) that affects the phenotype independently of the information content of the DNA'. On a certain level, he argued, DNA content and cell size must be causally related due to the physical impossibility of containing very large genomes within small cells. However, we do not yet have any direct experimental evidence in favour of the nucleotype theory, although there are some observational evidences. It is suggested that the nucleotype should be considered as setting minimum conditions or as exerting a very coarse control, on parameters at the cell level, while the genotype is to be considered as responsible for fine control of these features within these limits.

It has also been appreciated for a long time that the sizes of nucleus and cell on the one hand and cell division rates on the other hand are closely related. In diploid plants, a relationship really exists between the minimum mitotic cell cycle time, the interphase nuclear volume and the DNA content per cell and the size of the cell (van't Hof and Sparrow 1963). These authors further asserted that this relationship is such that if any one of these variables is known, an estimate can be made for the remaining two. Additional experiments revealed a relationship between DNA content and the duration of the *S* phase (Van't Hof 1965). Bennett (1977) showed a clear and strong correlation between genome size and the duration of meiosis in diploid flowering plants. However, it is impossible to increase greatly the C-value without also increasing the minimum time needed for cell division because of biophysical constraints. This is due to the fact that more DNA not only takes a longer time to replicate (i.e. prolongs *S* phase) but also affects

all stages of the cell cycle. This delay in cell cycle is ultimately responsible for the positive relationship between DNA content and cell size. An increase in DNA content beyond a certain point is not possible without also increasing the size of the nucleus and the cell (Krishnamurthy 2015).

3.2.5 Relative Importance of Cell Division and Cell Enlargement in Development

While discussing the relative importance of cell division and cell enlargement in plant growth and development, three aspects need critical consideration: (1) What causes a dividing cell to stop dividing and differentiating into a specialized cell type? As already discussed earlier the answer to this question is that a critical cell cycle factor is absent at the checkpoints, and consequently the cell will differentiate without dividing or progressing through *S* phase again. (2) Whether cell division is a prerequisite for cell enlargement and maturation events of specific cell types (Aloni 1987) and vice versa. According to Rost (1994), cell enlargement and differentiation into specific cell types do not specifically require cell division in a programming sense. Earlier, Dodds (1981a, b) made a similar conclusion. In the stressed cells of the intercalary meristems of rice and wheat leaves, modulations of the cell cycle do not precede any detectable change in cell growth, thus providing additional evidence for the fact that cell growth in plants is not the only driving force for cell division and vice versa. (3) Whether cell division and cell enlargement, independently and together, decide the shape, size and architecture of a plant organ and the whole plant. Is cell division informed by cell growth or is cell division the driving force for cell growth? Can cell division be reduced to the surveillance of cell growth? Since plant growth is determined by the number of cell divisions and the size of cells (Meyerowitz 1996), many plant biologists believe that cell division and cell expansion are more likely to be coordinated during plant organ growth. However, according to several others, the relationship between cell division and cell expansion in the growth and development of plant organs is disputable.

For a very long time, plant biologists generally believed that the shape (and size) of a plant organ is decided by the intensity and pattern of cell division seen in that plant organ during its development (Meijer and Murray 2001; Reinhardt and Kuhlemeir 2002). The relationship between cell division and organ shape/growth can be demonstrated by altering the number and intensity of cell division. Prior to the discovery and identification of genes affecting cell cycle/division, plant biologists mainly depended on external means to arrest cell cycle/division like chemical treatment, irradiation, etc. In recent years, cell cycle mutations and expression of cell division-affecting genes are being studied, particularly the latter in transgenic plants, in order to understand the role of cell division in plant growth and development. Mutations in *CLAVATA1* (*CLV1*) and *CLAVATA3* (*CLV3*) genes increase cell divisions; mutated *CURLY LEAF* (*CLF*) gene converts a determinate floral meristem into an indeterminate meristem with proliferative cell division activity. The overexpression of *AINTEGUMENTA* (*ANT*) promotes more cell divisions that increase the size of the organ. These examples show that cell divisions (and increase in cell number) bring about growth modifications. In view of the above, Meijer and Murray (2001) strongly emphasized that cell divisions are very important in plant growth and development and that they should not simply be regarded as a secondary consequence of growth.

On the contrary, some studies on leaf development have shown that changes in the rates, planes and patterns of cell division do not affect final leaf shape. For example, the new leaf primordia of irradiated wheat seedling can be initiated in the absence of cell divisions (Haber 1962; Foard 1971), although the leaf primordium was limited in size and had an abnormal appearance. Similarly, tobacco plants expressing a dominant negative mutant gene cdc2a of *Arabidopsis* had reduced cell numbers (due to reduced cell divisions), but showed no change in development (Hemerley et al. 1995). Already existing leaf primordia with downregulated cell cycle activity

(Hemerley et al. 1995) and ones with a mutation in the *TANGLED* gene of maize that interferes with correct cell-plate orientation (Smith et al. 1996) can develop almost normal leaf shapes. Plant development was also normal in *Arabidopsis* plants overexpressing a mitotic cyclin, despite increased root growth (Doerner et al. 1996). The extracellular protein expansin, which regulates cell wall extensibility, is expressed both in elongating and meristematic tissues, and it has been shown that local induction of an expansin gene in the apical meristem is sufficient to induce organ formation, whereas local induction of cell division did not induce organogenesis. Also, mutants such as *angustifolia* and *rotundifolia* of *Arabidopsis*, whose organ shape is altered, appear to be compromised in cell expansion, rather than in cell division. These observations were employed to emphasize that plant organs like a leaf can acquire their shapes independent of the patterns/intensity of cell divisions (Kaplan and Hagemann 1991; Lyndon 1998). Apparently, according to these investigators, cell expansion rather than the pattern of cell division is responsible for primordium initiation and final shape and size of the organ (Reinhardt et al. 1998); also, according to these investigators, cell division in the absence of coordinated expansion will only lead to subdivision of an existing volume. Moreover, a precise control of cell expansion is required at all stages of development of any organ, such as a leaf.

The importance or otherwise of cell number (i.e. more cell divisions) in embryo development has been discussed by Krishnamurthy (1988, 1994). It was generally believed that a progressive increase in cell number may be important in the histogenic differentiation in the developing embryo, since the various histogenic regions do not appear simultaneously, but progressively, in the developing embryo. Analysis of previous literature, however, shows no such relation between cell number and differentiation in embryo. In developing seeds of *Argemone mexicana* subjected to colchicine treatment, cell divisions continue in the globular embryos, resulting in larger globular embryos with many more cells at a stage when, in normal seeds, they would have transformed themselves into chordate embryos even with a smaller number of constituent cells being produced. In certain citrus varieties, the triploid embryos obtained after cross do not differentiate a plumule-radicle axis, despite continued mitotic activity in the embryo. In the 'reduced mutant' of tomato, histogenic differentiation in the embryo is either poor or absent although the embryo often develops without cotyledons and without organized radicle/shoot meristems through continued mitotic divisions. These examples suggest that mere cell divisions and increase in cell number are not enough to get an organized embryo at any stage of its development. This is perhaps true for any developing plant organ.

The investigation of Wang et al. (2000) showed that the expression of the plant cell cycle regulators results in the modification of all three processes: cell division, cell expansion and growth of plant/organ. This investigation indicated that a certain amount of uncoupling between cell size (i.e. expansion) and cell division (i.e. cell number) exists. If one considers that there exists a normal balance/coordination between cell size and cell division in wild-type plants, then this balance must have been altered and a new balance must have been reached in their study material due to *1CK1* gene overexpression. On the contrary, there was no significant effect on cell size due to overexpression of cyclin. It is, therefore, conceivable, according to these authors, that there must be a limit on the capacity of a plant to use cell size increase to compensate for the growth caused by reduced cell divisions (i.e. cell number), and beyond that limit, a reduced cell number would inevitably lead to growth inhibition. Thus, according to these authors, cell division and cell enlargement are both very vital for growth and development.

Meijer and Murray (2001) have accepted Green's (1976) opinion that cell division and cell expansion are different phases of a continuous developmental process and that both contribute to normal development and elaboration of plant organs (Dupuy et al. 2010). In both cellular and organismal theories of plant growth and

development, some degree of coupling between the genome and physics of growth is inescapable (Dupuy et al. 2010), and this makes it difficult to prefer one theory over the other, and the key to distinguish between them lies in understanding the precise nature of coupling between genetic and biophysical processes in living cells (Krishnamurthy 2015). Observational data have strongly indicated that cell expansion becomes dominant over cell division sooner or later (Donnelly et al. 1999), and, in time replaces it entirely. Although cell division as such does not contribute significantly to the volume of the growing plant organ, addition of cells is a primary necessity for the development of multicellular plants. Thus, cell enlargement determines the final size of the plant/plant organ, and it serves as the transition event between the stage of cell division and stages of maturation of plant/organs.

3.3 Differentiation, Dedifferentiation, Redifferentiation and Transdifferentiation

In biology the word 'differentiate' means *to make* or *become different* from the original/initial during development. Hence, differentiation is a process by which initial/original cells undergo structural and functional specialization and thus result in distinct and specialized cell types. There are 10–30 different cell types in plants such as parenchyma, collenchyma, sclerenchyma, tracheary elements, sieve elements, etc. The initial cells are said to be *undifferentiated*. The structural specialization of cells during differentiation involves the cytoplasm, nucleus and/or the cell wall; structural specialization of cells leads to or becomes the cause of functional specialization. In parenchyma cells there is very little differentiation because there are very little changes in cytoplasm, nucleus or cell wall. In collenchyma cells, there is no or very little change in cytoplasm or nucleus, but the cell wall undergoes extended primary thickening. In sieve elements, the nucleus is lost, cytoplasm undergoes a number of changes, and cell wall develops specialized sieve areas/sieve plates. In sclerenchyma, tracheary elements and cork cells, both the cytoplasm and nucleus are lost due to *programmed cell death* (*PCD*), and the wall undergoes secondary thickening due to lignin in the first two cell types and suberin in the third cell type. These cells undergo a unique type of differentiation by which they die to do their functions, while in other cell types, death happens after cells complete their functions. Based on the above account, it is evident that some cells like parenchyma cells are very less differentiated during their differentiation, some like collenchyma cells a little more differentiated and that at the end of the spectrum are the lignified and suberized cells that have undergone *terminal differentiation* (Krishnamurthy 2015).

Parenchymatous and collenchymatous cells, under normal circumstance, do not undergo cell division, but can resume division activity under certain conditions such as wounding, infection or in vitro culture; if given the requirements and proper conditions, these cells can even regenerate the whole plant. This property is known as *totipotency*. This capacity to resume meristematism and to act as initials to form other cell types is called *dedifferentiation*. The capacity of an already differentiated cell type like parenchyma or collenchyma to produce another cell type (but much more differentiated than them) is called *redifferentiation*; redifferentiation often involves changes in size and shape. If a fully differentiated living cell like a leaf mesophyll cell directly transforms itself, without undergoing any change in size or shape, into a tracheary element or a sieve element by PCD, the process is called *transdifferentiation*. Terminally differentiated sclerenchyma and tracheary elements, as well as the enucleate sieve elements, do not have the capacity to redifferentiate or dedifferentiate, i.e. they are not totipotent. All the above discussed concepts are shown diagrammatically in Fig. 3.10.

There are a few events that are closely related to the concept of differentiation. *Determination* (McDaniel 1984) is one of the important aspects

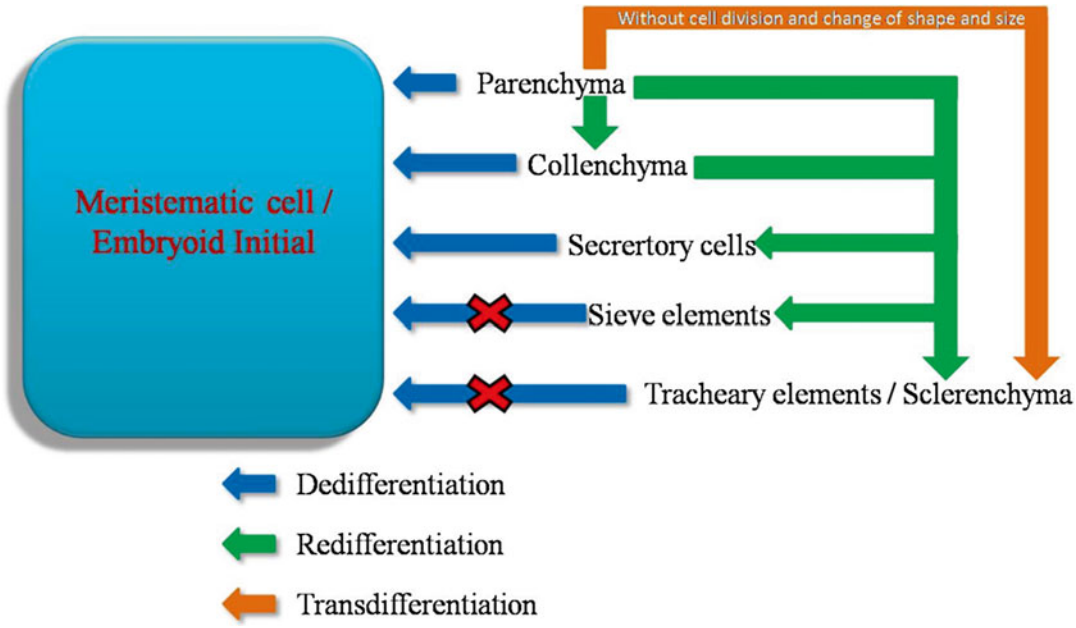


Fig. 3.10 Concepts of differentiation, dedifferentiation, redifferentiation and transdifferentiation. *X* indicates the inability of a cell type to get back to meristematism, and the lengths of *blue arrows* indicate the relative ease with which different mature cell types can dedifferentiate, the *shorter arrow* with greater ease (Krishnamurthy 2015)

related to differentiation. It means a progressive *commitment* of an initial cell to a specific course of differentiation/development that brings about a gradual weakening or loss of capacity to resume meristematism. Some cell types are determined earlier, and some more completely than other cell types. *Competence* refers to the ability of an initial to develop into a particular cell type in response to a specific signal, such as light or a chemical. This implies that a competent cell is capable of recognizing the right or even the wrong signal and translates it or fails to translate it into a particular response. Invariably competence begins to act at the checkpoint in the initial cell's cell cycle. The differentiating cell is a product of gene expression, but its fate, i.e. what kind of cell type will it become, is mainly determined by its *position* in a developing tissue/organ. Even though distinct cell lineages may be established, as, for example, in a developing embryo or in the derivatives of the root apical meristem, it is the position of a cell and not its lineage that determines its fate. At the time of division of an initial (in apical meristems or in lateral meristems like vascular cambium), it is often impossible to predict which of the two resultant daughter cells would 'inherit' the function of the initial and which would become the derivative cell. It is also known that a given initial may be replaced by a cell that through prior history would be rightly classified as a derivative derived from an initial (Zagórska-Marek and Turzanska 2000). *Chimaeras* have provided a strong evidence for it. Laser ablation experiments on *Arabidopsis* root apical meristems have shown that ablated cells are replaced by cells from other lineages and that the so replaced cells differentiate according to their new position. *Position effect* has been shown to be largely controlled by *positional information* that is probably conveyed by cell-to-cell signalling events; this is probably mediated by transmembrane receptor kinases or through plasmodesmata.

3.4 Pattern Formation

The concept of *pattern formation* was first established in the fruit fly *Drosophila* and was initially applied to animal developmental biology. Soon this concept began to be applied for plants as well. Pattern formation in plant development shows many mechanistic similarities to that in animals, although there are some very fundamental differences between the two. Pattern formation may be defined as that developmental process by which cells become organized into specifically patterned tissues or their progenitors, which in turn get organized into higher levels of patternized organization such as organs and whole plants. Since development, as already discussed, is gradual and progressive, pattern formation is also gradual and progressive. The production of patterned tissue from cells is called *histogenesis*, while the production of an organ from a patternized aggregation and spatial and locus-specific orientation of different tissues is called *organogenesis* (Krishnamurthy 2015). Histogenetic pattern (or tissue pattern) may be organized by a single cell type, as, for example, by parenchyma forming the cortex or pith, and lead to homogeneous simple tissue, or by a number of cell types to form a heterogeneous histogenetic pattern (complex tissues such as xylem, phloem or epidermis). Pattern formation is first observed in the embryo, which signals the basic rudiment of the body plan; this subsequently leads to the generation of the structurally complex patterns of different organs. For the successful establishment of body plan, pattern formation expects that each cell must behave, as discussed earlier, in a manner appropriate for its position in the embryo, so that the correct, differentiated, cell types arise at the correct locations and cells of the same type form regionally correct structures. The process, by which cells become specialized cell types in tune with their position, is called *regional specification*. This allows cells to adopt the correct spatial organization. Hence, regional specification is an essential requirement for pattern formation. The first event related to patterning that takes place in the embryo is *axis specification*, i.e. the establishment of the principal body axis with two distinct poles (Krishnamurthy 2015).

3.5 Morphogenesis

3.5.1 Definition and Importance

Morphogenesis means the creation of form and, by implication, the differentiation of the associated internal structural features. As already stated, some people, particularly some plant physiologists, consider development and morphogenesis as having the same meaning, but many others consider that there are subtle distinctions between the two, and that ‘development’ is a more inclusive term that includes morphogenesis as well; yet others like Wardlaw (1968) consider morphogenesis as including development. Plants and the structures of which they are made of are dynamic three-dimensional geometric entities having specific configurations, a physical reality resulting from genetic constitution and all chemical, physical and other processes that are involved in development under specific environmental conditions (Wardlaw 1968). Form, i.e. size, shape, disposition and structural basis of these three, is affected in various ways and at diverse stages of ontogeny based on functional requirements and thus strongly indicates the plasticity apparent in the developmental process. The importance of Galileo’s *principle of similitude* is recognized in morphogenesis. This states that changes in size (and form) of living organisms or their parts have unavoidable mathematical, physical and physiological underpinnings. Hence, form and attainment of form (=morphogenesis) have to be interpreted in terms of physics, chemistry and mathematics, although it is equally important that the acceptable interpretations (based on the above three disciplines) should be essentially biological ones.

Morphogenesis begins with an organized basic entity such as a meristematic initial, zygote, spore or any other diaspore. Morphogenesis involves all the activities that are involved throughout the ontogeny from this basic organized entity to the specific form and structure that are functionally effective and adaptive, which are characteristic of the adult state of the plant or its organs (Krishnamurthy 2015). Hence, many consider morphogenesis as evident at all levels of biological organization: cell, tissue, organ or whole plant levels. Others consider morphogenesis as essentially a cellular process where cell wall properties, membrane permeability, hydrostatic pressure, cell expansion and proliferation rates are not only genetically regulated, but are physically coupled across the system. Hence at cellular level, morphogenesis includes changes in size and shape, their adherence, establishment of new contacts with other cells, changes in cell wall and/or cell organelles, their quiescence or division activity or even their death due to programmed cell death. At higher levels of biological organization, morphogenesis includes the behaviour of many different cell types that help to shape the tissues and the organs. It is to be emphasized here that earlier in plant development, morphogenesis may be instrumental in *driving* the developmental programmes, while later in development, morphogenesis may be regarded as a response to developmental programmes (Krishnamurthy 2015).

3.5.2 Diffusion Reaction Theory and Positional Theory of Morphogenesis

Diffusion reaction theory is the most accepted theory to explain morphogenesis at all levels of biological organization. Recently (2014) this theory has been validated. The theory was proposed by Turing (1952) and hence is also called Turning's theory of morphogenesis. As per this theory, at any level of organization in any biological system, the chemical substance(s) called morphogen(s) is (are) initially distributed in a homogeneous manner, but a regular and patternized diffusion and distribution of this (these) morphogen(s) (leading to a biochemical pattern) may eventually result, affording the basis for the inception of a histological and a morphological pattern. In other words, according to this theory, the system (cell, tissue, organ or the whole plant) is considered to be a complex, specific, diffusion reaction system, which functions in conformity with the laws of physical chemistry, physics and mathematics to help in the patternized distribution of morphogen(s). Turing's theory further emphasizes that when small, random deviations in an initially homogeneous field are reinforced by feedback, derivations from the initial concentration of the morphogen(s) can form a stable pattern of peaks and troughs (Krishnamurthy 2015). Such a de novo formation of discrete pattern from a uniform field is analogous to many purely physical systems, such as the formation of a branched river from a uniform drainage field. The identity of the morphogen(s) is highly debated, but many now agree that morphogens are signalling molecules (Tello 2007). The diffusion of morphogen follows the ordinary laws of diffusion (Fick's Law), i.e. the morphogen moves from a region of greater concentration to regions of less concentration, and also that the diffusion is proportional to the 'diffusibility' of the morphogen. Thus, a gradient in morphogen is created by the diffusion.

According to some investigators, the formation of gradients in the morphogen can be explained by the alternative *positional theory* (Kerzberg and Wolpert 1998). According to this theory, the propagation of the morphogen(s) is dependent on the closeness of cells and that it happens along cell membranes between cells in contact with one another. Once the morphogen arrives at the cell surface, it gets bound to receptors and other kinds of molecules (Tello 2007). In contrast to the diffusion reaction theory which considers slow degradation of products of the morphogens and reversible binding, the positional theory does not consider degradation of products (Kerzberg and Wolpert 1998). Several mathematical models have been proposed so far to explain the distribution of morphogens taking into account diffusion, degradation and reversible binding processes.

3.5.3 Morphogenesis at Cell Level

Morphogenesis at the cell level forms the basis for morphogenesis at the other higher levels of biological organization. The successive morphogenetic processes leading to fully developed cell types after derivation from initials include the following (Wardlaw 1968; Krishnamurthy 2015):

1. Establishment of polarity: Polarity, as already discussed, is important in the morphogenesis of zygotes, spores, trichoblast mother cells, stomatal meristemoid mother cell, microspores, megaspore mother cell, etc. It is closely linked to the asymmetric cell division, which often happens in polarized cells.
2. Sequential synthesis of general and specific proteins, including enzymes, of which some are associated with specific organelles: As an example one can cite the secretory idioblasts which synthesize specific proteins and enzymes associated with ER, Golgi bodies and plasma membrane (and also with cell walls).
3. Specific development of particular organelles: As an instance, one may cite the differentiation of amyloplasts in the columella cells of the gravisensing root caps, of chloroplasts in leaf mesophyll cells, elaioplasts in endosperm cells of some fat-storing seeds, etc.
4. Vacuolation of cytoplasm: Vacuolation forms an important aspect in the morphogenesis of several cell types that include storage parenchyma cells and the so-called 'ergastic substance'-containing cells, fusiform initials of vascular cambium, rib meristem cell, etc.
5. Physical, chemical and structural changes in the cell wall: The physical changes include changes in texture, thickness, development of plasticity and elasticity, tensile properties (rheological properties), etc. Thick primary cell walls, in characteristic patterns of uneven thickness due to cellulose, pectin and cellulose deposition, are important in collenchyma cells, which help these cells in elongation, and at the same time provide mechanical strength. The chemical changes include the addition of cell wall polymers as constituents of primary or secondary walls. A detailed list of chemicals found in cell walls is found in Chap. 2 of this volume. Lignification is very vital for the morphogenesis of tracheary elements, sclereids and sclerenchyma fibres (Hepler et al. 1970; Wardrop 1981; Savidge 1996; Kaliamoorthy and Krishnamurthy 1998). In TEs, once sufficient expansion of the cell is over, first secondary cell wall made up of cellulose and other polysaccharides and structural proteins is laid in characteristic patterns, over which lignin is then deposited on the inside. Lignification first appears on the tangential walls and then on the radial walls. The secondary wall made up of polysaccharides is first laid and then lignification takes place over it. The two were believed by some to be closely interrelated and that the latter is required for the former (Siegel 1956; Northcote 1995), although others negated this (Smart and Amrhein 1985; Ingold et al. 1988). In vitro grown xylogenic calli subjected to treatment with metabolic inhibitors such as 2-aminoindan-2-phosphonic acid (AIP), a potential inhibitor of L-phenyl ammonia lyase (PAL); 2,3,5-triiodobenzoic acid (TIBA), an inhibitor of auxin transport; nifedipine and lanthanum chloride; calcium channel blockers; etc. were used to resolve this issue (Kaliamoorthy and Krishnamurthy 1998; Christopher and Krishnamurthy 2004). All these inhibitors completely prevented lignification, although polysaccharide secondary wall deposition (in characteristic patterns) was unaltered. Hence, the latter is not dependent on the former process, while the reverse is true. Secondary wall formation and lignification events are likely to be similar in sclerenchyma also.

Cutinization and cuticularization are important in morphogenesis of epidermal cells of aerial organs of plants. These regulate transport of water, solute and gas exchange and protect the inner tissues. Similarly, suberization is equally important in the cells constituting cork and exodermis. For example, in the roots of several plants, suberized cells of exodermis are interrupted by non-suberized *passage cells* that allow water transport (Fig. 2.19).

One typical example to stress the importance of structural changes in cell morphogenesis is exhibited by *transfer cell type*. Transfer cells are characterized by *cell wall ingrowths*, which often develop relatively late in cell maturation on the original primary cell walls, making some investigators to consider the wall as secondary (Pate and Gunning 1972). The plasma membrane of the concerned cell also extends along with these wall ingrowths, even if the latter are highly tortuous and branched. This distinct cell wall membrane apparatus is bordered by a cytoplasm with a dense population of mitochondria and conspicuous ER. Transfer cells have a greatly increased surface area of their wall/membrane apparatus (nearly 47 % increase). Two common types of wall ingrowths are seen in transfer cells, the *reticulate* and *flange* types. In the former, the wall ingrowths repeatedly branch and fuse laterally to form a complex network of different morphologies, while in the latter the ingrowths are curvilinear and rib-shaped projections (Talbot et al. 2002). The two types may be seen in the same plant and sometimes in the same transfer cell. The transfer cells are believed to play an important role in the short distance transport/transfer of solutes, especially sucrose (Gunning 1977), and hence, the presence of these cells is often correlated with the presence of intense inward (intake) or outward (secretion) solute flux directions across the plasma membrane. Transfer cells have been reported in a wide range of locations in the plant body, from bryophytes to flowering plants: in minor veins of leaves and cotyledons, in leaf traces in the nodal region (in both associated with xylem and phloem tissue), placental cells of ovary, central cells, synergids and sometimes antipodal cells of embryo sac, aleurone cells of cereal seeds, endosperm cells (especially haustoria), chalazal cells of ovules, root nodule cells that harbour bacteroids, nutritive tissue of insect galls, root-knot nematode-induced giant cells, various categories of secretory cells like nectaries, salt glands, glands of carnivorous plants, *Azolla*, and coralloid roots of cycads, cyanobacteria-containing cells of some bryophytes, foot region of the sporophytes of some bryophytes, etc. (Gunning and Pate 1969; Krishnamurthy 2015).

6. Differential growth of cells: A detailed account on this aspect has been given in an earlier section of this chapter.
7. Changes in the nucleus—in number per cell, ploidy and DNA content: An increase in nuclear number per cell, often known as *coenocytic* condition, is a characteristic indicator of morphogenesis in certain types of cells. Invariably nuclear number is proportional to the size of the cell. Cells that increase in size, especially differentiating fibres, vessel elements (Venugopal and Krishnamurthy 1984), fusiform initials of vascular cambium, laticifers, endothelial cells, endosperm haustoria, embryo suspensor cells, chalazal chamber of helobial endosperm, anther tapetum, secretory cells, fungal hyphae, etc. show more than one nucleus. Increased nuclear number is believed to be due to the increased transcriptional requirements of larger cells. A detailed coverage on the importance of polyploidy, of polyteny, and increased DNA content has already been made in this chapter.
8. Programmed cell death (PCD): It is a very important morphogenetic event that is vital to the development and function of certain cell types of plants. PCD may involve isolated individual cells in a developing tissue, a group of cells or almost all cells of a tissue/organ and selectively eliminate them in order to maintain the structural and functional integrity of the concerned tissue/organ/whole plant. There has been only a few scattered reports on PCD in plants and animals till about two decades, but in the last two decades, over 40,000 publications have appeared on this topic (including a number of books) emphasizing the 'contemporary fascination' that it enjoys among researchers (Krishnamurthy et al. 2000; Gray 2004). However, compared to animals, research on PCD in plant cells is fewer. The term 'programmed cell death' was introduced by Lockshin and Williams (1965). PCD is an active physiological process, accompanied by

morphologically visible changes leading to the selective elimination of unwanted cells in multicellular organisms. It involves the dying cell's own machinery as an 'executioner', a sort of suicide. It is reported to play an important role in cell number, recycling and turnover, tissue homeostasis, cell specialization, tissue sculpting and pattern formation, hypersensitive reactions of cells to biotic/abiotic stresses, defence reactions and in maintaining the overall integrity of the plant, all of which are very vital in plant morphogenesis and development. Particularly important is its role in cell specialization. PCD should not be confused with *necrosis* as the former is energy dependent, physiologically active and genetically programmed with the active involvement of regulatory genes, stimulating events, signalling pathways and morphologically expressed distinctive features; necrosis is a non-physiological process dissociated from developmental and morphogenetic events, involving cell swelling, lysis and leakage of cell contents (Krishnamurthy et al. 2000; Krishnamurthy 2015).

The categories of cells that undergo PCD in plants are the following (Krishnamurthy et al. 2000; Krishnamurthy 2015): (1) Cells that have already served their functions. To this category belong root cap cells, cells of senescing leaf, floral parts and other plant organs that are about to abscise off, vegetative cell of pollen/pollen tube, synergids, antipodals, anther tapetal cells and anther wall cells, etc. (2) Cells that are unwanted from the moment they were produced. To this category belong cells of staminodes and pistillodes of respectively female and male flowers, non-functional megaspores in monosporic and bisporic embryo sacs, non-functional microspores of members of Cyperaceae and Epacridaceae, etc. (3) Cells that undergo terminal differentiation like xylem tracheary elements (TEs), sclerenchyma cells and cork and other suberized cells (like exodermal cells). The most salient feature of these cell types is that they die to function, while other cells die after completing their functions. The other characteristic feature of these cell types is that they develop morphological and chemical changes in their cell walls. A subcategory of this includes cells that undergo 'partial' death during their development. The phloem sieve elements belong to this category; they lose their nuclei and some other cytoplasmic organelles. The functional life of these cells, however, is fixed (from a few days up to a few years). A similar cell type in animals is the red blood corpuscles. (4) Cells that are subjected to *hypersensitive reactions* (*HR*) due to biotic and abiotic stresses. Here, cells undergo PCD in the region of actual stress impact, so that the impact/effect and stress factor do not spread to surrounding regions and that they get isolated/restricted. (5) Cells that are present in wrong places. As examples may be cited the cells that die to form lobes in leaves and leaflets of compound leaves or to result in holes as in the lamina of *Monstera* and *Croton* species.

Although PCD of both plant and animal cells seems to be programmed, there are several operational differences between the two (Krishnamurthy et al. 2000); hence, it appears to be premature to assume that the two are similar. *Apoptotic bodies* of animal cells, which are small bits on DNA and degraded cytoplasm around it that undergo PCD and which are extruded out of the cell by *exocytosis* before the cell itself is eliminated, have not been demonstrated so far in plant PCDs. Neither DNA laddering nor the specific involvement of effector *caspases* were seen in plant PCDs; instead only a smearing of DNA materials (as shown by banding techniques) and the involvement of only cysteine and serine proteases have been reported in plants. The involvement of regulators and adaptors known in animal PCD is likely to be either absent in plant PCDs or the latter may involve altogether different sets of regulators and adaptors. The presence of a cell wall in plant cells is a crucial factor in the exhibition of at least some of these differences between plant and animal PCDs. The vacuoles and the lysozymes contained in them are vital in plant

PCD and carry out degradation and consumption of cytoplasm (*vacuolar autophagy*). However, there are certain common events that are shared by plant and animal PCDs. These include cytoplasmic condensation and shrinkage, cell shrinkage, production of reactive oxygen species (ROS), increase in cytosolic calcium, phosphorylation/dephosphorylation changes, chromatin condensation, presence and activation of endonucleases, etc. (Krishnamurthy et al. 2000).

The differentiation of tracheary elements (TEs) and sclerenchyma involves a unique mechanism of PCD. Since these cells die to function, the cytological changes accompanying PCD in TEs are directed towards making them persist and function almost till the end of the plant part/plant. The cells do not shrink but often enlarge and elongate to varying degrees during differentiation. Their nuclei are not only metabolically very active almost till the final stages of differentiation, but also often undergo mitotic division or endoreduplication/polyteny (Gahan 1981; Venugopal and Krishnamurthy 1984). They are transcriptionally very active almost till the end of TE differentiation and are responsible for the production of several chemicals including those that form part of the persisting specialized secondary walls. Transcripts of several genes including those of TED2, TED3 and TED4 genes are expressed. Recent data suggest the involvement of 380–500 specific genes in differentiating TEs and nearly 400 polypeptides have been identified (Fukuda and Komamine 1993; Fukuda 1997). Based on gene expression PCD in differentiating TEs has been classified into three stages (Fig. 3.11). For example, the expression of tubulin genes begins very early and continues until enough amount of tubulin is formed. Cortical microtubules are initially random and even-spreading but then rapidly change in orientation from longitudinal to transverse to facilitate expansion of radial walls. During secondary wall formation microtubules get concentrated in bands at the site of secondary polysaccharide wall deposition. During this stage there is not

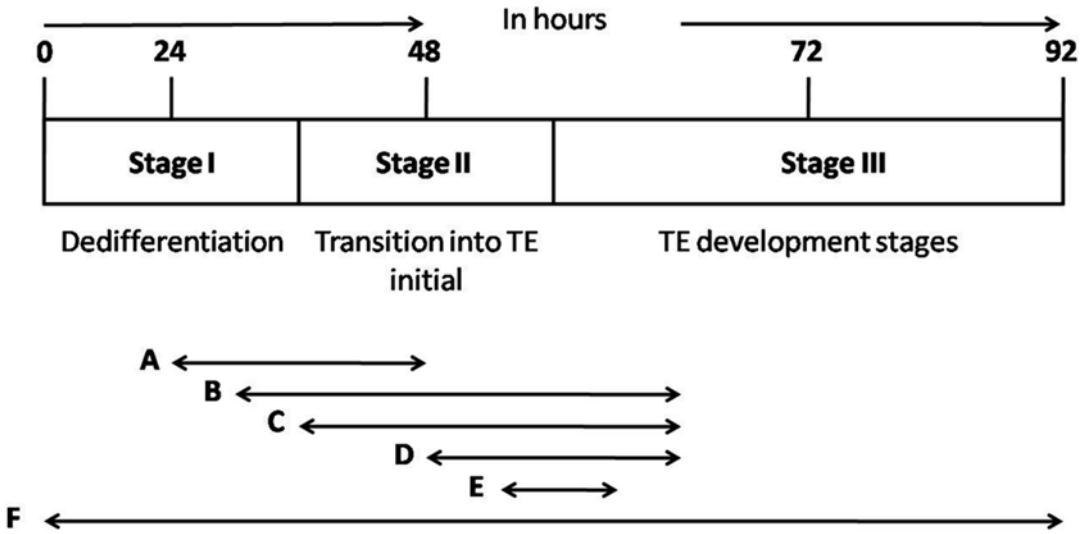


Fig. 3.11 Stages in the transdifferentiation of leaf mesophyll cells into tracheary elements (TE) and the localization patterns of the transcripts of involved genes. (**a**) Genes operating during dedifferentiation up to the initiation of transition to TE. (**b**) Genes operating during dedifferentiation up to the middle of TE development. (**c**) Genes operating right from transition of mesophyll cells into TE up to the middle of TE development. (**d**) Genes operating from the transition of mesophyll cell into TE initial up to the middle of TE development. (**e**) Genes unique to the early stages of TE development from the transformatted leaf mesophyll cell. (**f**) Genes operating throughout stages I–III of TE development from leaf mesophyll cells (Based on Fukuda 1997)

only a rapid increase in brassinosteroids but also the operation of calcium/calcium-calmodulin system. The presence of some types of enzymes in these cells is perhaps related to the clearing/cleaning up of the cytoplasmic debris and recycling them for use in organizing the secondary wall. Significant changes in enzymes such as xylan synthase, PAL, 4-coumarate coenzyme A ligases, peroxidases, cysteine and serine proteases, nucleases, etc. are noticed. A 40 KDa serine protease is secreted during secondary wall synthesis and this is believed to coordinate between secondary wall synthesis and PCD in the developing TEs of *Zinnia elegans*. But a study of an *Arabidopsis* mutant indicates that the two processes are independently regulated, which is perhaps true for TE differentiation in vivo. There are reports of endonucleases and TUNEL-positive reactions in PCD of TEs. Fukuda (1997) considers these endonucleases as of the general nucleases category.

3.5.4 Morphogenesis at Tissue Level

Tissues and tissue systems are very characteristic of each and every plant organ. They are known for their precise regularity, variety and geometrical symmetry patterns (Krishnamurthy 2015). These tissue patterns are structurally and functionally very important for the various plant organs and have been regarded as the result of an evolutionary division of labour in the interest of the functional economy of the plant (Wardlaw 1968). However, some researchers suggest that the concept of *functional harmony* should replace the earlier concept of division of labour although these two concepts are not mutually exclusive. In tissue morphogenesis, there are innumerable molecular and physical interactions between its different constituent cells, which proliferate and differentiate in their own characteristic and precise sequence of events. Thus, tissue morphogenesis is a process in which regular order prevails to give rise to a holistic tissue pattern (Krishnamurthy 2015). We may say that a particular cell in a tissue differentiates as it does not only because it is part of the whole tissue but also because of its precise positional location in the tissue. Two very important aspects of tissue differentiation, where like or unlike cells come together to organize a tissue, are attainment of specific shapes by the different cells and the development of *apoplastic* and *symplastic* domains. Cohesion between cells, one of the very important requirements of multicellular condition (Raven 1986) and of a tissue, is attained by the development of specific cell shapes and of specialized intercellular apoplastic matrix material, the middle lamella that binds the cells of a tissue. Communication and transport between different cells of a tissue are obtained by the development of apoplastic extracellular matrix (cell wall) and intercellular spaces and the symplastic plasmodesmata.

3.5.4.1 Cell Shape in a Tissue

Isolated protoplasts of any cell and many isolated cells such as spores, pollen grains and many unicellular algae and fungi are often spherical, because the sphere has the least surface area for any given volume. Also, a spherical form is always obtained whenever any body occurs ‘free’ from others (like a soap bubble, a raindrop, any celestial body like a planet) (D’Arcy Thompson 1942). On the contrary, if cells are closely grouped together into a tissue, they often lose their spherical shape and assume a polyhedral shape, i.e. they come to possess many sides or facets like soap bubbles in foam or lead shots subjected to surface tension pressure. An alternate theory for the attainment of cell shape is proposed (Korn 1984) because of two problems in D’Arcy Thompson’s hypothesis: (1) the soap bubble appearance of a cell is true only for some types of parenchyma cells, and (2) soap bubbles and compressed lead shots undergo slippage in order to assume states with minimum free surface energy, while plant cells are more or less fixed together with very little freedom to slide. Accordingly, it was proposed that cell shape in a tissue is mainly determined by developmental algorithms and that a cell is an ensemble of rods (edges), sheets (facets) and vertices assembled into distinct shapes. Due to positional specificity, each edge, sheet and vertex ‘has both structure in

terms of occupancy of space as well as one of several different states for growth or separation' (Korn 1984).

Whichever explanation is accepted, it should be understood that the three-dimensional shape of plant cells is essentially controlled by the cell wall. When several cells are packed together and when all the cells are subjected to uniform stress from within or without, the points of contact between the cells will be extended into lines, thus converting the spherical cell into a multifaceted cell. It was earlier assumed that a cell in a tissue would come to possess a rhombic dodecahedron or garnet form with 12 lozenge-shaped equilateral facets. However, it was found that in a relatively homogeneous parenchymatous tissue, each cell has around 14 facets, all bounded by vertices with 120° angles with neither the quadrilaterals nor the hexagons being necessarily plane. This structure is called *orthictetrakaidecahedron*. This is the most ideal packing figure not only because it can be repeated without leaving any small space, but also because it requires a minimum of materials for partition of space; in addition, it has an economy of surface in relation to volume. However, plant cells rarely approach this ideal form (Matzke 1940) and may show a variable number of facets (less than or more than 14) as well as variation in the size of the different facets. The fusiform initials of vascular cambium may have 8–32 facets. In complex tissues like xylem and phloem, the different facets of a single cell may often be in contact with different types, both spatially and temporally, and each facet will have its own chemistry and function different from its adjacent facet.

3.5.4.2 Intercellular Spaces

One of the very important apoplastic entities in plant tissues is the presence of intercellular space (I-spaces) system. In a real situation, even the parenchyma cells that are closest to a group of soap bubbles are partially separated from one another by the specific separation of vertices, edges and some facets to form the I-spaces but at the same time exhibiting the greatest economy of surface in relation to volume (Krishnamurthy 2015). In other words, during I-space formation, the polygonal cells have their internal corners rounded off, and where angles are rounded off, the cell walls tend to split apart from one another; thus, each cell seems to withdraw as far as it can into a sphere, the 'evolutionarily original' shape. This morphological differentiation of I-space is invariably accompanied by many structural and chemical specializations in I-space itself or immediately around it during later stages of tissue specialization. Thus, the formation of I-spaces is an event of great importance in tissue specialization (Knox 1993; Krishnamurthy 2000).

I-space occurrence is known in many plant tissues such as ground parenchyma, collenchymas, vascular cambium, xylem, phloem and leaf mesophyll and fruit pericarp. Based on origin, I-spaces may be *schizogenous* (as detailed in the previous paragraph), *lysigenous* (due to disintegration of cells) or *rhexigenous* (formed due to cell rupture during expansion). Based on function, I-spaces may be secretory (secreting gum, resin or other materials) or nonsecretory. Based on whether or not they contain solid, liquid or gaseous contents, I-spaces are of the *filled type* or *open type* (Roberts 1990). I-spaces that originate very early due to the leaving of a space where the division wall meets the parent wall are schizogenous and become either the filled or open type depending upon local requirements. The open-type I-spaces often have electron-dense material that occupies their corners (Roberts 1990). The materials that are present in filled-type I-spaces are derived from the cell walls or cytoplasm of cells that surround these I-spaces and include one or more of the following: cellulose microfibrils, β-1,3 glucans, esterified and non-esterified pectins, xyloglucans or other hemicelluloses, calcium ions, jasmonates, arabinogalactan proteins, proline-rich proteins, enzymes like ribonuclease, esterases, acid phosphatases, peroxidases, β,1-3-glucanases, chitinases, receptor kinases, oligosaccharides, etc. (Krishnamurthy 2000). These substances are not only tissue specific but also time specific, sometimes in the same I-space. In view of the above, the functions of the I-space must also be very diverse, in addition to the regular function of aeration: intercellular signalling

and communication, transport of diverse chemicals (including defence chemicals) between cells both in health and disease, initiation of lignification in TEs and sclerenchyma, pollen-pistil interactions and control of incompatibility/compatibility, and promotion of symbiotic/mutualistic interactions between rhizobia, mycorrhizal fungi and cyanobacteria on the one hand and the host root on the other. (Krishnamurthy 2000).

3.5.4.3 Symplastic Domains

A brief mention on plasmodesmata (PD) and their structure was provided in Chap. 2 of this volume. There would be no development and function in multicellular plants if they do not rely on cell-to cell communication through these symplastic plasmodesmata. In the embryos of higher plants, all cells are initially interconnected through PD and integrated into a single symplast (Gail McLean et al. 1997). However, as the plant develops from the embryo, individual cells or groups of cells become isolated probably by the loss of functional PD. For instance, in a seedling, the root meristem and root elongating zones are symplastically isolated from one another, the rhizodermis is symplastically isolated from the underlying cortical cells, root hair cells are isolated from other adjacent cells and so on. The loss of connection through degradation of PD happens via ubiquitin-mediated proteolysis of plasmodesmal components. This symplastic isolation allows subsets of cells or cell groups to function as distinct compartments; thus, the mature plants can best be described as a mosaic of symplastic domains (Gail McLean et al. 1997). The frequency, distribution and functional ability of PD decide the extent of communication/transport needed within and between these symplastic domains.

PD function is decided by its *size exclusion limit* (*SEL*), i.e. the size of the plasmodesmata controls the passage of molecule across it. SEL is now accurately calculated by confocal laser microscopy. However, it has been found that in response to physiological, developmental and/or environmental changes, the permeability of PD between cells can be either downregulated to decrease or prevent symplastic transport between cells or upregulated to allow movement of large molecules (Gail McLean et al. 1997). More than 100 proteins (ranging in size from 10 to 100 kDa) have been reported to be selectively transported from companion cells to enucleate sieve tube elements. Symplastic communication has been strongly implicated in regulating meristematic activity. Disruption of PD between cells of fern prothallus induces each cell to develop into a separate prothallus; this probably implies that a signal transmitted through PD inhibits cell division and directs all cells of prothallus to develop as a coordinated unit. During wounding or infection, PD is plugged by callose so as to isolate the wounded cell/tissue. The formation of new PD and symplastic domains is also vital for plant development. For example, carpels of some taxa establish new PD after post-genital fusion in order to facilitate maturation of vascular tissues. PD is also involved in the movement of developmental regulators between cells. The maize homeodomain protein *KNOTTED1* (*KN1*) (a transcription factor), the *Antirrhinum DEFICIENS* and *GLOBOSA MADS*-box family proteins and the *Antirrhinum* floral regulator protein *FLORICOLA* are shown to move through plasmodesmata from cell to cell (Krishnamurthy 2015).

3.5.4.4 Morphogenesis of Epidermal Tissue

The epidermis of the aerial parts of plants is basically derived from the outermost layer of the shoot apical meristem (L1 tunica). Once derived, its *promeristem* is called *protoderm*. In roots, its origin is either from dermatogen or from outermost layer of *periblem*. Hence, it is often called rhizodermis or epiblem or non-technically as root epidermis based on only topography. Excepting for the root hair cell, the root epidermis is histologically simple and hence, here, only the aerial epidermal tissue is discussed. Normally the epidermis expands through anticlinal division only, but in some taxa like *Ficus*, members of Pittosporaceae, Piperaceae, Begoniaceae, etc. periclinal divisions lead to the formation of a *multiple* (or *multiseriate*) *epidermis* (although even here the outermost layer alone shows the

characteristics of the normal epidermis) (Fig. 2.14). The epidermis is a complex tissue system with diverse cell types like ordinary epidermal cells, *stomata*, glandular and/or non-glandular *trichomes*, *crystal cells* (with silica, calcium oxalate or calcium carbonate), cells with *ectodesmata* or *trichodes* (that help in absorption, excretion, etc.), *hydathodes* (water stomata), *bulliform cells* (which help in folding of leaves and sleep movements), etc. Hence, the morphogenesis of these different cell types from a more or less simple protoderm is a matter of great importance. The selection of specific protoderm cells and their subsequent commitment to specific fates (i.e. to become different cell types) are often very well coordinated. This involves specific spacing in the development of not only different entities under the same cell type but also between entities of different cell types. This issue is discussed here with reference to the spacing of stomata and trichomes.

The epidermal tissue represents one of the simplest tissue patterns in which a minimum distance is maintained between differentiated cell types in a two-dimensional sheet of cells (Wolpert 1971; Larkin et al. 1997). This spacing is controlled either during the selection of the precursors of the cell types in the protoderm or regardless of the position of the precursor cells in the protoderm (Sachs 1978). In a number of dicots, the spacing of stomata (and trichomes) on the epidermis appears to be random (whether it is really random needs to be proved beyond doubt), but appears to be more ordered in other dicots and many monocots. The ordered spatial pattern of stomata relies on a highly ordered series of cell divisions, usually asymmetric. The *cell lineage theory* was proposed to explain this spatial order, based mainly on stomatal ontogeny in *Tradescantia*, where not only initials but also stomata are regularly spaced in distinct longitudinal files along the entire length of the leaf. According to this theory, there is the likely involvement of strict cell lineage during the first division of the stomatal initial; even laser ablation in developing stomata does not induce stomatal differentiation in neighbouring epidermal cells. Studies on stomatal mutants *too many mouths* (*tmm*) and *four lips* (*flp*) of *Arabidopsis* support this theory. In these mutants normal stomatal development is affected to result in clustering or pairing of stomata. In the mutant *stomatal density and distribution 1-1* (*sdd-1-1*), a two- to fourfold stomatal density is seen. *SDD 1-1* gene perhaps regulates the number of epidermal cells entering into the stomatal pathway, thus controlling the spatial pattern. According to the opponents of the cell lineage theory, the facts that occasional separation of adjacent stomata through variable number of cells and the lack of periodicity in the cell files lacking stomata across the width of the leaf do not support the cell lineage theory (Croxdale et al. 1992).

The *lateral inhibition theory* maintains that the spatial distribution of stomata relies on interactions/signalling between the different developing stomatal initials. Some kind of inhibitory effect from already existing stomatal initials prevent the formation of new stomata within the effective radius of the inhibitory substance. Lateral, instead of radial, inhibition (instead of cell lineage mechanism) is reported to operate in *Tradescantia*, according to some. Many botanists believe in this theory. The *cell cycle-dependent mechanism* emphasizes that stomatal patterning is coupled to cell cycle events of a group of initial cells as they are displaced through a specific leaf area. An analysis of arrested stomata indicated that these inactivated stomatal initials are not randomly located but are often closely associated with another adjacent stoma (Boetsch et al. 1995). On a whole leaf basis, stomatal patterning based on the cell cycle stage of clusters of initial cells has found support in the presence of stomatal complexes and stomatal precursor cells in linear discrete groups throughout the leaf length and differentiation of groups of cells in near synchrony in plants like *Tradescantia* (Chin et al. 1995). Clearly, mechanism of stomatal spacing is still not yet clear, although it is likely that it may involve more than one mechanism. A similar discussion and conclusion are applicable to the spacing of trichomes in the epidermal tissue (Larkin et al. 1996, 1997).

The genetic basis of trichome development has been studied in *Arabidopsis*, which has a three-branched unicellular trichome (Fig. 3.12). The

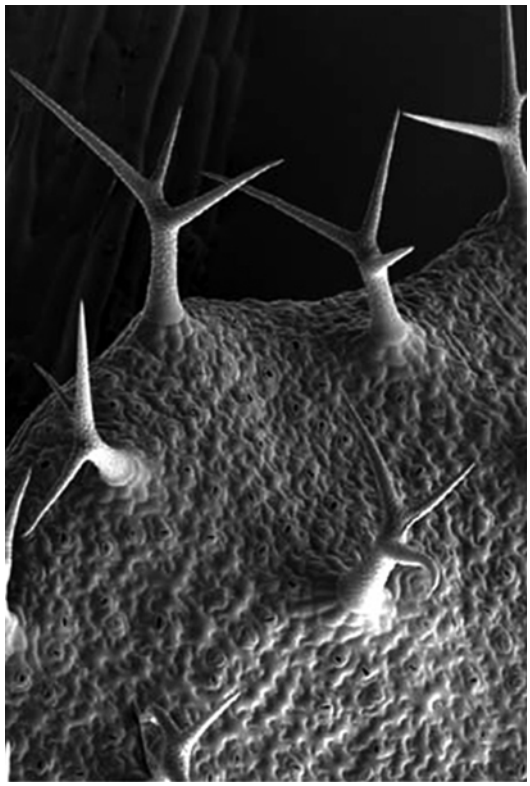


Fig. 3.12 *Arabidopsis thaliana*. Unicellular, three-branched trichomes (Photograph courtesy of Kim Findlay, John Innes Centre, UK)

single nucleus of the trichome primordium undergoes endoreduplication, and its DNA content increases 16-fold before settling down at the branching point of the trichome. In this plant more than 70 trichome mutants have been known and at least 21 genes are involved in the execution of the development of trichome. Of these 21, two genes, *GLABRA 1* (*GL1*) and *TRANSPARENT TESTA GLABRA1* (*TTG1*), are necessary for the specification of the trichome cell. *GLABRA3* (*GL3*) and *KAKTUS* (*KAK*) genes are involved in the progressive development of the trichome by controlling the enlargement of the committed cell; *GL2* also controls enlargement of trichome cell. The *DISTORTED* (*DIS*), *GNARLED* (*GRL*) and *KLINKER* (*KLK*) genes control the growth in length of the trichome. *ANGUSTIFOLIA* (*AN*), *STICHEK* (*STI*) and *ZWISCHEL* genes control the branching of the trichome. Two genes are known as negative regulators of trichome patterning: *TRIPTYCHON* (*TRY*) and *CAPRICE* (*CPC*); both act on together. These gene products are small clusters of about four trichomes. The *gl1* and *ttg* phenotypes have no trichomes. *GL1* gene specifies cell fate, maintains cell differentiation and triggers trichome development in cells that normally do not form trichomes; the last effect is seen when the gene is overexpressed in a *try* mutant with clusters of trichomes. Thus, trichome development is the result of an interaction between positive (*GL1*) and negative (*TRY*) gene regulators. One of the functions of the *TTG* gene in wild-type plants is to prevent adjacent epidermal cells from becoming trichomes; probably it interacts with *GL1*.

Regarding spatial patterning of root hairs on roots, three basic types can be recognized (Evert 2006). In type I, which is seen in most dicots and some monocots, any root epidermal cell can potentially become a root hair. Hence, the root hairs are apparently randomly distributed. In type II, which is seen in Nymphaeaceae and some monocots, root hairs are derived from the smaller *trichoblast* of a symmetrically derived root epidermal cell (as already discussed). The trichoblast is located at the proximal end, i.e. away from the root apex, as in some monocots or at the distal end of the initial cell as in some sedges and grasses. In type III, the root epidermal cells are arranged in vertical files, composed entirely of short hair cells or long non-hair cells. This type is seen in some dicots (Acanthaceae, Aizoaceae, Basellaceae, Brassicaceae, etc.). In *Arabidopsis* hair and non-hair, cell types are specified in a distinct position-dependent pattern, i.e. root hairs are always positional over the junction of anticlinal (radial) walls between two cortical cells, as discussed already. Several genes are involved in root epidermal cell patterning: *TTG1*, *GL2*, *WER* and *CPC*. In *ttg1*, *gl2* and *wer* mutants, all epidermal cells produce root hairs indicating that their wild-type genes are negative regulators of root hair development. The *cpc* mutant does not produce root hairs, but in transgenic plants overexpressing C*PC,* all root epidermal cells produce hairs. Hence, *CPC* is a positive regulator of root hair development. The expression of *CPC* is regulated by *TTG1* and *WER*, while *CPC* promotes root hairs by regulating *GL2*.

3.5.4.5 Sclerenchyma Tissue

Sclerenchyma tissue is composed of several types of sclereids and fibres (Evert 2006; Krishnamurthy 2015). Sachs (1972) and Aloni (1976, 1978) have shown that fibre strand development in stem is dependent on stimuli emanating in young leaf primordia and removal of the latter in pea plant failed fibre differentiation. Likewise, if the position of the young leaves is experimentally changed, there is a corresponding change in the locus of fibre strand differentiation in the subjacent part. In *Coleus* primary phloem fibre induction is polarized in a downward direction from the leaves to the roots. The effect of young leaves can be replaced by exogenous supply of IAA in combination with GA3 (Aloni 1979). In *interfascicular fiberless 1*(*ifl1*) and *revoluta* (*rev*) mutants, fibres do not develop in the interfascicular region, thus emphasizing the genetic basis of fibre development (Zhong and Ye 1999). These mutant genes perhaps have their effect on polar transport of hormones along the interfascicular region. Surgical experiments, wounding and removal of portions of organs provide evidence of the positional control of sclereid development. Auxin concentration in leaves influences sclereid differentiation; higher concentration suppressed sclereid development, while low concentration affected lignification.

3.5.4.6 Vascular Tissue

The *vascular tissues* contain xylem and phloem, the former transporting water and sap and the latter organic substances. In intact plants, the two vascular tissues occur in close proximity to one another with variable relative arrangement between the two such as collateral, bicollateral, concentric or radial. Vascular tissues are produced from procambium in primary plant organs (=primary vascular tissues), from vascular cambium in organs undergoing secondary growth (secondary vascular tissues) and from parenchyma in wounds and in in vitro cultured explants (=regenerative vascular tissues) (Aloni et al. 2006). The morphogenesis of vascular tissue from all the above sources involves integrated changes since both xylem and phloem are complex tissues with diverse cell types, each of which requiring specific inputs. There is a need to understand the hormonal mechanism that regulates the formation of the pattern of the primary vascular system in roots, stems and leaves. Most studies on vascular patterning and differentiation were made on shoots and in in vitro cultured explants. However, most studies have focused their main attention only on the tracheary elements of xylem and sieve elements of phloem tissue.

The prospective procambial and vascular cambial zones are indicated by strong esterase activity (Gahan 1981), which is perhaps the earliest marker. However, whether this marker provides evidence for determination (or commitment) or for competence of the vascular meristems is not clear (Savidge 1985). On the basis of surgical experiments on castor, in which a part of the cambial cylinder and derivative cells were surgically removed and replaced keeping xylem side outward and phloem inward (i.e. reversing their earlier position), Siebers (1971) concluded that the sites of cambial initiation and the polarities of tissue differentiation from the cambium were already determined. On the contrary, studies on cultural callus showed that phloem formed was always associated with xylem (Krishnamurthy and Kaliamoorthy unpublished data), thus proving that newly produced cells respond to factors that regulate differentiation pathways. The derivatives of the cambium are competent but not determined for any specific differentiation processes in contiguous tissues (Krishnamurthy 2015). In other words, the cells are *competent but not determined* for any specific vascular differentiation pathway that the derivatives of the vascular cambium are competent but not determined is also evident from the fact that they normally differentiate into a variety of cell types in both xylem and phloem (Savidge 1985). Savidge (1985) has also stated that the differentiation of different cell types from vascular meristems depends on the relative concentration of auxins, gibberellins and ethylene. This has also been demonstrated in callus grown in vitro (unpublished observation of Krishnamurthy and Kaliamoorthy).

The hormonal control of vascular differentiation and regeneration in both stem and root follows similar general principles, which provide an explanation of how the vascular system is induced. Severing of vascular continuity through V-shaped cuts (Krishnamurthy 2015) or through root-knot nematodes (Krishnamurthy, unpublished data) has demonstrated the conferment of competence on the neighbouring parenchyma cells near the cut to differentiate into new xylem and phloem elements that bypass the wound and link up the severed vascular tissue below and above the wound. Removal of leaves above the wound prevents such regeneration, but replacement of the excised leaves again promotes regeneration showing that growth hormones, particularly IAA, are very important in this process and that these hormones are produced by the leaves above and supplied basipetally. The basipetal wave of xylem and phloem regeneration from parenchyma cell supports this contention. Based on experiments such as the above, the *canalization hypothesis* was proposed. As per this hypothesis, the flow of growth hormones through the parenchyma cells prior to their differentiation as TEs or sieve elements provides the signal for the ordered pattern of vascular tissue differentiation. After some parenchyma cells differentiate into vascular tissue elements, they become preferential conduits for further hormone transport and begin to act as sinks to attract additional hormone molecules. This directs new stream of hormones from young leaves (or the root apical meristem) towards the differentiating vascular tissues, thus causing new vascular elements to be formed towards the preexisting vascular strand (Sachs 1981). Baima et al. (1995) studied the expression pattern of an *Arabidopsis* gene that encodes homeodomain protein during revascularization in wounded transgenic tobacco internodes. This experiment showed the presence of the transgene in single cells or clusters of cells soon after the wounding and subsequently in cells along the path of vascular elements formed. The expression of this gene was activated by growth hormones (auxin) in leaf tissues. Baum et al. (1991) have shown that not only auxins but also cytokinins are needed to increase the regeneration of xylem elements, especially above and below the wound. Cytokinins by themselves do not induce vascular tissues, but in the presence of IAA, cytokinins promote vascular differentiation and regulation (Aloni et al. 2006). Cytokinins control cell division in vascular meristems and affect the amount of xylem fibres. They also increase the number of phloem and xylem strands around a wound. Vessels in the secondary xylem are induced by polar IAA streams moving in the vascular cambium; especially, IAA increases vessel width and decreases vessel density with increasing distance from the leaves. In tissue cultures, low IAA concentrations induced sieve elements but not tracheary elements, while high IAA concentrations resulted in the differentiation of both phloem and xylem.

References

Aloni R (1976) Polarity of induction and pattern of primary phloem fiber differentiation in *Coleus*. Am J Bot 63:877–889

Aloni R (1978) Source of induction and sites of primary phloem fibre differentiation in *Coleus blumei*. Ann Bot 42:1261–1269

Aloni R (1979) Role of auxin and gibberellins in differentiation of primary phloem fibres. Plant Physiol 63:609–614

Aloni R (1987) Differentiation of vascular tissues. Annu Rev Plant Physiol 38:179–204

Aloni R, Aloni E, Langhans M, Ullrich CI (2006) Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. Ann Bot 97:883–893

Avers CJ, Grimm RB (1959) Comparative enzyme differentiations in grass roots II Peroxidase. J Exp Bot 10:341–344

Baima S, Nobili F, Sessa G, Lucchetti S, Ruberty I, Morelli G (1995) The expression of the *Athb-8* homeobox gene is restricted to provascular cells in *Arabidopsis thaliana*. Development 121:4171–4182

Baluška F, Salaj J, Mathur J, Braun M, Jasper F, Šamaj J, Chua NH, Barlow PW, Volkmann D (2000) Root hair formation: F-actin-dependent tip growth is initiated by local assembly of profilin-supported F-actin meshworks accumulated within expansin-enriched bulges. Dev Biol 227:618–632

Barlow PW (1994) From cell to system: repetitive units of growth in the development of roots and shoots. In:

Iqbal M (ed) Growth patterns in vascular plants. Dioscorides Press, Portland, pp 19–58
Baum SF, Aloni R, Peterson CA (1991) Role of cytokinin in vessel regeneration in wounded Coleus internodes. Ann Bot 67:543–548
Benfey PN, Linstead PJ, Roberts K, Schiefelbein JW, Haüser MT, Aeschbacher RA (1993) Root development in *Arabidopsis* four mutants with dramatically altered root morphogenesis. Development 119:57–70
Bennett MD (1971) The duration of meiosis. Proc R Soc Lond B 178:277–299
Bennett MD (1972) Nuclear DNA content and minimum generation time in herbaceous plants. Proc R Soc Lond B 181:109–135
Bennett MD (1977) The time and duration of meiosis. Phil Trans R Soc Lond B 277:201–277
Bennett MD (1987) Variation in genomic form in plants and its ecological implications. New Phytol 106:177–200
Bennett MD, Smith JB, Ward J, Jenkins G (1981) The relationship between nuclear DNA content and centromere volume in higher plants. J Cell Sci 47:91–115
Bennett MD, Heslop-Harrison JS, Smith JB, Ward JP (1983) DNA density in mitotic and meiotic metaphase chromosome of plants and animals. J Cell Sci 63:173–179
Berthold G (1886) Studien über Protoplasmamechanik. A. Felix, Leipzig
Boetsch J, Chin J, Croxdale J (1995) Arrest of stomatal initials in *Tradescantia* is linked to the proximity of neighbouring stomata and results in the arrested initials acquiring properties of epidermal cells. Dev Biol 168:28–38
Boniotti MB, Griffith ME (2002) "Cross-talk" between cell division cycle and development in plants. Plant Cell 14:11–16
Brett C, Waldron K (1990) Physiology and biochemistry of plant cell walls. Unwin Hyman, London
Brown RC, Lemmon BE (1991) Pollen development in orchids. 3. A novel generative pole microtubule system predicts unequal pollen mitosis. J Cell Sci 99:273–281
Brown RC, Lemmon BE (1992) Pollen development in Orchids. 4. Cytoskeleton and ultrastructure of the unequal pollen mitoses in *Phalaenopsis*. Protoplasma 167:183–192
Brown RC, Lemmon BE (1994) Pollen mitosis in the slipper orchid *Cypripedium fasciculatum*. Sex Plant Reprod 7:87–94
Cai G, Casino CD, Romagnoli S, Cresti M (2005) Pollen cytoskeleton during germination and tube growth. Curr Sci 89:1853–1860
Capron A, Okresz L, Genschik P (2003a) First glance at the plant APC/C, a highly conserved ubiquitin–protein ligase. Trends Plant Sci 8:83–89
Capron A, Serralbo O, Fulop K (2003b) The Arabidopsis anaphase- promoting complex or cyclosome: molecular and genetic characterization of the APC2 subunit. Plant Cell 15:2370–2382
Chin J, Wan Y, Smith J, Croxdale J (1995) Linear aggregations of stomata and epidermal cells in Tradescantia leaves evidence for their group patterning as a function of the cell cycle. Dev Biol 168:39–46
Christopher FS, Krishnamurthy KV (2004) Tracheary element differentiation in vitro – effect of certain calcium channel blockers. In: D'Souza L, Anuradha M, Nivas S, Hegde S, Rajendra K (eds) Biotechnology for a better future. SAC Publications, Mangalore, pp 51–81
Cleary AL (1995) F-action redistributions at the division site in living *Tradescantia* complexes as revealed by microinjection of rhodamine-phalloidin. Protoplasma 185:152–165
Cosgrove DJ (1987) Wall relaxation and the driving forces for cell expansive growth. Plant Physiol 84:561–564
Cosgrove DJ (1997) Relaxation in a high-stress environment: the molecular bases of extensible cell walls and cell enlargement. Plant Cell 9:1031–1041
Croxdale J, Smith J, Yandell B, Johnson JB (1992) Stomatal patterning in tradescantia: an evaluation of the cell lineage theory. Dev Biol 149:158–167
Cutter EG, Feldman LJ (1970a) Trichoblasts in *Hydrocharis*, I. Origin, differentiation, dimensions and growth. Am J Bot 57:196–201
Cutter EG, Feldman LJ (1970b) Trichoblasts in *Hydrocharis*. II. Nucleic acids, proteins and a consideration of cell growth in relation to endopolyploidy. Am J Bot 57:202–211
D'Amato F (1998) Chromosome endoreduplication in plant tissue development and function. In: Bryan JA, Chiatante D (eds) Plant cell proliferation and its regulation in growth and development. Wiley, Chichester, pp 153–166
D'Arcy Thompson W (1942) On growth and form, 2nd edn. Cambridge University Press, Cambridge, UK
De Veylder LJ, Joubes J, Inze D (2003) Plant cell cycle. Annu Rev Plant Biol 54:235–264
Den Boer BGW, Murray JAH (2000) Triggering the cell cycle in plants. Trends Cell Biol 10:245–250
Dewitte W, Murray JAH (2003) The plant cell cycle. Annu Rev Plant Physiol Plant Mol Biol 54:235–264
Di Laurenzio L, Wysocka-Diller J, Malamy JE, Pysh L, Helariutta Y, Freshour G, Hahn MG, Feldmann KA, Benfey PN (1996) The *SCARECROW* gene regulates an asymmetric division that is essential for generating the radial organization of the *Arabidopsis* root. Cell 86:423–433
Dodds JH (1981a) The role of the cell cycle and cell division in xylem differentiation. In: Barnett JR (ed) Xylem cell development. Castle House Publications Ltd, Kent, pp 153–167
Dodds JH (1981b) Relationship of the cell cycle to xylem cell differentiation: a new model. Plant Cell Environ 4:145–146
Doerner P, Jorgenesen JE, You R, Steppuhn J, Lamb CJ (1996) Cyclin expression limits root growth and development. Nature 380:520–523
Dolan L, Janmaat K, Willemsen V, Linstead P, Poethig S, Roberts K, Scheres B (1993) Cellular organization of

the *Arabidopsis thaliana* root. Development 119:71–84
Donnelly PM, Bonetta D, Tsukaya H, Dengler R, Dengler NG (1999) Cell cycling and enlargement in developing leaves of Arabidopsis. Dev Biol 215:407–419
Dupuy L, Mackenzie J, Haseloff J (2010) Coordination of plant cell division and expansion in a simple morphogenetic system. Proc Natl Acad Sci U S A 107:2711–2716
Dyer AF (1976) Modifications and errors of mitotic cell division in relation to differentiation. In: Yeoman MM (ed) Cell divisions in higher plants. Academic, New York, pp 199–249
Errera L (1888) Űber zellformen und seifenblasen. Bot Centralbl 34:395–398
Evert RF (2006) Esau's plant anatomy. Wiley, New York
Foard DE (1971) The initial protrusion of a leaf primordium can form without concurrent periclinal cell divisions. Can J Bot 49:1601–1603
Fowler JE, Quatrano RS (1997) Plant cell morphogenesis: plasma membrane interactions with the cytoskeleton and cell wall. Annu Rev Cell Dev Biol 13:697–743
Fukuda H (1997) Tracheary element differentiation. Plant Cell 9:1147–1156
Fukuda H, Komamine A (1993) Changes in the synthesis of RNA and protein during tracheary element differentiation in single cells isolated from the mesophyll of Zinnia elegans. Plant Cell Physiol 24:603–614
Gahan PB (1981) Biochemical changes during xylem element differentiation. In: Barnett JR (ed) Xylem cell development. Castle House Publications Ltd, Tunbridge Wells Kent, pp 168–191
Gail McLean B, Hempel FD, Zambryski C (1997) Plant intercellular communication via plasmodesmata. Plant Cell 9:1043–1054
Gallagher K, Smith LG (2000) Roles of polarity and nuclear determinants in specifying daughter cell fates after an asymmetric cell division in the maize leaf. Curr Biol 10:1229–1232
Gray J (2004) Programmed cell death in plants. Blackwell, London
Green PB (1976) Growth and cell pattern formation on an axis. Critique of concepts, terminology and modes of study. Bot Gaz 137:187–2002
Green PB (1980) Organogenesis–a biophysical view. Annu Rev Plant Physiol 31:51–82
Gunning BES (1977) Transfer cells and their roles in transport of solutes in plants. Sci Progr Oxf 64:539–568
Gunning BES, Pate JS (1969) "Transfer cells". Plant cells with wall in growths, specialized in relation to short distance transport of solutes–their occurrence, structure, and development. Protoplasma 68:107–133
Haber AH (1962) Non-essentiality of concurrent cell divisions for degree of polarisation of leaf growth. I. Studies with radiation-induced mitotic inhibition. Am J Bot 49:583–589
Hemerly A, Engler DA, Bergounioux C, Van Montagu M, Engler G, Inze D, Ferreira P (1995) Dominant negative mutants of the Cdc2 kinase uncouple cell division from iterative plant development. EMBO J 14:3925–3936
Hepler PK, Fosket DE, Newcomb EH (1970) Lignifications during secondary wall formation in *Coleus*: an election microscopic study. Am J Bot 57:85–96
Heyn ANJ (1931) Der Mechanismus der Zellstreckung. Re Trav Bot Neerl 28:113–244
Hofmeister W (1863) Zusätze und berichtigungen zu den 1851 verőffentichen untersuchungen der entuicklung hőherer kryptogamen. Jahrbucher fur Wissenschaft und Botanik 3:259–293
Houssa C, Bernier G, Pieltain A, Kinet JM, Jacqmard A (1994) Activation of latent DNA-replication origins. A universal effect of cytokinins. Planta 193:247–250
Huntley RP, Murray JAH (1999) The plant cell cycle. Curr Opin Plant Biol 2:440–446
Ingold E, Sugiyama M, Komamine A (1988) Secondary cell wall formation: change in cell wall constituents during the differentiation of isolated mesophyll cells of *Zinnia elegans* to tracheary elements. Plant Cell Physiol 29:295–303
Ishida T, Fujiwara S, Miura K, Stacey N, Yoshimura M, Schneider K, Adachi S, Minamisawa K, Umeda M, Sugimoto K (2009) Sumo E3 ligase HIGH PLOIDY2 regulates endocycle onset and meristem maintenance in *Arabidopsis*. Plant Cell 21:2284–2297
Jackson JP, Lindroth AM, Ca OX, Jacobson SE (2002) Control of CPNPG DAN methylation by the KRYPTONITE histone H3 methyltransferase. Nature 416:556–560
Kaliamoorthy S, Krishnamurthy KV (1998) Secondary wall deposition in tracheary elements of cucumber grown in vitro. Biol Plantarum 41:515–522
Kaplan DR, Hagemann W (1991) The relationship of cell and organism in vascular plants. Bioscience 41:693–703
Kende H, Zeevaart JAD (1997) The five "classical" plant hormones. Plant Cell 9:1197–1210
Kennard JL, Cleary AL (1997) Pre-mitotic nuclear migration in subsidiary mother cells of *Tradescantia* occurs in G1 of the cell cycle and requires F-actin. Cell Motil Cytoskeleton 36:55–67
Kerszberg M, Wolpert L (1998) Mechanisms for positional signalling by morphogen transport: a theoretical study. J Theor Biol 191:103–114
Knox JP (1993) Cell surface glycoproteins and plant morphogenesis. In: The extra cellular matrix of plants–molecular, cellular and development biology. Keystone Symposium, Santa Fe New Mexico
Korn RW (1984) Cell shapes and tissue geometries. In: Barlow PW, Carr DJ (eds) Positional controls in plant development. Cambridge University Press, Cambridge, pp 219–245
Krishnamurthy KV (1988) Endosperm controls symmetry changes in the developing embryos of angiosperms. Proc Indian Acad Sci (Pl Sci) 98:257–259
Krishnamurthy KV (1994) The angiosperm embryo: correlative controls in development, differentiation, and

maturation. In: Iqbal M (ed) Growth patterns in vascular plants. Dioscorides Press, Portland, pp 372–404
Krishnamurthy KV (2000) Intercellular spaces: the apoplastic domains of multicellular plants. J Plant Biol 27:213–222
Krishnamurthy KV (2015) Growth and development in plants. Scientific Publishers, Jodhpur
Krishnamurthy KV, Krishnaraj R, Chozhavendan R, Samuel Christopher F (2000) The programme of cell death in plants and animals–a comparison. Curr Sci 79:1169–1181
Larkin JC, Young N, Prigge M, Marks MD (1996) The control of trichome spacing and number in Arabidopsis. Development 122:997–1005
Larkin JC, Marks MD, Nadeau J, Sack F (1997) Epidermal cell fate and patterning in leaves. Plant Cell 9:1109–1120
Larkins BA, Dilkes BE, Dante RA, Coelho CM, Woo YM, Liu Y (2001) Investigating the hows and whys of DNA endoreduplication. J Exp Bot 52:183–192
Lima MF, Eloy NM, Pegoraro C, Sagit R, Rojas C, Bretz T, Vargas L, Elofsson A, Oliveira AC, Hemerly AS, Ferreira PCG (2010) Genomic evolution and complexity of the Anaphase-promoting Complex (APC) in land plants. BMC Plant Biol 10:254
Lintilhac PM, Vesecky TB (1984) Stress-induced alignment of division plane in plant tissues grown in vitro. Nature 307:363–364
Liso R, Calabrese G, Bitonti MB, Arrigoni O (1984) Relationship between ascorbic acid and cell division. Exp Cell Res 150:314–320
Liso R, Innocenti AM, Bitonti MB, Arrigoni O (1988) Ascorbic acid-induced progression of quiescent centre cells from G1 to S phase. New Phytol 110:469–471
Liso R, De Tullio MC, Ciraci S, Balestrini R, La Rocca N (2004) Localization of ascorbic acid, ascorbic acid oxidase, and glutathione in roots of *Cucurbita maxima* L. J Exp Bot 55:2589–2597
Lloyd C, Buschmann H (2007) Plant division: remembering where to build the wall. Curr Biol 17:R1053–R1055
Lockshin R, Williams C (1965) Programmed cell death. IV. The influence of drugs on the breakdown of the intersegmental muscles of silk moths. J Insect Physiol 11:803–809
Lynch TM, Lintilhac PM (1987) Mechanical signals in plant development: a new method for single cell studies. Dev Biol 181:246–256
Lyndon RF (1998) The shoot apical meristem-its growth and development. Cambridge University Press, Cambridge, UK
Matzke EB (1940) What shape is a cell? Teach Biol 10:34–40
Mayer U, Büttner G, Jürgens G (1993) Apical-basal pattern formation in the Arabidopsis embryo: studies on the role of gnom gene. Development 117:149–162
McDaniel CN (1984) Competence, determination, and induction in plant development. In: Malacinski (ed) Pattern formation. A primer in developmental Biology. Macmillan, New York, pp 393–412
McQueen-Mason SJ (1995) Expansins and cell wall expansion. J Exp Bot 46:1639–1650
Meijer M, Murray JAH (2001) Cell cycle controls and the development of plant form. Curr Opin Pl Biol 4:44–49
Meyerowitz EM (1996) Plant development: local control and global patterning. Curr Opin Genet Dev 6:475–479
Mirnov V, De Veylder L, Van Montagu M, Inzé D (1999) Cyclin–dependent kinases and cell division in plants-the nexus. Plant Cell 11:509–521
Nagl W (1981) Polytene chromosomes in plants. Int Rev Cytol 73:21–53
Northcote DH (1995) Aspects of vascular tissue differentiation in plants; parameters that may be used to monitor the process. Int J Plant Sci 156:245–256
Pate JS, Gunning BES (1972) Transfer cells. Annu Rev Plant Physiol 23:173–196
Peters JM (2006) The anaphase promoting complex/cyclosome: a machine designed to destroy. Nat Rev Mol Cell Biol 7:644–656
Potters G, Heremans N, Bellone S, Caubergs RJ, Trost P (2004) Dehydroascorbate influences the plant cell cycle through a glutathione-independent reduction mechanism. Plant Physiol 134:1479–1487
Potters G, Pasternak TP, Guisez Y, Jansen MA (2009) Different stresses, similar morphogenic responses: integrating a plethora of pathways. Plant Cell Environ 32:158–169
Rao KV, Krishnamurthy KV (1976) Angiosperms. Viswanathan and Co, Madras
Raven JA (1986) Evolution of plant life forms. In: Givnish TJ (ed) On the economy of plant form and function. Cambridge University Press, Cambridge, pp 421–492
Reinhardt D, Kuhlenier C (2002) Plant architecture. EMBO Rep 3:846–851
Reinhardt D, Wittmer F, Mandel T, Kuhlemeie C (1998) Localized upregulation of a new expansin gene predicts the site of leaf formation in the tomato meristem. Plant Cell 10:1427–1437
Roberts K (1990) Structure at the plant surfaces. Curr Opin Cell Biol 2:920–928
Rost TL (1994) Root top organization and the spatial relationships of differentiation event. In: Iqbal M (ed) Growth patterns in vascular plants. Dioscorides Press, Portland, pp 59–76
Sachs J (1878) Űeber die Anordnung der zellen in jüngsten pflanzentheilen. Arb Bot Inst Wurzburg 2:46–104
Sachs T (1972) The induction of fibre differentiation in peas. Ann Bot 36:189–197
Sachs T (1978) The development of spacing patterns in the leaf epidermis. In: Subtelny S, Sussex IM (eds) The clonal basis of development. Academic, New York, pp 161–183
Sachs T (1981) The control of patterned differentiation of vascular tissues. Adv Bot Res 9:151–262
Savidge RA (1985) Prospects for manipulating vascular cambium productivity and xylem-cell differentiation. In: Cannel MGR, Jackson JE (eds) Attributes of trees

as crop plants. Institute of Terrestrial Ecology, Natural Environment Research Council, Abbots Ripton, Huntingdon, PEI7 2LS, pp 208–227
Savidge SA (1996) Xylogenesis, genetic and environmental regulation–a review. IAWA J 17:269–310
Scheres B, Wolkenfelt H, Willemsen V, Tenlouw M, Lawson E, Dean C, Weisbeek P (1994) Embryonic origin of the *Arabidopsis* primary root and root meristem initials. Development 120:2457–2487
Scheres B, Di Laurenzio L, Willemsen V, Hauser MT, Janmaat K (1995) Mutations affecting the radial organization of the *Arabidopsis* root displays specific defects throughout the embryonic axis. Development 121:53–62
Schiefelbein J, Galway M, Masucci J, Ford S (1993) Pollen tube and root hair tip growth is disrupted in a mutant of *Arabidopsis thaliana*. Plant Physiol 103:979–985
Schopfer P (2009) Mechanical signals in *Arabidopsis* stress or strain? Sci Online Biol 2:33–39
Siebers AM (1971) Differentiation of isolated interfascicular tissue of *Ricinus communis*. L Acta Bot Neerl 20:343–355
Siegel SM (1956) The biosynthesis of lignin; evidence for the participation of cellulose as sites for oxidative polymerization of eugenol. J Am Chem Soc 78:1753–1755
Sinnott EW (1960) Plant morphogenesis. McGraw-Hill, New York
Smart CC, Amrhein N (1985) The influence of lignification on the development of vascular tissues in *Vigna radiata* L. Protoplasma 124:87–95
Smith LG (2001) Plant cell division: building walls in the right places. Nat Rev Mol Cell Biol 2:33–39
Smith LG, Hake S, Sylvester AW (1996) The *tangled-1* mutation alters cells division orientations throughout maize leaf development without altering leaf shape. Development 122:481–489
Stebbins GL, Jain SK (1960) Developmental studies of cell differentiation in the epidermis of monocotyledons. I. *Allium*, *Rhoeo*, and *Commelina*. Dev Biol 2:409–426
Strange C (1992) Cell cycle advances. Bioscience 42:252–256
Swamy BGL, Krishnamurthy KV (1975) Embryo sac ontogenies in angiosperms-an elucidation. Phytomorphology 25:12–18
Sylvester AW, Smith L, Freeling M (1996) Acquisition of identity in the developing leaf. Annu Rev Cell Dev Biol 12:257–304
Talbot MJ, Offler CE, Mc Curdy DW (2002) Transfer cell wall architecture: a contribution towards understanding localized wall deposition. Protoplasma 219:197–209
Tello JI (2007) Mathematical analysis of a model of morphogenesis: steady states. pp 1–8. XX Congreso de Ecuaciones Diferenciales y Aplicaciones X Congreso de Matemática Aplicada, pp 115–152, Sevilla, 24–28 Septiembre 2007
Théry M, Bornens M (2006) Cell shape and cell division. Curr Opin Cell Biol 18:648–657
Turing AM (1952) The chemical basis of morphogenesis. Phil Trans Roy Soc B 237:37–72
Twell D (1992) Use of a nuclear-targeted β-glucuronidase fusion protein to demonstrate vegetative cell-specific gene expression in developing pollen. Plant J 2:887–892
Twyman RM (2003) Developmental Biology (reprinted). Viva Books Private Limited, New Delhi
Van't Hof J (1965) Relationships between mitotic cycle duration, S period duration and average rate of DNA synthesis in root meristem cells of several plants. Exp Cell Res 39:48–58
Van't Hof J, Sparrow AH (1963) A relationship between DNA content, nuclear volume, and minimum mitotic cycle time. Proc Natl Acad Sci U S A 49:897–902
Venugopal N, Krishnamurthy KV (1984) Multinucleate condition in the differentiating secondary xylem vessel elements of Dalbergia sissoo. Roxb Curr Sci 53:43
Wang H, Zhar Y, Gilmer S, Whitwill S, Fowke LC (2000) Expression of the plant cyclin–dependent kinase inhibitor ICk1 affects cell division, plant growth and morphology. Plant J 24:613–623
Wardlaw CW (1968) Morphogenesis in plants–a contemporary study. Methuen, London
Wardrop AB (1981) Lignification and xylogenesis. In: Barnett JB (ed) Xylem cell development. Castle House Publications, Kent, pp 115–152
Wolpert L (1971) Positional information and pattern formation. In: Mosocona AA, Monroy A (eds) Current topics in developmental biology. Academic, New York, pp 183–224
Zagórska-Marek B, Turzanska M (2000) Clonal analysis provides evidence for transient initial cells in shoot apical meristems of seed plants. J Plant Growth Reg 19:55–64
Zhong R, Ye ZH (1999) *IFL1*, a gene regulating interfascicular fiber differentiation in *Arabidopsis*, encodes a homeodomain–leucine zipper protein. Plant Cell 11:2139–2152

Meristems and Their Role in Primary and Secondary Organization of the Plant Body

4

K.V. Krishnamurthy, Bir Bahadur, S. John Adams, and Padma Venkatasubramanian

Abstract

This chapter deals with meristems and their importance in the organization of the primary and secondary plant body. The meristem concept is explained with particular reference to initials, stem cells and permanency of initials. A classification of meristems is provided, followed by the organization of SAM, RAM and vascular cambium. The genetic basis of the organization and behaviour of these three meristems is dealt with in detail along with their hormonal control. An account on intercalary meristem, metamers and modules, origin of nodes and internodes, axillary buds, apical dominance, primary and secondary thickening meristems and phellogen is also provided.

Keywords

Axillary bud • Genetic control of meristems • Intercalary meristem • Lateral roots • Metamer • Phellogen • Quiescent centre • Root apical meristem (RAM) • Shoot apical meristem (SAM) • Vascular cambium

4.1 Introduction

An adult vascular plant is a complex chimera with contiguous cells and tissues of varied structure, symplastic domains, functions and ploidy organized into distinct axial (roots and stems) and appendicular organs (leaves and flowers). Some kind of complementary physiological mechanisms must operate, whereby the mutualistic relationships of the cells, tissues and organs during development are regulated by an overriding holism and by a pervasive tendency towards

K.V. Krishnamurthy (✉) • S. John Adams
P. Venkatasubramanian
Center for Pharmaceutics, Pharmacognosy and Pharmacology, School of Life Sciences, Institute of Trans-Disciplinary Health Science and Technology (IHST), Bangalore, Karnataka, India
e-mail: kvkbdu@yahoo.co.in

B. Bahadur
Sri Biotech Laboratories India Limited, Hyderabad, Telangana, India

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_4,

homeostasis (Wardlaw 1968). Hence, the adult plant is often considered as a complex of several reaction systems operating at different levels (cell, tissue and organ levels) and at different topographic locations in order to maintain the integrity of the whole plant, both structurally and functionally. The basic defining morphological feature of the adult plant is its architecture which is defined as the three-dimensional organization of the plant body (Reinhardt and Kuhlemeir 2002). Although architecture is easily noticed and striking in the shoot system, a similar, although in a less-striking, form may occur in the root system. In tropical tress 23 basic architectural patterns have been recognized (Hallé et al. 1978); it is likely that an equal or more number of architectural patterns might occur in temperate trees and in herbaceous taxa of all regions (Krishnamurthy 2015). These various architectural patterns are due to differences in the branching patterns which in turn are brought about by the differential behaviour of the apical meristems of the main stem and branches as well as by the number and differential developmental behaviour of the axillary meristems. A variation in root architecture in a similar way is the result of differential behaviour of the main root apical meristems and the lateral root meristems.

4.2 Concept of Meristem

During early embryogenesis, cell divisions occur almost throughout the embryo, but as the embryo matures, the addition of new cells to the embryo body is gradually restricted to certain of its regions only. This restriction of cell divisions to specific regions of the plant body continues as the embryo, via a seedling, becomes the adult plant. The, thus formed, *growth centres* remain embryonic and are maintained as such almost throughout the life of the plant. Hence, the adult plant body becomes a composite mixture of embryonic and matured tissues. These growth centres continue to add new cells to the adult plant body and are called *meristems*. This fact made Bower (1947) to say as follows: 'the life of the higher plants may be described as an *indefinitely continued embryology*, the increase in the number of parts being in a geometric ratio'. This restriction of cell divisions to specific meristems in the adult plant body is an evolutionarily advanced feature, since in many lower plants almost all cells are involved in cell division. Thus, in higher plants there is a clear-cut division of labour between the different parts of the plant body. The localization of growth centres (meristems) is an important feature of distinction between plants and animals. The term 'meristem' is derived from the Greek word 'meristos', which means 'division'. The tissue that organizes the meristem is called *meristematic tissue*. There are cells in each meristem that maintain it as a perennial source of new cells. These cells are called *initials*. Their derivatives give rise to mature cells of the plant body with or without intervening cell divisions in them.

The concept of initials needs to be discussed at this juncture. A given initial in any meristem produces two daughter cells after division, out of which one continues to act as an initial while the other invariably becomes the precursor of an adult cell. The concept of initials and derivatives often goes with a qualification, i.e. the two are not inherently different from one another, but that the two do have some differences, when we speak of them with specific reference to some categories of meristems such as vascular cambium (see for more details, a later page in this chapter). Unlike the derivative cell, the initial must recreate complex patterns of transcriptional activity with each mitotic division and self-renewing. But, how this is moulded and maintained is not very clear. Initials also are characterized by very infrequent divisions (low mitotic index) and prolonged division cycle when compared to their derivatives. It is commonly believed that many cells of the meristem serve as initials, mainly because of their specific location in the meristem and not because of their inherent properties. In animals, a fixed category and numbers of initial cells are formed during embryonic development itself, but in the adult animal, the tissues and organs are maintained throughout the animal's life by populations of such cells that reside within these tissues and organs. These cells are termed *stem cells* (Weizman 2000). Animal stem cells

are often compared to plant's initials and several biologists, especially in the last 15 years, have adopted the term 'stem cells' for initials (Veit 2006) or to their most recent derivatives. There is often a confused usage of both these terms together, while describing the shoot apical meristem. For instance, Fletcher (2004) states as follows: 'The stem cells are not permanent initials'. Laufs et al. (1998) stated as follows: 'it is now widely assumed that central cells function as stem cells and serve as initials or source of cells for the two other zones of the shoot apical meristem'. Bowman and Eshed (2000) mention, 'the central zone acts as a reservoir of stem cells ….. It should be noted that these cells do not act as permanent initials….'. Vernoux et al. (2000) said as follows: 'It is now generally accepted that the central zone acts as a population of stem cells…. and generating the initials for the other zones, while maintaining itself'. Meyerowitz (1997), while characterizing the shoot apical meristem as a 'group of stem cells', designated its central zone as the 'zone of initials'. It is not clear from the above whether stem cells are equivalent to initials or stem cells give rise to initials or stem cells are permanent while initials are not and vice versa. The authors of this chapter advocate the use of the more appropriate and conserved term 'initials' (Evert 2006; Krishnamurthy 2015), although, while quoting, other researchers use 'stem cells' employed by them. It should, however, be stated here that more and more people are using the stem-cell terminology and the word 'initial' is fast disappearing from meristem literature. In fact, meristems are now called stem-cell niches (Scheres 2007; Bitonti and Chiappetta 2011).

Another important aspect that needs to be discussed here relates to the *permanency of the initials*: whether the initials that make up a meristem at any one particular time continue to remain as initials till the entire life span of the meristem? Some experiments carried out by Ball and his associates (Soma and Ball 1963) and Newman (1965) on shoot apical meristems (SAMs) have indicated that it is not so. Ball and his co-workers did two types of experiments to come to this conclusion. In one, they put India ink dot on the surface at the tip of the SAM and traced its fate through time-lapse photography. They found the dot got split into several dots, got radially displaced and finally formed parts of the internode or leaf primordia. The carbon dot eventually got totally lost from the tip of the SAM. In another experiments, they slightly injured the surface and subsurface cells of SAM through a microneedle and traced the fate of these injured cells. As expected, they also got displaced from SAM and formed part of internode or leaf after some time. These results indicated that the cells of SAM (i.e. initials) are not permanently located in the meristem but are replaced by newer initials. Newman (1965) also concluded that no cell in SAM is a permanent cell of the meristem. The meristem can be compared to an office manned by a number of initials. Once the people of an office get retired or transferred, their place is taken over by other people. In a similar way, the office of the meristem is manned by different sets of initials at different times. Newman, in fact, compared the SAM to a fountain of water, in which water coming out of the single nozzle (pore) (compared to the central mother cell zone) splits to radiating pattern (comparable to the derivative cells of the meristem), thereby implying that neither the water coming out of the nozzle (i.e. initials of meristem) nor the water that gets split in a radiating manner (i.e. derivative cells) is a permanent feature of the nozzle (i.e. meristem) but gets replaced every time by fresh water (i.e. fresh initials). The concept of nonpermanency of initials can be extended to other types of meristems as well, although no experiment has been so far carried out on them (Krishnamurthy 2015).

After a thorough review of literature on meristems as well as by doing investigations on meristems, Swamy and Krishnamurthy (1978) have shown that meristems, whether apical or lateral, are always constituted of a relatively quiescent group of cells and that the actively dividing cells are located around this group in apical meristems or located in files of cells between actively dividing files of cells in lateral meristems. Two physiological features characterize this quiescent zone: the extended mitotic cycle and the low mitotic index of its constituent cells. The quiescent

zone/file of cells not only forms the ultimate source of all dividing cells of the meristem but also acts as a reservoir of cells to aid in the reconstruction of the meristem if the dividing cells are damaged for some reason (Krishnamurthy 2015).

4.3 Classification of Meristems

Four principal categories of meristems are recognized in higher vascular plants: *apical* (in the shoot and root apices), *lateral* (vascular cambium and cork cambium or *phellogen*—both running parallel to the long axis of the stem and root of gymnosperms and dicots), *intercalary meristem* [a group of meristematic cells intercalated between mature tissues and derived either from shoot apical meristem (*residual* type) or from dedifferentiated mature cells (*resumptive* type)] and *diffuse* meristems (which are highly localized, temporary groups of cells that divide in all planes). Apical and intercalary meristems promote the longitudinal growth (growth in length) of the plant organ, while lateral meristems promote growth in girth or latitudinal growth. Apical meristems are small and lateral meristems are thin, but together they produce the entire plant, with several thousand times more mass/volume than them.

Many developmental botanists distinguish the meristematic initials and their most recent derivatives from their partially differentiated but still dividing cells under the name *promeristem* (Evans and Barton 1997; Barton 1998), *protomeristem* or *primary meristem*. Promeristem is classified further into the following categories based on the tissue region produced from it: *protoderm* (that gives rise to the epidermis), *procambium* or *provascular tissue* (that gives rise to primary vascular tissues) and *ground meristem* (that gives rise to ground tissue). Promeristems often exhibit different but characteristic patterns of cell division and growth depending on the plant organ in which they are present. Consequently, they often get special names such as the *mass meristems* (undergo cell divisions in all planes), the *rib meristem* (which through regular transverse divisions produce parallel, longitudinal files of cells) and the *plate meristem* (in which cell divisions are primarily anticlinal to result in flat structures like leaf, petal, sepal etc.)

4.4 Shoot Apical Meristem (SAM)

The SAM is the most distal region (above the youngest leaf primordium) of the shoot apex of the main shoot as well as of the branches. The SAM ranges in width from about 40 μm as in *Luzula* and *Syringa* to about 2,300 μm as in *Cycas revoluta*, with most species having a width range of 120–300 μm. The width may also differ depending on the age of the plant, with embryonic and seedling apices having less width than those of adult plants. The height of the SAM (as measured from the base of the youngest leaf primordium to the summit of SAM) also varies greatly. SAM of water plants like *Elodea* and *Hydrilla* has the greater height. In all plants, the height varies with the *plastochronic changes* (the changes that happen in SAM during the period between the formations of two successive leaf primordia) with the least height during early leaf primordial initiation and the maximum height sometime after leaf initiation.

4.4.1 Organization of SAM

Vascular plants have two basic categories of SAM, one with a single large and morphologically and cytologically distinct *apical cell* and the other with a multicellular SAM. Single apical cells characterize many pteridophytes like *Equisetum* and many ferns. The apical cell is superficially located and more commonly inverted pyramidal in shape (Fig. 4.1). The base of the pyramid is towards the surface of the apex, while the other three facets are 'embedded' in the body of the shoot apex. Its derivatives are formed in an ordered pattern from three of its inner facets. In some water ferns, the apical cell is three sided, and new cells are cut off from two of its facets only to result in bilaterally symmetrical shoots. The term *merophyte* is often used to refer to the immediate derivatives of the apical cell.

Although the apical cell was considered by early plant morphologists to be the ultimate source of all cells of the shoot, some others concluded that the apical cell is active only in young plants.

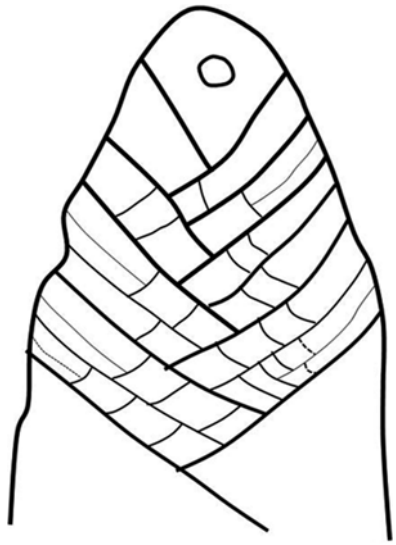


Fig. 4.1 Diagrammatic representation of L.S. of shoot apex of *Equisetum* showing the apical cell and planes of its division and formation of derivatives (merophyte) (Krishnamurthy 2015)

However, radiolabelled DNA precursor supply to SAM with single apical cells showed that the apical cell divides mitotically. However, it is relatively quiescent when compared to its immediate derivatives.

In taxa like *Selaginella*, the SAM appears to have more than one apical cell arranged in a single anticlinal row. While some consider it as a transitional SAM between SAM with single apical cell and SAM which is multicellular, others consider it only as SAM with a single apical cell. Multicellular SAM occurs in *Lycopodium*, *Isoetes*, a number of ferns, gymnosperms and angiosperms. Two major theories have been proposed to explain the organization of the multicellular SAMs. One is the *tunica-corpus* theory proposed by Schmidt (1924), according to which the SAM has a superficial mantle of cells constituting the *tunica,* which encodes an inner *core* (Fig. 4.2). The number of tunica layers varies from one to three (L1, L2 and L3) depending on the species. Generally, all cells of tunica divide only anticlinally, and because of this feature, only the layered structure of the tunica is maintained. The cells of the corpus divide in all planes, thereby adding to the bulk of the SAM. It has

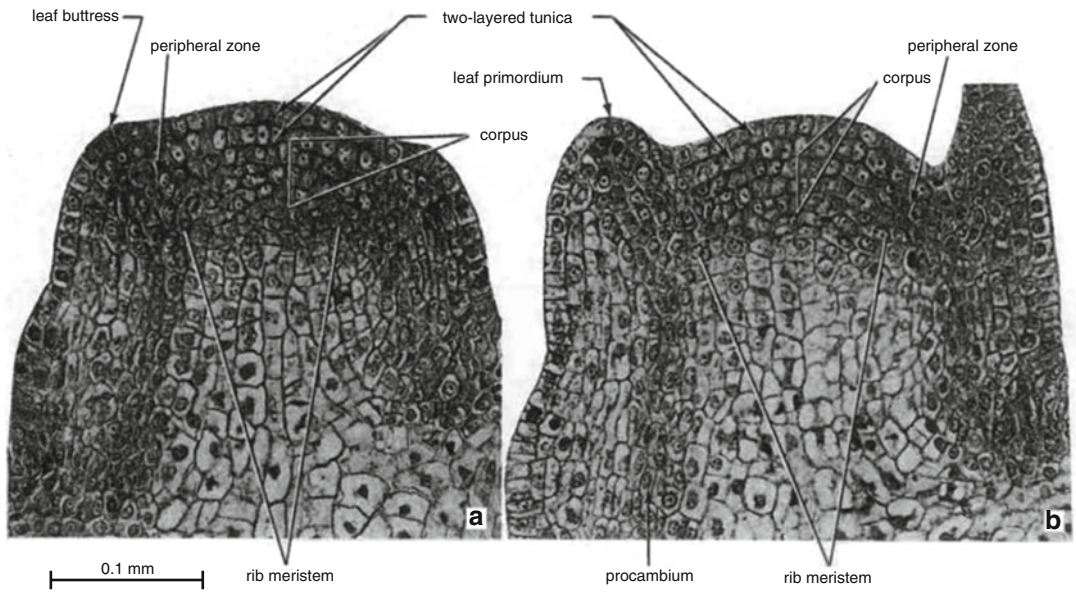


Fig. 4.2 L.S. of shoot apices of *Solanum tuberosum* exhibiting tunica-corpus organization (After Sussex 1955)

been suggested that each layer of tunica arises from a small group of separate initials, and the corpus has its own initials beneath those of the tunica. Clonal analyses made on naturally occurring and experimentally induced *chimeras*, as well as on polyploidy induced by colchicine on SAM cell layers, are cited as providing a strong evidence for the one to three initials in each tunica layer. The main merit of the tunica-corpus concept is that it is very simple and explains the organization of SAM only without implying any relation to histogenesis in the derivatives of SAM, although the epidermis of subjacent shoot is derived from L1 layer of tunica. The tunica-corpus organization could not be found in the SAM of gymnosperms other than that of in *Gnetum* and *Ephedra*.

Foster (1938) proposed the *cytohistological zonation concept* to explain the organization of SAM of gymnosperms (Fig. 4.3). This concept is based not only on the planes of division in cells of different zones of SAM but also on the degree of cytochemical differentiation and the extent (mitotic index) and duration (mitotic cycle) of cell divisions of the constituent cells in different zones. Thus, this concept recognizes different cytohistological zones in the SAM. Subsequently, similar zones have been recognized in angiosperm SAM. Cytohistological zones were then superimposed on tunica-corpus organization.

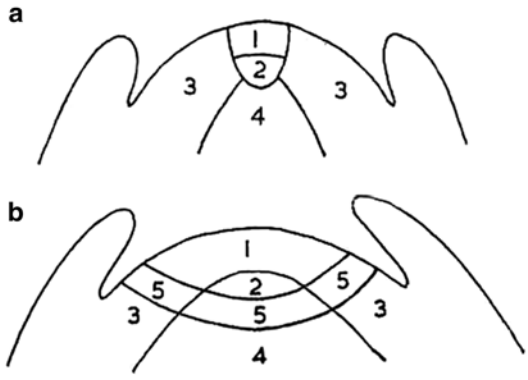


Fig. 4.3 (**a**, **b**) Diagrammatic representation of zonation patterns in the shoot apical meristems. *1* Apical initials, *2* central mother cell zone, *3* peripheral zone, *4* rib meristem, *5* cambium-like transition zone (Krishnamurthy 2015)

The zones initially recognized and subsequently confirmed in angiosperms are *apical initials*, *central mother cell zone*, *peripheral zone* and *rib meristem* (Fig. 4.3). Rib meristem lies below the central mother cell zone or is separated from it, in some cases by a very narrow *transition zone*. The apical initials are believed to contribute cells to the surface layer by anticlinal divisions and to the central mother cell zone by periclinal division. The central mother cell zone gives rise to the rib meristem (and transition zone) as well as to the peripheral zone (also called by some *flank meristem* or *flank zone*). The peripheral zone gives rise to the leaf, axially buds and the peripheral parts of the stem, while the rib meristem gives rise to the pith. The zonation concept underwent changes and reinterpretations by the French botanists, who recognized the following zones: the *waiting meristem* (*méristéme d'attente*) or *apical zone* (zone *apicale*), the *initiating ring* (*anneau initial*) and *medullary meristem* (*méristéme medullaire*). The first zone is equivalent to Foster's apical initials and central mother cell zone, the second to flank zone and the third to rib meristem. However, renewed cytological, cytochemical, ultrastructual and autoradiographic studies as well as surgical experiments and in vitro culture studies have helped to characterize the features of the cells of each zone. Also, investigators outside France reaffirmed the zones recognized by Foster (1938).

The central mother cell zone is the most important zone of SAM. It is the ultimate progenitor of all other zones of SAM. It has the following cytological characteristics: largest cells in SAM, vacuolated, fairly thick-walled, low RNA content, least RNA synthesis, filamentous mitochondria, large nucleoli, undifferentiated plastids, the least mitotic index (i.e. very few cells were in division at any time of observation) and the greatest cell doubling time (i.e. prolonged cell cycle) which ranged, depending on plants, from >40 to 250 h. Its cells are least affected, when SAM is damaged by environmental or other factors even when other cells are damaged and hence are able to regenerate the SAM. Some investigators even go to the extent of saying that the stem cells (initials of SAM) are located in the

upper region of central mother cell zone (Pautler et al. 2013) (but in fact, all its cells are the true initials). In contrast, the flank zone has the following contrasting cytological characteristics: smallest cells in SAM, free of vacuoles, thin walled, high RNA content, greatest RNA synthesis, normal mitochondria, small nucleoli, the greatest mitotic index (i.e. almost all its cells divide) and the least cell doubling time which ranges, depending on the plant, from 11 to 137 h, i.e. approximately two times quicker than in central mother cell zone. The other two zones are intermediate between these two zones with reference to their cytological characteristics (Krishnamurthy 2015).

4.4.2 Elaboration of SAM

A number of studies have been done on the origin of SAM in the developing embryo and its subsequent elaboration during its growth into the adult plant. For instance, the primary SAM in *Arabidopsis* is reported to become apparent relatively late in embryogenesis, after the cotyledons are initiated (Barton and Poethig 1993). However, a critical analysis of embryogenesis in plants revealed that the SAM is initiated in the midglobular embryo stage itself in the form of *epiphysis* (Swamy and Krishnamurthy 1977; Krishnamurthy 1994, 2015; Raghavan 2000), which is the embryonic equivalent of the central mother cell zone of the adult SAM. Very cleat structural, cytological and cytochemical differences are seen between the epiphysis and the cells around them. These differences are noticed even before the heart-shaped stage, which represents the stage of initiation of the two cotyledons. In other words, the cotyledons are the first organs formed by the epiphysis and its surrounding cells that together constitute the embryonic SAM. The viability of the embryonic SAM is associated with a change from globular to cordate shape of the embryo. Further differentiation in SAM is temporarily halted when it becomes dome shaped just before its dormancy as the seed containing it also becomes dormant (Krishnamurthy 2015). However, in many taxa such as legumes, this embryonic SAM is active and produces a number of leaf primordia before becoming dormant. It becomes again active with seed germination, gets elaborated and starts exhibiting the different cytohistological zones.

4.4.3 Control of SAM Activity

4.4.3.1 Genetic Control

The establishment of SAM requires the activity of *SHOOT MERISTEMLESS* (*STM*) gene in *Arabidopsis*, which is reported to be first expressed in one or two cells of the late globular embryo in the epiphyseal region. The *stm* mutations result in loss of function, i.e. these mutants produce seedlings with normal root and hypocotyl but lack SAM. *STM* mRNA is detected in the central mother cell zone and peripheral zone of the adult SAM but not in the developing leaf primordia. *STM* gene is, thus, required for the activity of SAM and the formation of leaf primordia. The expression of *STM* gene throughout the SAM but not in the leaf founder cells (see more information on founder cells in Chap. 6) prevents the apical meristem dome from premature differentiation by repressing the leaf primordium-specific regulator *ASYMMETRIC LEAVES1* (*AS1*) (Fig. 4.4) (see also Byrne et al. 2000). A well-defined SAM is also absent in the 'buds' regenerated on cultured explants of the *stm* mutants indicating that this gene controls both the embryonic and adventitious SAMs. The STM gene of *Arabidopsis* has been cloned and its predicted amino acid sequence shows a strong homology to the homeodomain (coded by the homeobox gene) of the *KNOTTED1* (*KN1*) gene of maize, the initial expression of the transcripts of which is seen in the early differentiating SAM of maize embryos and continues to be seen during postembryonic stages as well in the adult plant SAM. In rice, a gene similar to the *KN1* called *Osh 1* (for '*Oryza sativa* homeobox') is found; the mutation in this gene causes clump formation by SAM, and probably this gene maintains cells of SAM in the undifferentiated state. The *no-apical-meristem* (*nam*) mutation in *Petunia* species causes the non-differentiation of

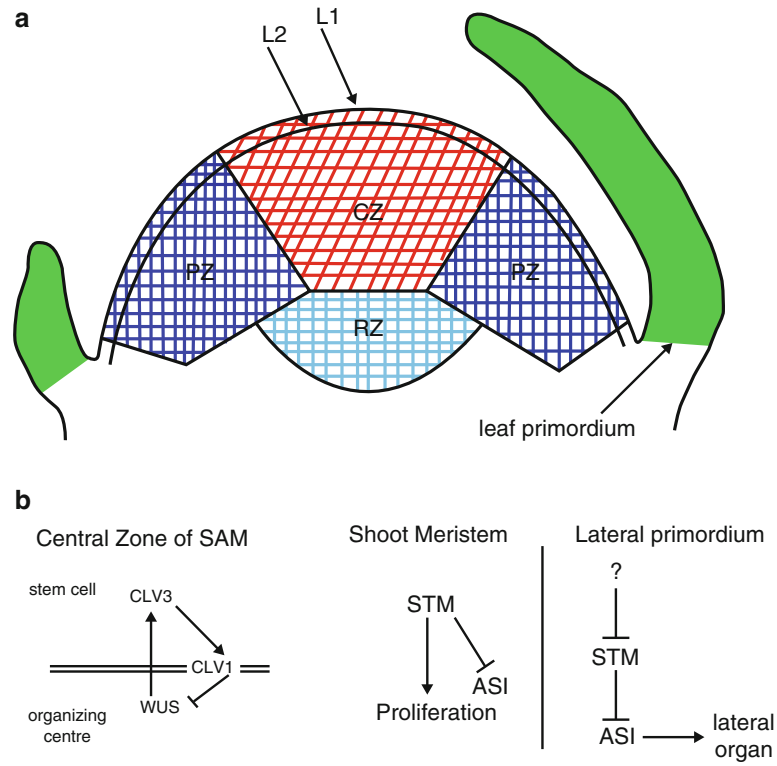


Fig. 4.4 (**a**) Schematic diagram of the *Arabidopsis* shoot apical meristem (*SAM*) with developing lateral organs. The infrequently dividing central zone (*CZ*) contains organizing centre with overlying stem cells. Frequently dividing cells in the peripheral zone (*PZ*) give rise to lateral organs, whereas divisions below the rib zone (*RZ*) contribute to growth of the shoot axis. (**b**) Regulatory pathways active in the shoot meristem. Shoot stem cells are maintained by the *WUS-CLV* feedback loop. WUS expression in organizing centre confers stem-cell identity. The *CLV3* ligand secreted by stem cells is thought to bind to the receptor *CLV1* which in turn represses *WUS* expression (denoted by T-bar). *STM* maintains proliferation in shoot meristem by repressing expression of *AS1*. *STM* is repressed (denoted by T-bar) in lateral primordia permitting activation of *AS1* expression that is required for lateral organ development (Vijayaraghavan et al. 2005)

SAM in embryos. It is believed that the novel protein encoded by *NAM* gene functions in intercellular signaling, the absence of which probably inhibits cell division around the epiphysis during transition from cordate to torpedo embryos. A putative *STM* orthologue has been expressed in the SAM of poplar and it is named *ARBORKNOX1* (*ARK1*) (Groover et al. 2006). *KNOX* group of genes such as the above are reported to bind directly and either activate or repress GA biosynthesis genes, thereby modifying the levels of GA in SAM and boundary regions. They are also reported to regulate cytokinin biosynthesis, as discussed in the next section (Pautler et al. 2013). Another mutant, similar to *stm,* is *zwille* (*zll*). This also does not express the differentiated characteristics of a SAM in that the initiation of the meristem is blocked at the torpedo-shaped stage of the mutant embryo. The *zll,* like *stm*, is likely to control both embryonic and adventitious SAM (in cultures). A closely similar mutation, referred to as *pinhead* (*pnh*) affects the SAM during embryogenesis, as well as the axillary bud meristem, but not SAM formed in cultured explants. In addition to *STM* gene, *WUSCHEL* (*WUS*) gene is required for the maintenance of initials' function in SAM. In *wus* mutants, the initials undergo differentiation into derivative mature cells and no longer remain as initials. *WUS* expression begins at 16-celled stage of the embryo in advance of

STM expression. In the fully developed SAM, *WUS* expression is restricted to a small group of central cells between the L3 layer (the initial layer of the corpus) and the rib meristem, and this expression persists throughout shoot development. *WUS* is not expressed within the initials but the signaling must occur between L3 and central cells. Further studies indicate that a short 57 bp *cis*-acting element in WUS promoter mediates the effects of diverse regulatory pathways controlling *WUS* expression (Baurle and Laux 2003). Thus, *WUS* is a positive regulator of SAM maintenance. It is likely that *WOX4* of rice may be similar to *WUS* as a positive regulator of SAM maintenance.

Mention must also be made of *MGOUN* (*MGO*) gene of *Arabidopsis*, which, like *WUS*, contributes to the precise definition of SAM. Cell divisions in SAM of *mgo* mutant embryos are distorted to some extent as in the SAM of *stm* mutant embryos leading to the formation of a larger-than-normal SAM. In these embryos, the corpus (central mother cells) region undergoes cell proliferation to form a fasciated structure. Hence, it may be stated that *WUS* and *MGO* genes are particularly required for maintaining the structural and functional integrity of SAM, specifically its central mother cell zone. However, we do not yet know whether the central mother cell zone and peripheral zone of SAM are differentially regulated. In addition to *STM* and *WUS* genes which regulate differentiation in SAM, there are two other *Arabidopsis* genes that regulate SAM size by repressing the overactivity of initials. These are *CLAVATA* (*CLV*) genes (Fig. 4.4b) (*CLV1*, *CLV2* and *CLV3*) and *EXTRACOTYLEDON* (*XTC*) genes. Mutation in these genes increases the production of more cells in the central mother cell zone and thereby its size (Fletcher 2002). This accumulation of cells is due to a failure of differentiation of flank meristem cells. *CLV3* expression is mainly restricted to L1 and L2 layers and to a few L3 cells in the central zone of SAM and probably marks the initials in these layers. *CLV1* expression underlines the L1 and L2 layers. As already mentioned, *WUS* gene expression is in the cells of central mother cell zone (the 'organizing cells' of the entire SAM) which confers initial cell identity to the overlying neighbouring cells, and the signals from *CLV1*/*CLV3* regions act negatively to dampen such activity. *CLV3* protein secreted by the initials in the apex moves through the apoplast, binds to a *CLV1*/*CLV2* receptor complex and causes the downregulation of *WUS*, maintaining the appropriate amount of activity of initials throughout development. In other words, *CLV* genes are negative regulators of SAM maintenance. Thus, a negative feedback loop exists between *CLV-WUS* which maintains a balance between the proliferation of promeristem cells and their loss through differentiation and the initiation of leaves in the flank meristem (Fletcher 2002, 2004; Schoof et al. 2000; Ha et al. 2010; Aichinger et al. 2012). The genetic relationship and molecular function of *FON1* and *FON2* in rice plant are very similar to those of *CLV1* and *CLV3* (Pautler et al. 2013).

Many more genes are needed for the correct organization of SAM in the embryo as well as its elaboration in the adult plant of *Arabidopsis*. The *EMBRYONIC FLOWER* (*EMF*) regulates the typical tunica-corpus organization right from the cordate embryo stage onwards; its effect is seen in adventitious SAM of callus. The meristem directly forms the reproductive meristem. The other genes of *Arabidopsis* involved in regulating SAM are *ULTRAPETALA 1 and 2* (*ULT 1 and 2*), *HANABA TARANAU* (*HAU*), *HAIRY MERISTEM* (*HAM*), *PHAVOLUTA* (*PHAV*), *PHABULOSA* (*PHAB*), *REVOLUTA* (*REV*), *CORONA* (*CAN*), *POUNDFOOLISH, STIP, FASCIATA1 (FAS1)* etc. The *FLATTENED SHOOT MERISTEM* (*FSM*) of rice controls size and shape of SAM; in *fsm* mutant, the SAM is flatter and smaller.

Some of the above genes are included under class III homeodomain-leucine zipper (*HD-ZIP III*) genes. These are believed to be targets of miRNAs 165 and 166. Overexpression of miRNA 166 by activation tagging results in downregulation of the *ATHB9*/*PHV*, *ATHB-14*/*PHB* and *ATHB-15* genes and concomitantly causes an enlargement of SAM (Zhou et al. 2007). It was further shown that overexpression of miRNA 165 causes a drastic reduction in the transcript levels of all five *HD-ZIP III* genes in *Arabidopsis*

(*IFL1/REV*, *ATHB-9/PHV*, *ATHB-14/PHB*, *ATHB-15*, *ATHB-15/CNA/ICU4*). The miRNA 165 overexpressors display prominent phenotypes reminiscent of loss-of-function mutants of *revphbphv* and *rev/ifl1*, including loss of SAM (Zhou et al. 2007).

4.4.3.2 Hormonal Control

The SAM, besides being controlled by genes, is controlled by hormones (Veit 2009). The SAM shows a characteristic placement along the proximo-distal polar axis. This polarized placement that is evident from the embryo onwards depends on the complex patterns of hormonal synthesis, transport and accumulation, particularly of auxin. Relatively low levels of auxin are a prerequisite for the normal organization and activity of SAM, probably through the effect of auxin on the expression of *CUC* genes and the activation of *STM* gene. Localized elevation in auxin levels, particularly in the flank meristem, triggers leaf initiation, probably accompanied by the downregulation of *KNOX* genes in leaf founder cells (see Chap. 5 in this volume). How auxin regulates *KNOX* gene expression in SAM is not yet clear. Low auxin levels in SAM may be related to the relatively nonpolarized cell growth in the corpus region. There is also suppression in GA activity in SAM and this suppression may be regulated by *KNOX* genes. Cytokinins are also important in regulating the SAM. Reduction in cytokinin level suppresses SAM activity, while an elevated level promotes SAM activity. This is evident from an analysis of *ABPHY1* gene in maize, which codes for an A-type cytokinin response regulator. Loss of *ABPHY1* function leads to great enlargement of SAM through a cytokinin-mediated cell proliferation (Giulini et al. 2004). The relationship between cytokinins and SAM activity is also revealed by an analysis of *KNOX* phenotypes. This gene expression results in a rapid increase in cytokinin levels through an isopentenyl transferase enzyme that mediates cytokinin synthesis. Available data suggest that *KNOX* genes and cytokinin mutually reinforce SAM identity. A genome-wide binding profile for *KN1* was recently identified (Bolduc et al. 2012). This study revealed that *KN1* targets auxin, cytokinin, GA and brassinosteroid hormone pathways. In *Arabidopsis*, *WUS* promotes cytokinin signaling by repressing the A-type genes *ARABIDOPSIS RESPONSE REGULATOR7* (*ARR7*) and *ARR15*, whereas cytokinin positively regulates the expression of *WUS* (Gordon et al. 2009). The importance of *LONELY GUY* (*LOG*) gene function in SAM organization of *Arabidopsis* is known very recently. The biologically active form of cytokinin, which is probably catalysed by *LOG4* expression in the SAM epidermis, acts as a positional cue for patterning the *WUS* expression domain (Chickarmane et al. 2012).

4.4.4 Autonomy of SAM

An important question relevant to SAM is whether it can function independently of its subjacent regions of the stem. To answer this question, two different experimental approaches had been followed: surgical experiments and in vitro culture of isolated SAMs. It is evident from such experimental studies that the SAM, to a very large extent, is autonomous and that it does not need signals from other parts of the plant to direct its functional activity (Smith and Murashige 1970; Soma and Ball 1963). A critical analysis of the results of surgical experiments indicated that the central mother cell zone is the most important region of the SAM and that any surgically removed portion of SAM without at least a few cells of central mother cell zone will not regenerate the SAM (Swamy and Krishnamurthy 1978; Krishnamurthy 2015). The cultured SAM explants first establishes a bipolar axis through the initiation of one or more root primordial before again producing stem tissues.

4.4.5 Evolution of SAM

The structure of SAM differs among plant groups, thus suggesting its probable independent evolution in lycophytes, ferns, gymnosperms and angiosperms. Several parameters such as structure, gene expression patterns, leaf primordia initiation patterns etc. have been used as the basis for such an evolutionary analysis.

One such parameter employed is the distribution and pattern of plasmadesmal (PD) network in the SAM (Imaichi and Hiratsuka 2007). An analysis of this parameter was made in the SAMs of 17 families and 24 species of angiosperms, gymnosperms and pteridophytes. The SAMs of angiosperms and gymnosperms have low PD density per unit area, with no difference between SAMs showing tunica-corpus organization and those showing cytohistological zonation patterns. On the contrary, SAMs of ferns (including *Psilotum* and *Equisetum*) have average PD densities (more than three times higher). Interestingly, the lycopods have both fern (in *Selaginella*) and seed-plant (Lycopodiaceae and Isoetaceae)-type PD densities. In other words, SAMs with single and plural initial cells have, respectively, the fern- and seed-plant-type PD distribution, indicating their probable independent evolution. Further, the fern- and seed-plant-type PD patterns are equivalent, respectively, to the lineage-specific network (LPD) (i.e. PDs formed in expanding cell plates during cytokinesis) and interface-specific PD network (IPD) (i.e. PDs having primary and secondary origins with the latter inserted into already existing walls) proposed earlier by Cook et al. (1996). The coordinated growth of the plant requires cell-to-cell signaling and transport of regulatory proteins and/or mRNAs, and hence PD distribution densities and patterns in apical meristems with single and plural initial cells assume great importance.

4.5 Root Apical Meristem (RAM)

In contrast to SAM, the RAM is subterminal, does not produce appendicular organ like leaves, does not undergo periodic changes in size due to plastochronic changes, produces endogenous root branches beyond the region of active growth (unlike exogenous origin of branches in stem very near the apical meristem) and does not produce nodes and internodes. Generally, the root may or may not have a specific protoderm (or *dermatogen*), the promeristem of epidermis (the outermost layer of root is often called *rhizodermis* or *piliferous* layer). Friedman et al. (2004) raised three basic questions regarding the roots: (1) Are roots of different land plant lineages homologous? (2) Are the developmental gene programmes that give rise to roots and shoots the same? (3) Do the shared features of histogenesis in roots and shoots arise from common developmental programmes first expressed in land plant sporophytes prior to the origin of roots? All these three queries also reflect on RAM as it gives rise to roots. The first question is still unresolved. Notwithstanding the fact that the RAM and SAM differ, as stated above, cladistic analyses (Kendrick and Crane 1997; Veit 2006) support the homology of the two meristems. Both have a centrally located 'quiescent centre zone', as emphatically shown even as early as 1978 by Swamy and Krishnamurthy; also, recent molecular and genetic analysis have shown the existence of similar mechanisms of operation in the two meristems. Some believed that the shoot apex was transformed into a root apex early during the evolutionary history of vascular plants, as supported by investigations of mutations affecting both RAM and SAM and by the similarities in the expression of *SCR* and *WOX* genes in both RAM and SAM (Jiang and Feldman 2005). There are others who believe in the de novo origin of root.

4.5.1 Organization of RAM

Like SAM, RAM is also of two basic categories in vascular plants: with a single apical cell (Fig. 4.5) and a multicellular RAM. RAM with single apical cell is seen in many ferns, while the second type of RAM is seen in lycophytes (other than *Selaginella*), a few ferns, gymnosperms and angiosperms. In most cases of RAM with an apical cell, the cell is tetrahedral and cuts off segments on three lateral (proximal) facets to produce the body of the root; the rootcap is produced from either the fourth facet of the apical cell distally or from a separate meristem formed very early in root ontogeny.

In the multicellular RAM of a number of plants, the body of the root arises from a massive meristem comprising of three of four precursor tissue regions or *histogens* (Hanstein 1868, 1870). Each of these

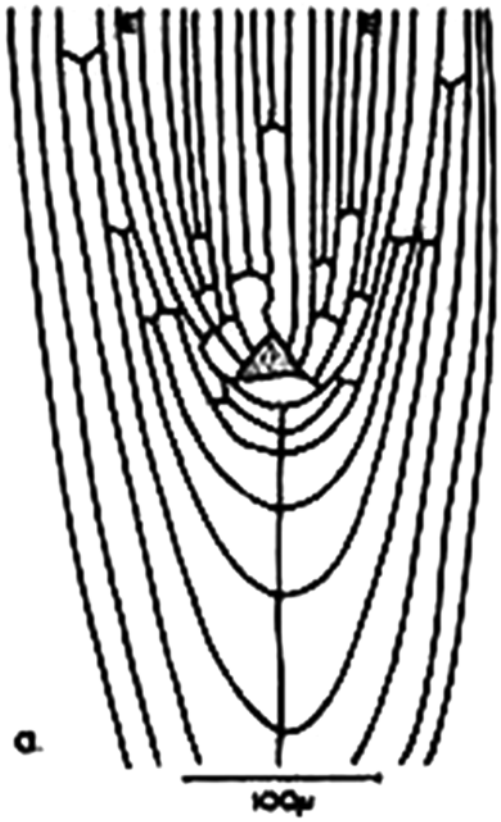


Fig. 4.5 L.S. of root apex of *Marsilea* showing apical cell and body-cap organization and T-division patterns in its derivative cells (Clowes 1961)

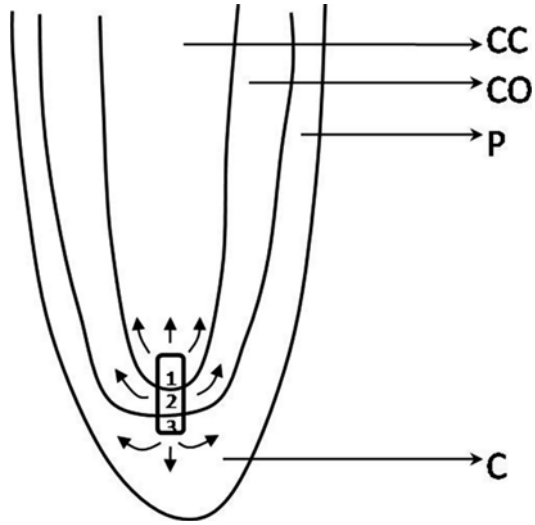


Fig. 4.6 Diagrammatic L.S. of the root apex interpreted as per histogen theory. *1* Plerome, *2* periblem, *3* dermatogen, *C* rootcap, *CC* central cylinder, *P* piliferous layer (Krishnamurthy 2015)

histogens begins with one to many initials at the apex arranged in superposed tiers (Fig. 4.6). The histogens are called dermatogen (precursor of the root dermal layer), *periblem* (precursor of cortex), *plerome* (precursor of stele and pith, if present) and *calyptrogen* (precursor of rootcap). In RAM of some plants, the dermatogen is absent and the surface layer is formed from periblem. In some gymnosperms and angiosperms, all tissue regions of the adult root or all except the central cylinder appear to arise from a common meristematic zone; such a RAM was called *open type* (Guttenberg 1960) and the ones with separate initials belong to the *closed type* (see Heimsch and Seago 2008). The distinction between open and closed types of RAM is not always clear, sometimes even in the same plant at different developmental stages; sometimes a transition type of RAM is seen in some taxa such as *Allium cepa*. Clowes (1981) and Deysson (1980) distinguished open and closed types of RAM in a slightly different way. According to them, a closed RAM has a distinct initial layer for the rootcap, while an open RAM has interchange of cells between cortex and cap.

Whatever may be the category of RAM, whether with single apical cell or multicellular or whether of open, closed or transitional type, the RAM shows the body (körper) or cap (kappe) type of cell divisions (Schüepp 1917). The planes of those cell divisions (= *formative divisions*) that are responsible for the increase in the number of vertical files in the actively dividing region of the RAM are very important to indicate the body or cap category. Many of the files divide into two, and where they do so, a cell divides transversely; then, one of the two new cells divide longitudinally and each daughter cell of this division becomes the source of a new file. This combination of the transverse and longitudinal divisions results in an approximately inverted T- or Y-shaped wall pattern, and hence, such divisions are called *T-divisions* (Fig. 4.5). The directions of the top stroke (horizontal bar) of the T vary in different root regions. Often, in the rootcap, particularly in the columella region, it is directed towards the base of the root (cap-type division),

while in the body of the root, it is towards the apex (body-type division). In some RAM, there is a clear boundary between the 'body' and 'cap'; in others such a boundary is not clear.

4.5.2 Quiescent Centre Concept

This is the most important concept relating to the organization of RAM. Clowes (1961) first discovered a *quiescent centre* (*QC*) in RAM in 1954, and this discovery completely revolutionalized our understanding of the behaviour of RAM. According to Clowes, there is a *minimal constructional centre* in the RAM, which largely ceases to be mitotically active, and this centre was labelled by him as QC. The regular presence of this in RAM was proved by conventional and cytochemical staining methods, surgical experiments, irradiation and on root apices fed with radiolabelled chemicals involved in DNA synthesis followed by autoradiography (Fig. 4.7).

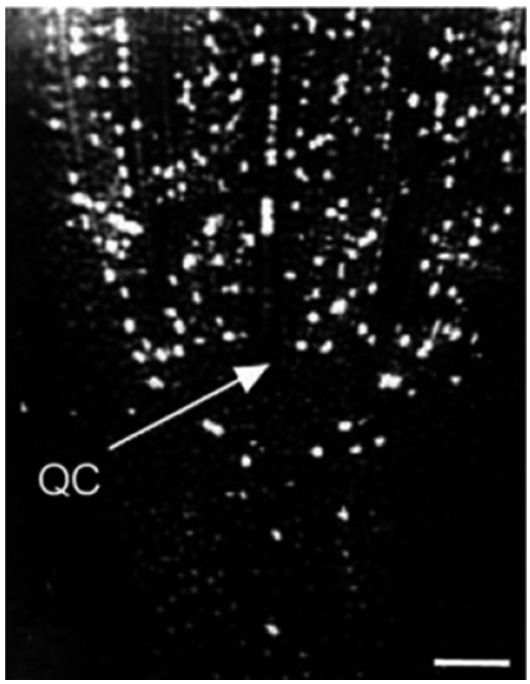


Fig. 4.7 Autoradiograph of longitudinal sections through the *Allium cepa* RAM of seedlings exposed to [Me-3H] thymidine for 24 h: root grown in water. Figure shows the lack of DNA synthesis in Quiescent centre (QC) (Liso et al. 1988)

Mitotic activity was noticed only in cells located on the margins of QC. The QC in the RAM of a mature root is hemispherical (*Zea mays*) or discoid in shape and contains as few as four cells as in *Arabidopsis* to more than a thousand as in some very broad roots. In *Zea mays*, it has almost 1,500 cells and in *Helianthus* about 800 cells. The report of its absence in the very thin roots of rice is erroneous as QC is an integral part of each and every RAM, however small it may be. Similarly, the report of origin of QC twice in primary roots, once in embryo and then during germination with an intervening absence in the mature embryo (see Evert 2006) is also erroneous since QC occurs since its inception as hypophysis in the globular embryo throughout the life of the root (Swamy and Krishnamurthy 1975; Krishnamurthy 1994, 2015).

The relatively inactive state (in terms of cell division) of the QC does not mean that its cells have become permanently inactive mitotically. QC cells do divide, although infrequently. It serves to renew the more actively dividing regions around the QC with its supply of new cells, since the dividing regions have an unstable population of cells that are displaced from time to time and need to be replenished with newer cells. By labelling nuclei with radiolabelled DNA precursors and by blocking cell cycle at metaphase with inhibitor chemicals, one can obtain quantitative data on the duration of *mitotic cycle* (*MC*) as well as number of cells actually undergoing cell division (i.e. *mitotic index*, MI) in the QC and other regions of RAM. These data indicate that the cells of QC divide approximately ten times slower than the adjacent dividing cells (Krishnamurthy 2015). Pulse labelling with tritiated thymidine has shown that the differences in the duration of mitotic cycles are largely caused by differences in the duration of *G1* phase of cell cycle. In other words, the MC takes a very long time to get completed in QC cells. The MI is also very low in the QC, i.e. only a few cells of QC divide and even these cells take a very long time to complete their division. The MC duration of QC cells of investigated taxa ranges from around 170 h to about 520 h in contrast to cells of other regions of RAM whose MC ranges from about 12 h to about 45 h.

The paucity of the mitotic activity of the cells of QC led Clowes (1961) to suggest that the actual initials of RAM are located just outside the QC, all around it. And he designated these initials as *promeristem* of RAM. However, Swamy and Krishnamurthy (1975), Barlow (1978) and Steeves and Sussex (1989) have argued that it would be more appropriate to consider the slowly dividing and sparsely dividing cells of the QC themselves as the ultimate source of cells to the entire root apex; they are the initials which are strikingly similar to those of central mother cell zone of the SAM. Hence, QC itself is to be considered as the promeristem. Some botanists like Kuras (1978) consider the root promeristem as comprising the QC as well as its immediate, actively dividing derivatives. Even today there is no uniformity in the use of terms and in the meanings attributed to these terms by different investigators, although the majority agrees that the QC is the promeristem.

Since QC has cells with low MI and since even its dividing cells have a prolonged MC, the QC may also be considered as a reservoir of cells to renew the RAM, if RAM becomes dormant due to unfavourable environmental conditions or becomes damaged due to various reasons. In fact, QC cells have been shown to enter into active division activity during recovery from a period of dormancy through cell cycle changes. The root apical meristem is occasionally damaged as the root forces its way through the resisting soil or damaged due to soil biota. When this damage occurs, cells of QC divide and once again organize the RAM with all its component zones including a QC. QC cells are not easily damaged by dormancy and other physical factors affect only dividing cells when they are in *S* and *G2* phases. Some of the cyclin-dependent kinases (CDKs) share a conserved PSTAIAR motif (stand for proline, serine, threonine, alanine, isoleucine and arginine sequence). A high level of this protein (CDK) has been identified by immunofluorescence studies in the QC of maize. This finding has raised the possibility that the PSTAIAR proteins might enable QC cells in readiness to enter the cell cycle after an extended interphase (Krishnamurthy 2015).

Based on an analysis of growth patterns in root apices, Barlow (1973) and Clowes (1984) have suggested that quiescence at a particular location of RAM results from antagonistic directions of cell growth in various parts of the meristem, the rootcap or calyptrogens being the most important in suppressing growth. Clowes (1978a, b) argued that the origin of QC during embryogenesis coincides with the appearance of the rootcap. If the cap is surgically removed or otherwise damaged, the QC gets activated to give rise to a new cap meristem and hence a new cap), following which quiescence again resumes in QC. This behaviour prompted Barlow and Adam (1989) to suggest that the activation of QC, after damage or removal of cap, results from an interruption or modification of signaling between the rootcap or its meristem and the QC. This signaling, according to these authors, is more likely to be a hormone, an auxin. It was further suggested that the origin and maintenance of QC in maize root apices are due to polar auxin supply and that the rootcap or its initial plays an important role in regulating the polar auxin transport towards the root tip (Kerk and Feldman 1994). High levels of auxin bring about elevated levels of ascorbic acid oxidase (AAO) resulting in the depletion of AA within QC, i.e. QC has a more oxidizing environment. Since AA is required for *G1* to S phase transition in the cell cycle in RAM (Liso et al. 1984, 1988), Kerk and Feldman (1995) proposed that depletion of AA in root apices may be responsible for the formation and maintenance of the QC. Subsequently, Kerk et al. (2000) reported that in maize AAO also oxidatively decarboxylates auxin; this is another mechanism for the regulation of auxin levels in QC and other RAM regions. An intact rootcap is vital for this physiological process to occur. There are some problems in the above attractive explanation: cells in the QC do divide, although in a prolonged manner. How do these dividing cells selectively escape when dividing cells (particularly at the margins of QC) are affected by the depletion of AA? There are roots without rootcap but not without QC. Moreover QC has its origin in hypophysis of the globular embryo stage when a rootcap or its initial is non-existent. Hence, it is

more likely that QC controls the rootcap (and other RAM tissues) and not vice versa. Moreover, it is stated in the earlier paragraph and emphasized long back by Swamy and Krishnamurthy (1975, 1978) that the QC is not only an integral part of RAM but also the ultimate source of all cells of RAM and that its prolonged MC and low MI is a built-in safeguard to regenerate the RAM if its meristematic cells are damaged by irradiation, mechanical damage, carbohydrate starvation, exposure to low temperature or exposure to ascorbic acid. But unlike the other factors mentioned above, those roots treated with ascorbic acid retained a minimal QC size or minimal constructional centre. The importance of QC is also brought to light by laser ablation studies on *Arabidopsis*. When all four QC cells are ablated, they were replaced by the initials of the root central cylinder (Scheres and Wolkenfelt 1998). Ablation of one QC cell causes cessation of cell division and the progression of differentiation in the columella and cortical initials with which it is in contact with. The major role of QC is thus to keep the initials in the dividing state. High meristematic activity around QC of *Arabidopsis* has been shown to be triggered by glutathione, a diffusible redox agent; this functions along with AA to maintain the cell's redox equilibrium. Dividing cells show an increased level of glutathione in contrast to a low level in QC. Thus, pool sizes of AA and glutathione and control of mitotic activity are linked in RAM. Some researchers believe that QC itself may be the site of hormone synthesis and this itself might prevent the QC cells from active mitosis. This is proved by the fact that exposure of maize RAM to TIBA, an inhibitor of auxin transport, causes a burst in mitotic activity in QC.

How does the QC originate and get elaborated as the plant develops from the zygote? As early as 1975 itself, Swamy and Krishnamurthy (1975) have analysed in detail the origin of QC in tap (primary), lateral and adventitious roots. Unfortunately this work was overlooked by most western botanists. In primary root (radicular root), QC originates as hypophysis, a cell first demonstrated and named by Hanstein (1870). It gets differentiated at the micropylar pole of the globular embryo with the simultaneous appearance of epiphysis on the exactly opposite pole of the embryo. Although there are slight variations in the origin of hypophysis (see details in Swamy and Krishnamurthy 1975; Krishnamurthy 1994, 2015) as traced by different embryologists, it was categorically demonstrated that the hypophyseal cell and its immediate derivatives remain histologically conspicuous by relatively poor stainability and a relatively 'dormant' and quiescent state. During seed germination, this histological distinction remains unchanged, although the size of hypophysis and its derivative cells slightly increase through slow addition of cells. Thus, the hypophysis and QC are a continuum, and two separate terms are not necessary and QC or QC initials should replace hypophysis (Swamy and Krishnamurthy 1975; Krishnamurthy 1994, 2015). The so-called hypophysis has the reduced capacity to incorporate radiolabelled DNA precursor and low poly(A)-RNA as revealed by in situ hybridization using [^{3}H] poly(U) as a probe (see Raghavan 2000). The same cells of hypophysis that fail to bind [3h] poly(U) do not incorporate [^{3}H] thymidine in the QC of seedling in *Capsella bursa-pastoris*. The hypophyseal origin of QC is also demonstrated by the *hobbit* (*hbt*) mutant of *Arabidopsis*. This mutant has an aberrant hypophysis specification during embryogenesis and its seedling lacks a recognizable QC (Willemsen et al. 1998). A precise delineation of cell determinants associated with the nondividing cells of QC, the dividing cells of dermatogen and the cells of the growing root must be taking place during the initial divisions of the hypophyseal cell (Krishnamurthy 2015). Hence Aida et al. (2004) must be wrong in believing that the four-celled *Arabidopsis* QC is not composed of stem cells; in fact, all these four cells are primordial stem cells (see earlier discussion on the concept of initials in this chapter). This conclusion of Aida et al. (2004) has also been questioned by Jiang and Feldman (2005), who regard these four cells as structural initials (stem cells) as opposed to the surrounding, more actively dividing functional initials cells.

The origin of QC in lateral and adventitious roots is shown to be de novo (Clowes 1961;

Swamy and Krishnamurthy 1975; Krishnamurthy 1994, 2015). In lateral roots, Clowes (1961) demonstrated that the origin of QC coincided with the arrest of DNA synthesis in the concerned cells after the lateral root initiation proceeds for a time. The de novo origin of QC in the adventitious roots has been traced through development studies made on *Commelina benghalensis* (Swamy and Krishnamurthy 1975; Krishnamurthy 2015).

As already discussed, the presence of QC is very vital for the effective functioning of the root, particularly in deciding the indeterminacy of root growth. If QC is lost, the RAM becomes determinate. This has been demonstrated in root primordia that are transformed into thorns as in some palms such as *Cryosophila* and some bamboos and in the cactus *Pachycereus* (Rodriguez-Rodriguez et al. 2003). In this cactus, immediately after germination, the primary root becomes determinate but instead several lateral roots emerge. This primary root loses its QC in a molecular mechanism similar to that noticed in *Arabidopsis HOBBIT* or *PINOID* mutants. The unpublished observations of one of the authors of this article, Krishnamurthy, indicate that the RAM becomes arrested in growth and stubby in mycorrhizae-infected roots of *Lycopodium* and *Pinus* essentially due to the disorganization of QC. However, little is known of the mechanisms leading to QC disappearance and determinate root growth.

4.5.3 Genetic and Hormonal Control of RAM Activity

The formation of hypophysis/QC is influenced by mutants in *MONOPTEROS* (*MP*) (Berleth and Jürgens 1993), *BODENLOS* (*BDL*) (Hamann et al. 1999), *SHORT-ROOT*, *SCARECROW*, *PLETHORA* (*PLT*) and *HOBBIT* genes. Some molecular genetic analysis made on RAM revealed what may be analogous to *WUS* activity in the QC that is noticed in SAM (Kamiya et al. 2003; Haecker et al. 2004). Artificial activation of this *WUS*-like gene promotes RAM, while overexpression of CLV-like peptides leads to depletion of RAM (Hobe et al. 2003; Fiers et al. 2005).

Many root-specific genes that are transcribed in the cortical derivatives have been known and identified. The one fairly well-studied gene is *Axis-abundant 92* (*AX-92*) of water-imbibed seeds of *Brassica napus*; this has been isolated from a cDNA library made to poly(A)-RNA. The transcripts of this gene are detected in the ground meristem and cortex of seedling root but are not present in significant amounts either in rootcap or the vascular tissues. Hence, it defines initial cortical parenchyma differentiation events and is first expressed in the cortical region of torpedo embryo radicle of transgenic plants (Deitrich et al. 1992). In contrast to this, transcripts of one of maize *ZRP3* (for '*Zea* root preferential') cDNA clones are found in several inner cortical layers, while transcripts of another clone of *ZRP4* are expressed in endodermis and three to four exodermal layers of outer cortex. The maximum expression of *ZRP3* gene is confined to a height of 2 cm while that of *ZRP4* to a height of 8 cm from the root tip. A *ZRP3* homologue is known in bean root, and this is expressed in cortical meristem with a gradually decreasing gradient from root tip towards root body (Choi et al. 1996).

In addition to genetic control, RAM activity is also controlled by plant growth regulators. In *Arabidopsis*, specification of RAM in embryo occurs as a consequence of *PIN1*- and *PIN4-dependent* accumulation of auxin. The auxin in preglobular embryos originates either in the suspensor or in the embryo sac (Friml et al. 2003), but by globular stage, the expression pattern of *PIN*-dependent transporters changes and auxin production begins. With further development of embryos, auxin production continues and increases at their apical ends. Genes mediating auxin response include *BDL* (which affects auxin sensitivity), *MP* (which affects polar auxin transport) and *AUXIN RESISTANT1* (which affects auxin response), the mutants of all of which do not form an embryonic root. *HOBBIT* mutants have incorrect hypophysis (QC) development and a reduction in auxin reporter gene expression and accumulate the *AXR3/IAA7* repressor of auxin responses. Some information on the importance of auxin on QC/RAM of adult roots was already provided. The apico-basal polarity of

RAM depends on the complex pattern of synthesis, transport and accumulation of growth regulators, mainly auxins, right from the embryo stage onwards. High auxin levels in the central basal domain of the *Arabidopsis* embryo trigger the expression of *PLT1* and *PLT2* genes, which are *AP2* class transcription factor genes. These genes along with *SCR* and *SHR* genes that control radial patterning help in the establishment of an organized RAM with the correct positioning of QC (Jiang and Feldman 2005). These are expressed as early as eight-celled embryos. *PLT* and *SCR*/*SHR* operate via parallel pathways that appear to converge to a subset of target QC-specific promoters (Fig. 4.8). High *PLT* expression promotes stem-cell fate (QC), while the lower and lowest expression promotes derivative cell proliferation and differentiation, respectively (Galinha et al. 2007). Through a feedback mechanism, PLT proteins promote *PIN* expression and maintain stem-cell niche. A histone acetyltransferase activity, general control nonrepressed protein5 (GCN5), is also necessary to promote *PLT2* expression (Fig. 4.8) (Kornet and Scheres 2009). CDKB1 and CDKB2 and CYCB1;1 (cell cycle kinases) are likely to be putative targets of *PLT2*. *PLT1* and *PLT2* induce the expression and/or accumulation of *HIGH PLOIDY2* (*HYP2*), a nuclear-located ligase, which is expressed in the dividing cells of RAM, and a negative regulator of endoreduplication. Thus, auxin maintains root meristem homeostasis through *PLT1/2* expression and high *HYP2* expression, which prevents endoreduplication and promotes cell proliferation.

The GRAS family transcription factors *SCR* and *SHR* are required for correct function of RAM; *SHR* is expressed in provascular cells adjacent to QC and endodermal initial layer (Fig. 4.8), where it interacts with *SCR*. This interaction results in the activation of target genes required for QC identity, stem-cell niche homeostasis as well as for cell divisions in RAM (Bitonti and Chiappetta 2011). Recently, a number of putative SCR-interacting proteins have been identified (Fig. 4.8), which specifically interact with their N-terminal domain. Their c-terminal domain interacts only with SHR. The former

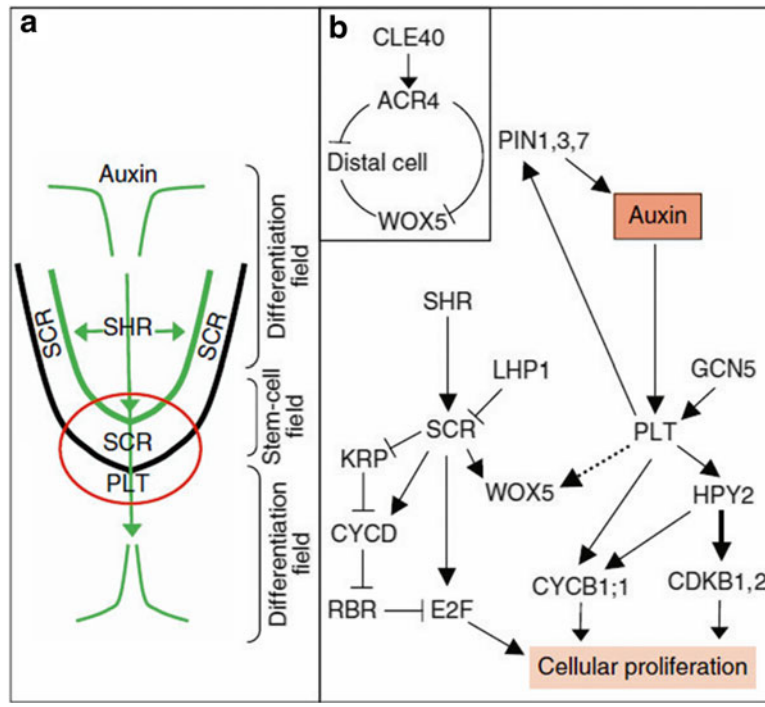


Fig. 4.8 Scheme depicting (**a**) auxin polar flux (Adapted from Vernoux and Benfey 2005) and (**b**) its interaction with a transcriptional network in root patterning (see text for further details). *Arrows* indicate positive regulation; *barred lines* indicate negative regulation (After Bitonti and Chiappetta 2011)

interaction is found to be versatile and is necessary to repress further cell divisions, while the latter is necessary to activate asymmetric cell division. Among the SCR-interacting proteins, LIKE HETEROCHROMATIN PROTEIN1 (LHP1) is highly expressed in the root elongation zone. LHP1 plays a role in cortex formation by acting together with *SCR* in preventing further asymmetric cell divisions. Thus, it can be concluded that the stem-cell niche can be identified as the domain where the highest expression levels of *PLT1*, *SHR* and *SCR* overlap. Perhaps all these (*PLT*, *SHR*, *SCR*) do not control the same target genes with the possible exception of *WOX5*. *WOX* expression is confined to QC by maintaining the stem-cell state and most likely involved in the control of QC-specific gene expression.

It was already mentioned that a feedback loop exists in SAM between *WUS* and *CLV* genes. The closest homologues of *CLV* in RAM are members of *CLV3/ENDOSPERM SURROUNDING REGION* (*CLE*) family. Some of the *CLE* genes like *CLV3*, *CLE19* and *CLE40* act to reduce RAM size, while others (*CLE41*) promote cell proliferation in stele. *CLE40* controls cell division in the distal root meristem, and the CLE40 protein is believed to be secreted from columella cells into QC and represses *WOX5* expression therein (Fig. 4.8). Probably this effect is brought through CLE40's putative receptor, ARABIDOPSIS CRINKLY4 (ACR4), which is mainly expressed in the distal meristem and locally restricts cell division activity interfering with columella stem-cell maintenance (Bitonti and Chippetta 2011) (Fig. 4.8). The *CLE40*/*WOX5* pathway of RAM parallels *CLV3*/*WUS* pathway in SAM.

Cytokinins are also implicated in RAM activity. A decreased cytokinin signaling leads to enhanced root meristem growth, while elevated cytokinin levels suppress RAM activity. This is evident from an analyses of *PASTICCINO* (*PAS*) 1, 2, 3 phenotypes that show suppression of cytokinin responses. Probably PAS gene controls the functional balance between cytokinin and auxin (Viet 2006). More recent work has indicated an antagonistic, and at times transient, interaction between auxin in determining positional information specifying cell fate or histogenesis in developing RAM (Bitonti and Chiappetta 2011). Cytokinins promote cell differentiation at the boundaries between the division and elongation zones by suppressing auxin signaling and transport, while auxin promotes cell division by suppressing cytokinin signaling (Ruzicka et al. 2009). Increased cytokinin levels reduce root meristem size and inhibit root growth, by modulating *PIN* expression and hence auxin distribution. This interplay depends on the convergence of both cytokinins and auxin on the same target gene, *SHORT HYPOCOTYL* (*SHY2*), which encodes an IAA class repressor protein of the auxin signaling pathway (Fig. 4.9). *SHY2* prevents the activation of auxin-responsive genes by negatively regulating *PIN1,3,7*. *SHY2* controls auxin and cytokinins in opposite ways. Auxin drives SHY2 protein degradation through SCF ^{TIRI}, while cytokinins promote SHY2 expression through AHK3/ARR1 (a signal pathway). Ethylene and auxin and brassinosteroids also interplay. Both these interplays

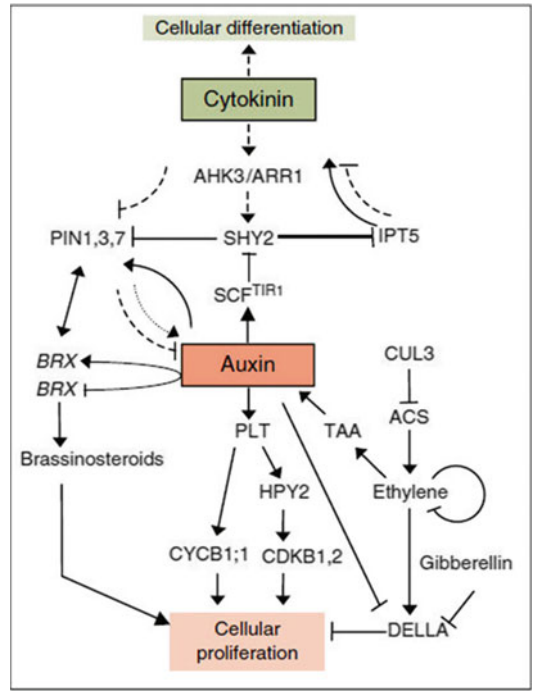


Fig. 4.9 Scheme depicting hormone interaction in transcriptional network underlying root patterning (see text for further details). *Arrows* indicate positive regulation; *barred lines* indicate negative regulation (Bitonti and Chiappetta 2011)

affect cell proliferation in RAM (Fig. 4.9) (Binonti and Chiappetta 2011).

4.5.4 Autonomy of RAM

The autonomy of RAM has been investigated using surgical experiments as well as in vitro culture of isolated RAM. Culture studies involving progressively shorter lengths of root apex as well as surgical experiments have shown that the RAM is autonomous to a great extent and that it has an inherent capacity to organize itself. Culture of longer segments of root apex of pea plant do much better than shorter ones, although the latter's growth can be improved through suitable alteration of the nutrient medium through vitamins, sucrose and micronutrients at the appropriate ratio. Culture of QC (about 1,500 cells) of maize roots regenerated a well-organized root of normal structure on a culture medium supplemented with kinetin and IAA (Feldman and Torrey 1976). Surgical experiments have shown that even very thin longitudinally split RAM portions can regenerate a whole RAM if they contain at least a few QC cells (Swamy and Krishnamurthy 1975).

4.6 Intercalary Meristem

Intercalary meristem (*IM*) was defined already. IM is more common in monocots than in dicots or other vascular plants, and it plays an important role in the longitudinal growth of internode and floral stalk or scape (Swamy and Krishnamurthy 1979). The detached IM category is more common and is found in internodes of grasses and many other monocots, and in *Equisetum*, while the resumptive type is seen in the gynophore of peanut plant. It may be located in the internodal region just above the node (as in many grasses and *Equisetum*), a little away from the node, i.e. not at the base of the internode but a little removed from the base (*Avena* and *Triticum*) or just below the ovary in the pedicel's uppermost region in gynophore of peanut or in the upper region of the scape. Initially cell division is seen throughout the internode/floral axis but then becomes gradually restricted to specific regions of internode/floral axis. IM should not be confused with the *uninterrupted meristem* (*UM*) that Fisher and French (1978) speak of. UM, according to these authors, is a region of cell division derived from the subapical meristem of shoot apex that progressively gets confined to the upper part of the internode or floral/reproductive stalk and that it is continuous with the subapical meristem (i.e. uninterrupted) and not isolated from the apex by mature tissues. Swamy and Krishnamurthy (1979), however, have shown that UM is not continuous from subapical meristem as there are mature nodal tissues that separate it from the subapical meristem. Hence, they advocated that only two types of meristematic activity are involved in internodal ontogeny: (1) subapical meristematic activity of the rib meristem that is seen in all plants with a distinct internode to a variable extent and the wave of differentiation of the internodal tissues is acropetal with progressive restriction of the division activity to the more and more apical regions of the internode before it finally ceases and (2) the IM activity where the IM is either derived from subapical meristem, with gradual restriction of division activity to any region of the internode/floral axis/spike axis where it is very much prolonged contributing significantly to internodal tissues, or derived by dedifferentiation of mature tissues and contribute division activity for a very long time. UM, thus, is a quickly fading subapical meristematic activity, while IM is a prolonged subapical division activity restricted to a particular locus of the internode.

4.7 Development of Stem

The primary body of the stem is essentially contributed by SAM, IM and UM activities of shoot.

4.7.1 Metamers and Modules

There are three levels of morphological organization in multicellular plants: *cell*, *metamer* and *module (*Fig. 4.10) (Barlow 1994). The cell is the first level and its autoreplication results in the

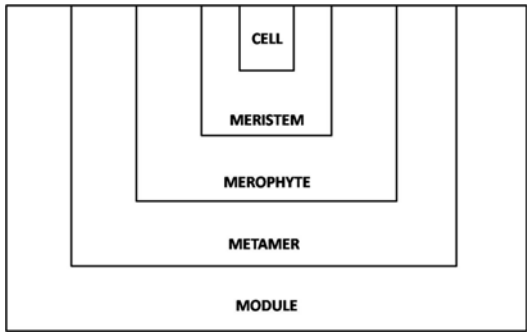


Fig. 4.10 The hierarchical composition of a module illustrating its interrelations to the lower levels of cell, merophyte and metamer (Modified from Barlow 1994; Krishnamurthy 2015)

Fig. 4.11 Long and short shoots of *Ginkgo biloba*; here short shoots are lateral (Robert W. Ridge 1987. Reproduced with permission from International Christian University)

replenishment of meristems which constructs the second level, the metamer. The metamers together organize the third, more complicated, level, the module. All these three levels/units are related to one another in a hierarchical fashion. Modules in combination organize the vast variety of shoot (and root) systems. The shoot metamer or *phytomer* is a unit that has one quadripartite set of leaf, node, internode and bud and represents the fundamental unit of shoot construction. The term *merophyte* (Douin 1923) is applied to each daughter cell derived from the single apical cell of lower vascular plants. Though the anatomical constitution of a metamer may vary according to the phyllotactic patterns of the plant, conceptually, the respective parts of a metamer per se, rather than their construction that is crucial for the definition of a metamer. A module is an axis formed of metameters. The growth of the module will be seen as long as the main SAM is active, while branching of the module is effected by axillary meristems. In many temperate tree taxa, the sequence of metamers is arranged into morphologically dissimilar groups because of dormancy, with some modules fully developed and others partially. These seasonally produced units of growth are called *submodules*; these have definite metameric structure but are only a part of the complete module. Fully developed modules of some temperate trees can be *long shoots* and *short* or *dwarf* shoots (sometimes also called *spur shoots*). The long shoots are vegetative while short shoots often bear reproductive organs. The short shoots may be *terminal short shoots* (derived from terminal SAM, with lateral shoots becoming prominent) as in *Terminalia* or *lateral short roots* as in *Ginkgo* (Fig. 4.11) and *Pinus*. The short shoots often have poor subapical rib meristem activity (hence internodes are telescoped) and usually do not bear lateral branches.

4.7.2 Origin of Nodes and Internodes

The groups of cells that ultimately form part of the node and internode are specified simultaneously with the initiation of leaf primordia on the SAM. The morphological definition of node and internode is decided, at least partly their topographical relationship with the adjacent leaf primordium. Hence, the plastochronic changes in the SAM have a greater significance in defining node and internode than merely deciding leaf production (Swamy and Krishnamurthy 1974). The histological definition, as different from spatial definition, of prospective node and internode has largely gone unnoticed (Barlow 1994). For instance, the appearance of tannin idioblastic cells in *Sambucus racemosa* is restricted only to the internode, and hence its location in the shoot apex will distinguish the internode from node (Zobel 1989a, b), where otherwise it is difficult. Hence, node and internode are not simply

passively defined by their position relative to the accompanying leaf primordium but are defined before the primordium becomes visible (Swamy and Krishnamurthy 1974; Lyndon 1987; Barlow 1994). Once it is delineated, the internodal growth is contributed not only by subapical rib meristematic activity (seen in all plants, although to a variable extent) but also by IM and, to a limited extent, by UM, as already discussed. The subapical meristem is recognized as one of the five morphogenetic zones in the shoot apex (Fig. 4.12) by Wardlaw (1957, 1968). The final form and behaviour of the internode is largely decided by this meristem (Romberger 1963; Swamy and Krishnamurthy 1979). In some taxa, this meristematic activity extends from 1.9 to 18.1 cm below the apical dome depending on the taxon and may extend to the seventh internode from top or even beyond. The plane of cell division in this meristem is almost exclusively transverse. The activity of this meristem is almost absent or extremely limited in acaulescent and rosette plants and short shoots, and GA is implicated in deciding the extent of this activity (Sachs et al. 1959, 1960; Sachs and Lang 1961). The nodal region, from the beginning, does not elongate and the constituent cells are also telescoped and very short. In monocots, it is marked by a nodal plexus, from the beginning, which is made of anastomosic provascular/vascular tissue. The internode consists of a dermal layer, a cortex and a stele (with or without pith) with a ring of collateral (xylem and phloem on the same radius) vascular bundles in dicots and gymnosperms, while in monocots, the dermal tissue is followed by a ground tissue in which vascular bundles are embedded. There are significant variations from this basic plan in respect to tissue distribution, stele types, distribution of vascular bundles and structure of vascular bundles (Evert 2006; Krishnamurthy 2015).

The node is the region from where leaf traces originate, and a gap or *lacuna* (= parenchymatous region) is seen in the stele where a leaf trace departs from the leaf. Based on the number of gaps associated with each leaf, the nodes are usually classified into *unilacunar*, *trilocular* and *multilacunar* types. Monocot nodes have a nodal plexus and leaf gaps cannot be recognized. More details on these nodal types and their phylogenetic and taxonomic significance can be seen in Krishnamurthy (2015).

4.7.3 Procambialization and Primary Vascularization in Stem

The most prominent feature of nodal and internodal differentiation in the shoot apex is the blocking out of the *procambium* from which the primary vascular tissues are derived. The height at which this blocking out (as different from ground tissue) happens varies, but in most taxa, it

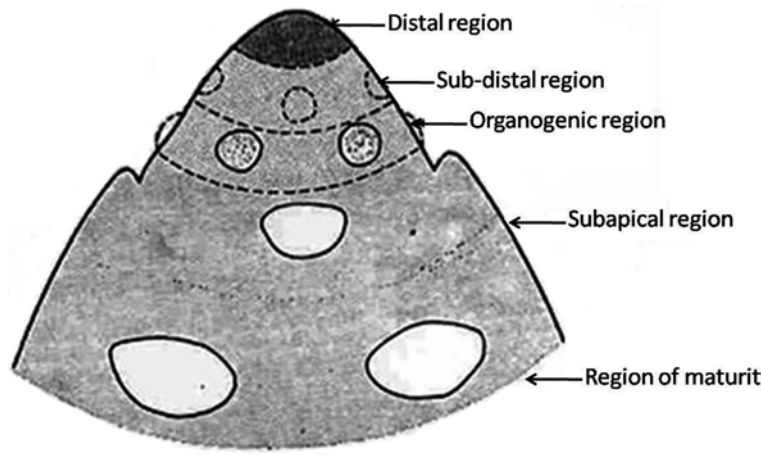


Fig. 4.12 Diagrammatic representation of a shoot apex as a system of contiguous interrelated zones (Based on Wardlaw 1957)

is stated to happen at almost the level of the youngest leaf primordium. Some people speak of a progenitor for procambium called by names such as *meristem ring*, *prodesmogen* or *residual meristem*, but it is very difficult to distinguish this progenitor from procambium and also because the progenitor is stated to give rise to interfascicular parenchyma as well. The actual timing, location and identification of procambialization are matters of great dispute mainly because of interpretational problems and also because of the actual definition of procambial tissue (Esau 1943, 1965). Procambium in this location is usually identified by the small cross-sectional area, dense and vacuole-less cytoplasm and elongated cells, but these have been contested as not unique features of procambium by many researchers. It is very disappointing, and often frustrating, that till date we do not have a meaningful concept of procambium and unique markers for it. Most people associate procambium with the leaf primordia, mostly influenced by the researches of Professor Esau. These people go to the extent of asserting the entire procambial system and the primary vascular tissues derived from it as being made of leaf traces. Leafless taxa also have vascular bundles in their stems; also removal of leaf primordial in taxa with leaves also does not prevent vascular tissue formation in stems (Krishnamurthy 2015).

The other problem in procambialization relates to its wave of differentiation. Most people speak of a basipetal longitudinal wave extending from leaf downwards into the stem. There is no real proof of it although basipetal wave is observed in monocots for lateral traces. In the junction region between the stem and leaf, the so-called procambial strand is made of vacuolated parenchyma cells (and not procambial cells) which connect stem procambium with leaf procambium. The xylem elements that differentiate in this transition region is also morphologically very different and are unlike primary xylem tracheary elements that normally differentiate from procambium. From the transition region, procambialization into the leaf is acropetal, but is basipetal in the internode. The transverse wave of procambial differentiation is both centripetal and centrifugal so as to increase the transactional area of its occupation; surrounding non-procambial cells also contribute to the procambial mass.

The longitudinal wave of primary vascularization from the procambium follows the wave described for procambialization. The transverse wave is bidirectional in each procambial strand with the protophloem elements arising first towards periphery of the strand and then extending towards the centre of the strands while the protoxylem elements arising first in the strands towards the centre of the stem and extending towards the periphery of the strand. The metaxylem and metaphloem meet at the centre of the procambial strand, often leaving a few cells of procambium in the middle in dicots and gymnosperms but often not in monocots; however, these procambial cells become parenchymatous. The concepts of protoxylem and metaxylem (and protophloem and metaphloem) are often disputed (Easu 1965; Krishnamurthy 2015), since all possible distinctions proposed between them so far have exceptions.

4.7.4 Axillary Buds and Branches

In some lower vascular plants, bisection occurs in the SAM itself to result in true *dichotomous branching*. When a branch occurs laterally, at or near the shoot apex, from the axillary buds, the branching is called *monopodial*, and this is the most common type of branching in seed plants. In many angiosperms, the SAM of the main stem becomes a floral meristem, and its function is taken over by an axillary meristem which again ends after some time in a floral meristem and this process continues. This type of branching is called *sympodial* branching (Reinhardt and Kuhlemeir 2002). Genes regulating sympodial branching have been recorded. In the tomato mutant *self-pruning* (*sp*), the sympodial branching units are successively reduced. The dominant *SP* gene is orthologous to *CEN* and *TFl* genes of *Antirrhinum* and *Arabidopsis,* respectively.

Details on the histological origin and structure of axillary buds can be found in Evert (2006) and Krishnamurthy (2015). Details on additional or

accessory axillary buds and adventitious buds are provided in Krishnamurthy (2015). Experimental evidence indicates that the axillary buds are determined by the 'subtending' leaves (Snow and Snow 1942). If a leaf primordium is removed surgically before its 'axillary' bud is initiated, the bud would fail to develop, but even if a small portion of the base of the leaf primordium remains after surgical removal, the axillary bud is triggered to develop (Snow and Snow 1942). This observation is supported by an analysis of the *Arabidopsis phabulosa-1d* (*phb-1d*) mutant, where the abaxial (lower surface) leaf fate is transformed into adaxial (upper) leaf fate resulting in axillary bud development on the underside angle that the leaf makes with the stem instead of the normal upper side angle of the leaf (McConnell and Barton 1998); this emphasizes that the basal region of the adaxial leaf fate plays an important role in bud development. Transcripts of members of a family of maize genes called *KNOX* and *ROUGH SHEATH* (*RS*) are expressed in the axillary bud (and internode) near the base of leaves. *KN1*, *KNOX* and *RS* genes together are reported to predict the sites of axillary bud and its associated metameric components.

Another important phenomenon associated with axillary buds and branching is *apical dominance*. It is commonly observed that the actively growing terminal bud often slows down or even inhibits the development of the axillary (= lateral) buds into branches and that if it is curtailed through pruning, axillary buds sprout into branches. This phenomenon is called apical dominance. This is intimately related to the branching habit of the plant. A very well-coordinated control of apical dominance and the activity of lateral buds is responsible for the evolution of the 23 basic architectural patterns recognized in tropical trees. Apical dominance is strictly enforced in tall and unbranched plants like coconut and papaya but is highly flexible in branched and bushy taxa. Auxin, cytokinin and a few other less characterized chemical factors have been shown to mediate apical dominance through their regulation on axillary buds. Application of auxin on excised terminal bud region prevents axillary bud development and brings back apical dominance. Similarly, application of cytokinin to axillary buds of an apically dominant plant released their inhibition to develop into branches (Cline 1991) showing the interplay of auxin and cytokinin in apical dominance. Transgenic tobacco plants incorporated with a chimeric gene that encodes for the enzyme isopentenyl monophosphatase show a vast increase in cytokinin and the extensive development of lateral branches (Li et al. 1992). Transgenic *Petunia* incorporated with an IAA biosynthesis gene promoted apical dominance by preventing branches (Sitbon et al. 1992). The presence of a third hormone that plays a central role in apical dominance by regulating axillary bud dormancy is based on studies made in *Arabidopsis*, rice and pea mutants with increased branching. The existence of this signal, an unknown carotenoid-based hormone, was proved by reciprocal grafting experiments. This hormone moved acropetally from the roots into the shoot. The levels of root-synthesized terpenoid hormones called *strigolactones* (*SLs*) were reduced in these mutants and an exogenous application of SLs rescued the shoot branching phenotypes. Hence, SLs are a novel and specific inhibitor of axillary bud outgrowth. Not much is known about the receptor of SL, but it is likely that *DWARF14* gene product may be the receptor (Pautler et al. 2013).

Some information on genetic control of apical dominance and branching is now available. The maize mutant *barren stalk 1* (*ba1*) encodes a basic helix-loop-helix (bHLH) transcription factor that is required to establish axillary meristems (AMs) in vegetative (and reproductive) stages (Gallavotti et al. 2004). Compared to teosinte, the closest wild relative of maize, and in the *teosinte branched1*(*tb1*) mutant, most nodes are branched, while in wheat, branching is limited to occasional nodes and hence apical dominance is present (Reintardt and Kuhlemeier 2002). Some parallels are there between *TB1* gene, that suppresses branching (Doebley et al. 1997) and the *CYCLOIDEA* (*CYC*) gene of *Antirrhinum majus,* that suppresses the growth of floral organs. In rice, when *lax1* mutant is combined with the *monoculm* (*moc1*) mutant (double mutant), vegetative AMs are completely abolished and highly

reduced in number in *lax1 lax2* double mutants (Tabuchi et al. 2011). The *GRASSY TILLERS1* (*GT1*) mutant has reduced AMs (and hence tillers) in rice. The *lateral suppressor* (*ls*) mutant gene of tomato also suppresses axillary bud development into branches.

4.8 Development of Root

Whether one accepts the QC as the promeristem, supports the view that the meristematic initials are present along the margins of QC or considers both the QC and the meristematic initials as the promeristem, there is no difference of opinion regarding the fact that the root tissues are developed from this region. Cells in this part of RAM are believed to be controlled by their position and positional information from their more mature derivatives rather than by any intrinsic characteristic when they give rise to the different regions of the root. This is proved by laser ablation experiments carried out in *Arabidopsis* RAM (Scheres and Wolkenfelt 1998; Van den Berg et al. 1997). When any cell of the different histogens or the QC is selectively ablated, an adjacent cell takes over its role; however, if derivatives of these initials are ablated, for example, cortical derivatives, the cortical initial was unable to generate the cortical cells including endodermis. This is contrary to the more traditional view that the RAM is an autonomous pattern-generating machine.

Both the root body and the rootcap are envisioned as consisting of files of cells emanating from the promeristem (Rost 1994). However, the limits of the classically recognized meristematic, elongation and maturation zones (Ivanov 1973) are not clear-cut, since all the tissue files of any of these three zones do not end at the same level in the root body. Cell division, cell expansion and cell maturation overlap not only in different tissue regions but also in the different cell files of the same tissue region and even in individual files. What regulates the differences between adjacent cell files and what regulates the spatial modulation of the transition points between cell division and expansion and between cell expansion and maturation (Ivanov 1973) are not clearly studied (Rost 1994). Transition points can move within a cell file relative to the growth rate of the root, since in fast-growing roots, differentiation events occur farther from the root tip than in slow-growing roots. Cell division activity in different tissue layers stopped at various distances from RAM, and it invariably extended for greater distance in the dermal layer than the remaining tissue regions. Attention was already drawn to the *formative divisions* or T-divisions (radial or periclinal divisions) that makes one cell file into two thus increasing the diameter of the root body. The other type of division is the *proliferative division* or transverse division which occurs within each file. Each file apparently maintains a more or less fixed number of proliferative divisions (Rost 1994). For instance, the tracheary element precursor cell in *Vicia faba* root divides five times, while parenchyma cells divide seven times. The proliferative divisions have at least two functions: (1) they provide a continuous supply of new cells to ensure the growth of the root, and (2) they coordinate cell length through the restriction of number of cell divisions in each file. Groups of cells of common ancestry, called *cell packets*, are often seen in the different files after proliferative cell division.

Usually, the cortex matures first; the prospective metaxylem cells can also be distinguished very close to the apex, especially in taxa without pith. The primary xylem and phloem differentiate at different radii, and in a mature root, they are arranged alternately as seen in T.S. The number of radiating groups of the exarch xylem and phloem may vary from two to several depending on the taxon and sometimes in the same taxon depending on the size of the root.

4.8.1 Lateral Roots

Lateral roots are endogenous in origin and are positionally related to pre-existing vascular tissues, especially opposite to protoxylem poles. They always arise far away from RAM, through periclinal cell divisions in pericycle, although in ferns they arise close to the RAM, through cell divisions in endodermis (not from pericycle).

The lateral root primordium is almost akin to the radicle, but the QC arises de nova in lateral roots. The lateral primordium pierces through the cortex before it emerges out. Auxin promotes lateral and adventitious root formation (Aloni et al. 2006). Exogenous application of auxins promotes lateral root initiation and development in many plants, especially in stem and root cuttings. Other than auxins, thiamine, nicotinic acid, adenine and one or a few micronutrients are needed for lateral root development. The importance of auxin is revealed by the study of the *Arabidopsis* mutants such as *superroot* (*sur*) and *aberrant lateral root formation* (*alf*). The former produces many adventitious roots from parts other than roots of seedlings due to an elevated level of auxin. The second mutant groups overproduce lateral roots (in *alf1-1*) or no lateral root at al1 (in *alf4-1*), while in *alf3-1* mutant, the growth of lateral root primordia produced by pericycle is arrested and they do not emerge out. These mutants can be rescued by auxin treatment. The other genes involved in lateral root ontogeny are genes that code for cell size regulatory proteins and cyclins such as *CDC2* and *CYC* (Martinez et al. 1992), while *CYC* gene promotes their growth out of the parent root cortex. Cytokinins inhibit lateral root formation and reverse the auxin effect. Ethylene promotes both lateral and adventitious roots.

4.9 Meristems Involved in Latitudinal Growth

Latitudinal growth is also called growth in girth or thickness and is seen in root and stem. It is normally seen in dictos and gymnosperms once after primary longitudinal growth is over, but in some monocot stems, a type of latitudinal growth is seen simultaneously with primary growth. This primary latitudinal growth is due to a meristem called *primary thickening meristem* (*PTM*). Secondary latitudinal growth takes place through a *secondary thickening meristem* (*STM*) in some monocot stems and through a *vascular cambium* (*VC*) in the stems and roots of dicots and gymnosperms.

4.9.1 Primary and Secondary Thickening Meristems (PTM and STM)

In many arborescent, semi-arborescent and a few herbaceous monocot taxa, PTM is found (DeMason 1994; Swamy and Krishnamurthy 1974, 1979); these plants lack a true vascular cambium. PTM is found often from the embryo stage onwards and continues till the vegetative life of the plant. PTM occurs at the shoot apex, but yet the meristem is classified as a lateral meristem; it is also a primary meristem as the name implies. The most characteristic feature of the taxa with PTM is that the shoot apex produces a very quick succession of leaf primordia even when the plants are still in the seedling stage so that the SAM appears to be sunken below the surrounding leaf primordial tissues. Also, the adult diameter of the plant is often obtained even in the seedling stage itself (Fig. 4.13). The PTM occurs at the bases of all these leaf primordia at progressively declining angles from the youngest to the oldest leaf primordia, thus making the central cylinder of the stem wide and the cortex narrow. The sequence of events in the organization of PTM can be divided into three stages that almost occur simultaneously. The first event is the initiation of PTM cell files in the crown, which happens immediately adjacent to the base of the SAM and must occur by anticlinal divisions in the youngest cell files. During the second event, there is elongation of these cell files by periclinal cell division activity in the PTM followed by cell enlargement along the length of the files. In the third event, there is a reorientation of the cell files to a horizontal plane. The cell files repeatedly cut off cells which contribute to increase the width of the stem. The period of maturation of growth in girth of stems is centrifugal in the central cylinder and centripetal in the cortex (DeMason 1994). The tissues derived from PTM include ground tissue and procambial strands.

A number of arborescent and semi-arborescent species of monocots show thickening growth by STM (Fig. 4.13). This has been wrongly called by many botanists vascular cambium. There is functional and developmental relationship

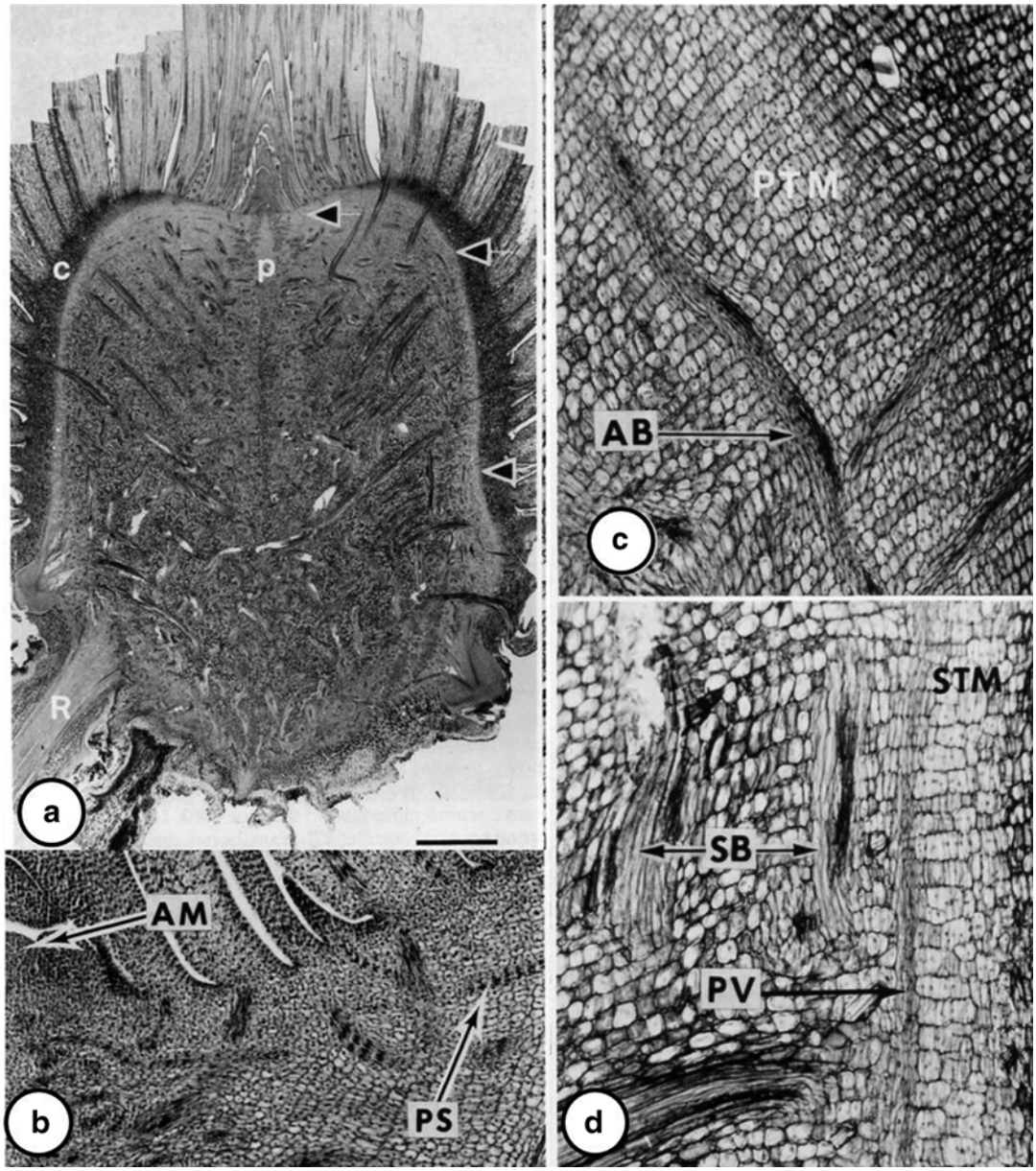


Fig. 4.13 *Yucca whipplei*. (**a**) Median L.S. of the apex of 1-year-old stem. *Arrows* indicate primary secondary thickening meristems; (**b**) L.S. of apical meristem and the subjacent primary thickening meristem; (**c**) meristematic activity confined to a recognizable zone of tangentially flattered cells; (**d**) secondary thickening meristem region. *AB* primary axial bundle, *C* cortex, *P* pith, *PS* procambial strand, *PTM* primary thickening meristem, *PV* secondary provascular strand, *SB* secondary axial bundle, *STM* secondary thickening meristem (Diggle and DeMason 1983)

between the PTM and STM (DeMason 1994). If the two meristems are developmentally related, then the function of the STM should be related to the function of PTM, PTM and STM should be longitudinally continuous in an actively growing stem and the STM should arise for the first time in the stem in relation to the PTM. Studies on taxa with STM have shown that all three criteria proposed above are fulfilled. Thus, the PTM and STM form a continuum. The STM produces

derivative cells mainly towards inside, although in some taxa, it may also contribute parenchyma cells towards outside. The derivatives produced towards inside develop into parenchyma cells as well as into vascular bundles or strands containing xylem and phloem that get embedded in the parenchyma tissue. In some taxa, the parenchymatous derivatives may become fully or partially differentiated into sclerenchyma, particularly around the vascular bundles.

4.9.2 Vascular Cambium

Vascular cambium (*VC*) is a typical lateral meristem that contributes to latitudinal growth or *secondary growth* of stem and root of gymnosperms and dicots. The reports of a VC in monocots (Rangarajan and Swamy 1980) and pteridophytes (Bhambie 1994; Cichan and Taylor 1990) and in the appendicular organs like leaf are erroneous since their vascular meristems have neither the typical structure nor the characteristic activity and behaviour of VC. The VC normally is bifacial (Larson 1994) and forms *secondary xylem* or *wood* on the inside and *secondary phloem* or *bast* on the outside (Fig. 4.14). The fusiform initials of only seed plants show anticlinal divisions, but not the so-called cambial initials of pteridophytes. The duration of production of wood and phloem by VC decides the degree of growth in girth of the plant axis, and this prodcution happens till the life of the tree to result in very massive stems as in some giant conifers and *Eucalyptus*. Secondary growth through VC involves highly plastic developmental processes, which are reflected through extensive anatomical and functional variations that are observed both within individual plants and among plants. The degree of VC activity in herbaceous taxa is very limited. In most taxa, there is only one cambial ring, but in some lianes and trees, more than one ring is formed to result in successive zones of wood and phloem.

4.9.2.1 Origin of Vascular Cambium

We must speak of origin of VC in any plant from two angles: in terms of evolutionary origin and in terms of physical origin. Although Cichan and Taylor (1990) have spoken of an independent origin of VC in arborescent lycopods, sphenopsids, *Rhacophyton* (all pteridophytes) and seed plants, for reasons mentioned in the previous paragraph, only seed plants should be considered as having a true VC. The fern *Botrychium* once considered to have a VC is now proved to be without it. Krishnamurthy (2005) has discussed in detail the various aspects of evolutionary origin of VC and had concluded that it evolved with the evolution of eustely, true arborescence and branching habit in vascular plants. The plants had tried different

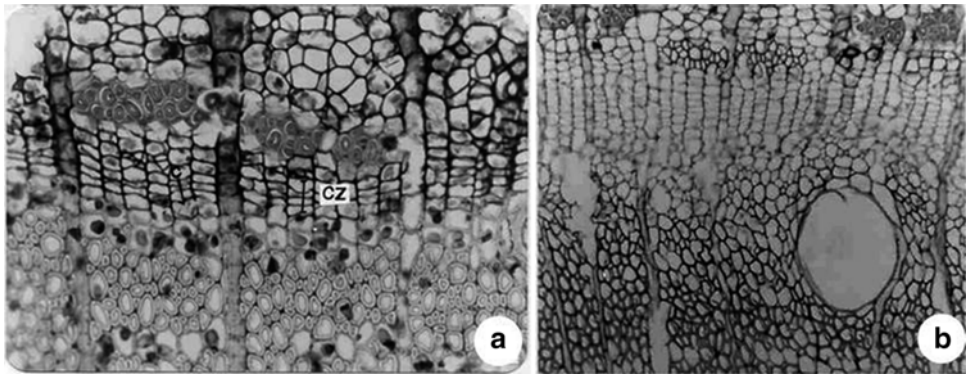


Fig. 4.14 (**a**) T.S. of stem of *Dalbergia sissoo* showing dormant cambial zone (*CZ*) with secondary xylem below and secondary phloem (with fibres) above cambial zone; (**b**) T.S. of stem of *Albizia amara* showing actively dividing cambial zone, secondary xylem (below cambium) and secondary phloem (above the cambium) (Photographs courtesy of Dr. N. Venugopal)

methods of meeting with arborescent habit, but selection pressures prevailed in retaining VC as the best suited to taxa with arborescent branching habit, i.e. gymnosperms and dicotyledons. The bifacial and true VC of extant seed plants may share a common evolutionary origin that predates the divergence of gymnosperms and angiosperms (Spicer and Groover 2010) and may even predate the origin of seed. Within angiosperms, results from molecular phylogenetic analysis and character state reconstructions support the idea that a VC is a feature of both basal angiosperms and early-diverging eudicots, but it is not clear whether VC had a single or multiple origins.

With reference to the physical origin of VC in gymnospermous and dicotyledonous taxa, the consensus of opinion (although wrong) among many plant morphologists is that it originates from procambium. They believed that after production of primary xylem and primary phloem, the leftover procambium of the vascular bundle gives rise to the VC after little or no modification. According to these people, procambium and VC are to be looked upon as two developmental stages of the same meristem (Esau 1965) and that separate terms like procambium and cambium are needed only for convenience (Sterling 1946). This is in spite of the fact that there are very distinct differences between procambium and VC in terms of structure and organization (Fahn et al. 1972; Swamy and Krishnamurthy 1980; Krishnamurthy 2015). All vascular plants have procambium, but only dicots and gymnosperms have a VC; then why all vascular plants do not have a VC? These people have not answered this question so far. In an elaborate analysis, Swamy and Krishnamurthy (1980) not only have given evidences for non-procambial origin of VC in both stems and roots but also have shown that procambium is not at all required for the origin of VC. They have also shown that in all instances, parenchyma cells give rise to VC (Swamy and Krishnamurthy 1980; Krishnamurthy 2015). The parenchyma cells of dictos and gymnosperms are more totipotent and require fewer inputs to redifferentiate into meristematic cambial initials than those of pteridophytes and monocots. They also need only very few changes to become cambial initials as they are structurally very close to fusiform initials of VC than the latter are to procambial cells. There are four types of VC, based on topography (Krishnamurthy 2015): (1) The primary vascular tissue has the form of an almost continuous vascular cylinder in the internode so that the interfascicular regions are either absent or extremely narrow. Hence, the VC forms a continuous cylinder so also the secondary vascular tissues formed from it. (2) The primary vascular tissue forms discrete strands/bundles, but the VC (due to conversion of interfascicular parenchyma cells into VC cells) and the secondary vascular tissues formed from it form a continuous cylinder. (3) The VC forms a continuous cylinder, but it cuts off secondary vascular tissues only in the fascicular regions but not in the interfascicular regions where only parenchyma cells are cut off. So both primary and secondary vascular tissues have the appearance of a system of discrete strands. (4) The VC does not form a continuous cylinder but occurs as strips in the fascicular region only; hence, secondary vascular tissue production is restricted only to the fascicular regions.

4.9.2.2 Structure of Vascular Cambium

The VC consists of two morphologically distinct types of initials: *fusiform* and *ray initials* (Fig. 4.15). These, respectively, give rise to the vertical and horizontal systems of secondary vascular tissues. The ratio of fusiform to ray initials varies in VC of different taxa (Iqbal 1994). In some taxa, ray initials are totally absent leading to secondary raylessness in the vascular tissues. The relative arrangement of the two types of initials in the VC also varies with taxon. In non-storeyed (also spelled as *non-storied*) or *non-stratified* type of VC (Fig. 4.15a), the fusiform and ray initials, as seen in TLS, have highly overlapping arrangement. In *storeyed* (also spelled *storied*) or *stratified* type of VC, both these initials or at least the fusiform initials have a more or less distinct tiered arrangement, as seen in TLS (Fig. 4.15b). While the former type is the predominant type seen in all gymnosperms and the majority of dicots, the stratified type is seen in some members of Malvaceae,

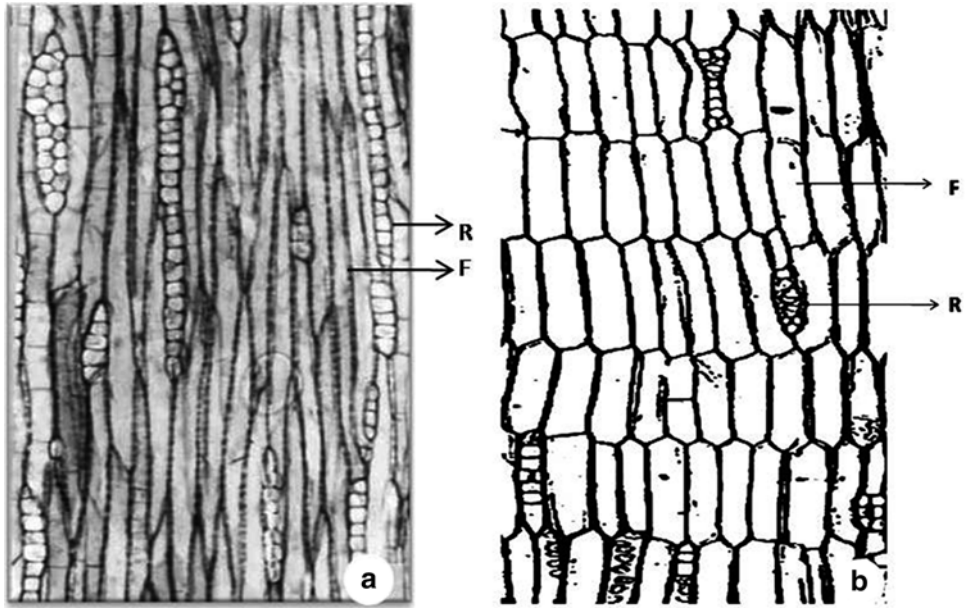


Fig. 4.15 (**a**) T.L.S. of non-storeyed vascular cambium of *Albizia amara*, (**b**) T.L.S. of storeyed cambium of *Aeschynomene aspera. F* fusiform initial, *R* ray initial (**a** Courtesy of Venugopal; **b** based on Phillipson et al. 1971)

Leguminosae, etc. Moreover, the fusiform initials in the stratified type are shorter (around 120 μm), while they are considerably longer in the non-stratified type (around 450 μm in dicots to up to 9,000 μm in some conifers). However, the length of the fusiform initial (called genetic length) varies in the same plant with age—shorter in young trees, reaching the maximum at around 60 years and then maintaining a constant length.

The fusiform initials, which form the vertical system of VC, are tangentially flattened, pointed at both ends and possesses between 8 and 32 facets (with an average of 18–21); each initial will be in contact with at least 14 other cells. The volume of these initials is tens or hundreds of times of the initial of SAM. The ratio between length and width varies from 30:1 to 600:1 depending on the taxon. These initials are highly vacuolated in order to minimize the amount of cytoplasmic materials synthesized and to reduce energy expenditures at each cell cycle. Thus, the vacuoles economically extend the reach of the cytoplasm. Ultrastructurally, the cells are similar to vacuolated parenchyma cells. The cytoplasm contains larger mitochondria, differentiated plastids, peroxisomes, ribosomes, lipid bodies, dictyosomes, ER, microtubules, lomasomes and storage substance. The cell walls have fibrillar architecture so as to enable the regulation of direction of growth in the expanding wall. The wall also meets the conflicting demands of cohesion and extensibility of the cells and their derivatives. Radial (R) walls are usually thicker than tangential (T) walls, particularly during dormancy of cambium and it is also beaded. The chemical composition of these walls is also slightly different since T-walls are produced afresh at each periclinal division and their expansion is limited, while R-walls remain plastic and undergo a constant radial extension. The cell walls of cambial zone exhibit a far greater radial enlargement (up to 100 times) than that of tangential enlargement. Radial walls have a cellulose skeleton embedded in xyloglucans and arabinogalacturan proteins, while T-walls have xyloglucans and very little or no acidic pectins and xylans. The ray initials form the horizontal or radial systems of VC. In TLS, they are of variable height depending on the taxon, with varied number of cells and thickness. They may be *uni-*, *bi-*,or *multiseriate* (Figs. 4.15 and 4.16) and may be *homo-* or *hetero-cellular* (respectively with only

Fig. 4.16 T.L.S of Cambial zone of *Gmelina arborea* showing multiseriate ray initial (R) (Photo by S. John Adams)

procumbent or *erect cells* or with both). The cells have shorter tangential than radial diameters (very clearly evident in RLS). Ray initials have well-developed cell-to-cell connections through plasmodesmata (more in tangential than in radial walls) and constitute the main symplastic route for horizontal transport of signal molecules and other chemicals.

One of the most debated aspects of VC is whether it has a single layer of initials or more than one layer, i.e. whether it is a cambial zone. The debate is mainly due to the fact that it is very difficult to identify a single initial layer even during the most dormant condition and even with more highly sophisticated instruments and techniques, when a maximum of four layers of cells are seen in the cambial zone (Fig. 4.14a). Even if a single initial layer is assumed to be present, it cannot be distinguished from its immediate derivative layer(s). This debate is made more complicated by the naming and semantic controversies, especially with reference to the application of the term 'cambium' to the component layers. For those who believe in the single initial layer concept, this term is applicable only to this initial layer, while for those who believe in the cambial zone concept, the term is applicable to the entire zone. However, the importance of VC should not be lost in the terminology conflict (Iqbal and Ghouse 1990).

4.9.2.3 Activity of Vascular Cambium

The fusiform initials cut off secondary xylem on the inside and secondary phloem on the outside through tangential, i.e. periclinal cell divisions. Simultaneously the ray initials cut off xylem and phloem rays. The mechanism of periclinal division in the fusiform initial has already been described in Chap. 3 of this volume. The volume of secondary xylem cut off by the VC ring is several times more than that of the secondary phloem. Hence, the extent of outward shift of the cambial ring is determined by the volume of secondary xylem added on the inside. This should be followed by appropriate increase in the circumference of the cambial ring with concomitant and proportionate increase in the number of fusiform and ray initials, and this increase in cell number is taken care by the anticlinal cell divisions of fusiform initials. The detailed mechanism of anticlinal division is described in Chap. 3 of this volume. Once derivatives are cut off on the two sides of the cambial ring, they differentiate into secondary xylem and phloem tissue. This differentiation occurs in three phases (Krishnamurthy 2015): (1) The phloem and xylem derivatives may divide further. (2) The cells that cease dividing usually enlarge in radial direction (and in the case of large vessel elements even in tangential direction) and start differentiation events specific to each cell type of xylem and phloem. (3) Phenomena such as second wall formation and lignification in tracheary elements and in phloem and xylem fibres, sieve area development in sieve elements, development of pits and plasmodesmata etc. take place subsequently.

Within limits of genomic control, VC operates under the influence of internal physiological processes and external environmental factors. The VC invariably becomes dormant during times of extreme cold (in winter) and hot temperatures (in

summer), respectively, in temperate and tropical taxa. The activation of VC, a deterministic process, begins when stress due to extreme temperatures is removed with the approach of favourable season. Thus, cambial activity is periodic rather than continuous. Even in those tropical environments where there are no extremes of temperature, the evergreen trees growing there do show some degree of unequal cambial activity, at least during 1 or 2 months in a year. In taxa with rhythmic cambial activity, the number of times the VC becomes dormant/active is one each in a year, but in certain taxa, more than one dormant/active period may be seen within a year, depending on prevailing environment. During the dormant period, the cambial zone is reduced to three or more commonly four layers, and one of these layers is likely to be an initial layer and the one or two layers inside should be xylem derivative(s) and the one or two layers outside should be phloem derivative(s). The cells of this dormant cambial zone store a number of chemical substances and have no or fewer vacuoles. The cytoplasmic organelles become less abundant or poor; the ER invariably becomes smooth and the plasmalemma is thrown into folds. The water content is also greatly reduced and growth inhibitors accumulate.

There is a close relationship between bud break and cambial reactivation in temperate trees and between flushing of new leaves and cambial reactivation in tropical trees. Hence, the number of times of cambial reactivation depends on the number of times of bud break/new leaf flushing noticed in a year. This means that initiation of longitudinal growth precedes the initiation of latitudinal growth. Most researchers believe that auxins produced during bud break and in the newly flushing leaves stimulate cambial reactivation through their effect on initiating cell divisions. In temperate trees, cambial reactivation is initiated just a week to 15 days before visible bud break, but in tropical trees, it happens just after leaf flushing. During cambial reactivation, the level of growth hormones increases in its cells. The initiation of periclinal divisions is usually preceded by cell enlargement with increase water content. The cell expansion is predominantly in the radial direction. Soon a broad cambial zone of several layers is formed (Fig. 4.14b). The cytology of active fusiform initials is already described. The storage products are exhausted in and around cambial zone and used up to form new cell materials during cambial reactivation; metabolic rate increases.

Cambial reactivation in tropical trees happens mostly only once in a year and, depending on the taxon, it happens between January and early July. The same species may show cambial reactivation at different periods within these months depending on its location. For example, in teak reactivation of cambium happens in early March or between early June and early July and in *Ricinodendron heudelotii* in February to March or in December. More than one cambial reactivation/dormancy period is seen in *Holarrhena floribunda* (February to March and September) and in *Psidium guajava* (March and July). There is also variation in the production period of secondary xylem and phloem. Their production need not be simultaneous as is generally believed by many.

One major result of the nonuniform activity of VC through a year or part thereof is the production o*f growth rings*. The differences noticed in the quality and/or quantity of xylem elements produced by the VC during its active period and during its approach to dormancy cause the visibility of growth rings. Usually evergreen trees with continuous cambial activity do not show growth rings and are characterized by *diffuse porous woods* in which the diameter of vessel elements (= pores), as seen in T.S., is of uniform size. On the contrary, in trees with seasonal cambial activity vessels, elements produced during active period are with greater diameter and the diameter gradually reduces towards the dormancy period to result in *ring porous woods*. This, along with other features such as lack of vessel production, production fibres with smaller transactional diameter, thick-walled fibres or only parenchyma, dense wood etc., may mark a growth ring; this part of the wood is often called *late wood* in contrast to *early wood* formed during very active period of cambium. The so-called *annual rings* are growth rings but not all growth rings are annual. False ‘growth’ rings may result

due to the effects of drought, frost, flooding, defoliation, air pollution etc. Hence, a critical study of growth rings of a tree (called *dendrochronology*) would throw a lot of light on past climates in any area.

A study of cambial variants (i.e. deviations from the normal presence of a single cambial ring and its normal activity of producing xylem inside and phloem outside in continuous cylinders) and of the structure of the secondary xylem and phloem produced by the VC is beyond the scope of this article, and a useful review of the same can be found in Evert (2006), Spicer and Groover (2010) and Krishnamurthy (2015). However, some discussion must be focused on *reaction wood* (*RW*). The formation of RW by VC is a very important mechanism that is vital for a free species to effectively function. As the main stem bends or as a plagiotropic branch grows, it might be expected to bend downwards because of its increased weight due to production of more and more twigs and leaves. Such a phenomenon is often resisted by the formation of RW (Krishnamurthy 2007). Trees with RW have an increased wood production on either the upper or lower side of the bent main trunk or leaning branches through increased cambial cell divisions. In conifers, RW is formed on the lower side of the leaning main stems and branches, and this wood by expansion (compressive strain) pushes such stems and branches more upright and maintains a more constant branch angle. This type of RW is called *compression wood* (CW). The tracheids of this RW are rounded in T.S. and with intercellular spaces (Fig. 4.17). The tracheid walls are abnormally thick and contain more lignin than normal wood and less cellulose than usual. These tracheids by unequal expansion bring about the upward push needed to make the leaning part erect. In dicots, RW is formed on the upper side of the leaning branches and bent stems and *contracts* (tensile strain) to *pull* the bent main stem erect and the branch towards the trunk by tension to enable the branches to maintain a constant angle. This type of RW is called *tension wood* (*TW*). TW becomes more pronounced at greater leans (10–20°) both through increase in *gelatinous fibres* (*G-fibres*) (Fig. 4.18), a special category of xylem fibres, and through more highly developed G-fibres in terms of the

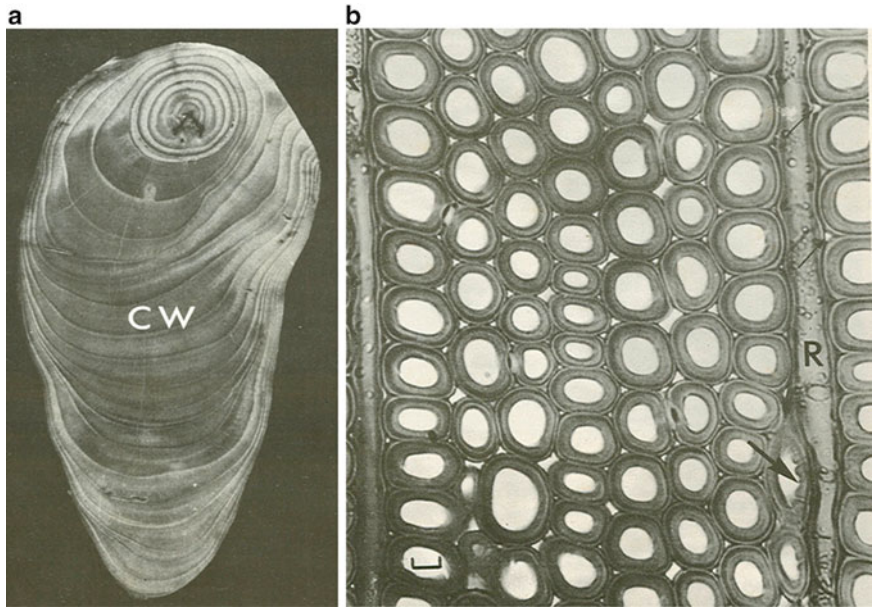


Fig. 4.17 Compression wood (*CW*) of *Picea abies*. (**a**) T.S. of whole wood; (**b**) a portion of the compression wood enlarged to show circular tracheids with intercellular spaces. R ray (Timell 1973)

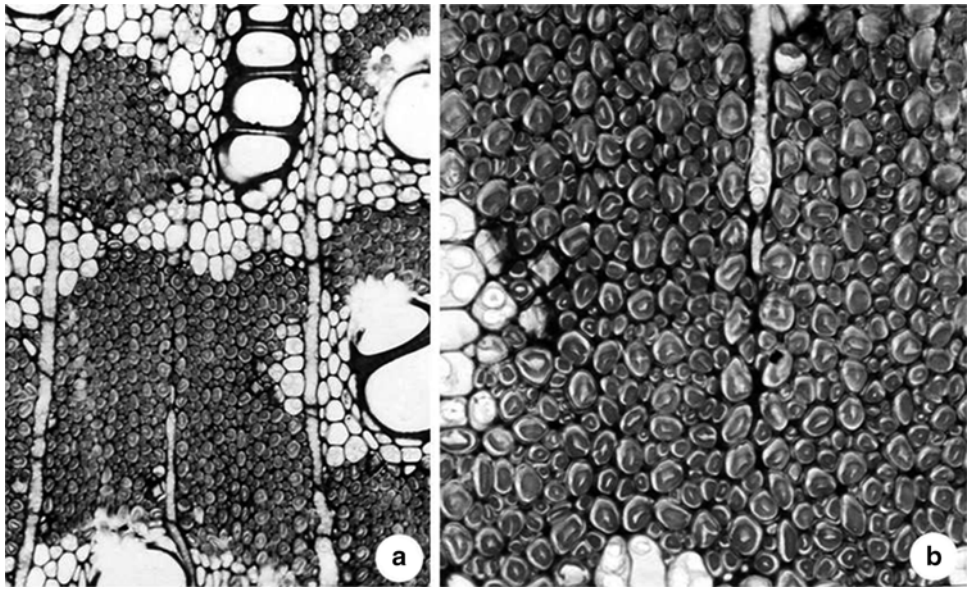


Fig. 4.18 T.S. of tension wood of *Cassia* sp. showing gelatinous fibres (**a**); portion of the same enlarged (**b**) (Krishnamurthy 2015)

thickness of the gelatinous layer (*sg-layer*). These fibres through their selective shrinkage bring about the correction needed in the leaning stem and bending branches. These fibres have secondary walls that are thicker than those in normal wood (NW) because they have an additionally, often convoluted, cellulose-rich sg-layer that additionally has carboxylated acidic polysaccharides. TW, unlike CW, is poor in lignin. Ten fasciclin-like arabinogalactan-rich proteins are specifically expressed in TW, but not in cambial zone. Additionally extensins (hydroxyproline-rich proteins) are localized immunocytochemically close to the inner part of the sg-layer of G-fibres (Hariharan and Krishnamurthy 1995; Lafarguette et al. 2004; Jothi et al. 2010). These structural stress proteins are likely to be linked to specific mechanical properties of TW. RW could be a gravitropic response and a mechanism to maintain the plagiotropic growth of branches at a constant angle or a response to tension and pressures resulting from bending or both, but gravity appears to be more important (Timell 1986; Krishnamurthy 2007), and this may be the cause for the strains that are developed and the bending movements that these strains generate. The functional importance of RW may be summarized as follows: Each and every tree has a specific architecture, particular branching pattern and specific branch angles which are all needed to have that particular crown geometry to optimize light capture and gas exchange. Any deviation in branch angle through added weight in the form of more twigs and leaves and bending would jeopardize this nice balance in crown geometry. Hence, RW is developed to correct this bending through extension of tracheids in the CW of conifers on the underside of such bending branches or through shrinking of G-fibres in the TW of dicots on the upper side. Correction is made every time the branch angle changes to added weight (Krishnamurthy 2007).

4.9.2.4 Genetics of Vascular Cambium

There is clear evidence for overlap in the genetic mechanisms controlling the SAM and vascular cambium (Groover 2005; Groover et al. 2006). This is evident from recent microarray analyses of gene expression that happens during cambial activity, which have revealed that key genes regulating SAM are also expressed in the cambial region or have paralogues that are expressed

there (Ko and Han 2004; Schrader et al. 2004). For instance, the class III homeodomain-leucine zipper (*PHA*/*PHV* and *ATHB-15*; *CORONA*) and *KANADI1* transcription factors responsible for abaxial-adaxial patterning in leaf primordia (operating in SAM), *SHORT-ROOT* (*SHR*) and potential orthologues of *AINTEGUMENTA* (*ANT*) and *PINHEAD* (*PNH*) are also expressed in the cambial region of poplar (Schrader et al. 2004; Spicer and Groover 2010). Similarly, putative *STM* orthologue called *ARBORKNOX1* (*ARK1*) and *BREVIPEDICELLUS* orthologue (*ARK2*) are known to be expressed in poplar (Groover et al. 2006; Spicer and Groover 2010). *ARK1* overexpression does not preclude secondary growth but serves to inhibit the onset and differentiation of secondary vascular tissues at the morphological level. Microarray analysis had helped to identify genes that got misregulated in response to *ARK1* overexpression. This analysis showed that 41 % of genes are up-regulated and that their proteins are involved in extracellular matrix (cell wall) synthesis or modifications, including proteins involved in cell identity and signaling, cell adhesion or cell differentiation. These gene expression differences are reflected in alterations of cell wall biochemistry and lignin composition in *ARK1* overexpression plants (Groover et al. 2006). Hence, *ARK1* is likely to act regulating cell fates of cambial derivatives through modification of the extracellular matrix. *ARK2* shows a broad expression pattern that includes not only the cambial zone but also developing secondary xylem and phloem (Du et al. 2009). *ARK2* expression levels are positively correlated with the width of the cambial zone and negatively correlated with the differentiation of lignified cell types in both secondary xylem and phloem fibres. The *Populus* orthologues of *Arabidopsis KANADI* genes *KAN1* and *KAN2* have the greater expression in secondary phloem and perhaps are required for phloem differentiation.

The patterning and polarity of cambial activity and secondary thickening are regulated by hormones and genetic mechanisms (Spicer and Groover 2010), although the details of this interplay are still poorly understood. While auxins were once proposed as simple morphogens that show a gradient across the cambial zone and its derivatives, a study of Nilsson et al. (2008) suggests that this attractive hypothesis may be an oversimplification or incorrect. Auxin-responsive genes were identified in *Populus* cambium using microarrays, but the expression levels of auxin-responsive genes across the cambial zone and its derivatives were poorly correlated with the auxin gradient. In addition, transgenic *Populus* that expresses a dominant mutant form of *PttIAA3* has altered auxin responses and has fewer cell divisions in the cambial zone and smaller lignified cell types in the wood. Hence, Nilsson et al. (2008) proposed that rather than acting as simple morphogens, auxins may regulate the expression of a few downstream regulators to affect key aspects of wood formation, particularly cell division in VC. Auxins, particularly their longitudinal gradients, also control the orientation of cambial initials and their derivatives as well as the relative rotation of cambial initials (Spicer and Groover 2010).

The class III HD-ZIP *Arabidopsis* orthologues in *Populus* such as *PHV*/*PHB*, *CAN* and *ATHB8* show the highest expression levels found in adaxial xylem tissue (Schrader et al. 2004). *Populus* plants expressing a dominant miRNA-resistant *Populus REV* transgene show patterning and polarity defects in wood and secondary phloem that include formation of ectopic cambia in the stem cortex which produce wood on the outside but not on inside. This class of genes is also involved in cambial initiation from parenchyma cells.

4.9.3 Phellogen

Phellogen or *cork cambium* is a lateral meristem closely related to VC. Phellogen and VC function in coordination with one another. Phellogen is located on the periphery of the axis of gymnosperms and dicots. Once the epidermal tissue are crushed by the VC derivatives, the phellogen has its origin in primary phloem, primary cortex, subhypodermis or even in the epidermis (before it is crushed). It generates through periclinal division

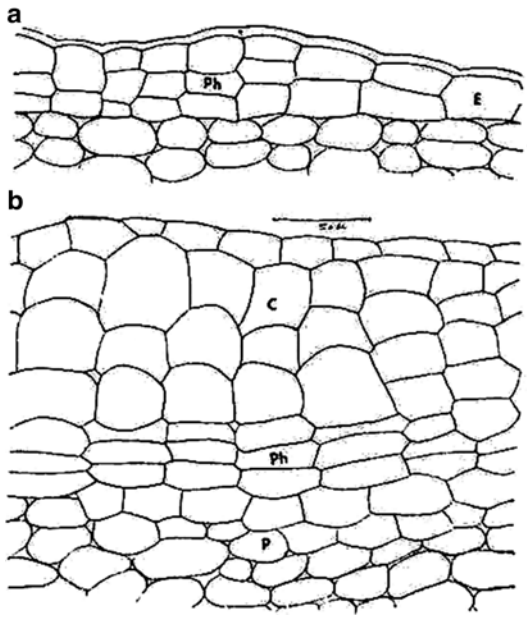


Fig. 4.19 (**a**) T.S. of the peripheral part of the stem of *Ipomoea* sp. showing the initiation of phellogen (*ph*) from epidermal tissue (*E*). (**b**) T.S. of the peripheral part of the stem of *Nyctanthes* sp. showing derivatives of phellogen (*ph*)-cork cells (*C*) towards outside and phelloderm (*P*) towards inside (Krishnamurthy 2015)

on its outside the suberized cork tissue or *phellem* and on the inside secondary cortex or phelloderm (Fig. 4.19). In many taxa, some of the outer derivatives of phellogen develop into *lenticels*, which are structures that facilitate gas exchange in place of stomata that are lost along with the epidermis. Phellogen may function perennially or is periodically replaced by successively developed phellogens derived from internal tissues. Like VC, there is controversy regarding its unilayered or zonate nature, again because of the difficulty in identifying the initial layer.

References

Aichinger E, Kornet N, Friedrich T, Laux T (2012) Plant stem cell niches. Annu Rev Plant Biol 63:615–636

Aida M, Beis D, Heidstra R, Willemsen V, Blilou I (2004) The *PLETHORA* genes mediate patterning of the *Arabidopsis* root stem cell niche. Cell 119:109–120

Aloni R, Aloni E, Langhans M, Ullrich CI (2006) Role of cytokinin and auxin in differentiation, lateral root initiation, root apical dominance and root gravitropism. Ann Bot 97:883–893

Barlow PW (1973) Mitotic cycles in root meristems. In: Balls M, Billett FS (eds) The cell cycle in development and differentiation. Cambridge University Press, Cambridge, pp 133–165

Barlow PW (1978) RNA metabolism in the quiescent centre and neighboring cells in the root meristem of *Zea mays*. Z Pflanzenphysiol 86:147–157

Barlow PW (1994) From cell to system: repetitive units of growth in the development of roots and shoots. In: Iqbal M (ed) Growth patterns in vascular plants. Dioscorides Press Portland, Oregon, pp 19–58

Barlow PW, Adam JS (1989) The response of the primary root meristem of *Zea mays,* L. to various periods of cold. J Exp Bot 40:81–88

Barton MK (1998) Cell type specification and self-renewal in the vegetative shoot apical meristem. Curr Opin Plant Biol 1:37–42

Barton MK, Poethig RS (1993) Formation of the shoot apical meristem in *Arabidopsis thaliana*: analysis of development in the wild type and in the shoot meristemless mutant. Development 119:823–831

Baurle I, Laux T (2003) Apical meristems: the plant's fountain of youth. Bioessays 25:961–970

Berleth T, Jürgens G (1993) The role of the monopteros gene in organizing the basal body region. Development 118:575–587

Bhambie S (1994) Secondary growth in pteridophytes. In: Iqbal M (ed) Growth patterns in vascular plants. Dioscorides Press Portland, Oregon, pp 185–210

Bitonti MB, Chiappetta A (2011) Root apical meristem pattern; hormone circuitry and transcriptional networks. In: Luttage U, Beyschiag W, Budel B, Francis D (eds) Progress in botany 72. Springer, Berlin, pp 37–71

Bolduc N, Yilmaz A, Meija-Grotewold E (2012) Unraveling the *KNOTTED1* regulatory network in maize meristem. Genes Dev 26:1685–1690

Bower FO (1947) The botany of living plants. Macmillan & Co Ltd, London

Bowman JL, Eshed Y (2000) Formation and maintenance of the shoot apical meristem. Trends Plant Sci 1:110–115

Byrne ME, Barley R, Curtis M, Arroyo JM, Dunham M, Hudson A, Martienssen RA (2000) *Asymmetric leaves1* mediates leaf patterning and stem cell function in *Arabidopsis*. Nature 408:967–971

Chicharmane VS, Gordon SP, Tarr PT, Heister MG, Meyerowitz EM (2012) Cytokinin signaling as a positional cue for patterning the apical-basal axis of the growing *Arabidopsis* shoot meristem. Proc Natl Acad Sci U S A 109:4002–4007

Choi DW, Song JY, Kwon YM, Kim SG (1996) Characterization of a cDNA encoding a proline-rich 14 kDA protein in developing cortical cells of the roots of bean (*Phaseolus vulgaris*) seedlings. Plant Mol Biol 30:973–982

Cichan MA, Taylor TN (1990) Evolution of cambium in geologic times- a reappraisal. In: Iqbal M (ed) The vascular cambium. Wiley, New York, pp 213–223

Cline MG (1991) Apical dominance. Bot Rev 57:318–358
Clowes FAL (1961) Apical meristems. Blackwell, Oxford
Clowes FAL (1978a) Origin of the quiescent centre in *Zea mays*. New Phytol 80:409–419
Clowes FAL (1978b) Origin of quiescence at the root pole of pea roots. Ann Bot 42:1237–1239
Clowes FAL (1981) The difference between open and closed meristems. Ann Bot 48:761–767
Clowes FAL (1984) Size and activity of quiescent centres of roots. New Phytol 96:13–21
Cook TD, Tilney MS, Tilney LG (1996) Plasmodesmal networks in apical meristems and mature structures: geometric evidence for both primary and secondary formation of plasmodesmata. In: Smallwood M, Knox JP, Bowles DJ (eds) Membranes: specialized functions in plants. BIOS Scientific, Cambridge, UK, pp 471–488
Deitrich RA, Radice SA, Harada JJ (1992) Downstream DNA sequences are required to activate a gene expressed in the root cortex of embryos and seedlings. Plant Cell 4:1371–1382
DeMason DA (1994) Stem thickening in monocotyledons. In: Iqbal M (ed) Growth patterns in vascular plants. Diosconides Press Portland, Oregon, pp 288–310
Deysson G (1980) Control of cell multiplication in root meristems of higher plants. Biol Cell 38:75–80
Diggle PK, DeMason DA (1983) The relationship between the primary thickening meristem and the secondary thickening meristem in *Yucca whipplei* Torr. I. Histology of the mature vegetative stem. Am J Bot 70:1195–1204
Doebley J, Stec A, Hubbard L (1997) The evolution of apical dominance in maize. Nature 386:485–488
Douin C (1923) Recherches sur le gamétophyte des Marchantiées. III.–Le thalle stérile des Marchantiées développement basilaire des feuilles et autres organs latéraux chez les Muscinées. Rev Gen Bot 23:487–508
Du J, Mansfield SD, Groover AT (2009) The *Populus* homebox gene *ARBORKNOX2* regulates cell differentiation during secondary growth. Plant J 60:1000–1014
Esau K (1943) Origin and development of primary vascular tissues in seed plants. Bot Rev 9:125–206
Esau K (1965) Vascular differentiation in plants. Holt Rinehart and Winston, New York
Evans MMS, Barton MK (1997) Genetics of angiosperm shoot apical meristem development. Annu Rev Plant Physiol Plant Mol Biol 48:673–701
Evert RF (2006) Esau's plant anatomy. Wiley, New York
Fahn A, Ben-Sasson R, Sachs T (1972) The relation between the procambium and the cambium. In: Ghouse AKM, Yunus M (eds) Research trends in plant anatomy. Tata McGraw-Hill, New Delhi, pp 161–170
Feldman LJ, Torrey JG (1976) The isolation and culture *in vitro* of the quiescent centre of *Zea mays*. Am J Bot 63:345–355
Fiers M, Golemiec E, Xu J, van der Geest L, Heidstra R, Stiekeme W, Liu CM (2005) The 14- amino acid CLV3, CLE19, CLE 40 peptides trigger consumption of the root meristems in Arabidopsis through CLAVATA2-dependent pathway. Plant Cell 17:2542–2553
Fisher JB, French JC (1978) Internodal meristems of monocotyledons: further studies and general taxonomic summary. Ann Bot 42:41–50
Fletcher JC (2002) The vegetative meristem. In: McManus MT, Veit BE (eds) Meristematic tissues in plant growth and development. Sheffield Academic Press, Sheffield, pp 16–57
Fletcher JC (2004) Stem cell maintenance in higher plants. In: Lanza R, Blau H, Melton D, Moore M, Thomas ED, Verfaille C, Weissman IL, West M (eds) Handbook of stem cells, vol 2, Adult and Fetal. Elsevier Academic Press, Amsterdam, pp 631–641
Foster AS (1938) Structure and growth of the shoot apex of *Ginkgo biloba*. Bull Torrey Bot Club 65:531–556
Friedman WF, Moore RC, Purugganam MD (2004) The evolution of plant development. Am J Bot 91:1726–1741
Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, Hamann T, Offringa R, Jürgens G (2003) Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. Nature 426:147–153
Galinha C, Hofhuis H, Luijten M, Willemsen V, Blilou I, Heidstra R, Scheres B (2007) *PLETHORA* protein as dose-dependent master regulators of *Arabidopsis* root development. Nature 449:1053–1057
Gallavotti A, Zhao Q, Kyozuka J, Meeley RB, Ritter MK, Doebley JF, Pè ME, Schmidt RJ (2004) The role of barren stalk1 in the architecture of maize. Nature 432:630–635
Giulini A, Waug J, Jackson D (2004) Control of phyllotaxy by the cytokinin-inducible response regulator homologue *ABPHYL1*. Nature 430:1031–1034
Gordon SP, Chickarmane VS, Ohno C, Meyerowitz EM (2009) Multiple feedback loops through cytokinin signaling control stem cell number within the *Arabidopsis* shoot meristem. Proc Natl Acad Sci U S A 106:16529–16534
Groover A (2005) What genes make a tree a tree? Trends Plant Sci 10:210–214
Groover AT, Mansfield SD, Difazio SP, Dupper G, Fontana JR, Miller R, Wang Y (2006) The *Populus* homebox gene *ARBORKNOX1* reveals overlapping mechanisms regulating the shoot apical meristem and the vascular cambium. Plant Mol Biol 61:917–932
Ha CM, Jun JH, Fletcher JC (2010) Shoot apical meristem form and function. Curr Top Dev Biol 91:103–140
Haecker G, Gross-Hardt R, Geiges B, Sarkar A, Breuninger H, Herrmann M, Laux T (2004) Expression dynamics of *Wox* genes mark cell fate decisions during early embryonic patterning in *Arabidopsis thaliana*. Development 131:657–668
Hallé F, Oldeman RAA, Tomlinson PB (1978) Tropical trees and foretes – an architectural analysis. Springer, Berlin
Hamann T, Mayer U, Jürgens G (1999) The auxin-insensitive bodenlos mutation affects primary root formation and apical –basal patterning in the *Arabidopsis* embryo. Development 126:1387–1395

Hanstein J (1868) Die Scheitelzellgrupe in Vegetationspunkt der Phanerogamen. Festchr Niederrhein Ges Natur Heilkunde 1868:109–134

Hanstein J (1870) Die Entwicklung der Keimes der Monokotylen und Dikotylen, In: Hanstein J (ed) Botanische Abhandlungen aus dem Gebiet der Morphologie und Physiologie, vol 1. pt. 1. Marcus, Bonn

Hariharan Y, Krishnamurthy KV (1995) A cytochemical study of cambium and its xylary derivatives on the normal and tension wood sides of the stems of *Prosopis juliflora* (SW.) DC. Beitr Biol Pflanzen 69:459–472

Heimsch C, Seago JL Jr (2008) Organization of the root apical meristem in angiosperms. Am J Bot 95:1–21

Hobe M, Muller R, Grunewald M, Brand U, Simon R (2003) Loss of CLE 40, a protein functionally equivalent to the stem cell restricting signal CLV3, enhances root waving in *Arabidopsis*. Dev Genes Evol 213:371–381

Imaichi R, Hiratsuka R (2007) Evolution of shoot apical meristem structure in vascular plants with respect to plasmodesmal network. Am J Bot 94:1911–1921

Iqbal M (1994) Structural and operational specializations of the vascular cambium of seed plants. In: Iqbal M (ed) Growth patterns in vascular plants. Dioscorides Press, Portland, pp 211–271

Iqbal M, Ghouse AKM (1990) Cambial concept and organization. In: Iqbal M (ed) The vascular cambium. Research Studies Press Ltd, Somerset, pp 1–36

Ivanov VB (1973) Growth and reproduction of cells in roots. In: Obrucheva NA (ed) Physiology of roots. Uniiti, Moscow, pp 1–40

Jiang K, Feldman LJ (2005) Regulation of root apical meristem development. Annu Rev Cell Dev Biol 21:485–509

Jothi R, Parthasarathy S, Krishnamurthy KV (2010) Computational internal sequence repeats analysis of accelerated evolution and the role of extensins under abiotic and biotic stresses. Open Access Bioinforma 2:157–168

Kamiya N, Nagasaki H, Morikami A, Sato Y, matsuoka M (2003) Isolation and characterization of a rice *WUSCHEL*-type homebox gene that is specifically expressed in the central cells of a quiescent centre in the root apical meristem. Plant J 35:429–441

Kenrick P, Crane PR (1997) The origin and early diversification of land plants- a cladistic study. Smithsonian Institute Press, Washington, DC

Kerk NM, Feldman LJ (1994) The quiescent centre in roots of maize: initiation, maintenance and role in organization of the root apical meristem. Protoplasma 183:100–106

Kerk NM, Feldman LJ (1995) A biochemical model for the initiation and maintenance of the quiescent centre: implications for organization of root meristems. Development 121:2825–2833

Kerk NM, Jiang K, Feldman LJ (2000) Auxin metabolism in the root apical meristem. Plant Physiol 122:925–932

Ko J, Han K (2004) *Arabidopsis* whole-transcriptome profiling defines the features of coordinated regulations that occur during secondary growth. Plant Mol Biol 55:433–453

Kornet N, Scheres B (2009) Stem-cell factors in plants: chromatin connections. Cold Spring Harb Symp Quant Biol 73:235–242

Krishnamurthy KV (1994) The angiosperm embryo: correlative controls in development, differentiation, and maturation. In: Iqbal M (ed) Growth patterns in vascular plants. Dioscorides Press, Portland, pp 372–404

Krishnamurthy KV (2005) Evolutionary origin of vascular cambium. In: Bahadur B et al (eds) Gleanings in botanical research current scenario. Dattsons, Nagpur, pp 413–443

Krishnamurthy KV (2007) In pursuit of understanding the tension wood riddle. J Ind Bot Soc 86:14–26

Krishnamurthy KV (2015) Growth and development in plants. Scientific Publishers, Jodhpur

Kuras M (1978) Activation of embryo during rape (*Brassica napus* L.) seed germination. I. Structure of embryo and organization of root apical meristem. Acta Soc Bot Pol 47:65–82

Lafarguette F, Leple JC, Dèjardin A, Laurans F, Costa G, Lesage-Decauses MC, Pilate G (2004) Popular genes encoding fasciclin-like arabinogalactan proteins are highly expresses intension wood. New Phytol 164:107–121

Larson PR (1994) The vascular cambium. Springer, New York

Laufs P, Jonak C, Traas J (1998) Cells and domains: two views of the shoot apical meristem in Arabidopsis. Plant Physiol Biochem 36:33–45

Li Y, Hagen G, Guilfoyle TJ (1992) Altered morphology in transgenic tobacco plants that overproduce cytokinins in specific tissues and organs. Dev Biol 153:386–395

Liso R, Calabrese G, Bitonti MB, Arrigoni O (1984) Relationship between ascorbic acid and cell division. Exp Cell Res 150:314–320

Liso R, Innocenti AM, Bitonti MB, Arrigoni O (1988) Ascorbic acid-induced progression of quiescent centre cells from G1 to S phase. New Phytol 110:469–471

Lyndon RF (1987) Initiation and growth of internodes and stem and flower frusta in *Silene coeli-rosa*. In: Atherson JG (ed) The manipulation of flowering. Butterworths, London, pp 301–314

Martinez MC, Jorgensen JE, Lawton MA, Lamb CJ, Doerner PW (1992) Spatial pattern of *Cdc2* expression in relation to meristem activity and cell proliferation during plant development. Proc Natl Acad Sci U S A 89:7360–7364

McConnell JR, Barton MK (1998) Leaf polarity and meristem formation in *Arabidopsis*. Development 125:2935–2942

Meyerowitz EM (1997) Genetic control of cell division patterns in developing plant. Cell 88:299–308

Newman IV (1965) Pattern in the meristems of vascular plants. III. Pursuing the patterns in the apical meristem where no cell is a permanent cell. J Linn Soc (Botany) 59:185–214

Nilsson J, Karlberg A, Antti H, Lopez-Vernaza M, Mellerowicz E, Perrot-Rechenmann C, Sandberg G, Bhalerao RP (2008) Dissecting the molecular basis of the regulation of wood formation by auxin in hybrid aspen. Plant Cell 20:843–855

Pautler M, Tanaka W, Hirano HY, Jackson D (2013) Grass meristems I: shoot apical meristem maintenance, axillary meristem determinancy and the floral transition. Plant Cell Physiol 54:302–312

Philipson WR, Ward MJ, Butterfield BG (1971) The vascular cambium. Chapman & Hall, London

Raghavan V (2000) Developmental biology of flowering plants. Springer, New York

Rangarajan R, Swamy BGL (1980) Studies on the procambium in certain organs of monocotyledons. I. Anther filaments of *Gloriosa superba.* L. In: Periasamy K (ed) Histochemistry, developmental and structural anatomy of angiosperms: a Symposium. P&B Publications, Tiruchirapalli, pp 201–219

Reinhardt D, Kuhleme C (2002) Plant architecture. EMBO Rep 3:846–851

Ridge RW (1987) Brief notes on *Ginkgo biloba*. http://mac122.icu.ac.jp/ginkgo/icho.html

Rodriguez-Rodriguez JF, Shishkova S, Napsucialy-Mendivil S, Dubrovsky JG (2003) Apical meristem organization and lack of establishment of the quiescent centre in Cactaceae roots with determinate growth. Planta 271:849–857

Romberger JA (1963) Meristems, growth and development in woody plants. Tech. Bull. 1293. US Dept. of Agriculture, The Forest Physiology Laboratory, Plant Industry Station, Beltsville, p 214

Rost TL (1994) Root top organization and the spatial relationships of differentiation event. In: Iqbal M (ed) Growth patterns in vascular plants. Dioscorides Press, Portland, pp 59–76

Ruzicka K, Simaskova M, Duclercy J, Petrasek J, Zazimalova E, Simon S, Friml J, van Montagu MCE, Benkova E (2009) Cytokinin regulates root meristem activity via modulation of the polar auxin transport. Proc Natl Acad Sci U S A 106:4284–4289

Sachs RM, Lang L (1961) Shoot histogenesis and the subapical meristem: the action of gibberellic acid, Amo-1618, and maleic hydrazide. In: Plant growth regulation. Fourth international conference on Plant Growth Regulation proceedings. Iowa State University Press, Ames, pp 567–578

Sachs RM, Bretz CF, Lang A (1959) Shoot histogenesis: the early effects of gibberellins upon stem elongation in two rosette plants. Am J Bot 46:376–384

Sachs RM, Lang A, Bretz CF, Roach J (1960) Shoot histogenesis: subapical meristem activity in acaulescent plant and the action of gibberellic acid and Amo-1618. Am J Bot 47:260–266

Scheres B (2007) Stem-cell niches: nursery rhymes across kingdoms. Nat Rev Mol Cell Biol 8:345–354

Scheres B, Wolkenfelt (1998) The *Arabidopsis* root as a model to study plant development. Plant Physiol Biochem 36:21–32

Schmidt A (1924) Histologische studien an Phanergamen vegetationspunkten. Bot Arch 8:345–404

Schoof H, Lenhard M, Haecker A, Mayer KF, Jurgens G, Laux T (2000) The stem cell population of *Arabidopsis* shoot meristem is maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. Cell 100:635–644

Schrader J, Nilsson J, Mellerowicz E, Berglund A, Nilsson P, Hertzberg M, Sandberg G (2004) A high-resolution transcript profile across the wood-forming meristem of poplar identifies potential regulators of cambial stem identity. Plant Cell 16:2278–2292

Schüepp O (1917) Untersuchungen über Wachstum und Formwechsel von Vegetationspunkten. Jb Wiss Bot 57:17–79

Sitbon F, Hennion S, Sundberg B, Little CHA, Olsson O, Sandberg G (1992) Transgenic tobacco plants overexpressing the *Agrobacterium tumefaciens iaaM* and *iaaH* genes display altered growth and indoleacetic acid metabolism. Plant Physiol 99:1062–1069

Smith RH, Murashige T (1970) In vitro development of the isolated shoot apical meristem of angiosperms. Am J Bot 57:562–568

Snow M, Snow R (1942) The determination of axillary buds. New Phytol 41:13–22

Soma K, Ball E (1963) Studies on the surface growth of the shoot apex of *Lupinus albus*. "Meristems and differentiation". Brookhaven Symp Biol 16:13–45

Spicer R, Groover A (2010) Tansley review. Evolution of development of vascular cambia and secondary growth. New Phytol 186:577–592

Steeves TA, Sussex IM (1989) Patterns in plant development, 2nd edn. Cambridge University Press, Cambridge, UK

Sterling C (1946) Growth and vascular development in the shoot apex of Sequoia sempervirens (Lamb) Endl III Cytological aspects of vascularisation. Am J Bot 23:35–45

Sussex IM (1955) Morphologenesis in *Solanum tuberosum* L.: apical structure and developmental pattern of the juvenile shoot. Phytomorphology 5:253–273

Swamy BGL, Krishnamurthy KV (1974) Studies in the developmental anatomy of *Commelina benghalensis* L. I. The shoot apex and the early delineation of the internode. J Indian Bot Soc 53:213–223

Swamy BGL, Krishnamurthy KV (1975) Certain conceptual aspects of meristems. I. On hypophysis and quiescent centre. Phytomorphology 25:60–65

Swamy BGL, Krishnamurthy KV (1977) Certain conceptual aspects of meristems. II. Epiphysis and shoot apex. Phytomorphology 27:1–8

Swamy BGL, Krishnamurthy KV (1978) Certain conceptual aspects of meristems. III. A model. Phytomorphology 28:1–7

Swamy BGL, Krishnamurthy KV (1979) Certain conceptual aspects of meristems. IV The internodal meristems concerned with longitudinal growth. J Madras Univ Sect B 42(2):22–28

Swamy BGL, Krishnamurthy KV (1980) On the origin of vascular, cambium in dicotyledonous stems. Proc Indian Acad Sci (Plant Sci) 89B:1–6

Tabuchi H, Zhang Y, Hattori S, Omae M, Shimizu-Sato S, Oikawa T (2011) *LAX PANICLE2* of rice encodes a

novel nuclear protein and regulates the formation of axillary meristems. Plant Cell 23:3276–3287

Timell TE (1973) Ultrastructure of the dormant and active cambial zones and the dormant phloem associated with formation of normal and compression woods in *Picea abies* (L.) Karst SUNY Coll. Envir Sci For Tech Rep 96:94

Timell TE (1986) Compression wood in gymnosperms, vol 3. Springer, Berlin

Van den Berg C, Willemsen V, Hendriks G, Weisbeek P, Scheres B (1997) Short-range control of cell differentiation in the Arabidopsis root meristem. Nature 39:287–289

Veit B (2006) Stem cell signaling networks in plants. Plant Mol Biol 60:793–810

Veit B (2009) Hormone mediated regulation of the shoot apical meristem. Plant Mol Biol 69:397–408

Vernoux T, Autran D, Traas J (2000) Developmental control of cell division patterns in the shoot apex. Plant Mol Biol 43:569–581

Vernoux T, Benfey PN (2005) Signal that regulate stem-cell activity during plant development. Curr Opin Genet Dev 15:388–394

Vijayaraghavan U, Prasad K, Meyerowitz E (2005) Specification and maintenance of the floral meristem: interactions between positively-acting promoters of flowering and negative regulators. Curr Sci 89:1835–1844

Von Guttenberg H (1960) Grundzüge der Histogenese höherer Pflanzen. I. Die Angiospermen. Handbuch der Pflanzenanatomie, Band 8, Teil 3. Gebrüder Borntraeger, Berlin

Wardlaw CW (1957) On the organization and reactivity of the shoot apex in vascular plants. Am J Bot 44:176–185

Wardlaw CW (1968) Morphogenesis in plants – a contemporary study. Methuen, London

Weizman IL (2000) Stem cells: units of development, units of regeneration and units in evolution. Cell 100:157–168

Willemsen V, Welkenfelt H, de Vrieze G, Weisbeek P, Scheres B (1998) The HOBBIT gene is required for formation of the root meristem in the Arabidopsis embryo. Development 125:521–531

Zhou GK, Kubo M, Zhong R, Demura T, Ye ZH (2007) Overexpression of miR165 affects apical meristem formation, organ polarity establishment and vascular development in *Arabidopsis*. Plant Cell Physiol 48:391–404

Zobel AM (1989a) Origin of nodes and internodes in plant shoot. I. Transverse zonation of apical parts of the shoot. Ann Bot 63:199–209

Zobel AM (1989b) Origin of nodes and internodes in plant shoot. II. Models of node and internode origin from one layer of cells. Ann Bot 63:209–220

Origin, Development and Differentiation of Leaves

5

K.V. Krishnamurthy, Bir Bahadur, S. John Adams, and Padma Venkatasubramanian

Abstract

Leaves are the most important organs of plants and carry out very vital physiological activities such as photosynthesis, respiration, transpiration, photoreception and synthesis and supply of signal compounds, including growth regulators. They are always associated with shoot apical meristems from which they arise. Leaves are arranged on the stem with very characteristic and non-mutagenic phyllotactic pattern characteristic of each plant. The various theories to explain this pattern are briefly described. The leaf primordia are initially with a leaf axis from which the lamina, petiole and phyllopodium regions of the mature leaf arise. This chapter deals with the genetic network that operates during various phases of leaf ontogeny. The genetic basis for shoot apical meristem (with indeterminate growth)–leaf primordium (with determinate growth) boundary is also discussed. This article also discusses the ontogenetic and genetic bases of the differences between simple and compound leaves. A short account each on heteroblasty, heterophylly, senescence and evolution of leaf is also provided.

Keywords

Dorsiventrality • Founder cells • Genes in leaf development • Heteroblasty • Heterophylly • Leaf primordium • Phyllotaxy • SAM–leaf primordium boundary • Senescence • Three-domain model • Two-domain model

K.V. Krishnamurthy • S. John Adams
P. Venkatasubramanian
Center for Pharmaceutics, Pharmacognosy and Pharmacology, School of Life Sciences, Institute of Trans-Disciplinary Health Science and Technology (IHST), Bangalore, Karnataka, India

B. Bahadur (✉)
Sri Biotech Laboratories India Limited, Hyderabad, Telangana, India
e-mail: birbahadur5april@gmail.com

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_5,

5.1 Introduction

The most important and characteristic organ of the shoot system of vascular plants is the leaf, borne on the stem. It is involved in the vital physiological processes of photosynthesis, respiration and transpiration. It is also the source of growth hormones involved in the control of photoreception, photomorphogenesis, flowering and cambial activity. The leaf is an appendicular organ. Only very rarely leaves are absent. The major questions that are often asked are why leaves are formed at all and why on stems but not on roots. We do not have definite answers to these questions, although some suggestive answers have been put forward and tested. One of the major suggestions proposed is that the surface of a shoot apex is under compression and hence tends to produce folds in the form of leaves. But surgical experiments carried out on SAM (shoot apex meristem) in the form of shallow incisions at the sites of next new leaf primordium showed that there is not much either of compression or tension on the surface of the SAM. Hence these questions remain still to be answered; however, the evolution of a flat organ like a leaf would be the best geometrically designed structure to optimise light harvest and gaseous exchange than a cylindrical stem. This chapter discusses the origin, development and differentiation of leaves.

5.2 Phyllotaxy

The regular order of leaf arrangement on the stem is known as *phyllotaxy* or *phyllotaxis* (Jean 1994). The most common arrangement is *spiral* in which the angle of divergence between successive leaves is about 137.5°. The spirally arranged leaves apparently look as alternately arranged leaves. The other types of leaf arrangement are *distichous*, with leaves disposed at almost 90° from each other and in pairs at each node; *opposite superposed*, with leaves in pairs in each node and with each pair aligned one above the other along the same horizontal axis; *opposite decussate*, with each successive pairs of laves in the node at right angles to the previous and next pairs; and *whorled*, with three or more leaves in a node and arranged in a circle. However, the majority of leaf arrangement patterns noticed in flowering plants are based on spiral (helical) phyllotaxy. The single spiral that can be drawn connecting through the centres of all leaves in the order of their succession is often called *genetic* or *ontogenetic spiral* (Fig. 5.1). The genetic spiral was used by botanists to determine the numerical value of phyllotaxis. Phyllotaxy is expressed by a fraction number, in which the numerator is the number of leaves intervening between two vertically superimposed leaves. One common fraction is 5/18. The other common fraction numbers seen in plants are 1/2, 1/3, 2/5 and 3/8, while the less common fractions are 5/13, 8/12, etc. This series of fractions belongs to the so-called Fibonacci summation series 0, 1, 1, 2, 3, 5, 8, 13, 21. The numerator and denominator of each succeeding fractions are the sum of the numerators and denominators of the two preceding fractions. Each of these fractions represents the angle intervening between the centres of successive leaf primordia. This angle is approximately 137.5° for all fractions, as already mentioned. Hence, with very few exceptions, all phyllotactic spirals are probably alike.

5.3 Leaf Initiation

It is often convenient to divide leaf ontogeny into three phases depending on the time at which different leaf features become determined (Sylvester et al. 1996; Poethig 1997). During the first phase, the leaf primordium is initiated from the SAM and acquires its identity. During the second phase, the major parts of the leaf become determined and the leaf gets its basic shape, and in the third phase, leaf histogenesis is completed.

Leaves always get initiated in association with a SAM. Yet, the role of SAM in leaf initiation is still debated by some researchers. Although some evidence is cited to show that SAM is not always actually required for leaf initiation, as, for example, leaves or leaf-like appendages are described to develop in the absence of SAM in plants like *Begonia* and watercress and in some calli, it is questionable for the following reasons: (1) whether such structures are true leaves or

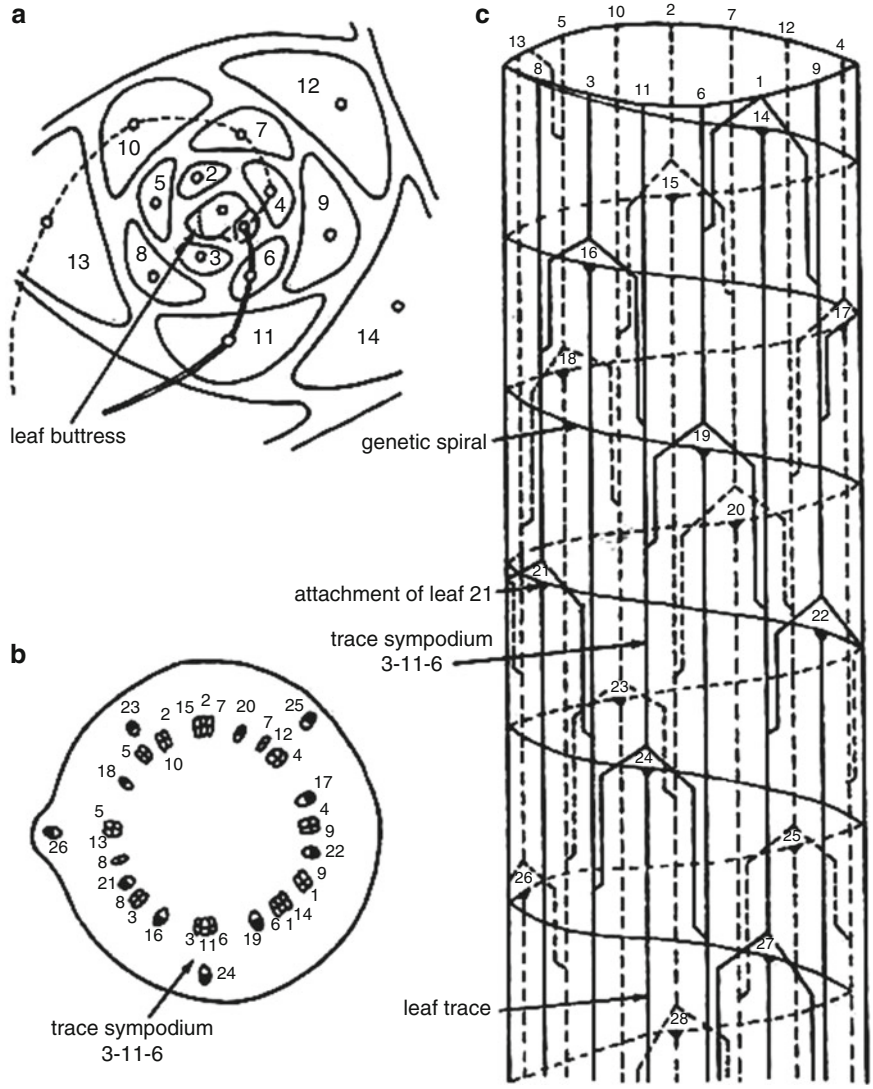


Fig. 5.1 Diagrams of the primary vascular system and phyllotaxis of *Hectorella caespitosa* in T.S. (**a**, **b**) and in three-dimensional view (**c**); (**a**) stem apex (*a*) and leaves 1–14, in each of which the midrib is indicated by a circle. The *two curved lines* (one broken, the other double) indicate a pair contact parastichies. (**b**) Stem cut near node of leaf 23. The traces cut above the level of their connections with other traces are indicated by *blackened* phloem regions. (**c**) Diagram showing interconnections of leaf traces (Skipworth 1962)

prophylls and (2) whether a SAM-like structure is really absent in these instances. Also, the report that the *Arabidopsis* mutant *PINHEAD* gene blocks the development of one or more leaf primordia (Poethig 1997) needs critical reexamination to verify whether these had initiation even before the SAM was blocked. However, these results on *Begonia* and cultures are believed to indicate that although leaf initiation may be facilitated by the unique physiology/structure of SAM, the meristem may not actively direct the process of leaf development. Poethig (1997) believes that the SAM is likely to represent a region of the plant body in which leaves might spontaneously organise themselves rather than a structural entity that produces leaf primordia. Poethig stated further that one of the functions of SAM 'may be to make the tissue that makes the leaves rather than to make the leaves by itself', but the fact that a SAM controls leaf initiation cannot be easily

disapproved, because the lack of epiphysis (the progenitor of SAM in embryo) prohibits the formation of the seed leaves (i.e. cotyledons).

5.3.1 Theories on Leaf Initiation

The sites where leaf primordia arise in the SAM are correlated with the phyllotaxis of the shoot. The mechanism behind orderly production of leaf primordia around the circumference of SAM has been a subject of great debate among botanists. Several theories have been proposed in this connection:

1. *Next* (*or first*) *available space theory*: This theory is essentially based on results obtained from surgical experiments made on shoot apices (Snow and Snow 1932). According to this theory, a new leaf primordium arises on the SAM when sufficient width and distance from the summit of the meristem are obtained. The leaf primordium must first be determined as a whole and not just at its centre, as expected by the repression theory (see below). Also, it is the whole of each existing primordium which restricts the space available for future leaf primordia (see Krishnamurthy 2015).
2. *Phyllotactic theory*: This theory was proposed by Plantefol (1946). According to this theory, a leaf-forming impulse moves acropetally along the foliar helix and causes the initiation of a leaf primordium at the SAM site where it ends (Fig. 5.2). In most dicots there are said to be two foliar helices starting with each of the two cotyledons, but this number is not invariable and can change during ontogeny. In monocots also there may be many foliar helices. Plantefol's theory is not supported outside France.
3. *Repulsion theory*: The *repulsion theory* of Richards (1948) is also called the *physiological field theory* (Wardlaw 1949). According to this theory, as each new leaf primordium is initiated in the SAM, it is surrounded by a physiological field within which the formation of another new leaf primordium is inhibited. Once the place of origin of the next primordium comes outside this field, it gets originated on the SAM. A kind of repulsion spreads from the summit of SAM and from the centre of each of the existing leaf primordia, and this prevents a new primordium from being formed. The factor behind this repulsion may be an inhibitor, or a competition for growth hormones might be present. However, there is no information available on this. Since this theory proposes that repulsion is emanating from specific sites mentioned above, the new leaf primordium about to be formed is also determined at a point in its centre. When the site of the leaf primordial centre is determined, the rest of the leaf base is then decided.
4. *Biophysical forces theory*: Green and his coworkers (Green and Lang 1981; Green 1986) proposed two biophysical mechanisms for the regulation of leaf primordia initiation. The first model suggests that the main event in leaf initiation is the production of a field of cells in which cellulose microfibrils are in a roughly circular arrangement. These circular microfibrils are believed to prevent cells from lateral expansion, thus forcing the primordium to expand out of the plane of the apical meristem. In a meristem with pre-existing cells, the site of the next leaf primordium is specified by the way in which these pre-existing leaves modify the cellulose pattern within cells of SAM. Green (1986) suggested the second model (often called the *biophysical forces theory*), according to which in the growing SAM biophysical forces determine the leaf primordium initiation sites. A new primor-

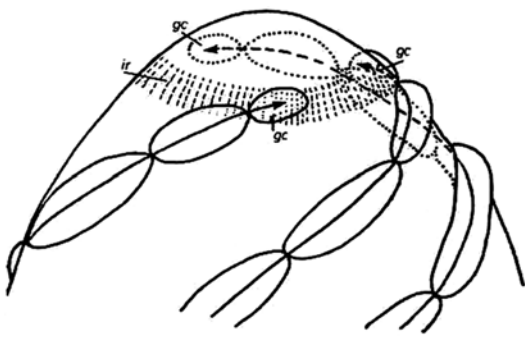


Fig. 5.2 Vegetative apex interpreted in accordance with the concept of foliar helices and contiguity. Three foliar helices are represented, each one proceeding from a generating centre *gc*, moving in the 'initiating ring' (anneau initial) *ir* (Plantefol 1947)

dium arises when a region of the tunica surface bulges or buckles, a condition brought about in part by a localised reduction in the L1's ability to resist pressure from the tissues below (Green 1999; see also Selker et al. 1992). Loops of cellulose microfibrils reinforce the walls of these tunica cells and create the favourable conditions for leaf initiation. The buckling creates local stress variations which are suggested to trigger the periclinal divisions required to initiate leaf primordia. This mechanism is supported in part by experiments in which localised application of *expansin* to SAM of tomato induced the formation of leaf-like outgrowths. Expansin is likely to promote cell wall extensibility in the outer cell wall layer of tunica resulting in outward bulging of the SAM tissue (Fleming et al. 1997). In situ hybridisation experiments done in tomato and rice plant have indicated that there was a specific expression of expansin genes at the sites of leaf primordia initiation (Reinhardt et al. 1998). The biophysical theories, thus, provide a mechanism not only for the spatial positioning of leaf primordia but also for the initiation of leaf primordia. The latter aspect is not explained by chemical theories (see below). On the contrary, there is no clear evidence to demonstrate that the orientation of cellulose microfibrils in the cells of SAM or in a leaf primordium regulates the rate of cell expansion. The second biophysical mechanism predicts that the leaf primordium is subjected to compression to start with, but most studies have shown that cells in the rudiment of leaf primordia are either under tension or show no evidence of being under any type of mechanical stress (Selker et al. 1992).

5. *Procambial strand theory*: This was proposed by Larson (1983). According to this theory, the procambial strands decide the location where a new primordium is to be initiated. Thus, phyllotaxy is correlated with the architecture of the vascular system of the stem. The developmental relationship between the leaves and the leaf traces in the stem (Esau 1965) suggests that procambial traces associated with the prospective leaf primordial sites apparently provide a transport route for auxin and/or other hormones that promote the initiation of leaf primordia. In *Arabidopsis*, the precocious leaf procambial traces are detected as a high-expression region of the *PINHEAD* (*PNH*) gene. This expression of this wild gene precedes the downregulating *STM* gene expression at the leaf primordial site and, hence, may be regarded as an early marker of leaf initiation than the loss of *STM* expression.

The procambial strand theory and other chemical theories such as repulsion theory for leaf initiation invoke the presence of chemicals, promoting and inhibiting, and their interaction, which is controlled by one of many diffusion–reaction mechanisms (Meinhardt 1984; Nelson and Dengler 1997). Several researchers have implicated the role of auxins in the regulation of phyllotaxis (see Cleland 2001; de Reuille et al. 2006; Jönsson et al. 2006; Braybrook and Kuhlemeir 2010). If the shoot apex of tomato is cultured on a synthetic medium containing auxin inhibitors, it does not produce any new leaf primordia (Reinhardt et al. 2000), but once auxin is supplied to it, its leaf-producing ability is restored. Stieger et al. (2002) proposed a model which proposes that auxin efflux carriers control the delivery of auxin to SAM, while influx and efflux carriers regulate its distribution within SAM; the model also proposes that the influx carrier presumably is needed for correct phyllotaxy. In *Arabidopsis*, because of spiral phyllotaxis, auxin distribution is unequal between left and right sides, resulting in asymmetric growth of leaf laminas; in a clockwise phyllotactic spiral pattern, the left side will grow more than the right side (Chitwood et al. 2012). This is followed by bulging of the leaf primordium along with the establishment of dorsiventrality. Cytokinins alone or along with auxin are also known to promote leaf initiation. The *Arabidopsis* cytokinin-resistant mutant *cyr1* produces few or no leaf primordia (Deikman and Ulrich 1995). The above-mentioned chemical models are very good at generating spatial patterns of leaf initiation, but the existence and identity of these chemical signals are not proved beyond reasonable doubt (Poethig 1997). It is not also clear whether these chemicals are promoters, inhibitors or both.

5.4 Early Histogenesis of Leaf Primordia

Leaf primordia are stated to come into being following a change in the orientation of cell enlargement and division in both the tunica and corpus of the flank meristem of SAM. This change results in the formation of a protrusion from SAM, called *leaf buttress* (Fig. 5.3), resulting in asymmetric or symmetric enlargement of SAM depending on alternate or opposite phyllotaxy. The interval between the initiation of two successive leaf primordia (or pairs of leaf primordia in opposite phyllotaxy) is called a *plastochron* (also spelled *plastochrone*), and the changes in the SAM during this interval are called *plastochronic changes*. The *maximal phase* is one when the leaf primordium is about to arise, while the *minimal phase* is one when there is no leaf primordial initiation on the SAM. The duration of plastochronic period depends on the plant and also, in the same plant, on environmental factors or on age of the plant. A *PLASTOCHRON1* (*PLA1*) gene has been known from rice plant, and this gene is implicated in the regulation of duration of vegetative phase by controlling the plastochron intervals. Some botanists use the term *phyllochron* to refer specifically the interval between the visible appearance or emergence of successive leaves.

Leaf, as already stated, gets initiated with an increase in frequency of periclinal divisions in the outer corpus cells of the flank meristem at the site of its initiation. Invariably the outer tunica layer does not undergo periclinal division but keeps pace with the emergence of leaf buttress through repeated anticlinal divisions. If the SAM has more than one tunica layer, the inner layer(s) may or may not participate in periclinal divisions; if not, they also undergo anticlinal divisions. Generally only a few cells of the flank meristem are involved in division to initiate the leaf primordium, and these cells are called *founder cells* by some botanists. However, botanists do not agree so far on the number of founder cells that initiate the leaf primordium as well as on the actual role of these founder cells in controlling the final shape of the leaf. Some even state that founder cells are formed from a few *pre-founder cells*. Tsukaya (2013) goes to the extent of equating the leaf primordium to the founder cells. The concept of founder cells is in contrast to the classical concept that the leaf primordium can be traced back to the activity of a single subepidermal cell. The founder cells concept is now mostly accepted. The founder cells concept believes that the leaf primordium is formed by the division of cells in at least three cell layers of SAM and that each layer is constituted of about 50–200 founder cells depending on the plant. For example, in *Arabidopsis* the leaf primordium in the embryonic SAM is formed by the divisions of as few as ten founder cells in each layer. While the leaf primordial protrusion results from a significant increase in cell division levels above those occurring in the SAM, initial cell division activity in the bulge of early leaf primordia is somewhat reduced in comparison with that following establishment of leaf blade/petiole junction region (Ichihashi et al. 2011). Some people have questioned the causal relationship between periclinal division and initiation of leaf primordia. The gamma plantlet of wheat studied by Foard (1971) throws doubts on this causal relationship. In this plantlet a polarised cell elongation unaccompanied by cell division accounts for the initial protrusion of the leaf primordium from the sides of SAM. Hence, it is emphasised that a slight shift in the polarity of expansion of a group of founder cells rather than a change in their division plane already determines the growth of the leaf primordium.

Fig. 5.3 L.S. of shoot apex of *Commelina benghalensis* showing initiation of leaf primordial

The founder cells in all taxa first give rise to the leaf axis that will form the petiole and midrib (main *costa*), and this leaf axis in turn will give rise to the lamina. The growth of the leaf axis was initially believed to be more apical to start with and then becomes diffuse sooner or later. But recent studies indicate a different situation, as described below. Cell to cell communication is an important component of leaf axis ontogeny. Based on the concept of Nath et al. (2003) that there is present a 'cyclic arrest front', a front in developing leaf primordial axis that distinguishes a meristematic (cell-proliferative) area from an area where cells exit the mitotic process and begin expansion and differentiation, there have been many studies of the ways in which this front in the leaf axis is regulated, particularly in leaf ontogeny. Tsukaya (2002) also proposed the existence of unknown cell to cell interactions in leaf primordial axis based on the concept of 'compensation', which is an abnormal increase in cell volume triggered by defects in cell proliferation in leaf primordia. Two important findings to date relate to the above two concepts: (1) initially, it was thought that the cyclic arrest front moves gradually from tips to bases of leaf primordia, but Kazama et al. (2010) demonstrated that this is not the case, and (2) an unknown cell to cell communication system indeed links the levels of cell proliferation with that of cell expansion in the leaf primordium (Kawade et al. 2010). In *Arabidopsis* the apical part of the leaf primordium is occupied by small, non-polarised cells with longitudinal, transverse and oblique arrangements of cross walls to the proximal–distal axis. The basal part of the leaf primordium has large, longitudinally polarised cells that are arranged parallel to the proximal–distal axis. Once apical and basal regions differentiate, cell division activity in the leaf primordium gets accelerated, and the narrowed appearance between the leaf blade and petiole becomes conspicuous. New cells are supplied apically from the junction region towards the tip for the construction of the leaf blade and towards the base for construction of the petiole. Chimeral analysis has indicated that particular cell populations at the junctions between petiole and leaf blade function as common sources, intercalary leaf meristems, for the bidirectional cell supply (Ichihashi et al. 2011). Cell divisions are predominantly anticlinal in the petiole-forming cell lineage, once a particular developmental stage of leaf primordium is reached (Horiguchi et al. 2011). In a number of monocots, the increase in length of the leaf axis is also contributed by an intercalary meristem located at the base of the axis.

5.5 Determination and Commitment of Leaf Primordia

Information on leaf primordia determination and commitment to develop into a leaf come from surgical experiments and tissue culture studies done on shoot apices, particularly of ferns. Such experiments also throw light on the autonomy of leaf primordia, once initiated. Culture of leaf primordia of *Osmunda cinnamomea* and a few other ferns that have already been determined as prospective leaves develops into small leaves, but in a developmental pathway akin to fronds on intact plants (Steeves 1959). This experiment proves that such leaf primordia are determined in advance over differentiation, thus proving their autonomy. The culture of still younger leaf primordia, however, did not result in leaves but in leafy shoots; here, the young leaf primordia acted like SAMs. Thus, no irreversible change has occurred in the leaf primordium at the time of its inception, but yet the primordium has acquired the ability to develop into a leaf, if the primordium has attained a sufficient size.

Surgical experiments have been done on fern shoot apices in which the centre of SAM or very young leaf primordia are isolated by incisions and allowed to continue development. These experiments have supported the view that the leaf primordium acquires autonomy only after sometime after inception. In general, in angiosperms, cultured leaf primordia and experiments in which leaf primordia have been removed show great degree of propensity to reorganise themselves and give rise to leaves; the commitment of leaf primordia to mature into leaves is more binding

in angiosperms than in ferns (Steeves and Sussex 1989). It should, however, be pointed out that leaf primordia are not determined at the level of the whole plant in taxa such as *Impatiens* in which floral meristem after initiation of flowers reversed to vegetative growth and leaf production due to altered photoperiodic treatments.

5.6 Post-initiation Development

Very detailed studies have been carried out on diverse taxa on leaf development and histogenesis after initiation of the leaf primordium. A critical review of leaf development in angiosperms reveals that there are two classical mechanisms on how leaves develop: (1) the final shape of leaf with its fully differentiated tissues is determined by a series of properly oriented cell divisions closely accompanied by appropriate patterns of cell elongation, and (2) once apical growth of leaf primordium (leaf axis) stops, the dorsiventral lamina is produced by specific *marginal meristems* and *submarginal meristems* along the lateral sides of leaf axis (Fig. 5.4). These were reported to act in diverse ways to produce the entire lamina in different taxa (Fig. 5.4). The most important function for these meristems is the determination of the basic number of laminal cell layers to be formed through characteristic planes of cell division.

Although the concepts of marginal and submarginal meristems have been followed for a very long time to explain the morphogenesis of lamina (Boyce 2007), a number of investigators have indicated that the zone occupied by these meristems is not a site of elevated mitotic activity; these people have raised questions regarding its importance in lamina development. For instance, Merrill (1986a, b) has emphasised that this zone may have a significant role during the initiation of lamina but not during the later stages of lamina development. This author has shown that the distinction in mitotic activity between this zone and the proximal zone is dampened subsequently and that the leaf primordial lamina becomes more uniformly meristematic. The quantitative investigations on pattern of cell division in the developing lamina of some species indicated that there was no preferential mitotic activity in the marginal meristematic zone, thus questioning its role in lamina formation (Poethig and Sussex 1985a; see also Donnelly et al. 1999). This was also shown in a clonal analysis of leaf development in tobacco in which radiation-induced chlorophyll mutations were induced. If marginal meristems are involved in lamina formation, sectors with chlorophyll mutations will be confined within compartment boundaries extending from laminal margin to midrib. But this was not noticed and mutant sectors were randomly distributed in the mature leaf. This diffuse pattern indicated that there was only a generalised and diffuse meristematic activity through the developing lamina. Hence, the role of marginal meristems, if any, was very minimal (Poethig and Sussex 1985b). A similar observation was made, based on clonal analysis of leaf development, in cotton (Dolan and Poethig 1991). It has also been known for some time that while the lamina development in many ferns with marginally ending dichotomous veins depends on the marginal meristem, development of angiosperm leaves with many higher-order vein reticulations and internally directed, free-ending internal veinlets depends on dispersed, non-marginal growth (Boyce 2007). In an unpublished work, one of the authors of this chapter, Krishnamurthy, has suggested that the marginal meristems of a developing leaf lamina are akin to the QC of RAM and SAM (see Chap. 4 of this volume). It was further suggested that although the marginal meristem is the ultimate source of all laminal tissue, its cells themselves divide very infrequently, and only their derivative cells have more division activity. If the marginal meristem is carefully removed from a developing leaf primordium, the further development of the lamina would be suppressed, although the leaf axis develops into the petiole and a midrib region. Hence, the role of a marginal meristem in lamina ontogeny cannot be totally ruled out as nil.

Some investigators advocated the presence of many more or less discrete developmental domains in a leaf primordium along the dorsiventral, centrolateral and proximodistal axes of the

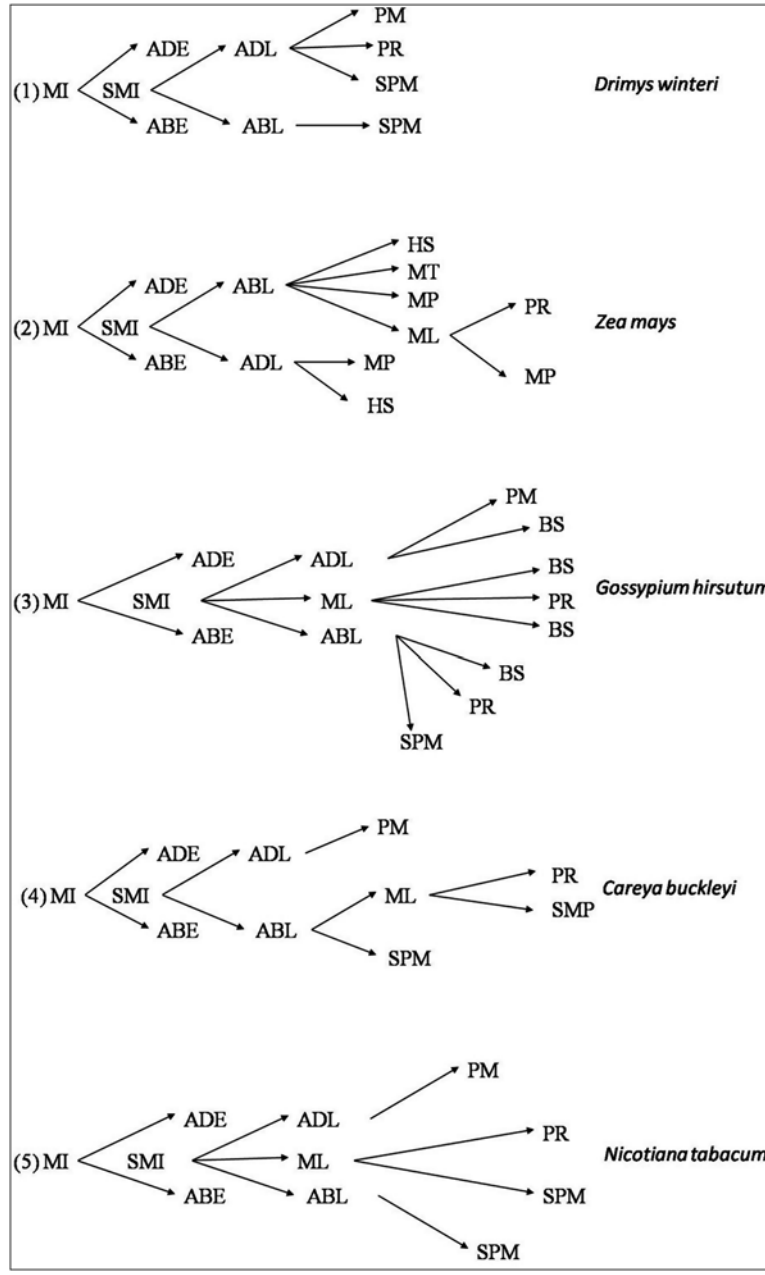


Fig. 5.4 Different patterns of leaf histogenesis from marginal meristem strip (*M1* marginal initial, *SM1* submarginal initial, *ABC* adaxial epidermis, *ADE* adaxial epidermis, *ABL* abaxial layer, *ADL* adaxial layer, *ML* middle layer, *SPM* spongy mesophyll, *PM* palisade mesophyll, *MP* mesophyll, *BS* bundle sheath, *PR* procambium, *HS* hypodermal sclerenchyma, *MT* midrib thickening)

developing leaf (see Poethig 1997). As soon as the leaf primordium emerges from SAM, the dorsiventral symmetry of its axis is very apparent because its adaxial side is generally flatter than its abaxial side. Later, the dorsiventral axis of the leaf is defined by the pattern of cellular differentiation, with some leaf tissues confined to its dorsal and some to its ventral sides. The defects in dorsiventrality (i.e. lack of either adaxial or abaxial identity) in leaves results in rod-, lotus leaf-, or trumpet-shaped leaves when the defect is partial. The first establishment of dorsiventrality is believed to depend on an unknown factor from the SAM. Not only the identity of this factor is unknown but also its presence is unconfirmed (Efroni et al. 2010). Some product of succinic

semialdehyde dehydrogenase (probably GABA or related compound), when absent, causes instability of the adaxial–abaxial (Ab-Ad) border in *Arabidopsis* (Toyokura et al. 2011). The demarcation of the leaf primordium into central and lateral domains is marked by the differentiation of a distinctive band of cells along the lateral margins of its primordium. This zone with this band of cells is called *blastozone* by Hagemann and Gleissberg (1996). It is so-named because the more classical term 'marginal meristem' implies features that are not observed in all species. Cells of the blastozone expand laterally to form the lamina (or pinnae in compound leaves), whereas the central region of the primordium differentiates into the midrib (or rachis). The proximodistal domains are defined by the way in which the blastozone develops. Along the axis, the developing leaf is usually divided into a distal region that produces the lamina, a proximal region that forms the petiole (where the lamina is either absent or highly reduced) and a basal region, which extends around the stem as *phyllopodium* to a greater or lesser extent.

5.6.1 Genetic Control of Leaf Development

Phyllotaxy is considered generally to be a non-mutagenic trait. Many regulatory genes that function in leaf formation and positioning are known, but intensive genetic screening has mostly failed to yield mutants that specifically affect phyllotaxis. In other words, there is so far no homeotic transformation of one phyllotaxy type into another type. Till now, information on the functions of several individual genes in the control of leaf initiation and development has accumulated. It is now time to focus on the roles of genetic networks and interactions among these genes to finally result in a leaf (Tsukaya 2013). Among genes that control leaf initiation, most are known in *Arabidopsis* and maize. The class 1 *KNOX* members (*SHOOT APICAL MERISTEMLESS*; *STM*; *AT1G62360*; *KNAT1*, *KNAT2*; *AT1G70510*; and *KNAT6*; *AT1G23380*) are key factors in the formation and maintenance of SAM activity (see Chap. 4 of this volume). Hence, their elimination or loss of impact from early leaf primordia is required. However, reactivation of class 1 *KNOX* genes in leaf primordium after its initiation is required for proper formation of complex leaves in most angiosperms (Bharathan et al. 2002; Uchida et al. 2007), although not required for simple leaves such as those of *Arabidopsis*. Hence, all leaf primordia initially downregulate class 1 *KNOX*. When expression is ectopic in the primordia, leaves become deformed, developing irregular lobes or deep serrations due to an abnormally increased potential for organogenesis. The downregulation of *KNOTTED1* (*KN1*) class of homeobox-containing plant genes that were originally identified in maize in fact provided an early molecular marker of leaf initiation in SAM (Brutnel and Langdale 1998; Sinha 1999). *KN1* gene is specifically downregulated at the site of leaf initiation in maize, and its expression is high in SAM itself. Hence, *KN1* gene may function to maintain SAM in an indeterminate state, and its downregulation at sites of future leaf primordia might assign the leaf's fate as a determinate organ. Downregulation of *STM1* and *KNOTTED1* class genes *KNAT1*, i.e., homologue of *KNOT1* in *Arabidopsis*, also marks the sites of initiation of leaf primordia. The *HBK1* gene expression found in SAM of *Picea alba* is analogous to *KN* gene of angiosperms. The following genetic factors are involved in the suppression of class 1 *KNOX* gene expression in leaf primordia: *ASYMMETRIC LEAVES1* (*AS1*; *AT2G37630*), *AS2* (*AT1G65620*), *BLADE-ON-PETIOLE1* (*BOP1*; *AT3G7130*), *BOP2* (*AT2G41370*), *SAWTOOTH1C* (*BEL1-LIKE HOMEODOMAIN* (*BHL*)*2*/*SAW1*; *AT4G36870*), *BHL4*/*SAW2* (*AT2G23760*), *JAGGED* (*JAG*; *ATIG68480*), *JAGGED LATERAL ORGANS* (*JLO*; *AT4G00220*), *TCP2* (*AT4G18390*), *TCP 10* (*AT2G31070*) (all in *Arabidopsis*), *ROUGH SHEATH2* (in maize) and *PHANTASTICA* (in *Antirrhinum*), among others (see full literature in Tsukaya 2013). Studies also indicate that the four vegetative *YABs* (*YABBY* genes) are essential in switching from the SAM programme to the leaf-specific programme in *Arabidopsis* because they translate dorsiventral polarity into activation on leaf lamina

programmes. The *forever young* (*fey*) mutant of *Arabidopsis* causes anarchic initiation of leaves (Callos et al. 1994). The *FEY* gene encoded a protein showing a significant homology to *nodulin* of legume root nodules and a limited homology to the enzymes reductases and dehydrogenases. The exact role of this protein is not clear, but it probably regulates leaf initiation by modification of a signal molecule. The founder cells that initiate leaf primordia express *ZWILLE/PINHEAD* (*ZW1/PNH*), *PINFORMED1* (*PIN1/AUX1*) and *REVOLUTA* (*REV*) genes. The boundary of founder cells, i.e., the leaf primordium, is characterised by the expression of genes like *CUC*. The polarised leaf primordium shows the expression of genes such as *AS1*, *AG2*, *LFY*, *ANT*, *CUC/PID/LAS*, *KANADI/YABBY* and *PHB/PHV/REV*. The *as1* shows stunted leaf blades with short petioles, while *as2* with elongated petiole. The *bop1 bop2* double mutant has normal leaf blade in the upper region but has abnormally prolonged morphogenesis in the lower leaf blade and petiole (Ichihashi et al. 2011).

Dominant mutations of *KN1* gene in maize also affect the development of lamina. These result in chaotic growth or knobs which are generally confined to the lateral veins in discrete regions of the leaf; these knobs can be traced to localised sites of extra cell division and expansions in the cell layers of leaf. Different stocks of *KN1* mutant show a few more morphological abnormalities. The expression of *KN1* gene in transgenic tobacco plants results in alterations of leaf size and shape. This gene is believed to function in keeping cells in the indeterminate pathway, an over-expression of this gene resulting in ectopic shoot formation on the leaf surface, in addition to leaf lobbing. The leaf also becomes radially symmetrical, a characteristic feature of the stem. Mention must be made of the *rs* mutants of maize, which show some abnormal characters in the leaves. These mutants have proliferated leaf sheath-like tissue at the base of the lamina and also have defects in vein morphology, proliferation of mesophyll tissue and abnormal cell shapes. Both *RS* and *KNOX* genes (another member of maize homeobox gene family), like the *KN1* gene, are not expressed in the leaf primordia but are expressed in SAM preceding leaf initiation and subsequently as a ring at the place of each leaf insertion (Jackson et al. 1994). It is interesting to note that in both *rs* mutants and wild-type maize, *KNOX* gene is initially repressed in the leaf founder cells of the flank zone of SAM. Thus, the acquisition of leaf cell fate depends upon the downregulation of KNOX-like genes, and the *RS* gene also plays a role in this event. From these results obtained on mutants, the general strategy of possible action is that the fate of cells of SAM may be defined by the independent or interactive functions of *KN1*, *KNOX*, *RS* and other similar genes.

Most mutant genes that affect leaf initiation also affect major aspects of post-initiation leaf development and morphology. There is great dispute regarding the role of cell division in leaf shape, while many stress its importance, as discussed earlier, others do not place much importance to cell division in leaf shape. A separation between cell division and laminal shape during lamina development has been demonstrated by a study of the *tangled1* (*tan1*) mutant of maize, where there is an absence of ordered cell divisions. These aberrant cell divisions cause a slowing down of the rate of growth of the leaf, although at the end the overall leaf shape is attained (Smith et al. 1996). These results show that precise and spatially oriented cell divisions appear to play a relatively minor role in the final shape of the leaf. The existence of genes in *Arabidopsis* mutants that control polarity-specific cell expansion respectively along width and length directions of the leaf to produce narrower and thicker or shorter and rounder than the wild-type leaves has been reported (Tsuge et al. 1996; Bohmert et al. 1998). One of these genes is *ARGONAUTE* (*AGO*) which controls lateral expansion of leaves through its unidentified soluble protein product (Bohmert et al. 1998). Another gene is *ROTUNDIFOLIA* (*ROT*) that controls the longitudinal growth of leaves; this gene belongs to a class of steroid-producing cytochrome P-450 (Kim et al. 1998). However, the actual mode of action of these protein products is not known.

The genetic basis of dorsiventrality or DV-axis formation is very complicated (see review by Kidner and Timmermans 2010; Nakata and Okada 2012). According to the *two-domain theory* of dorsiventrality, the leaf primordium is divided into adaxial and abaxial domains (Yamaguchi et al. 2012); these two domains are controlled by two distinct groups of regulators in *Arabidopsis*. The dorsiventral nature of the developing leaf is decided very early in leaf development, and the genetic basis for this is provided by a study of *phantastica* (*phan*) series of mutants of *Antirrhinum majus*. These mutants have a great variety of leaf morphology lacking dorsiventrality. These leaves apparently have a normal initiation, but during subsequent development concentric layers of cells, normally belonging to the ventral side, fill the leaf. An inductive influence, i.e., dorsalisation, of the *PHAN* gene product is absolutely needed to specify the identity of dorsal cell types of the developing leaf such as dorsal epidermis, palisade and spongy mesophyll (Waites et al. 1998). In *Nicotiana sylvestris* the *lam1* and *fat* mutations modify leaf morphology through control on orientation of cell divisions in the leaf primordia. In the first mutant, the leaves are without lamina from the beginning; while anticlinal divisions proceed normally in L1 and L2 layers of the primordium, periclinal divisions in L3 layer, needed for mesophyll development, are absent in this mutant. In the *fat* mutant, additional periclinal divisions take place in the prospective mesophyll cells during lamina formation and expansion, resulting in abnormally thick lamina. Hence, it is possible that *FAT* gene acts as a negative regulator of periclinal divisions (McHale 1992). The class III homeodomain–zinc finger (HD-Zip III) family [*PHABULOSA* (*PHB*), *PHAVOLUTA* (*PHV*) and *REVOLUTA* (*REV*; *AT5G60690*)] identifies the adaxial domain, while the *KANADI* (*KAN*) family (*KAN1*; *AT5G32240*) and *ETTIN* [*ETT/AUXIN RESPONSE TRANSCRIPTION FACTOR* (*ARF*)*3* (*AT2G33860*) and *ARF4* (*AT5G60450*)] of *Arabidopsis* identify the abaxial domain (see literature in Tsukaya 2013). The two domains suppress one another. There is also regulation of the above key factors by many other factors such as the AS1–AS2 protein complex. AS2 seems to stabilise Ab–Ad polarity in leaf primordia. Negative regulation of class 1 *KNOX* is also linked to the Ab–Ad regulation network via 'junction genes' or 'boundary genes' that express at organ boundaries (see later). The *phabulosa* (*phb*) mutant of *Arabidopsis* shows the transformation of abaxial leaf fates into adaxial leaf fates and the development of radially symmetrical, bladeless leaves, thus emphasising the view that juxtaposition of adaxial and abaxial cell fates is essential for the growth of the lamina (McConnell and Barton 1998). A mutation of *LEAFBLADENESS* (*LBL*) gene in *Zea mays* results in diverse leaf morphologies; one extreme manifestation of this is the production of radially symmetrical 'leaves' (Timmermans et al. 1998). Hence genes such as *PHAN*, *PHB* and *LBL* are necessary for the adaxial cell identity maintenance in the developing leaves.

In recent years, a new model called the *three-domain model* has been proposed by Nakata et al. (2012) (Fig. 5.5). These authors found that *PRESSED FLOWER* (*PRS*)*/WUSCHEL-RELATED HOMEOBOX* (*WOX*) *3* (*AT2G28610*) and *WOX1* (*AT3G18010*) genes are expressed in the mid-sectors of leaf primordia (Fig. 5.5). This 'middle domain' is a part of the adaxial domain, and *PRS*, *WOX1* and *FIL* are expressed there. Because the loss of function in both *PRS* and *WOX1* causes instability in the establishment of Ab–Ad polarity and because *KAN* family genes suppress the expression of *PRS* and *WOX1* (Nakata et al. 2012), this domain is important for establishment/maintenance of a previously recognised Ab–Ad axis in leaves. As per this model, the leaf primordium (from the top) is divided into an adaxial domain which expresses *AS2i* and HD-Zip III (after elimination by miR165/166 in the other domains), a middle domain expressing *PRS WOX1* and *FIL* and an abaxial domain, in strict sense, expressing *KAN* and *FIL*.

The SAM–leaf primordium boundary seems to have an important function in the control of leaf polarities. Many genes express at the boundary and regulate leaf organogenesis. Three gene families are involved: the *LATERAL ORGAN*

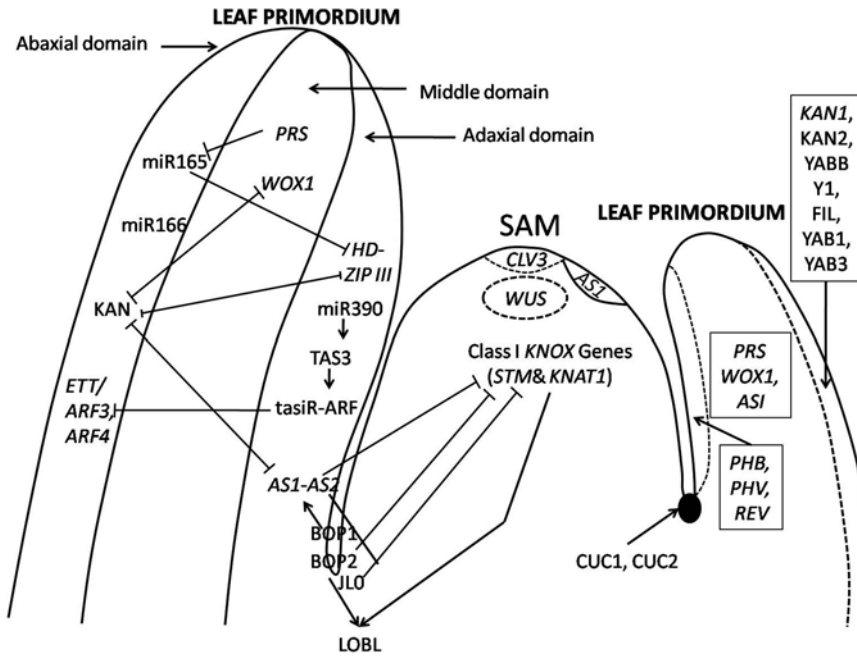


Fig. 5.5 Diagrammatic representation of genetic network operating in the shoot apical region that controls the initiation of leaf primordia and integrity of the SAM and decides the boundary between the *SAM* and *leaf primordia* and the dorsiventrality of the developing primordium according to the three-domain model. Genes that could not be shown in the *left side* primordium are shown in the *right side* leaf primordium. For more details, see the text (Figure reconstructed as per the data provided in Tsukaya 2013)

BOUNDARY (*LOB*) gene family, *BOB1* and *BOB2* and *CUC* gene family. *JAGGED LATERAL ORGANS* (*JLO*), a member of *LOB* family, suppresses class 1 *KNOX* genes such as *STM* and *KNAT1* in the basal parts of leaf primordium; if *JLO* activity is lost, SAM becomes inactive (Rast and Simon 2012). The *bob1 bob2* double mutant has ectopic lamina in the place of petiole; Ab-Ad polarity is disturbed in the *bob* mutant. How the boundary gene *CVC3* acts is not clear. More studies are needed on how the boundary between SAM and leaf primordia is decided.

5.7 Differentiation of Laminal Tissues

The mature leaf has an abaxial and adaxial epidermis, a mesophyll tissue in between the two epidermises and a vasculature. The two epidermises are derived through anticlinal divisions from the protoderm of the leaf primordium and are invariably single layered. The leaf epidermis is a complex tissue system and consists of various cell types, as detailed in Chap. 3 of this volume. The details of stomatal development involving asymmetric cell divisions are provided in Chap. 3 of this volume; the spacing of stomata and trichome in the epidermal tissue are also provided in that chapter. The mesophyll is of variable thickness depending upon the species.

To start with, the cells of mesophyll are very closely packed, but soon the cells enlarge unequally with the simultaneous development of large intercellular spaces. Along with it plastid differentiation takes place and most plastids become chloroplasts. The mesophyll may be uniformly of the *spongy* type as in many grasses or may be distinguished into a spongy and palisade region. The latter are formed on the upper side of the lamina in one or a few layers and are elongated perpendicular to the leaf axis. In isobilateral leaves, palisade mesophyll is seen on both upper and lower sides of the leaf. The spongy

mesophyll cells have irregular outlines and have very large intercellular spaces between them. Chloroplasts fill both types of mesophyll cells. Idioblasts and special cell types are found distributed here and there in the mesophyll. These include sclereids, fibres, crystal cells, tanniferous cells, secretory cells/tubes of various kinds, etc. Many vascular strands are found in distinct patterns in the mesophyll (see details later). The areas where vascular strands are found are called *costae* (singular: *costa*). The costal region in many taxa may have hypodermal collenchyma or sclerenchyma either on both upper and lower sides of the lamina or only on the upper side.

The pattern of vasculature (i.e. venation) in the leaf is very varied depending on the taxon. It is broadly classified into *reticulate* and *parallel types*, respectively, common in the leaves of dicots and monocots although with exceptions. The pattern and ontogeny of leaf venation appear to guide or limit leaf differentiation and function. All the other leaf tissues, mentioned above, differentiate in definite positions showing a spatial relationship to the vascular system and its pattern (see Krishnamurthy 2015). It is not clear how and where leaf venation pattern is created or revealed, as the data available so far are largely descriptive. A universal theory of leaf vascular pattern formation would have to account for (1) the continuous and acropetal formation (in some cases basipetal formation) of primary and secondary veins in many dicot leaves and (2) the formation of generally parallel vascular strands interconnecting them as in monocot leaves and (3) the simultaneous formation of minor veins in both dicots and monocots.

Till now, no single mechanism fully accounts for these disparate spatial and temporal patterns of venation seen in plants, but three general hypotheses, each based on a different approach, have been suggested so far to explain these patterns:

1. *Canalisation* or *signal flow hypothesis*: It is based primarily on experimental observations, particularly on experiments involving surgical/severing of vascular strands, with the implication of inductive effects of auxin on vascular formation (Sachs 1981, 1989, 1991a, b). A polar flow of auxin (signal molecule) induces the formation of vascular strands. The mechanism is the same as described for regeneration of vascular tissues in V-shaped wounds, the details on which are provided on Chap. 3 of this volume. The cells surrounding prospective vascular strands would be drained off auxin, and hence they would be prevented from differentiating into vascular tissues. This hypothesis readily accounts for the differentiation of open branching pattern of venation in dicot leaves. It was proposed by this hypothesis that localised point sources of auxin induce the formation of bridging vascular strands either through a changing spatial pattern of such sources or through alteration of location of such sources between sides of the meristematic region. This would explain the formation of reticulate minor veins. Neither of these, according to Nelson and Dengler (1997), is fully satisfactory and requires the progressive, rather than simultaneous, formation of minor veins. Also, the canalisation hypothesis does not explain adequately the initial formation of parallel longitudinal veins in monocot leaves (Nelson and Dengler 1997).
2. *Diffusion–reaction pattern hypothesis*: This hypothesis is based on computer modelling of interactions among hypothetical diffusible substances (Meinhardt 1996; Koch and Meinhardt 1994). This hypothesis makes use of Turing's diffusion–reaction systems, about which information has already been provided in Chap. 3 of this volume. According to Nelson and Dengler (1997), Meinhardt's hypothesis has several attractive features. If a morphogenetic field is enlarging isotropically, new peaks of activation of morphogen emerge at positions in which the inhibitor concentration is low, thereby preserving the average spacing of the system. Also, depending on the kinetics of interaction between activating and inhibiting molecules, different patterns may arise de novo. If, for instance, a small random increase in the concentration of an activating molecule in an initially homogeneous field induces its further increase through positive feedback, i.e., if autocatalysis is not saturated,

regularly spaced peaks are formed, but if autocatalysis is saturated and the production of inhibiting substance is limited, a stripe-like pattern results. Combining models of systems forming patches with those forming stripes builds a closed network, i.e., patches specify where no stripes are allowed (Fig. 5.6). Thus, stripes appear at the largest distance from other stripes (Meinhardt 1996). The longitudinal veins of monocots get differentiated in a homogeneous two-dimensional field and appear as a series of parallel stripes with new stripes intercalated between the pre-existing ones as they grow apart. New veins appear simultaneously and not progressively. This suggests that cells in a uniform field are induced to develop as vascular precursors in response to prepatterns formed by diffusing morphogens (see Nelson and Dengler 1997). This hypothesis also explains the simultaneous differentiation of minor vein networks and commissural veins of monocots. However, the major problem with this hypothesis is that so far no direct observational evidence is

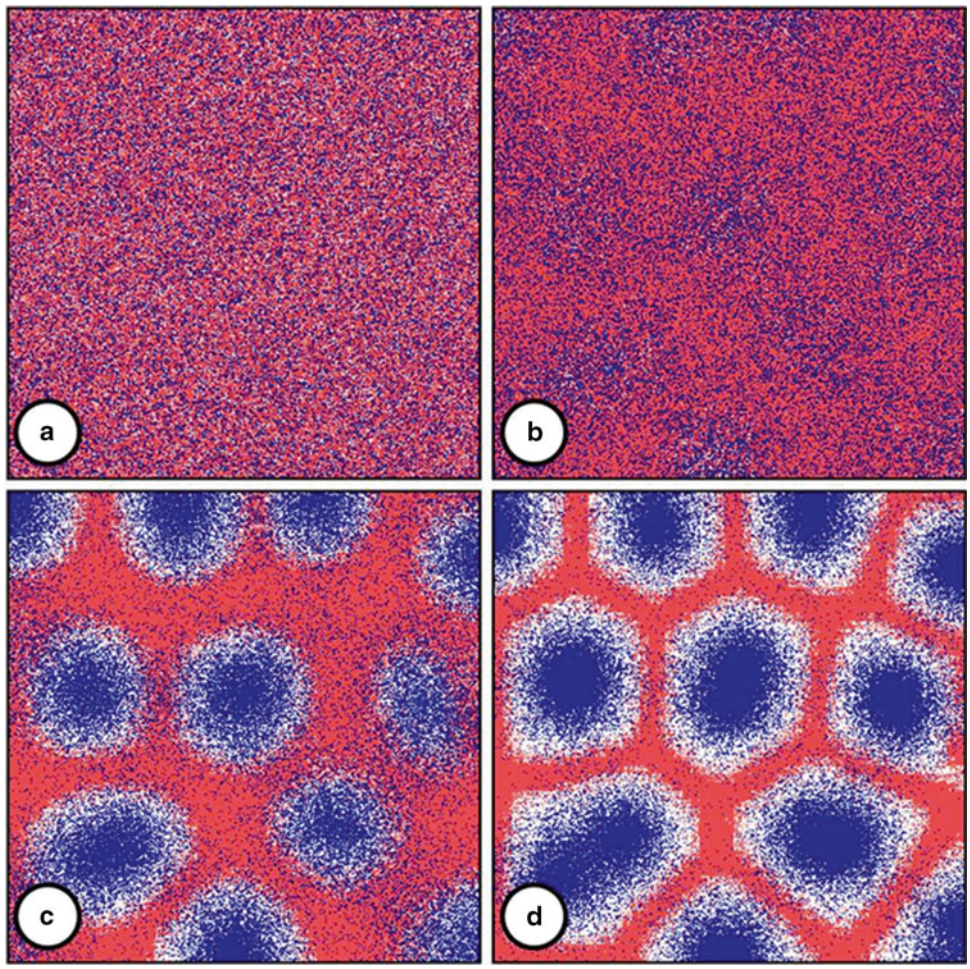


Fig. 5.6 Diffusion–reaction prepattern hypothesis to explain the progressive stages (**a-d**) in vascular pattern formation in the leaf lamina. Computer simulation of the system forming patches combined with a system forming stripes. The patches specify where no stripes are allowed, and thus stripes appear at the largest possible distance from other stripes (Figures: Courtesy of Dr. H. Meinhardt)

available for the morphogens that may act either as an activator or inhibitor, but it is believed that auxin or other hormones may be involved.

3. The third hypothesis speaks of the creation of leaf venation patterns based on mathematical modelling for self-organisation of two-dimensional space by using topological rules and fractal science in combination with estimates of physiology and transport requirements in a leaf (Kull and Herbig 1995).

5.8 Morphogenesis of Leaf Form/Size

There is great variation in the form, shape and size of leaves in vascular plants. Leaf size is largely determined by the behaviour of the cyclic arrest front, cell division and cell enlargement. Leaves are either *simple* or *compound* depending on whether a leaf has a single lamina or more than one (*leaflet*). Simple leaves may have a complete lamina or may have lobes/dissections to various extent, and these lobes are *pinnate* or *palmate* (arranged like a feather or like fingers of a palm). Compound leaves show much more diversity of form than simple leaves; these leaves may be pinnately compound or palmately compound. Pinnately compound leaves may be once pinnate, bipinnate or multipinnate depending on taxa. The main stalk of simple leaf is the *petiole*, while that of the compound leaf is the *rachis*.

Simple and compound leaves have an identical origin and early development (as detailed already), but differences between them set in only during lamina development. Hagemann (1973) proposed the concept of *meristem fractionation*, in which localised zones of meristematic activity become separated by regions of vacuolated cells to describe the mechanism giving rise to lamina lobes in simple leaves and leaflets in compound leaves. For example, the mitotic index of incipient leaf lobes within the lamina margin of *Tropaeolum peregrinum* is significantly higher than in adjacent prospective non-lobe region. However, some other botanists consider that lobes and leaflet primordia arise in close proximity to 'generative centres' located at distal or proximal regions of the incipient lamina. As per this view, the changes in shape associated with leaflet formation can be recognised in a uniformly meristematic zone even before 'fractionation' happens. While leaf lobes are usually formed as part of an already defined lamina margin, leaflets are usually formed directly on the axis of leaf primordium. Leaflet primordia and whole leaf primordia have similar forms initially and then undergo similar process of lamina formation. In compound leaves, the pattern of lamina formation is identical to that of simple leaves, but the timing is delayed (see Krishnamurthy 2015). *KN1* gene controls the development of lobed/dissected leaf of tomato (Hareven et al. 1996). The leaf generally has about nine lobes borne on a central midrib. The expression of this gene is associated with the development of a phenotype with about 2,000 lobes in the transgenic plant. Such an excessively dissected lamina is also observed in spontaneously occurring mutants *curl* (*cu*) and *mouse ears* (*me*) of tomato.

To understand the control of form of compound leaf, the pea plant is often used as a model system. The compound leaf of pea plant has a rachis on whose basal part is up to three pairs of opposite leaflets and distal part with up to four pairs of tendrils (all modified leaflets). Each leaf also has a pair of large stipules. Surgical experiments were carried out on the leaves at various developmental stages, and the results of these indicate a progressive determination of leaf form. About 30-μm-long young leaf primordia left on the bud after surgery are capable of partial regeneration; if the tip of longer primordia of about 70 μm is cut off, the remaining parts grow into stipules. If the two sides of this primordium are removed in the 70-μm-long primordium, the rachis alone is formed with the terminal tendril also formed from the leftover lower part. Through other surgical manipulations, it was possible to change a future leaflet into a tendril, or vice versa (Sachs 1969). From all these surgical experiments, it is clear that the main elements of leaf architecture are laid down early in leaf ontogeny, but the morphology of leaflets is determined later.

This conclusion is also supported by a study of leaf mutants in this plant. For instance, *afila* (*af*) mutant has no leaflets but a bunch of branched tendrils; the *tendrilless* (*tl*) mutant has only leaflets but no tendrils; the *pleofila* (*af*/*tl*) mutant has a highly branched system of vey small leaflets but no tendrils; the *unifoliate* (*uni*) mutant has one to three leaflets on very short stalks but no tendrils. A genetic analysis of the first three mutants has led to the view that *pleofila* represents the basic phenotype of pea plant, without interference from either *AF* or *TL* genes. From this basic phenotype, specific interaction between *AF* and *TL* genes is believed to determine pinna identity in the regions of the leaf corresponding to stipules, leaflets and tendrils. *UNI* gene forms a distinct class as it promotes compound leaf development through the maintenance of a transient state of indeterminacy in the leaf primordium (Lu et al. 1996; Hofer et al. 1997).

A critical review of studies made so far on compound leaves has revealed that there are two basic views on the origin of compound leaves: (1) irrespective of their complexity, all compound leaves are dissected forms of simple leaves, and (2) the leaflets of compound leaves develop by distinct genetic programmes different from those of simple leaves (see full literature in Efroni et al. 2010).

How is leaf index (i.e. leaf length × leaf width ratio) controlled? It was postulated by Tsuge et al. (1996) that leaf index is mainly decided by a balance between polar-dependent cell expansion systems. The gene *AN* regulates lateral expansion of leaf cells, while *ROTUNDIFOLIA3* (*ROT3*), as already indicated, regulates longitudinal cell expansion. This concept is supported by the histology of rheophytic ferns of tropical forests. These have narrow leaves when compared to the leaves of the closely related species. The narrow leaves of rheophytic angiosperms, on the contrary, are the result of altered distributions of leaf cells and not from polarity-dependent cell expansion. In *Arabidopsis*, leaf index is under the influence of four genes; they have their effects on cell numbers or cell shapes along two axes, the lateral and the longitudinal (see full literature in Tsukaya 2013). The *an* mutant has narrower leaves owing to a defect in leaf lamina cell divisions. *ROT4* influences leaf length through a negative effect on the cell proliferation zone in leaf primordia. *LONGIFOLIA* (*LNG*) (*AT5G15580*)/*LNG2* (*AT3G02170*) also control leaf length (and the leaves are also more slender). The *lng* has shorter leaves due to poor cell elongation. Although *an3* mutant has narrow leaves, *AN3* does not directly affect the direction pattern of cell division plane; rather loss of function in *AN3* specifically affects cell proliferation activity in phase II mitosis when longitudinal and oblique divisions increase at the expense of transverse divisions that are dominant in phase I. The molecular roles of genes other than *ROT3* and *AN3* in leaf morphogenesis remain enigmatic.

5.8.1 Heteroblastic Leaf Development

Studies on leaf morphogenesis have been made not only on single leaves but also at the whole plant level. Heteroblasty is one such phenomenon seen at the whole plant level. Heteroblasty means the presence of morphological difference between successively produced vegetative parts, particularly leaves, of a growing plant. Some degree of heteroblasty is seen in all plants in the vegetative parts, but it is very specifically evident through its effect on leaf development. Thus, there are differences between juvenile and adult plant leaves. Not only is there a gradual increase in size of leaf but also in complexity in the adult leaf form (see Krishnamurthy 2015). Heteroblasty has been studied genetically on the basis of mutations. The maize dominant mutations like *teopod* (*tp*) result in abnormal expression of juvenile characters such as the presence of epicuticular wax and delays in the appearance of epidermal trichomes (Poethig 1988a, b; Bongard-Pierce et al. 1996). Poethig (1988b) has shown that the x-ray-induced genetic mosaics expressing the wild-type allele of *TP1* gene in the mutant plants possess a mutant instead of a wild-type leaf phenotype. In this respect, the *TP1* gene shares the characteristic of non-cell-autonomous expression with *kn1* mutation. It is suggested that *tp* mutant

gene is likely to control the production/distribution of a diffusible chemical involved in the maintenance of juvenile status, but this idea has not yet been verified. In order to test whether *tp* mutation would alter the development of adult leaf through its effect on SAM or on specific cells of potential juvenile leaves, a comparative clonal analysis of cell lineages in both wild-type and mutant *tp2* plants has been done. The results showed that the *tp* mutation does not affect cell lineages of the juvenile nodes of the shoot that arise from embryonic SAM but extends the growth of cells near the apex of SAM. The apparent number of embryonic SAM cells that give rise to each metamer is also unaltered in the mutant. This observation suggests that the mutation fails to prolong the expression of juvenile features in the leaf by altering the patterns of growth and cell division within the SAM but changes the fate of cells normally meant for adult growth to promote juvenility (Dudley and Poethig 1991). In contract to *tp* mutation that prolongs juvenile phase, the *glossy15* (*gl15*) mutation shortens the juvenile phase and promotes early adulthood. *GL15* changes the juvenile leaf into adult form: it shows homology to homeotic genes that regulate floral organ identity in *Arabidopsis* (Evans et al. 1994). Subsequent to discovery of heteroblasty in *Arabidopsis* by Kerstetter and Poethig (1998), Poethig and his co-workers conducted detailed studies on heteroblastic mechanisms in this model plant (Wu and Poethig 2006; Wu et al. 2009; Li et al. 2011). Also, understanding of molecular mechanisms of heteroblasty has progressed rapidly in recent years (see Poethig 2010). Leaves are smaller and rounded and have trichomes only on the adaxial side, infrequent serrations or hydathodes along the margin and small numbers of large cells in the juvenile phase. Adult phase leaves are larger and elongated and have trichomes on both epidermises, frequent serrations or hydathodes and large numbers of small-sized cells. Two distinct genetic pathways are currently known to switch juvenile into adult leaves: (1) a pathway of miR156-mediated regulation of *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) genes and (2) a pathway of *tasiR-ARF*-mediated regulation of *ETT*/*ARF3* and *ARF4*, which also regulate dorsiventrality of leaves (Wu and Poethig 2006). Only the first pathway regulates cell size and number per blade, while both pathways regulate traits of gross morphology.

The endogenous hormonal basis of heteroblasty has been analysed in taxa like *Hedera helix* and *Centaurea solstitialis*. Studies made on the first species have shown that GA converts adult leaf form into palmately lobed juvenile leaf form. The juvenile plants of this species contain higher levels of GA-like substances in their apical buds in contrast to the apical buds of adult plants, although the levels of these growth regulators in the leaves of both are the same. A similar role for GA as a regulator of juvenile leaf development has been shown in other herbaceous and tree taxa. In the second taxon mentioned above, the juvenile leaves are simple and entire in contrast to increasingly lobed adult leaves. Leaf primordia of different ages of this taxon were excised and cultured in appropriate growth media containing GA, and it was found that the fifth and primordia older than fifth developed into juvenile leaves, but younger primordia did not survive. These studies also show that leaf-type variations are regulated by change in GA concentration, but these variations do not involve concomitant changes in the shoot apex; GA probably directly acts on the developing leaf primordia to induce juvenile features. GA action prevents the formation of lobes in the younger leaf primordia and the further growth of lobes in the older primordia.

5.8.2 Heterophylly

The production of leaves of different shapes in the same adult plant is called *heterophylly*. This is seen in some water plants in which, depending on the position of the shoot apex in relation to the water level, leaves with different morphologies are produced. Submerged portions have linear and narrow or highly dissected leaves, termed *water forms*, while aerial parts have broad/entire leaves, termed *land forms*. Since the SAM and the young leaf primordial produced by it are

structurally similar in both forms, differences must have been introduced only in later stages of leaf development. ABA has been shown to be a regulatory growth hormone in many heterophyllous aquatic plants. Culture of plants in appropriate concentrations of ABA promotes land form leaf morphology even in submerged shoot parts showing that ABA regulated aerial leaf form. ABA has been demonstrated in aerial shoots but not in submerged shoots of *Hippuris vulgaris*.

5.8.3 Light and Leaf Form

Environmental factors such as gravity and light control leaf form, shape and size. Gravity provides key information on the expected direction of illumination. The direction of light is important for the rosette life form of *Arabidopsis*. Three characteristics of light have been shown to control leaf form: irradiance, spectral composition and photoperiod (Dengler 1994). Leaf production, size, shape and anatomy are all affected by these light parameters. It has been demonstrated that total daily photon flux is more important than instantaneous photon flux in the modification of leaf growth indicating that irradiance levels are perceived through their effect on photosynthetic rates in expanded leaves and that carbohydrate level within the shoot will have important effect on the development of newly formed leaves. This may explain heteroblastic leaf shape changes which are accelerated by high irradiance. Reduced irradiance generally increases leaf area but decreases leaf thickness. These changes in leaf area and thickness occur during leaf development through changes in rates and duration of leaf development through changes in rates and duration of leaf expansion. The close relation between leaf area and cell number indicates that leaf area is controlled through regulation of cell division. There is not much evidence for the effect of altered spectral composition of light on leaf expansion. However low red/far red ratio induces larger leaf areas in some shade-tolerant terrestrial taxa; it also regulates leaf shape in at least two aquatic plants (Dengler 1994). Photoperiod affects leaf development through its effect on total daily photon flux. Altered phytochrome equilibria induced by photoperiodism induce leaf shape changes. These are probably the heteroblastic changes in leaf form that precede flowering in some taxa.

Work done on *Arabidopsis* has shown that the final leaf size and shape are adjusted to the intensity and direction of light. Under weak light, leaf blades are underdeveloped and elongation of petiole is promoted, a part of the 'shade-avoidance syndrome'. This syndrome is seen in a loss-of-function mutant of the photoreceptor gene *PHYTOCHROME B* (*PHY B*; *AT2G 18790*). Both blue and red lights are important in controlling shade avoidance (Kozuka et al. 2005). Blue and red lights have different effects on the control of leaf shape. Auxin and brassinosteroids contribute to shade-avoidance-dependent petiole elongation as revealed by the expression of genes in the end-of-day far red light treatment (see Tsukaya 2013). Leaves developing under high light intensity are thick, and these leaves are called sun leaves, while those under weak light are thin (as in dense forest floor plants) (= shade leaves). The thickness difference is mainly due to the light effect on leaf mesophyll tissue. Phototrop *2* (*PHOT2*, *AT5G58140*) is the major photoreceptor regulating elongation of mesophyll cells in the direction of leaf thickening. However, more research is needed to establish more precisely the relation between light and leaf form.

5.9 Senescence of Leaf

Once the leaves have reached a level of functional obsolescence, they are discarded by the plant through *leaf fall* or *abscission*. Leaf fall happens then and there in individual leaves in evergreen taxa, but in deciduous taxa most, if not, all leaves are shed at a time. Before fall, the leaves undergo senescence. Programmed cell death (PCD) is intimately involved in leaf senescence. This natural termination of the life of leaves as a result of senescence is often regarded as a normal feature of plant development, a sequel to the events of growth, morphogenesis, development and maturation (Greenberg 1996;

Krishnamurthy et al. 2000). Since senescence occurs in an orderly sequence in the life of a leaf and as an active degradation process, it is considered to be genetically controlled or programmed, i.e., it is a process of PCD (Kuriyama and Fukuda 2002). Leaf senescence is regulated by various chemical substances and environmental factors. Sometimes the term *ageing* is used to refer to leaf senescence, but the two terms are not synonymous, since ageing denotes the accumulation of changes that lower the vitality of a living entity without being lethal by themselves. Ageing may lead to senescence.

Senescing leaf cells are invariably characterised by a loss of chlorophyll, accumulation of coloured pigments, decline in nucleic acid content, membrane degradation and the consequential increase in the weakness of cells and an overall decline in metabolism. Many components of the transcriptional and translational systems lose their functions accompanied by a downregulation of a number of genes, particularly genes involved in photosynthesis. In contrast, over 100 senescence-associated genes have been identified to be upregulated in many plants (Jing et al. 2003); these genes perhaps are involved in the disassembly of macromolecules already present as well as in the transport of mobilised nutrients from the senescing tissues. There are also genes that induce the initiation of senescence. For instance, protease genes have been identified by subtractive hybridisation methods, as also genes for many enzymes involved in catabolism. Examples for the downregulation of genes involved in photosynthesis are provided by *Arabidopsis* and soybean. There are also reduced levels of *Rbcl* and *Rbcs* gene transcripts during senescence of soybean leaves along with a concomitant decrease in RUBISCO enzyme and chloroplast DNA. There is a rapid decline in cytokinin levels implicating its role in senescence; also externally supplied cytokinin delays senescence in leaves. A bacterial gene encoding for an enzyme called IPT, when introduced into a plant, enables the leaves of a transgenic plant to remain green for a longer period.

5.10 Evolution of Leaf

The phylogenetic mapping of leaves among land plants indicated that the leafy shoot systems have evolved at least five times over the course of evolution of Embryophyta, which includes bryophytes with so-called leaves, and Lycopsida, which have *microphylls*. Microphylls are leaves without leaf gaps and are seen in taxa with protostelic structure. These phylogenetic analyses have also shown that among euphyllophytes (all megaphyllous taxa including pteridophytes other than Lycopsida and seed plants), the leaves of seed plants and pteridophytes are not homologous. Molecular developmental data may provide useful information on the origin of leaves. For example, such data may help to assess the antecedent structure (a sterilised sporangium) of leaves of Lycopsida. Unfortunately, no study on the molecular basis of evolution of leaf development over the course of diversification of land plants has been thus far seriously undertaken. An analysis of homologous genes involved during the entire gamut of leaf development in angiosperm taxa and in other land plants may provide insights into the evolutionary origin of leaves and leaf-like structures. However, a study of the genetic basis of compound leaf development, particularly the basis of expression of *KNOX* genes, has provided mechanistic support for the leaf–shoot continuum model, which stresses that leaves, at least in euphyllophytes, are derived from cauline (i.e. axially indeterminate) organs (Bharathan and Sinha 2001). Thus, this provides support to the available paleobotanical evidences which suggest that the leaves of seed plants evolved from a radial indeterminate branched shoot system and were thus compound (see Efroni et al. 2010). The first evolved angiosperm leaves were simple suggesting that compound leaves must have evolved many times in angiosperms from ancestral simple leaves. The patterns of expression of *KNOX* gene in various *Lepidium* species that have either simple or compound leaves have shown the importance of the evolution of morphology of leaf types in a phylogenetic

context. *KNOX* gene expression certainly plays a very important role in leaf development, but the way in which its expression has been co-opted to form either compound or simple leaves can differ among plant species. Simple and compound leaves are believed to have evolved independently several times through alterations in different components of leaf genetic programmes. There appears to be fundamental difference between expression of *KNOX* genes in the compound leaves of ferns and of seed plants (Friedman et al. 2004). While *KNOX* gene expression is seen in the leaf primordium in extant seed plants (angiosperms and gymnosperms like *Picea* and *Welwitschia*), its expression is not downregulated in the fern *Anogramma chaeophylla*. This observation emphasises the independent origin of leaves in ferns and seed plants.

References

Bharathan G, Sinha NR (2001) The regulation of compound leaf development. Plant Physiol 127:1533–1538

Bharathan G, Goliber TE, Moore C, Kessler S, Pham T, Sinha NR (2002) Homologies in leaf form inferred from KNOX1 gene expression during development. Science 296:1858–1860

Bohmert K, Camus I, Bellini C, Bouchez D, Caboche M, Benning C (1998) *AGO1* defines a novel locus of *Arabidopsis* controlling leaf development. EMBO J 17:170–180

Bongard-Pierce DK, Evans MMS, Poethig RS (1996) Heteroblastic features of leaf anatomy in maize and their genetic regulation. Int J Plant Sci 157:331–340

Boyce CK (2007) Mechanisms of laminar growth in morphologically convergent leaves and flower traits. Int J Plant Sci 168:1151–1156

Braybrook S, Kuhlemeir C (2010) How a plant builds leaves. Plant Cell 22:1006–1018

Brutnel TP, Langdale JA (1998) Signals in leaf development. Adv Bot Res 28:161–195

Callos JD, DiRado M, Xu B, Behringer FJ, Link BM, Medford JI (1994) The forever young gene encodes an oxidoreductase required for proper development of the *Arabidopsis* vegetative shoot apex. Plant J 6:835–847

Chitwood DH, Headland LR, Ranjan A, Martinez CC, Braylook SA, Koenig D, Kuhlemeir C, Smith RS, Sinha NR (2012) Leaf asymmetry as a developmental constraint induced by auxin-dependent phyllotactic patterning. Plant Cell 24:2318–2327

Cleland RE (2001) Unlocking the mysteries of leaf primordial formation. Proc Natl Acad Sci U S A 98:10981–10982

de Reuille PB, Bohn-Courseau I, Liung K, Morin H, Carraro N, Godin C, Traas J (2006) Computer stimulations reveal properties of the cell-cell signaling network at the shoot apex in Arabidopsis. Proc Natl Acad Sci U S A 103:1627–1632

Deikman J, Ulrich M (1995) A novel cytokinin–resistant mutant of *Arabidopsis* with abbreviated shoot development. Planta 195:440–449

Dengler NG (1994) The influence of light on leaf development. In: Iqbal M (ed) Growth patterns in vascular plants. Dioscorides Press, Portland, pp 100–136

Dolan L, Poethig RS (1991) Genetic analysis of leaf development in cotton. Development 1(Suppl):39–46

Donnelly PM, Bonetta D, Tsukaya H, Dengler R, Dengler NG (1999) Cell cycling and cell enlargement in developing leaves of Arabidopsis. Dev Biol 215:407–419

Dudley M, Poethig RS (1991) The effect of a heterochronic mutation, *teopod 2*, on the cell lineage of the maize shoot. Development 111:733–739

Efroni I, Eshed Y, Lifschitz E (2010) Morphogenesis of simple and compound leaves: a critical review. Plant Cell 22:1019–1032

Esau K (1965) Vascular differentiation in plants. Holt, Rinehart and Winston, New York

Evans MMS, Passas HJ, Poethig RS (1994) Heterochronic effects of *glossy 15* mutations on epidermal cell identity in maize. Development 120:1971–1981

Fleming AJ, McQueen-Mason S, Mandel T, Kuhlemeir C (1997) Induction of leaf primordia by the cell wall protein expansin. Science 276:1415–1418

Foard DE (1971) The initial protrusion of a leaf primordium can form without concurrent periclinal cell divisions. Can J Bot 49:1601–1603

Friedman WF, Moore RC, Purugganam MD (2004) The evolution of plant development. Am J Bot 91:1726–1741

Green PB (1986) Plasticity in shoot development: a biophysical view. In: Jennings DH, Trewavas AJ (eds) Plasticity in plants. Company of Biologists Ltd., Cambridge, pp 211–232

Green PB (1999) Expression of pattern in plants: combining molecular and calculus–based biophysical paradigms. Am J Bot 86:1059–1076

Grèen PB, Lang LM (1981) Towards a biophysical theory of organogenesis: birefringence observations on regenerating leaves in the succulent, *Graptopetalum paraguayensis* E. Walther Plant 151:413–426

Greenberg JT (1996) Programmed cell death: a way of life for plants. Proc Natl Acad Sci U S A 93:12094–12097

Hagemann W (1973) The organization of shoot development. Rev Biol 9:43–67

Hagemann W, Gleissberg S (1996) Organogenetic capacity of leaves: the significance of marginal blastozones in angiosperms. Plant Syst Evol 199:121–152

Hareven D, Gutfinger T, Parnis A, Eshed Y, Lifschitz E (1996) The making of a compound leaf: genetic

manipulation of leaf architecture in tomato. Cell 84:735–744
Hofer J, Turner L, Hellens R, Ambrose M, Matthews P, Michael A, Ellis N (1997) UNIFOLIATA regulates leaf and flower morphogenesis in pea. Curr Biol 7:581–587
Horiguchi G, Nakayama H, Ishikawa N, Kubo M, Demura T, Fukuda H, Tsukaya H (2011) *ANGUSTIFOLIA3* plays roles in adaxial/abaxial patterning and growth in leaf morphogenesis. Plant Cell Physiol 52:112–124
Ichihashi Y, Kawada K, Usami T, Horiguchi G, Takahashi T, Tsukaya H (2011) Key proliferative activity in the junction between the leaf blade and the leaf petiole of *Arabidopsis thaliana*. Plant Physiol 157:1151–1162
Jackson D, Veit B, Hake S (1994) Expression of maize *KNOTTED* related home box genes in the shoot apical meristem predicts patterns in morphogenesis in the vegetative shoot. Development 120:405–413
Jean RV (1994) Phyllotaxis: a systemic study in plant morphogenesis. Cambridge University Press, Cambridge/New York
Jing H–C, Hille J, Dijkwel PP (2003) Ageing in plants: conserved strategies and novel pathways. Plant Biol 5:455–464
Jönsson H, Heisler MG, Shapiro BE, Meyerowitz EM, Mjolsness E (2006) An auxin-driven polarized transport model for phyllotaxis. Proc Natl Acad Sci U S A 103:1633–1638
Kawade K, Horiguchi G, Tsukaya H (2010) Non-cell-autonomously coordinated organ-size regulation in leaf development. Development 137:4221–4227
Kazama T, Ichihashi Y, Murata S, Tsukaya H (2010) The mechanism of cell cycle arrest front progression explained by a KLUM/CYP78 A5-dependent mobile growth factor in developing leaves of *Arabidopsis thaliana*. Plant Cell Physiol 51:1046–1054
Kerstetter RA, Poethig RS (1998) The specification of leaf identity during shoot development. Annu Rev Cell Dev Biol 14:373–398
Kidner CA, Timmermanns MCP (2010) Signaling sides: adaxial–abaxial patterning in leaves. Curr Top Dev Biol 91:141–168
Kim G-T, Tsukaya H, Uchimiya H (1998) The CURLY LEAF gene controls both division and elongation of cells during the expansion of the leaf blade in Arabidopsis thaliana. Planta 206:175–183
Koch AJ, Meinhardt H (1994) Biological pattern formation: from basic mechanisms to complex structures. Rev Mod Phys 66:1481–1507
Kozuka T, Horiguchi G, Kim G-T, Ohgishi M, Sakai T, Tsukaya H (2005) The different growth responses of the *Arabidopsis thaliana* leaf blade and the petiole during shade avoidance. Cell Physiol 46:213–223
Krishnamurthy KV (2015) Growth and development in plants. Scientific Publishers, Jodhpur
Krishnamurthy KV, Krishnaraj R, Chozhavendan R, Samuel Christopher F (2000) The program of cell death in plants and animals – a comparison. Curr Sci 79:1169–1181
Kull U, Herbig A (1995) Leaf venation of angiosperms – form and evolution. Naturwissenschaften 82:441–451
Kuriyama H, Fukuda H (2002) Developmental programmed cell death in plants. Curr Opin Plant Biol 5:568–573
Larson PR (1983) Primary vascularisation and siting of primordia. In: Dale JE, Milthorpe FL (eds) The growth and functioning of leaves. Cambridge University Press, Cambridge, pp 25–51
Li Y, Conway SR, Poethig RS (2011) Vegetative phase change is mediated by a leaf-derived signal that represses the transcription of miR156. Development 138:245–249
Lu B, Villani PJ, Watson JC, DeMason DA, Cokke TJ (1996) The control of pinna morphology in wild type and mutant leaves in the garden pea (*Pisum sativum* L). Int J Plant Sci 157:659–673
McConnell JR, Barton MK (1998) Leaf polarity and meristem formation in Arabidopsis. Development 125:2935–2942
McHale NA (1992) A nuclear mutation blocking initiation of the lamina in leaves of *Nicotiana sylvestris*. Planta 186:355–360
Meinhardt H (1984) Models of pattern formation and their application to plant development. In: Barlow PW, Carr DJ (eds) Positional controls in plant development. Cambridge University Press, Cambridge, UK, pp 1–32
Meinhardt H (1996) Models of biological pattern formation: common mechanism in plant and animal development. Int J Dev Biol 40:123–134
Merrill EK (1986a) Heteroblastic seedlings of green ash. II. Early development of simple and compound leaves. Can J Bot 64:2650–2661
Merrill EK (1986b) Heteroblastic seedlings of green ash. III. Cell division activity and marginal meristems. Can J Bot 64:2662–2668
Nakata M, Okada K (2012) The three-domain model: a new model for the early development of leaves in *Arabidopsis thaliana*. Plant Signal Behav 7:1423–1427
Nakata M, Matsumoto N, Tsugeki R, Rikirsch E, Laux T, Okada K (2012) Roles of the middle domain-specific *WUSCHEL-RELATED HOMEOBOX* genes in early development of leaves in *Arabidopsis*. Plant Cell 24:519–535
Nath U, Craeford BC, Carpenter R, Corn E (2003) Genetic control of surface curvature. Science 299:1404–1407
Nelson T, Dengler N (1997) Leaf vascular pattern formation. Plant Cell 9:1121–1135
Plantefol L (1946) Fondements d'une theorie phyllotaxique nouvelle. Ann Sci Nat Bot 7:153–220, 11th ser
Plantefol L (1947) Helices foliaires, point vegetatif et stele chez les dicotyledones. La notiond'anneau initial. Rev Gen Bot 54:49–80
Poethig RS (1988a) Heterochronic mutations affecting shoot development in maize. Genetics 119:959–973
Poethig RS (1988b) A non-cell-autonomous mutation regulating juvenility in maize. Nature 336:82–83
Poethig RS (1997) Leaf morphogenesis in flowering plants. Plant Cell 9:1077–1087

Poethig RS (2010) The past, present, and future of vegetative phase change. Plant Physiol 154:541–544
Poethig RS, Sussex IM (1985a) The developmental morphology of and growth dynamics of the tobacco leaf. Planta 165:158–169
Poethig RS, Sussex IM (1985b) The cellular parameters of leaf development in tobacco: a clonal analysis. Planta 165:158–169
Rast MI, Simon R (2012) *Arabidopsis JAGGED LATERAL ORGANS* acts with *ASYMMETRIC LEAVES2* to coordinate *KNOX* and *PIN* expression in shoot and root meristems. Plant Cell 24:2917–2933
Reinhardt D, Wittmer F, Mandel T, Kuhlemeier C (1998) Localized upregulation of a new expansin gene predicts the site of leaf formation in the tomato meristem. Plant Cell 10:1427–1437
Reinhardt D, Mandel T, Kuhlemeier C (2000) Auxin regulates the initiation and radial position of plant lateral organs. Plant Cell 12:507–518
Richards FJ (1948) The geometry of phyllotaxis and its origin. Symp Soc Exp Biol 2:217–245
Sachs T (1969) Regeneration experiments on the determination of the form of leaves. Israel J Bot 18:21–30
Sachs T (1981) The control of patterned differentiation of vascular tissues. Adv Bot Res 9:151–262
Sachs T (1989) The development of vascular networks during leaf development. Curr Top Plant Biochem Physiol 8:168–181
Sachs T (1991a) Cell polarity and tissue patterning in plants. Dev Suppl I:83–93
Sachs T (1991b) Pattern formation in plant tissues. Cambridge University Press, Cambridge, UK
Selker JML, Steucek GL, Green PB (1992) Biophysical mechanisms for morphogenetic progressions at the shoot apex. Dev Biol 159:29–43
Sinha N (1999) Leaf development in angiosperms. Annu Rev Plant Physiol Plant Mol Biol 50:419–446
Skipworth JP (1962) The primary vascular system and phyllotaxis in *Hectorella caespitosa* hook. N Z J Sci 5:253–258
Smith LG, Hake S, Sylvester AW (1996) The *tangled-1* mutation alters cells division orientations throughout maize leaf development without altering leaf shape. Development 122:481–489
Snow M, Snow R (1932) Experiments on phyllotaxis. 1. The effect of isolating a primordium. Philos Trans R Soc Lond B 221:1–43
Steeves TA (1959) The development of leaves in sterile nutrient culture. Proc IX Int Bot Congr 2:380
Steeves TA, Sussex IM (1989) Patterns in plant development, 2nd edn. Cambridge University Press, Cambridge, UK
Stieger PA, Reinhardt D, Kuhlemeier (2002) The auxin influx carries is essential for correct leaf positioning. Plant J 32:509–517
Sylvester AW, Smith L, Freeling M (1996) Acquisition of identity in the developing leaf. Annu Rev Cell Dev Biol 12:257–304
Timmermans MCP, Schultes NP, Jankovsky JP, Nelson T (1998) Leaf bladeless is required for dorsoventrality of lateral organs in maize. Development 125:2813–2823
Toyokura K, Watanabe K, Oiwaka A, Kusana M, Tameshige T, Tatematsu K, Matsumoto N, Tsugeki R, Saito K, Okada K (2011) Succinic semialdehyde dehydrogenase is involved in the robust patterning of *Arabidopsis* leaves along the adaxial-abaxial axis. Plant Cell Physiol 52:1340–1353
Tsuge T, Tsukaya H, Uchimiya H (1996) Two independent and polarized processes of cell elongation regulate leaf blade expansion in *Arabidopsis thaliana*. Development 122:1589–1600
Tsukaya H (2002) Interpretation of mutants in leaf morphology: genetic evidence for a compensatory system in leaf morphogenesis that provides a new link between cell and organismal theory. Int Rev Cytol 217:1–39
Tsukaya H (2013) Leaf development. The Arabidopsis Book 1–21
Uchida N, Brad T, Chung K-H, Sinha N (2007) Regulation of *SHOOT MERISTEMLESS* genes via an upstream-conserved noncoding sequence coordinates leaf development. Proc Natl Acad Sci U S A 104:15953–15958
Waites R, Selvadurai HRN, Oliver IR, Hudson A (1998) The *PHANTASTICA* gene encodes a MYB transcription factor involved in growth and dorsoventrality of lateral organs in *Antirrhinum*. Cell 93:779–789
Wardlaw CW (1949) Experiments on organogenesis in ferns. Growth 13(Suppl):93–131
Wu G, Poethig RS (2006) Temporal regulation of shoot development in *Arabidopsis thaliana* by miR156 and its taught SPL3. Development 133:3539–3547
Wu G, Park MY, Conway SR, Wang J-W, Weigel D, Poethig RS (2009) The sequential action of miR156 and miR172 regulates developmental timing in *Arabidopsis*. Cell 38:750–759
Yamaguchi T, Nukazuka A, Nukazaku A, Tsukaya H (2012) Leaf adaxial-abaxial polarity specification and lamina outgrowth: evolution and development. Plant Cell Physiol 53:1180–1194

Plant Biodiversity

6

T. Pullaiah, Bir Bahadur, and K.V. Krishnamurthy

Abstract

An overview of plant biodiversity is provided in this chapter. Details of genetic diversity, species diversity and ecosystem diversity are given. Hotspots of biodiversity and their details have also been given. Threats to biodiversity and methods of conservation are described.

Keywords

Plant biodiversity • Genetic diversity • Species diversity • Ecosystem diversity • Hotspots of biodiversity • Biodiversity conservation

6.1 Introduction

Planet Earth is endowed with a rich variety of life forms, and the teeming millions of these living organisms have been well knit by the laws of nature. The interdependence of the various life forms starting from the unicellular primary producers to the complexly built higher plants and animals is a unique feature of this green planet.

The word *biodiversity* was coined by Walter G. Rosen in 1986, and it is highly popularised during the recent times. Biodiversity, as this assemblage of life forms is referred to, has now been acknowledged as the foundation for sustainable livelihood and food security. Scientists have estimated that more than 50 million species of

T. Pullaiah (✉)
Department of Botany, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India
e-mail: pullaiah.thammineni@gmail.com

B. Bahadur
Sri Biotech Laboratories India Limited, Hyderabad, Telangana, India

K.V. Krishnamurthy
Center for Pharmaceutics, Pharmacognosy and Pharmacology, School of Life Sciences, Institute of Trans-Disciplinary Health Science and Technology (IHST), Bangalore, Karnataka, India

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_6,

plants and animals, including invertebrates and microorganisms, occur on Earth and hardly two million of them have been described by man so far. Scientists are also aware of the immense potentials of the various life forms especially in the context of recent advances made in science and technology. The incessant human assault on forests has left indelible scars on nature. One result of the United Nations Conference on Environment and Development held in Rio de Janeiro, Brazil, in June 1992 was a *Convention on Biological Diversity* which was signed by 187 countries.

6.1.1 Definition

Biological diversity refers to the variety and variability among living organisms and the ecological complexes in which they occur. Diversity can be defined as the number of different items and their relative frequency. For biological diversity, these items are organised at many levels ranging from complete ecosystems to the chemical structures that are the molecular basis of heredity. Thus, the term encompasses different ecosystems, species, genes and their relative richness and abundance.

6.2 Why Biodiversity Is Significant?

Broadly speaking biological diversity satisfies human needs in two different ways, direct and indirect. Much of the world's agricultural and pharmaceutical needs – from developing hybrid seeds to herbal cures – come from prime forests.

Biodiversity will not only help in increasing agricultural productivity but also in developing disease-resistant varieties. It was evident in the early 1970s that the epidemic called grassy stunt virus, which destroyed more than 1,60,000 ha of rice in Asia, could be controlled from a single sample of wild rice *Oryza nivara* from Central India, which was found to be the only known genetic source of resistance to the grassy stunt. Besides 20 major genes from wild for disease and pest resistance are used in rice improvement programmes.

Besides food and other basic needs, human health has gained priority in welfare programmes. Once, all medicines used to come from plant and animal resources. Worldwide medicines from plants are now worth 40 billion dollars a year. Even now 80 % of the people in the developing countries depend upon traditional medicines.

Indirect benefits include nutrient trapping, maintaining water cycles, soil production and protection of soil, absorption and breakdown of pollutants, provision of recreational, aesthetic, scientific, spiritual, etc.

It is estimated that more than 25 % of all medicines available today are derived from tropical plants. Over 40 % of all pharmaceuticals available in the USA depend on natural sources.

In 1960, a child contracting leukemia had one chance in five of survival. Since then scientists have developed a drug – vincristine – from a plant of the tropical forests. *Catharanthus roseus* (Syn. *Vinca rosea*), now allows a leukemia sufferer four chances in five of survival. The National Cancer Institute near Washington DC has screened 29,000 plant species for potential use against cancer. About 3,000 show preliminary promise, and at least 5 may come to rival vincristine. The institute believes that mass extinctions of species could represent a serious setback to the future of anticancer campaigns.

Among other medical products, the 'pill' that is swallowed by 80 million women each day contains sex hormone combinations derived from a Mexican forest yam (*Dioscorea mexicana*). Over-the-counter sales of the pill are now worth one million dollars a year.

6.3 Biodiversity in the World

6.3.1 Genetic Diversity

Genetic diversity refers to intraspecific diversity and is often measured in terms of total DNA content, genome size in terms of base pair numbers, number of genes and by some on the chromosome number, size and morphology.

Genetic diversity studies have been done not only on wild taxa but also on taxa that are domesticated/cultivated by humans. In fact more attention has been paid to the latter groups of taxa, particularly on agricultural and horticultural plants. Agriculture today is characterised by a sharp reduction in the diversity of cultivated plants. Out of an estimated total of 30,000 edible plant species, only 30 'feed the world', with the three major crops being maize (*Zea mays*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*) (FAO 1996). Genetic resources can be defined as all materials that are available for improvement of a cultivated plant species (Haussmann et al. 2004). Plant genetic resources are the biological basis of food security and, directly or indirectly, support the livelihoods of every person on Earth. Plant genetic resources for food and agriculture (PGRFA) consist of diversity of seeds and planting material of traditional varieties and modern cultivars, crop wild relatives and other wild plant species. These resources are used as food, feed for domestic animals, fibre, clothing, shelter and energy. The conservation and sustainable use of PGRFA are necessary to ensure crop production and meet growing environmental challenges and climate change. The erosion of these resources poses a severe threat to the world's food security in the long term.

Countries are fundamentally interdependent with regard to plant genetic resources and in particular for crop genetic resources which have been systematically developed, improved and exchanged without interruption over millennia. Food and agriculture production are dependent on genetic resources domesticated elsewhere and subsequently developed in other countries and regions. Continued access to plant genetic resources and a fair and equitable sharing of the benefits arising from their use are therefore essential for food security.

Much of the spectacular success in plant variety development of the rich industrialised countries in the north are attributed to the richness of genetic diversity at the centres of origin and primary diversity of economic species located in the poorer developing countries of the south. While the genetic indebtedness of the north to the south is widely recognised, sharing of economic benefits accruing from genetic wealth is still a matter of debate and discussion.

The advent of the era of molecular biology and recombinant DNA research has brought home the point that all forms of genetic diversity have potential commercial value and therefore needs protection. The basic feedstock for biotechnology industry is biodiversity. This is why in the global biodiversity convention, the linkages between the two have been stressed.

Worldwide 1,308 gene banks are registered in the WIEWS (World Information and Early Warning System on PGR) database (http://apps3.fao.org/wiews) and conserve a total of 6.1 million accessions, including major crops, minor or neglected crop species, as well as trees and wild plants. Of the 30 main crops, more than 3.6 million accessions are conserved ex situ.

6.3.2 Species Diversity

On this planet Earth there are about 30 million insects; 15,210 mammals, reptiles and amphibians; 9,225 birds; 21,000 fishes; about 4,80,000 plants; and 3 million other invertebrates and microorganisms. Many among them have not been identified. For example, out of 30 million insects only 7,51,000 have been identified. Figures for other organisms identified are (total number of species in brackets): mammals, reptiles, and amphibians 14,484 (15,210); birds 9,040 (9,225); fish 19,056 (21,000); plants 3,22,311 (4,80,000); and other invertebrates and microorganisms 2,76,594 (3,000,000), making a total of 1,392,485 (33,525,435) (Table 6.1). The number of angiospermous species in different countries is given in Table 6.2.

Most of the 1,700 million hectares of tropical forests, rich in biodiversity, are located in poor countries. While such forests covered barely 7 % of the land surface, they harbour half of the species of the world's flora and fauna. For instance, in a 15-ha patch of rainforest in Brunei, 700-odd species of trees have been identified, as many as in all of North America.

Table 6.1 Estimated number of species in the world

Group	Total number of species	Number of identified species	Percentage of the identified over the total species
Mammals, reptiles and amphibians	15,210	14,484	95
Birds	9,225	9,040	98
Fish	21,000	19,056	90
Plants	4,80,000	3,22,311	67
Insects	300,00,000	7,51,000	3
Other invertebrates and microorganisms	30,00,000	276,594	9
Total	335,25,435	1,392,485	4

Table 6.2 Number of angiospermous species in different countries

Country	Angiospermous species
Brazil	55,000
Columbia	45,000
Ecuador	29,000
China	27,000
Mexico	25,000
Australia	23,000
South Africa	21,000
Indonesia	20,000
Venezuela	20,000
Peru	20,000
India	17,000

Species Richness Species richness has become an important component of biodiversity assessment. Now it is very commonly used as a synonym of species diversity. Similarly, global biodiversity is very often considered in terms of global number of species in each of the different taxonomic groups. In other words, measures of biodiversity for particular areas, habitats or ecosystems are often largely reduced to a straightforward measure of species richness (Krishnamurthy 2003). Although species richness data may provide relatively little ecologically significant information, in practice such data are the most easily derived. Thus, they are perhaps the most useful indices for comparisons of diversity on a larger geographical scale. Moreover, at present species richness data are the only type of information available for most areas of the world. Such data are also important for prioritising conservation strategies since they allow identification of geographic regions of the world with exceptional or with very poor diversity.

One of the major limitations with species diversity measures is that they treat all species (even within a specific group of organisms) equally, i.e. they take no account of differences between species in relation to their place in a natural hierarchical system. A taxic diversity approach, therefore, is based on the view that 'individual species vary enormously in the contribution they make to diversity because of their taxonomic position'. Taxonomically isolated species or species of taxonomically isolated genera are of very great value (e.g. *Ginkgo biloba*) in diversity assessment in an area. An area containing taxonomically diverse species is considered to have greater diversity than an area with closely related species in equal numbers.

Species Abundance As just mentioned, simple species richness data may have very limited ecological value. More meaningful are measures of species abundance, especially relative abundance of different species in an area. In general, as remarked earlier, the more equally abundant the various species in an area or ecosystem, the more diverse it is considered. Species abundance data will provide meaningful interpretation of population size of any species in any area. Population is the basic unit of studying genetic diversity in a species taxon.

6.3.3 Ecosystem Diversity

The wide variety in physical features and climate situations have resulted in a diversity of ecological habitats like forests, grasslands, wetlands, coastal and marine ecosystems and desert ecosystems, which harbour and sustain the immense biodiversity.

6.3.3.1 Forest Ecosystem

A forest ecosystem is a natural woodland unit consisting of all plants, animals and microorganisms (biotic components) in that area functioning together with all the nonliving physical (abiotic) factors of the environment.

6.3.3.1.1 Types of Forests

Forests can be classified in different ways and to different degrees of specificity. One such way is in terms of the 'biome' in which they exist, combined with leaf longevity of the dominant species (whether they are evergreen or deciduous). Another distinction is whether the forests are composed predominantly of broadleaf trees, coniferous (needle-leaved) trees or mixed.

Boreal forests occupy the subarctic zone and are generally evergreen and coniferous.

Temperate zones support both broadleaf deciduous forests (e.g. temperate deciduous forest) and evergreen coniferous forests (e.g. temperate coniferous forests and temperate rainforests). Warm temperate zones support broadleaf evergreen forests, including laurel forests.

Tropical and subtropical forests include tropical and subtropical moist forests, tropical and subtropical dry forests and tropical and subtropical coniferous forests.

Physiognomy classifies forests based on their overall physical structure or developmental stage (e.g. old growth vs. second growth).

Forests can also be classified more specifically based on the climate and the dominant tree species present, resulting in numerous different forest types (e.g. ponderosa pine/Douglas-fir forest). A number of global forest classification systems have been proposed, but none has gained universal acceptance. UNEP-WCMC's forest category classification system is a simplification of other more complex systems (e.g. UNESCO's forest and woodland 'subformations'). This system divides the world's forests into 26 major types, which reflect climatic zones as well as the principal types of trees. These 26 major types can be reclassified into 6 broader categories: temperate needle leaf, temperate broadleaf and mixed, tropical moist, tropical dry, sparse trees and parkland and forest plantations. Each category is described as a separate section below.

6.3.3.1.1.1. Temperate Needle Leaf Forests

Temperate needle leaf forests mostly occupy the higher latitude regions of the Northern Hemisphere, as well as high altitude zones and some warm temperate areas, especially on nutrient-poor or otherwise unfavourable soils. These forests are composed entirely, or nearly so, of coniferous species (Coniferophyta). In the Northern Hemisphere pines (*Pinus*), spruces (*Picea*), larches (*Larix*), firs (*Abies*), Douglasfir (*Pseudotsuga*) and hemlocks (*Tsuga*) make up the canopy, but other taxa are also important. In the Southern Hemisphere, most coniferous trees (members of the Araucariaceae and Podocarpaceae) occur in mixtures with broadleaf species and are classed as broadleaf and mixed forests.

6.3.3.1.1.2. Temperate Broad Leaf and Mixed Forests

Temperate broad leaf and mixed forests include a substantial component of trees in the Anthophyta. They are generally characteristic of the warmer temperate latitudes but extend to cool temperate ones, particularly in the Southern Hemisphere. They include such forest types as the mixed deciduous forests of the USA and their counterparts in China and Japan; the broadleaf evergreen rainforests of Japan, Chile and Tasmania; the sclerophyllous forests of Australia, central Chile, the Mediterranean and California; and the southern beech *Nothofagus* forests of Chile and New Zealand.

6.3.3.1.1.3. Tropical Moist Forests

There are many different types of tropical moist forests, although most extensive are the lowland evergreen broad leaf rainforests, for example, várzea and igapó forests and the terra firma forests of the Amazon basin; the peat swamp forests and dipterocarp forests of Southeast Asia; and the high forests of the Congo Basin. Forests located on mountains are also included in this category, divided largely into upper and lower montane formations on the basis of the variation of physiognomy corresponding to changes in altitude.

6.3.3.1.1.4. Tropical Dry Forests

Tropical dry forests are characteristic of areas in the tropics affected by seasonal drought. The seasonality of rainfall is usually reflected in the deciduousness of the forest canopy, with most trees being leafless for several months of the year. However, under some conditions, e.g. less fertile soils or less predictable drought regimes, the proportion of evergreen species increases and the forests are characterised as 'sclerophyllous'. Thorn forest, a dense forest of low stature with a high frequency of thorny or spiny species, is found where drought is prolonged and especially where grazing animals are plentiful. On very poor soils, and especially where fire is a recurrent phenomenon, woody savannas develop (see Sect. 6.3.3.1.1.5).

6.3.3.1.1.5. Sparse Trees and Parklands

Sparse trees and parkland are forests with open canopies of 10–30 % crown cover. They occur principally in areas of transition from forested to non-forested landscapes. The two major zones in which these ecosystems occur are in the boreal region and in the seasonally dry tropics. At high latitudes, north of the main zone of boreal forest or taiga, growing conditions are not adequate to maintain a continuous closed forest cover, so tree cover is both sparse and discontinuous. This vegetation is variously called open taiga, open lichen woodland and forest tundra. It is species poor, has high bryophyte cover and is frequently affected by fire.

6.3.3.1.1.6. Forest Plantations

Forest plantations, generally intended for the production of timber and pulpwood, increase the total area of forest worldwide. Commonly monospecific and/or composed of introduced tree species, these ecosystems are not generally important as habitat for native biodiversity. However, they can be managed in ways that enhance their biodiversity protection functions and they are important providers of ecosystem services such as maintaining nutrient capital, protecting watersheds and soil structure as well as storing carbon. They may also play an important role in alleviating pressure on natural forests for timber and fuel wood production.

6.3.3.2 Grasslands

Grasslands, which are also known at steppes, prairies, pampas and savannas in various parts of the world, are vegetation types with predominance of grass and grass-like species.

Grasslands are an important part of the Earth's many ecological communities, originally covering as much as 25 % of the Earth's surface. They have provided expansive grazing land for both wild and domesticated animals and offered flat areas that have been ploughed to grow crops. Grasslands occur in areas with hot summer temperatures and low precipitation. Areas with less rainfall are deserts and areas with more rainfall tend to be forested. There are two broad types of grasslands in the world: tropical savannah and temperate grassland.

6.3.3.2.1 Tropical Savannah

Tropical savannah occurs in Africa, Australia, South America and Indonesia. Rainfall of 50–130 cm a year is concentrated in 6–8 months with drought the rest of the year. Soils are usually very thin, supporting only grasses and forbs (flowering plants), with only scattered trees and shrubs. Differences in climate and soils create many variations in the plant communities and animal species throughout the savannah. In many areas, the grasslands have been burned to maintain a healthy grass crop for grazing animals. In some areas the savannah has been expanded by

cutting the forest and burning the area each year to prevent the return of trees.

6.3.3.2.2 Temperate Grasslands

Temperate grasslands have less rainfall (25–90 cm) than tropical grasslands and a much greater range of temperatures from winter to summer than savannah. There are two broad types of grasslands in temperate latitudes: prairie and steppe.

6.3.3.2.3 Prairie Grasslands

Prairie grasslands are found across the globe. They have a variety of names in other parts of the world: pampas in South America, veldt in South Africa and puszta in Hungary. These areas have deep, rich soils and are dominated by tall grasses; trees and shrubs are restricted to river valleys, wetlands and other areas with more moisture. Over the years the native grass species on the extensive areas of level ground have been ploughed and fields seeded. Many of these grasslands have been lost to cereal crops.

6.3.3.2.4 Steppe Grasslands

Steppe grasslands receive only 25–50 cm of rainfall each year and the grasses are much shorter than those on prairie grasslands. They are also not as widespread, occurring only in Central and Eastern Europe, Northern Eurasia and Western North America.

6.3.3.3 Wetland Ecosystem

Wetlands are transitional zones that occupy intermediate position between dryland and open water. These ecosystems are dominated by the influence of water; they encompass diverse and heterogenous habitats ranging from rivers, flood plains and rainfed lakes to swamps, estuaries and salt marshes.

Wetlands are productive ecosystems which serve as habitat for a variety of plants and animals. Wetlands perform essential functions including flood control, natural sewage treatment, stabilisation of shorelines against wave erosion, recharging of aquifiers and supporting rich biodiversity. Many wetlands serve as the winter habitats for migratory birds.

Many of the wetland areas have been drained and reclaimed for agricultural and urban expansion. Siltation problems particularly in shallow wetlands are also subjected to the stresses such as agricultural run-offs, pesticides and construction of dams and barrages.

Wetlands are found throughout the world except in Antarctica. The world has 7–9 million km^2 of wetland which is 4 to 6 % of the land surface. 56 % of the 4–6 % of land surface is found in the tropical and subtropical regions. In 1987 Matthews and Fung estimated the extent of wetlands in the world by climatic zones they found: polar/boreal 2.7 million km^2, temperate 0.7 million km^2, subtropical/tropical 1.9 million km^2, rice paddies 1.5 million km^2 and total wetland area 6.8 million km^2.

6.3.3.3.1 Major Wetland Regions of the World

South America – The Orinoco River Delta of Venezuela covers 36,000 km^2 and is dominated by brackish shoreline by mangrove forests. It enters the Caribbean.

The Llanos: Located on the western part of the Orinoco River found in western Venezuela and northern Colombia covers 450,000 km^2. It is one of the largest inland wetland areas of South America. Winter wet season with a summer dry season gives rise to yearly flooding. Dominated by savannah grasslands and scattered palms.

The Amazon River: Covers 300,000 km^2, 3,000 km long and the river floods 5–15 m high yearly. It is considered as the world's single largest river, with a flow that results in about one-sixth to one-fifth of all the fresh water in the world.

The Pantanal: One of the largest wetlands in world located in southwestern Brazil. Covers 140,000 km^2 four times the size of the Florida Everglades, with about 131,000 km^2 of that area flooded annually. It is a haven to 650–700 species of birds.

6.3.3.3.1.1. Europe

1. Mediterranean Sea Deltas: The Rhone River Delta created France's most important wetland, the Camargue. Covers about 9,000 km^2. Highly affected by a hot, dry summer and

cool, wet winters. Home to one of the world's 25 major flamingo nesting sites.
2. Coastal marshes of Northern Europe: Extensive salt marshes and mud flats are found along the Atlantic Ocean and the North Sea coastlines of Europe. Mostly grass-type marshes.
3. Rhine River Delta: It is a major transportation artery in Europe. Much of the Netherlands is on the Rhine River Delta.
4. Peatlands: About 3.46 million km^2 of northern boreal and subarctic peatland exist, more than half of the world wetlands. Predominately found in the Old World, Ireland, Scandinavia, Finland and Russia. Mostly made up of decomposed sphagnum moss.

6.3.3.3.1.2. Africa

An abundance of wetlands is found in sub-Saharan Africa such as the Congo River swamps, Inner Niger Delta, Sudd of the Upper Nile and the Okavango Delta.

Sudd of the Upper Nile: Rainforest where the Blue and White Nile meet in the southern Sudan.

Nile Delta: It used to be a huge delta; the land has been converted to farm land. It no longer floods due to the Aswan Dam.

Okavango Delta: A vast number of rivers, channels, island and lagoons are diverted to the Okavango Delta. Along the coast, there are many mangrove forests.

6.3.3.3.1.3. Australia

Wetlands are distinctive due to seasonal dryness from high evaporation rates and low rainfall. Saline wetlands and lakes are common as a result of the high evaporation rates.

6.3.3.3.1.4. New Zealand

It is one location in North Island which has all seven types of wetlands. It has lost 90 % of its wetlands. South Island receives 2–10 m annually of rain and has several types of wetlands.

6.3.3.3.1.5. Asia

Most of its wetlands have been converted for agriculture. South Asia and Southeast Asia have the biggest wetlands. Some of the major rivers are the Indus, Ganges, Chao Phraya, Mekong and Red. The Mekong begins at the Tibet Plateau and runs through China, Laos, Cambodia and Vietnam draining 625,000 km^2. It is estimated that 20 million people receive their protein from fishing in these areas.

China: Approximately 650,000 km^2 of wetlands and Pearl and Yangtze River Deltas. Of that, 250,000 km^2 have been reserved.

6.3.3.4 Coastal and Marine Ecosystem

The coastal-marine ecosystem refers to the marine region extending from the 'upper tidal limits out across the continental shelf, slope and rise'; it thus includes rocky shores, sandy beaches, kelp forests, subtidal benthos and the water column over the shelf, slope and rise. Marine ecosystems cover approximately 71 % of the Earth's surface and contain approximately 97 % of the planet's water. They generate 32 % of the world's net primary production. The coastal-marine system generally encompasses the exclusive economic zones of nations and is approximately 200 nautical miles wide with a 440,000 km long continental profile. The coastal-marine system remains largely neglected despite its very huge productivity. Hayden et al. (1984) described 21 types of oceanic and coastal-marine realms and 45 coastal provinces.

6.3.3.4.1 Mangroves

Mangroves are salt-tolerant ecosystems in tropical and subtropical regions. These ecosystems are largely characterised by assemblage of unrelated tree genera that share the common ability to grow in saline tidal zones. The evergreen broad-leaved trees of mangrove forests are highly adapted to the stresses of flooding and salinity.

Mangroves are various types of trees up to medium height and shrubs that grow in saline coastal sediment habitats in the tropics and subtropics. The remaining mangrove forest areas of the world in 2,000 were 137,760 km^2. The mangrove biome is a distinct saline woodland or shrub land habitat characterised by depositional coastal environments, where fine sediments

(often with high organic content) collect in areas protected from high-energy wave action. Mangroves dominate three-quarters of tropical coastlines. The saline conditions tolerated by various mangrove species range from brackish water, to pure seawater, to water concentrated by evaporation, to over twice the salinity of ocean seawater. Healthy mangrove forests provide a vast array of important co-benefits to coastal communities around the world. These benefits include ecosystem services such as a rich cultural heritage, the protection of shorelines from storms, erosion or sea-level rise, food from fisheries, maintenance of water quality and landscape beauty for recreation and ecotourism. In a 'Blue Carbon' context, these ecosystems also store and sequester potentially vast amounts of carbon in sediments and biomass.

6.3.3.5 Desert Ecosystems

Deserts are arid regions, generally receiving less than 10 in. of precipitation a year, or regions where the potential evaporation rate is twice as great as the precipitation. Desert ecosystem is characterised by low precipitation, arid lands, with expanse of sands, rock or salt, which are largely barren except for sparse or seasonal vegetal cover.

The world's deserts are divided into four categories. Subtropical deserts are the hottest, with parched terrain and rapid evaporation. Although cool coastal deserts are located within the same latitudes as subtropical deserts, the average temperature is much cooler because of frigid offshore ocean currents. Cold winter deserts are marked by stark temperature differences from season to season, ranging from 100 °F (38 °C) in the summer to 10 °F (−12 °C) in the winter. Polar regions are also considered to be deserts because nearly all moisture in these areas is locked up in the form of ice.

Subtropical deserts include Sahara desert (3.5 million sq mi in Morocco, Western Sahara, Algeria, Tunisia, Libya, Egypt, Mauritania, Mali, Niger, Chad, Ethiopia, Eritrea, Somalia), Arabian desert (1 million sq mi in Saudi Arabia, Kuwait, Qatar, UAE, Oman, Yemen), Kalahari desert (2,20,000 sq mi in Botswana, South Africa, Namibia), Australian deserts (Gibson – 1,20,000 sq mi; Great Sandy – 1,50,000 sq mi; Simpson and Sturt Stony – 56,000 sq mi), Mojave (54,000 sq mi, USA), Sonoran (1,20,000 USA Mexico), Chihuahuan (1,75,000 sq mi in Mexico, USA) and Thar desert (over 1,75,000 sq mi in India and Pakistan). Cool coastal deserts include Namib (13,000 sq mi in Angola, Namibia, South Africa) and Atacama (54,000 sq mi in Chile). Cold winter deserts include the Great Basin and Colorado Plateau in USA; Patagonian in Argentina; Karakum in Uzbekistan and Turkmenistan; Kyzyl kum in Uzbekistan, Turkmenistan and Kazakhstan; and Iranian desert and Gobi desert in China and Mongolia, while Polar deserts include Arctic and Antarctic.

6.4 Hotspots of Biodiversity

Biodiversity hotspots are a method to identify those regions of the world where attention is needed to address biodiversity loss and to guide investments in conservation. They are first developed by Norman Myers in 1988 to identify tropical forest 'hotspots' characterised both by exceptional levels of plant endemism and by serious levels of habitat loss. Myers subsequently updated the concept in 1990, adding eight hotspots, including four in Mediterranean regions. Conservation International adopted Myers' hotspots as its institutional blueprint in 1989, and in 1996, the organisation made the decision to undertake a reassessment of the hotspots concept, including an examination of whether key areas had been overlooked. Three years later an extensive global review was undertaken, which introduced quantitative thresholds for the designation of biodiversity hotspots.

To qualify as a hotspot, a region must meet two strict criteria: it must contain at least 1,500 species of vascular plants (>0.5 % of the world's total) as endemics, and it has to have lost ≥ 70 % of its original native habitat.

Myers et al. (2000) gave the details of 25 'hotspots' of global biodiversity (Table 6.3).

Table 6.3 Hotspots of biodiversity of the world

Hotspot	Original extent	Plant species	Endemic plants
Tropical Andes	1,258,000	45,000	20,000
Mesoamerica	1,155,000	24,000	5,000
The Caribbean	263,500	12,000	7,000
Brazil's Atlantic forest	1,227,600	20,000	8,000
Choco/Darien/Western Ecuador	260,000	9,000	2,250
Brazil's Cerrado	1,783,200	10,000	4,400
Central Chile	300,000	3,429	1,605
California Floristic Province	324,000	4,426	2,125
Madagascar	494,150	12,000	9,704
Eastern arc and coastal forest of Kenya/Tanzania	30,000	4,000	1,500
Guinean forests of West Africa	1,265,000	9,000	2,250
Cape floristic province	74,000	8,200	5,682
Succulent Karoo	112,000	4,849	1,940
Mediterranean Basin	2,362,000	25,000	13,000
Caucasus	500,000	6,300	1,600
Sundaland	1,600,000	25,000	13,000
Wallacea	347,000	10,000	1,500
Philippines	300,800	7,620	5,832
Indo-Burma	2,060,000	13,500	7,000
South Central China	800,000	12,000	3,500
Western Ghats/Sri Lanka	182,500	4,780	2,180
SW Australia	309,850	5,469	4,331
New Caledonia	18,600	3,332	2,551
New Zealand	270,500	2,300	1,865

Currently, 34 biodiversity hotspots have been identified, most of which occur in tropical forests. Between them they contain around 50 % of the world's endemic plant species and 42 % of all terrestrial vertebrates but have lost around 86 % of their original habitat.

Hotspots are not formally recognised or governed areas. However, the identification of these areas as hotspots increases the likelihood of conservation investment. In addition, other designations for biodiversity conservation are likely to be present within these broad areas which may have more formal management structures. For example, the average protected area coverage of hotspots, based on IUCN protected area categories I–VI, is 10 % of their original extent.

6.5 Species Extinction

During the next 20–30 years, the world could lose more than a million species of plants and animals – primarily because of environmental changes due to humans. At 100 species per day, this extinction rate will be more than 1,000 times the estimated 'normal' rate of extinction. The list of lost, endangered and threatened species includes both plants and animals. About 10 % of temperate region plant species and 11 % of world's 9,000 bird species are at some risk of extinction. In the tropics, the destruction of forests threatens 1,30,000 species which live nowhere else. Harvard biologist E.O. Wilson estimates that the chopping down of tropical

forests leads to extinction of at least 50,000 species every year – about 140 every day.

In the west during the nineteenth-century industrial revolution, forest destruction for timber industry and hunting led to extinction of several of its important wildlife species. Wolves from Scotland, bear and lynx from Switzerland, European bison from Poland, bottle-nosed dolphin from Netherlands and northern bald ibis from Spain are a few of the several species Europe has lost. These countries are now interested in spending heavily to bring back the species they have lost. It is wise to learn from the hard lessons learnt by these countries.

Presently, approximately 80 % of the world food supply is provided by fewer than two dozen species of plants and animals. In the process of depending upon such a few number of species, we are also (1) narrowing the genetic diversity of crops that we depend upon, (2) changing diverse natural areas in monocultures, (3) reducing the numbers of actual and potential ancestors of crops and domestic animals which may provide genetic material to develop new strains or races and (4) undermining the food security for a growing population.

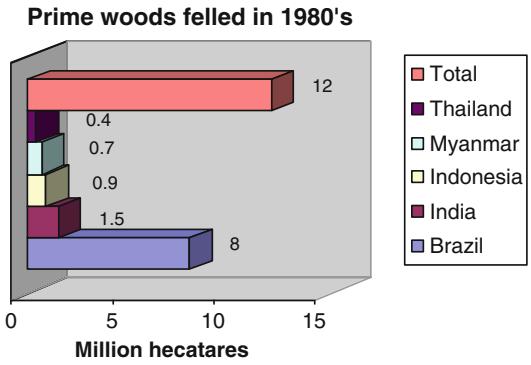
Most of the agricultural crops currently being cultivated have been selected for a particular geographic area. These cultivars may not be as productive or even viable if the climate changes and if new pests or diseases evolve. This makes even more pronounced the need to preserve genetic diversity needed to find food species, which can adopt to new conditions.

6.6 Threats to Ecosystem

Over 1.6 billion people depend directly on forests for their livelihoods across the world, and forests play a crucial role in the Earth's life support system – including the global carbon and hydrological cycles. Some areas of the world are experiencing net gains in forest cover, through the natural expansion or reforestation efforts. However, deforestation in some areas of the world continues apace, impacting livelihoods and the global climate. The UN estimates that 18 % of global carbon dioxide emissions stem from deforestation and forest degradation in developing countries. Avoiding deforestation and degradation is therefore a priority in reducing greenhouse gas emissions.

Forests cover 31 % of the world's land surface, just over 4 billion hectares. (One hectare = 2.47 acres.) This is down from the pre-industrial area of 5.9 billion hectares. According to data from the UN Food and Agriculture Organization, deforestation was at its highest rate in the 1990s, when each year the world lost on average 16 million hectares of forest – roughly the size of the state of Michigan. At the same time, forest area expanded in some places, either through planting or natural processes, bringing the global net loss of forest to 8.3 million hectares per year. In the first decade of this century, the rate of deforestation was slightly lower, but still, a disturbingly high 13 million hectares were destroyed annually. As forest expansion remained stable, the global net forest loss between 2000 and 2010 was 5.2 million hectares per year.

Mountain ecosystem takes the major negative impact of unplanned development, opening of roads, degradation of catchment areas and resultant landslides and erosion. The major threats faced by the forest ecosystem are commercial clear felling and selective clear felling; conversion for agriculture, settlements and roads; inundation for development projects like multipurpose river valley projects, shifting cultivation and conversion to monoculture; army operations; grazing; mining; fire wood collection; introduction of exotics, fire and pollution, development projects, conversion for agriculture and tree plantations; and introduction and spread of exotics.



Grasslands are one of the most threatened ecosystems. Apart from commercial pressures, they come under pressure from grazing, fire and pollution,

The lakes, marshes, river systems and other wetlands are threatened mainly by domestic pollution from untreated sewage, industrial pollutants and toxic effluents; agricultural run-offs containing residues of pesticides and chemical fertiliser; and excessive siltation from degraded catchments. Excessive withdrawal of water from the water bodies for industry, irrigation or domestic use; dredging and reclamation of water bodies; excessive fishing; and building of dams, jetties and canals are other factors adversely affecting the wetlands. A number of wetlands are reported to be seriously threatened.

Mangroves are subjected to serious threats due to their reclamation for urban development, waste disposals, oil spillage, etc. Coral reef ecosystems are threatened because of mining, blasting, dredging, collection of reef biota, coastal clearance for development, sewage disposal, discharge of effluents from industries and thermal power plants, chemical pollution and oil spillage.

The world's deserts with a high livestock population face heavy biotic pressure. Besides, expansion of mining, urbanisation and industrialisation also poses threat to this ecosystem. The expanding salt extraction has resulted in widespread disturbance in salt deserts. In the cold desert, a major destructive factor is road construction, which in turn leads to landslides and soil erosion. Other threats are overgrazing and excessive collection of fuel wood. Desertification and land degradation per se pose a potential threat to biodiversity.

Table 6.4 Categories of fundamental human factors contributing to the erosion of biological activity

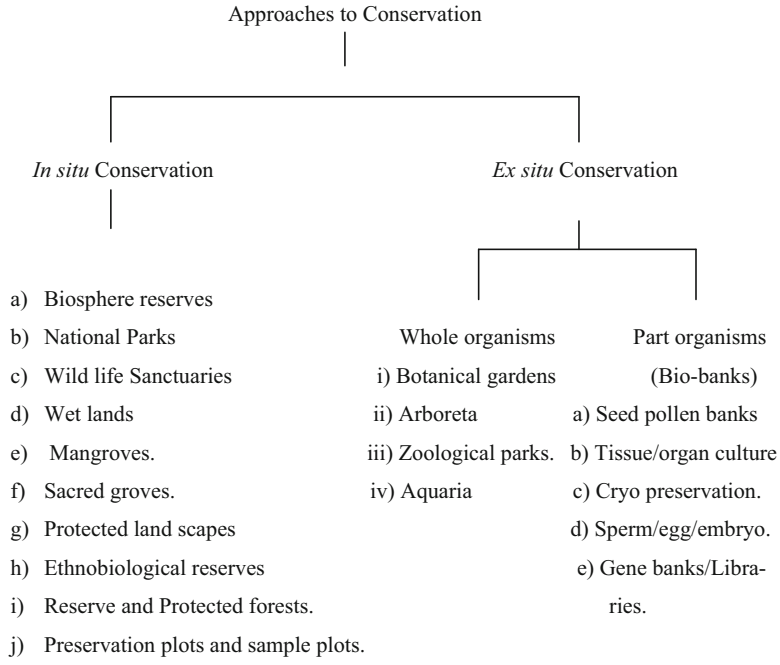
Factor	Example of impact on conversion
Population growth	Demographic pressure
Poverty	Hunger, deforestation, trading of species in danger of extinction, lack of popular support
Bad perception	Desire of quick results and negation of failures in the long term
Anthropocentrism	Absence of support for nonutilitarian causes
Cultural transitions	Unsustained management of resources during colonisation and quick social changes
Economy	Absence of planning as a result of the internationalisation of markets and erratic price of goods
Implementation of policies	Social crisis, wars, corruption, non-fulfilment of law

Source: Pullaiah (2012)

Factors contributing to the loss of biodiversity are given in Table 6.4.

6.7 Conservation of Biological Diversity

Biological diversity can be preserved for posterity in two ways – in situ and ex situ. Ex situ maintenance of species is provided by botanic gardens, Zoos and aquaria and of gene pools by germplasm banks (seed stores, in vitro collections and field gene banks) and grass-root collections of plant cultivars and animal breeds. Botanic gardens probably have a greater capacity with respect to plant species. But clearly it is possible to maintain ex situ only a tiny fraction of the world's species.



In situ conservation is done by protecting areas rich in biodiversity. These include biosphere reserves, national parks, sanctuaries, etc. The concept of biosphere reserves is the brainchild of Man and Biosphere Programme of UNESCO. The primary objective of this concept is to save, for the present and future use, the diversity and integrity of biotic communities of plants and animals within natural ecosystems and to safeguard the genetic diversity of species on which their continuing evolution depends. Such reserves are to comprise of terrestrial and marine ecosystems and to coincide with national parks and sanctuaries.

6.7.1 In Situ Conservation

Areas of natural habitats/ecosystems protected under in situ conservation are called protected areas. Today, there are over 9,832 protected areas including 1,508 national parks, of approximately 9.25 million km^2 or about 8.2 % of the Earth's land surface. A further 40,000 smaller protected areas cover another 5 % of the land area. The goal recommended by IUCN, however, is preservation of a cross section of all major ecosystems to the extent of 13 million km^2 or about 10–12 % of the Earth's surface.

6.7.1.1 Some Biosphere Reserves of the World

1. Rainforest reserves – Albert National Park, Congo, and King George V Park, Malaysia
2. Grassland reserves – Savannah Steppe (Africa, Georgia, USA), and Prairie (Oklahoma and Montana, USA)
3. Desert reserves – Arizona Desert, South African Desert and Nevada Desert
4. Tundra reserves – Lapland Reserves, Finland

These reserves aim at conserving the biological diversity and genetic integrity of plants, animals and microorganisms in their totality as part of the natural ecosystems, so as to ensure their self-perpetuation and unhindered evolution of living resources.

6.7.2 In Situ Conservation of Plant Genetic Resources

Plants are the basis of life on planet Earth. Plants are the only organisms capable of using the solar energy for transforming the carbon dioxide in the air into organic substances. Consequently, plants produce food for all and reduce the global warming. Agriculture, with nurturing and utilising plant diversity, plays a key role in feeding millions and protecting our natural resources and the environment.

Plant diversity on Earth is represented by an estimated 300,000 species of higher plants. However, only about 7,000 species have been domesticated and cultivated by humans over the millennia for food and feed. Today, our nutrition anywhere in the world is supplied by a mere 30 plant species because they provide 95 % of dietary energy and protein.

While the number of cultivated plant species is relatively small and seemingly insignificant, nature has evolved an extraordinary intraspecific genetic diversity in crop plants and their wild relatives. For example, the number of rice land races in India is estimated to be 50,000 and wild rice about 200. Add to that 20 new improved varieties released each year. It is this diversity within species that allows for the cultivation of crops across different regions and in different situations such as weather and soil conditions. These invaluable and irreplaceable plant resources are called plant genetic resources (PGR). They form the basis of all crop varieties that are bred to produce more, withstand stresses and yield quality output.

Plant genetic resources for food and agriculture (PGRFA) have been systematically collected and exchanged for some 500 years. Conservation focuses explicitly on maintaining the diversity of the full range of genetic variation within a particular species or taxa. Plant genetic resources can be conserved both in situ and ex situ.

The main reasons for conserving PGRFA are to ensure the future adaptability of cultivars and wild populations, to preserve data and traits that ensure sustainable agriculture, to promote the use of genetic resources in commerce and biotechnology and to conserve genetic diversity for cultural reasons.

Ex situ conservation of genetic resources entails conservation of biological diversity components outside their natural habitats. The main storage infrastructures for such conservation techniques are gene banks; millions of accessions are now stored in hundreds of gene banks around the world for conservation and utilisation purposes.

In situ conservation of genetic resources means the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticates or cultivated species, in the surroundings where they have developed their distinctive properties. Common approaches for in situ conservation are Genetic reserve conservation and on-farm conservation.

6.7.3 Ex Situ Conservation

To complement in situ conservation, attention has been paid to ex situ conservation measures. Some taxa 'extinct in the wild' but conserved in the botanic gardens is given in Table 6.5.

Table 6.5 Some taxa 'extinct in the wild' but conserved in the botanic gardens (data from IUCN/WCMC and Maunder 1997)

Taxon	Native country
Anthurium leuconeura	Mexico
Arctostaphylos uva-ursi ssp. *loebreweri*	USA
Bromus verticillatus	UK
Calandrinia feltonii	Falkland Island
Ceratozamia hildae	Central America
Commidendrum rotundifolium	St. Helena
Cosmos atrosanguineus	Mexico
Erica verticillata	South Africa
Encephalartos woodii	South Africa
Franklinia alatamaha	USA
Graptopetalum bellus	Mexico
Helichrysum selaginoides	Tasmania
Lysimachia minoricensis	Minorea
Opuntia lindheimeri	USA (Texas)
Paphiopedilum delenatii	Vietnam
Sophora toromiro	Easter Island
Tecophilaea cyanocrocus	Chile
Trochetiopsis erythroxylon	St. Helena
Tulipa sprengeri	Turkey
Dombeya acutangula	Rodrigues
D. mauritiana	Mauritius
Vernonia shevaroyensis	S. India

6.8 Climate Change and Biodiversity

Levels of greenhouse gases in the atmosphere are rapidly increasing, warming the Earth's surface and lower atmosphere. Higher temperatures lead to climate change that includes effects such as rising sea levels, changes in precipitation patterns that can produce floods and droughts and the spread of vector-borne diseases such as malaria. Some areas may benefit from changes in the climate. Others, including those in least developed countries and small island developing states, may suffer greatly.

There is ample scientific evidence that climate change affects biodiversity. Climate change, according to the Millennium Ecosystem Assessment, is likely to become the dominant direct driver of biodiversity loss by the end of the century. It is already forcing biodiversity to adapt either through changing habitat, life cycles or development of new physical traits. This, in turn, will affect vital ecosystem services for all humans, such as air and water purification, pollination and production of food, decomposition and nutrient cycling, carbon sequestration, etc. Change in phenology leads to loss of synchrony between species.

Biodiversity can also help reduce the effects of climate change. The conservation of habitats, for example, can reduce the amount of carbon dioxide released into the atmosphere. Moreover, conserving mangroves can reduce the disastrous impacts of climate change such as flooding and storm surges. If we act now to mitigate greenhouse gas emissions and identify systems-based adaptation priorities, we can reduce the risk of species extinctions and limit damage to ecosystems. We can preserve intact habitats, especially those sensitive to climate change, improve our understanding of the climate change-biodiversity relationship and view biodiversity as a solution to climate change.

In marine environments, plankton species have been shifting geographically and so have fish species. In America's great estuary, the Chesapeake Bay, the southern boundary of the eel grass (*Zostera marina*) community, an important element in the ecology and productivity of the bay, has been moving steadily northward. Eel grass has a distinct upper temperature limit and as the bay has warmed the area in which it grows has decreased by 25 % as a consequence. Range shift of Edith's checkerspot butterfly (*Euphydryas editha*) moving northward and up in altitude and as the bay has warmed the area in which it grows has decreased by 25 % as a consequence population present population extinct. Tropical ecosystems are affected as well. Costa Rica's legendary Monteverde Cloud Forest is experiencing more frequent dry days as climate change raises the altitude at which clouds (virtually the sole source of moisture for cloud forests) form. It is believed that the golden toad (*Bufo periglenes*), which has not been seen in Monteverde for about 20 years, is the first species to be driven to extinction by climate change.

6.9 The Convention and Indigenous and Local Communities

The international community has recognised the close and traditional dependence of many indigenous and local communities on biological resources, notably in the preamble to the Convention on Biological Diversity. There is also a broad recognition of the contribution that traditional knowledge can make to both the conservation and the sustainable use of biological diversity, two fundamental objectives of the convention.

The Conference of the Parties has established a working group specifically to address the implementation of Article 8 (j) and related provisions of the convention. This working group is open to all parties, and indigenous and local communities' representatives play a full and active role in its work. Traditional knowledge is considered a 'cross-cutting' issue that affects many aspects of biological diversity, so it will continue to be addressed by the Conference of the Parties and by other working groups as well. In particular, in decision VII/19D, the Conference of the Parties requested the Ad Hoc Open-ended Working Group on Access and Benefit-sharing with the collaboration of the Ad Hoc Working Group on Article 8 (j) and related provisions to

elaborate an international regime on access to genetic resources and benefit sharing with the aim of adopting an instrument/instruments to effectively implement the provisions in Article 15 and Article 8 (j) of the convention and the three objectives of the convention. This is an ongoing priority of the convention.

6.10 Biodiversity Prospecting

Bioprospecting is an umbrella term describing the process of discovery and commercialisation of new products based on biological resources. Bioprospecting often draws on indigenous knowledge about uses and characteristics of plants and animals. In this way, bioprospecting includes biopiracy, the exploitative appropriation of indigenous forms of knowledge by commercial actors, as well as the search for previously unknown compounds in organisms that have never been used in traditional medicine.

Natural organisms have evolved a staggering variety of chemical compounds to escape predators, capture prey, enhance reproductive success and fight infection. Some of these chemical compounds have proved to be of great value when adapted for industrial, agricultural and pharmaceutical uses. In the USA for instance, nearly 25 % of prescription medicines contain active ingredients derived from plants, while many other drugs are synthesised to replicate or improve naturally produced molecules. Today we treat leukemia with medicines derived from rosy periwinkle of Madagascar and the bark of the pacific yew tree is the source of a promising treatment for ovarian cancer.

6.11 International Organisations Involved in Biodiversity Conservation

6.11.1 IUCN: International Union for Conservation of Nature

IUCN – International Union for Conservation of Nature helps develop conservation science, manages field projects all over the world and brings together players from different domains and sectors to develop and implement policy, laws and best practice. The International Union for Conservation of Nature and Natural Resources is the most important world body of 74 sovereign states, 105 government agencies, 674 nongovernmental organisations and 32 affiliates that is concerned with the conservation of nature worldwide. The headquarters of the IUCN is situated at Gland, Switzerland. Anyone who is interested in conservation should be aware of the activities of the IUCN, its organs and publications. www.iucn.org

The Species Survival Commission (SSC) is one of the six volunteer commissions within the IUCN, with the mission to conserve biological diversity by developing and executing programmes to study, save, restore and manage wisely the species and their habitats. SSC is the source of information for IUCN on the conservation of species. On behalf of the IUCN, the SSC delivers and promotes its knowledge, advice and policies to those who can influence the implementation of conservation action. The SSC has its headquarters at the IUCN in Switzerland.

6.11.2 Conservation International

Global Conservation Fund finances the creation, expansion and long-term management of priority areas for conservation. Conservation International aims to protect life on Earth and to demonstrate that human societies will thrive when in balance with nature. It works with governments, nonprofit organisations, universities, businesses and local communities in priority regions to strengthen conservation efforts. www.conservation.org

6.11.3 World Wildlife Fund

The largest multinational conservation organisation in the world, WWF works in 100 countries and is supported by 1.2 million members in the USA and close to 5 million globally. The World

Wildlife Fund has derived a system called the 'Global 200 Ecoregions', the aim of which is to select priority ecoregions for conservation within each of 14 terrestrial, 3 freshwater and 4 marine habitat types. They are chosen for their species richness, endemism, taxonomic uniqueness, unusual ecological or evolutionary phenomena and global rarity. All biodiversity hotspots contain at least one of the global 200 ecoregions. www.worldwildlife.org

6.12 Biodiversity-Related Conventions

Six international conventions focus on biodiversity issues: the Convention on Biological Diversity (year of entry into force: 1993), the Convention on Conservation of Migratory Species, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1975), the International Treaty on Plant Genetic Resources for Food and Agriculture (2004), the Ramsar Convention on Wetlands (1971) and the World Heritage Convention (1972). Each of the biodiversity-related conventions works to implement actions at the national, regional and international level in order to reach shared goals of conservation and sustainable use. In meeting their objectives, the conventions have developed a number of complementary approaches (site, species, genetic resources and/or ecosystem based) and operational tools (e.g. programmes of work, trade permits and certificates, multilateral system for access and benefit sharing, regional agreements, site listings, funds).

The six biodiversity-related conventions are as follows.

6.12.1 Convention on Biological Diversity

The objectives of the CBD are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising from commercial and other utilisation of genetic resources. The agreement covers all ecosystems, species and genetic resources.

The Convention on Biological Diversity (CBD) was formed at a meeting in Rio de Janeiro in 1992 and came into force, with a membership of 133 countries, in December 1993. CBD aims to protect the world's biological resources from further erosion or at least to slow that rate of erosion down. Till CBD came into force, living organisms were considered a common heritage of the humankind, but CBD accepts them as a sovereign property of the nation states. CBD is to promote conservation of biological diversity, a sustainable use of its components and equitable sharing of the resultant benefits. Thus, there is a difference in the objectives of the IUCN and the CBD, though basically both strive to conserve the world's biological resources.

The Convention on Biological Diversity has three main goals:

1. Conservation of biological diversity (or biodiversity)
2. Sustainable use of its components
3. Fair and equitable sharing of benefits arising from genetic resources

2010 was the International Year of Biodiversity. The Secretariat of the Convention on Biological Diversity is the focal point for the International Year of Biodiversity. The 11th Conference of Parties (COP) to the Convention on Biological Diversity was held in Hyderabad. On 22 December 2010, the UN declared the period from 2011 to 2020 as the UN Decade on Biodiversity. They, hence, followed a recommendation of the CBD signatories during COP10 at Nagoya in October 2010.

6.12.2 Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)

The CITES aims to ensure that international trade in specimens of wild animals and plants does not threaten their survival. Through its three appendices, the convention accords varying degrees of protection to more than 30,000 plant and animal species.

CITES is an international agreement between governments. Its aim is to ensure that international trade in specimens of wild animals and plants does not threaten their survival. CITES has established the international legal framework for the prevention of trade in endangered species and for an effective regulation of trade in others. Member states respect the recommendations of CITES presented in CITES Appendices and implement restriction on the trade of the listed species. CITES Appendix I lists species that are threatened while Appendix II includes the species that may become threatened with extinction if trade is not regulated. Those in Appendix III are species that require watching. Depending upon the need, species may be shifted from one to another Appendix. The CITES Appendices are periodically reviewed, the latest being the outcome of the Tenth Conference of the Parties (all those concerned with trade, governments, NGOs and conservation experts) in June 1997 in Harare, Zimbabwe.

Recently, CITES and TRAFFIC together resolved to work closely with traditional medicine communities to (a) eventually eliminate illegal trade in endangered species of medicinal plants, (b) ensure that the appropriate national legislation is in place to control trade in parts and derivatives of CITES listed species, (c) strengthen enforcement efforts, (d) promote forensic identification techniques and (e) investigate the use of substitutes and artificial propagation. www.cites.org.

Trade Records Analysis of Fauna and Flora in Commerce (TRAFFIC) is the body that monitors the volume of trade in endangered species and works in co-ordination with CITES and SSC, to assess the impact of trade, the objective being to manage trade sustainably.

6.12.3 Convention on the Conservation of Migratory Species of Wild Animals

CMS or the Bonn Convention aims to conserve terrestrial, marine and avian migratory species throughout their range. Parties to the CMS work together to conserve migratory species and their habitats by providing strict protection for the most endangered migratory species, by concluding regional multilateral agreements for the conservation and management of specific species or categories of species and by undertaking cooperative research and conservation activities.

6.12.4 The International Treaty on Plant Genetic Resources for Food and Agriculture

The objectives of the Treaty are the conservation and sustainable use of plant genetic resources for food and agriculture and the fair and equitable sharing of the benefits arising out of their use, in harmony with the Convention on Biological Diversity, for sustainable agriculture and food security. The Treaty covers all plant genetic resources for food and agriculture, while its multilateral system of access and benefit sharing covers a specific list of 64 crops and forages. The Treaty also includes provisions on Farmers' Rights

6.12.5 Convention on Wetlands (Popularly Known as the Ramsar Convention)

The Ramsar Convention provides the framework for national action and international cooperation for the conservation and wise use of wetlands and their resources. The convention covers all aspects of wetland conservation and wise use, recognising wetlands as ecosystems that are extremely important for biodiversity conservation in general and for the well-being of human communities.

The Ramsar List of Wetlands of International Importance now includes 2,122 sites (known as 'Ramsar Sites') covering 205,366,160 ha (507,470,800 acres) up from 1,021 sites in 2000. The nation with the highest number of sites is the UK at 169; the nation with the greatest area of listed wetlands is Canada, with over 130,000 km^2

(50,000 sq mi), including the Queen Maud Gulf Migratory Bird Sanctuary at 62,800 km^2 (24,200 sq mi). The Ramsar definition of wetlands is fairly wide, including 'areas of marine water the depth of which at low tide does not exceed six meters' as well as fish ponds, rice paddies and salt pans. Presently there are 168 contracting parties, up from 119 in 1999 and from 21 initial signatory nations in 1971. The state parties meet every 3 years as the Conference of the Contracting Parties (CCP), the first held in Cagliari, Italy, in 1980. Amendments to the original convention have been agreed to in Paris (in 1982) and Regina (in 1987).

6.12.6 World Heritage Convention (WHC)

The primary mission of the WHC is to identify and conserve the world's cultural and natural heritage, by drawing up a list of sites whose outstanding values should be preserved for all humanity and to ensure their protection through a closer cooperation among nations.

The 156 biodiversity World Heritage sites cover a total land area of 1.1 million km^2, i.e. nearly 0.8 % of the global land surface, or 6.6 % of the total extent of the world's terrestrial protected areas. Generally speaking, biodiversity World Heritage sites are very large protected areas, often involving multiple component parts in serial sites. The existing network of biodiversity World Heritage sites encompasses many outstanding protected areas that represent a wide range of global biodiversity conservation priorities. Biodiversity World Heritage sites 'represent' 31 (89 %) of the 35 biodiversity hotspots and all five high-biodiversity wilderness areas, 97 (68 %) of the 142 Global 200 terrestrial priority ecoregions, 72 (31 %) of the 234 Centres of Plant Diversity and 83 (38 %) of 218 Endemic Bird Areas

References

FAO (1996) Report on the state of the world's plant genetic resources for food and agriculture. Prepared for the international technical conference on Plant Genetic Resources, Leipzig, 17–23 June 1996, Rome

Hausmann BIG, Parzies HK, Presterl T, Sušić Z, Miedaner T (2004) Plant genetic resources in crop improvement. Plant Genet Res 2:3–21

Hayden BP, Ray GC, Dolan R (1984) Classification of coastal and marine environments. Environ Conserv 11:199–207

Krishnamurthy KV (2003) An advanced textbook on biodiversity: principles and practices. Oxford & IBH Publishing Co., New Delhi

Myers N (1988) Threatened biotas: 'hotspots' in tropical forests. Environmentalist 8:187–208

Myers N (1990) The biodiversity challenge: expanded hotspots analysis. Environmentalist 10:243–256

Myers N, Mittermeirs RA, Mittermier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403(6772):853–858

Pullaiah T (2012) An overview on biodiversity and conservation perspectives. Bioherald 29:1–14

Fungi: An Overview

7

M.A. Singara Charya

Abstract

Fungi play an important role in the management of nutrient cycles and providing continued benefit to humankind. Most fungi are composed of hyphae, which are a source for its absorption of food. Basically, the fungal cell wall is made up of chitin. Fungi are chemoheterotrophs and adopted parasitic/saprophytic/symbiotic ways of nutrition. During the availability of sufficient nutrition, fungi prefer asexual mode of reproduction, and in unfavorable situations, it switches over to sexual mode of reproduction. The dikaryon stage in the life cycle is dominant in Basidiomycotina while sexual reproduction is never observed in Deuteromycotina. Fungi have a great economic value and utilized for centuries both industrially and commercially. Its role in medicine, food, textiles, and vitamins is well known. They play positive and negative role in agriculture; while they are responsible for nutrient recycle in the soil, soil fertility, and symbiotic associations, they cause damage to agriculture by many fungal diseases and losses. The fungal enzymes are attained a greater importance in paper and pulp, coal liquefaction, food processing, fuel alcohol, baking and brewing, wine making, and pharmaceutical and leather processing industries. Recently, the significance of fungi in the development of biopesticides/biofungicides has also gained momentum to discourage chemical pesticides to conserve the precious biodiversity.

Keywords

Fungal hyphae structure • Chitin • Classification • Reproduction • Nutrition • Deuteromycotina • Fungal enzymes • Fungal diseases

M.A. Singara Charya (✉)
Department of Microbiology, Kakatiya University,
Warangal 506 009, Telangana, India
e-mail: singaracharyam@gmail.com

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_7,

7.1 Introduction

The Kingdom Fungi (according to the Five-Kingdom concept of Whittaker 1969) includes some of the most important organisms, both in terms of their ecological and economic roles. By breaking down dead organic material, fungi continue the nutrient cycle through ecosystems. In addition, most vascular plants could not grow without the association of symbiotic fungi, or mycorrhizae, that inhabit their roots and supply essential nutrients. Other fungi provide numerous drugs (such as penicillin and other antibiotics); foods like mushrooms, truffles, and morels; and the bubbles in bread, champagne, and beer (Alexopolous et al. 1996). A fungus is a member of a large group of eukaryotic organisms that include microorganisms such as yeasts and molds as well as the more familiar mushrooms. These organisms are classified as a kingdom, Fungi, which are separate from plants, animals, protists, and bacteria. One major difference is that fungal cells have cell walls characterized by the presence of chitin unlike the cell wall of higher plants and even bacteria. The true fungi have their evolutionary origins within the chytrids. In addition to the true fungi, a number of other evolutionary lineages have produced fungus-like organisms (Lene 2010). The most similar are the Oomycetes, a lineage that is related to diatoms and brown algae – all being members of the stramenopiles. Other fungus-like organisms include amoeboid slime molds. The true fungi are heterotrophic organisms (Webster 1970). The cytoplasm is enclosed within a chitinous cell wall. While the majority of species grow as multicellular filaments called hyphae, with all of the hyphae together forming the mycelium, some species (such as yeasts) also grow as single cells. Sexual and asexual reproduction of the fungi is common via various kinds of spores, often produced on specialized structures like ascus or basidium. Yeasts, molds (molds), and mushrooms are common examples of fungi. True fungi lack flagella, but the chytrid ancestors are unicellular organisms that swim with the aid of flagella (Burnett 1976). Occurring worldwide, most fungi are largely invisible to the naked eye, living for the most part in soil, dead matter, and as symbionts of plants, animals, or other fungi. They perform an essential role in many ecosystems in decomposing organic matter and are indispensable in nutrient cycling and exchange (Hawksworth et al. 1983). Some fungi become noticeable when fruiting, either as mushrooms or molds. Fungi are sources of antibiotics used in medicine and for various enzymes such as cellulases, pectinases, and proteases important for industrial use or as active ingredients of detergents. Many fungi produce bioactive compounds called mycotoxins, such as alkaloids and polyketides that are toxic to animals including humans. Some fungi produce hallucinogenic effects. Several species of the fungi are significant pathogens of humans and other animals, and huge losses of crops worldwide due to wide variety of fungal diseases or food spoilage caused by fungi can have a large impact on human food supply and local economies. With an estimated 1.5 million species, fungi represent one of the largest branches of the Tree of Life. They have an enormous impact on human affairs and ecosystem functioning, owing to their diverse activities as decomposers, pathogens, and mutualistic symbionts. And perhaps more than any other group of nonphotosynthetic organisms, fungi are essential biological components of the global carbon cycle (Barnett et al. 2000). Collectively, they are capable of degrading almost any naturally occurring biopolymer and numerous human-made ones. As such, fungi hold considerable promise in the development of alternative fuels, carbon sequestration, and bioremediation of contaminated ecosystems. The use of fungi for the continued benefit of humankind, however, requires a better understanding of how they interact in natural and synthetic communities. The ability to sample environments for complex fungal metagenomes is rapidly becoming a reality and will play an important role in harnessing fungi for industrial, energy, and climate management purposes. However, our ability to accurately analyze these data relies on well-characterized, foundational reference data of fungal genomes.

7.2 Structure of Fungal Hyphae

Most fungi grow as hyphae (vegetative structure), which are cylindrical, threadlike structures 2–10 μm in diameter and up to several centimeters in length (Fig. 7.1). Hyphae grow at their tips (apices); new hyphae are typically formed by emergence of new tips along existing hyphae by *branching*, or occasionally growing hyphal tips bifurcate giving rise to two parallel-growing hyphae. The combination of apical growth and branching leads to the development of a mycelium, an interconnected network of hyphae. Hyphae can be either septate or coenocytic: septate hyphae are divided into compartments separated by cross walls (internal cell walls), the septa, that are formed at right angles to the cell wall giving the hypha its shape, with each compartment containing one or more nuclei; coenocytic hyphae are not compartmentalized. Septa have pores that allow cytoplasm, organelles, and sometimes nuclei to pass through. Each hypha is essentially a tube – consisting of a rigid wall and containing protoplasm, tapered at its tip, and this is the region of active growth. Septa (cross walls) possess at regular intervals along the lengths of the hyphae. In others, cross walls form only to isolate old or damaged regions of a hypha or to isolate reproductive structures. Some septa possess one of more pores – such septa divide up the hyphae into a series interconnected hyphal compartments, rather than separate, discrete cells. But these structures are simply the large, macroscopic fruiting bodies produced by some groups of fungi. The actively growing and reproductive structures of most species are microscopic, and although most fungi are mycelial (filamentous), there are some exceptions to this growth form. Figure 7.1 shows the structure of hyphae and cell wall. The fungal plant body can be mycelia, unicellular primitively branched as in chytrids, unicellular as in *Saccharomyces* or dimorphic.

7.2.1 Mycelia (Filamentous)

Most fungi are composed of microscopic filaments called hyphae (Fig. 7.2), which branch to eventually form a network of hyphae, called a mycelium (colony). The mycelium extends over or through whatever substrate the fungus is using as a source of food. Each hypha is essentially a tube, containing protoplasm surrounded by a rigid wall. Depending upon the species, the protoplasm may form a continuous, uninterrupted mass running the length of the branching hyphae, or the protoplasm may be interrupted at intervals by cross walls called septa. Septa divide up hyphae into individual discrete cells or interconnected hyphal compartments. Hyphae exhibit apical growth (i.e., they elongate at their tips) and are capable of growing indefinitely, provided that environmental conditions remain favorable for growth. Hyphae may initially develop from a germ tube (a short, immature hypha) that emerges from a germinating spore. Spores are the microscopic dispersal or survival propagules produced by many species of fungi. Although most fungi are mycelial (filamentous), the following represent exceptions to this growth form:

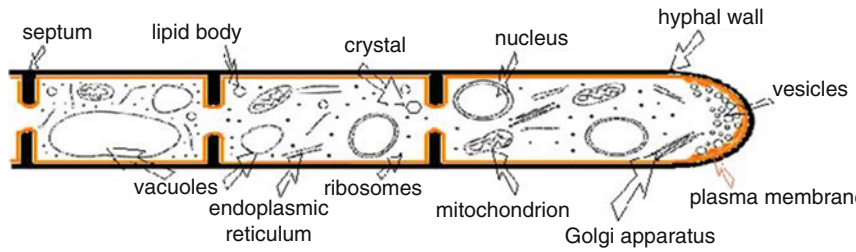


Fig. 7.1 Ultrastructure of a septate hypha

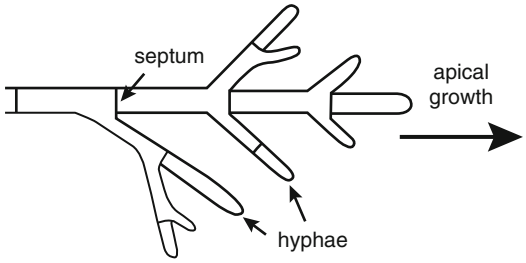


Fig. 7.2 Mycelia (filamentous)

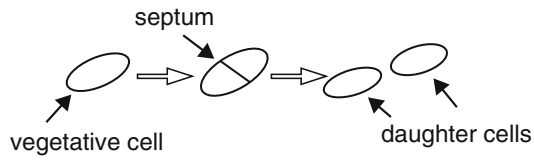


Fig. 7.3 Yeast showing binary fission

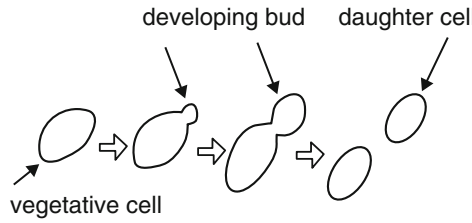


Fig. 7.4 Yeast showing budding

7.2.2 Unicellular and Primitively Branched Chytrids (*Chytridiomycota*)

Fungi belonging to the *Chytridiomycota* exist as either single round cell (unicellular species) or primitively branched chains of cells (Fig. 7.3). In either case, the fungus may be anchored to its substrate by rhizoids.

7.2.3 Yeasts (Unicellular)

Yeasts, which are used in a variety of commercially important fermentation processes (e.g., bread making, brewing beers and wines), are capable of reproducing asexually and sexually (Fig. 7.4).

1. Budding (e.g., *Saccharomyces cerevisiae*) or
2. Binary fission (splitting into two equal halves; e.g., *Schizosaccharomyces pombe*)

7.2.4 Dimorphic

Some fungi are dimorphic and capable of alternating between a mycelial growth form and a unicellular yeast phase. This change in growth form is often in response to some change in environmental conditions. This dimorphism is exhibited by several species of fungi that are pathogenic in humans, e.g., *Paracoccidioides brasiliensis*.

7.2.5 The Plasma Membrane

The plasma membrane is closely associated with the hyphal wall and in some regions may even be firmly attached to it – making it difficult to plasmolyze hyphae. Each hyphal cell or compartment normally contains one or more nuclei. In species whose septa possess a large central pore, the number of nuclei within a hyphal compartment does not remain static because the nuclei are able to pass between adjacent compartments, via the central septal pore. Other cytoplasmic organelles are those commonly found in all eukaryotic cells. The growing tip is structurally and functionally very different from the rest of the hypha, and its cytoplasm appears more dense. There are no major organelles at the extreme tip, but at the extreme tip, there is an accumulation of membrane-bound vesicles – the apical vesicular cluster – which plays an important role in apical growth. Vacuoles may be visible in subapical hyphal compartments – although small at first, they grow larger and merge with one another; they store and recycle cellular metabolites, e.g., enzymes and nutrients. In the oldest parts of the hypha, the protoplasm may break down completely, due either to autolysis (self-digestion) or in natural environments heterolysis (degradation due to the activities of other microorganisms). Septa (cross walls) can be seen by light microscopy, but electron microscopy has revealed that several different types of septa exist among the major taxonomic groups of fungi.

7.2.6 Oomycota and Zygomycota

In general, the hyphae of fungi belonging to these groups are not regularly septate, but septa in the form of complete cross walls are formed to isolate old or damaged regions of the mycelium or to separate reproductive structures from somatic hyphae.

7.2.7 Ascomycota and Some Mitosporic Fungi

Fungal hyphae belonging to these groups possess perforated septa at regular intervals along their length. The septum consists of a simple plate with a relatively large central pore (50–500 nm diameter) – this permits cytoplasmic streaming (the movement of organelles including nuclei) between adjacent hyphal compartments. Cytoplasmic streaming enables subapical and intercalary (central) compartments of young hyphae to contribute toward growth of the hyphal tip – transporting nutrients and essential enzymes to the apex – so maximizing the capacity for somatic growth. Associated with each septum are spherical, membrane-bound organelles called the Woronin bodies that are composed of protein and remain close to the septal pore and tend not to be disturbed by the cytoplasmic streaming taking place. They are larger in diameter than the septal pore and are, therefore, capable of blocking the pore and also will block the septal pore if the adjacent hyphal compartment is damaged or aging and becoming highly vacuolated. Not all fungi belonging to the Ascomycota possess Woronin bodies – those that do not often possess large hexagonal crystals of protein in the cytoplasm that are capable of serving the same function, i.e., they can seal the septal pores of damaged or aging hyphae.

7.2.8 Some Other Mitosporic Fungi

A number of mitosporic fungi possess septa with a single central pore, similar to that observed in the Ascomycota, but other mitosporic fungi may possess multiperforate septa. The septa of *Geotrichumcandidum* possess characteristic micropores (ca 9 nm diameter). The number of pores in each septum can vary up to a maximum of ca. 50. These micropores allow cytoplasmic continuity between adjacent hyphal compartments but are too small to allow cytoplasmic streaming to occur to the extent observed in fungi possessing larger septal pores.

7.2.9 Basidiomycota

The most complex type of septum is found in fungi belonging to the Basidiomycota. Each septum is characterized by a swelling around the central pore (dolipore) and a hemispherical perforated cap (parenthosome) on either side of the pore. The perforated parenthosome allows cytoplasmic continuity but prevents the movement of major organelles. The plasma membrane lines both sides of the septum and the dolipore swelling, but the membrane of the parenthosome is derived from endoplasmic reticulum.

7.2.10 Functions of Septa

The septa supports structurally with the platelike cross walls along the tubelike structure (hypha) that will help to stabilize it. Acting as the first line of defense when part of a hypha is damaged – large-pored septa that have Woronin bodies or large proteinaceous crystals associated with them have the advantage that cytoplasmic streaming can occur between adjacent compartments. But at the same time, a mechanism exists for rapidly sealing the septal pore under conditions of stress thereby helping protect the mycelium. Septa can isolate adjacent compartments from one another so that different biochemical and physiological processes can occur within them – these may result in differentiation of the hyphae into specialized structures, such as those associated with sporulation.

7.3 Fungal Cell Wall

The functions of the cell wall are the following: (1) protects the underlying protoplasm, (2) determines and maintains the shape of the fungal cell or hypha, (3) acts as an interface between the fungus and its environment, (4) acts as a binding site for some enzymes, and (5) possesses antigenic properties – which allow interactions with other organisms (Fig. 7.5).The chemical composition of the wall is with polymeric fibrils, chitin, cellulose (in the Oomycota), amorphous matrix components, glucans, proteins, lipids, and heteropolymers (mixed polymers) of mannose, galactose, fucose, and xylose (Gander 1974). The types and amounts of these various components vary among different groups of fungi and may even vary during the life cycle of a single species.

In general, the inner part of the wall consists of polymeric fibrils embedded in an amorphous matrix, and this is covered by further layers of matrix material. At the hyphal tip, the wall is thinner and simpler in structure, consisting of only two layers – an inner layer of fibrils embedded in protein and outer layer of mainly protein. Extra layers of wall material are deposited in the lateral walls behind the extending apex – strengthening the wall as the hypha matures. In the oldest parts of the hyphae (and in many fungal spores), lipids and pigments are deposited in the wall; lipids serve as a nutrient reserve and help prevent desiccation, and pigments, such as melanin, help protect the protoplast against the damaging effects of UV radiation.

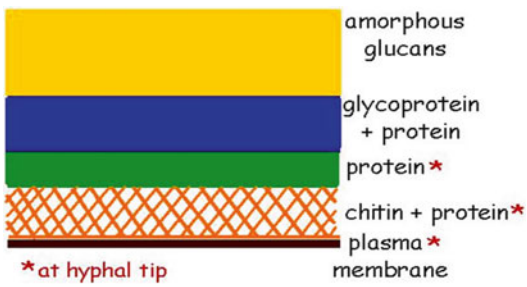


Fig. 7.5 Diagrammatic representation of sectional view of mature lateral wall of hyphae of *Neurospora crassa* (*Source*: http://www.fungionline.org.uk/1intro/1intro_char.html)

7.4 Fungal Nutrition

All fungi are chemoheterotrophic (chemoorganotrophic) – synthesizing the organic compounds they need for growth and energy from preexisting organic sources in their environment, using the energy from chemical reactions. Since their protoplasm is protected by a rigid wall, fungi must obtain their nutrients by the process of absorption (Jennings 1995). Small molecules (e.g., simple sugars, amino acids) in solution can be absorbed directly across the fungal wall and plasma membrane. Larger, more complex molecules (e.g., polymers such as polysaccharides and proteins) must be first broken down into smaller molecules, which can then be absorbed. This degradation takes place outside the fungal cell or hypha and is achieved by enzymes which are either released through or are bound to the fungal wall. Because these enzymes act outside the cell, they are called extracellular enzymes. Since water is essential for the diffusion of extracellular enzymes and nutrients across the fungal wall and plasma membrane, actively growing fungi are usually restricted to relatively moist (or humid) environments. Although fungi are similar to plants in many ways, they do not have chlorophyll, the green pigment that enables plants to make their own food with the aid of sunlight (photosynthesis). Fungi release digestive enzymes that decompose things around them, turning them into food. The fungus then absorbs the dissolved foods through the walls of its cells. Fungi have adapted various ways of doing this; these are the following:

7.4.1 Parasitic Fungi

Several species of fungi exist as parasites, feeding on live hosts, which might be animals, plants, or even other fungi. Some of these parasitic fungi damage our crops, sicken farm animals,

and harm or completely destroy trees. Dutch Elm disease, caused by the fungus *Ophiostoma ulmi*, destroyed hundreds of millions of Elm trees worldwide. The rice blast fungus *Magnaporthe oryzae* can devastate rice crops. The following are the serious diseases to humans: *aspergilloses*, *candidiases*, *coccidioidomycosis*, *cryptococcosis*, *histoplasmosis*, *mycetomas*, and *paracoccidioidomycosis*.

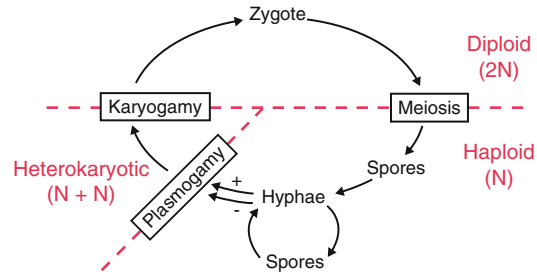


Fig. 7.6 Generalized life cycle of fungi

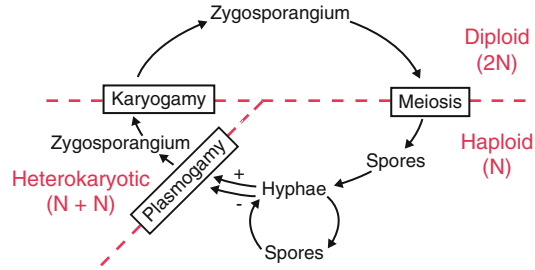


Fig. 7.7 Diagrammatic presentation of the fusion of two hyphae leads to the formation of a *zygosporangium*

7.4.2 Saprobes or Saprophytes

These break down dead organisms and substances that contain organic compounds and feed on them when they have rotted. Humans welcome saprobes and also fear them. They are useful decomposers of organic material, but also damage wood products and spoil our food. When ships used to be made of wood, they were often rendered unusable by wood-digesting saprobes (polypores).

7.4.3 Symbiosis

This is when one living thing builds up a relationship with another for the mutual survival of both. Some fungi form mycorrhizae which enhance a plant root's capacity to absorb nutrients. The plant synthesizes nutrients the fungus needs and exchanges these nutrients for minerals the fungus absorbs from the soil – i.e., the plant and the fungus trade nutrients. Some leaf-cutting ants eat nothing but a type of fungi that lives in their nests. The fungi live on nothing but the leaves the ants carry in for them. If the ant starved the fungi and killed them, the ant would have no food and would die; if the fungi found a way of poisoning the ants and killing them off, the fungi would have no food and would die. They both depend on each other for survival.

7.5 Fungal Reproduction

Fungi generally undergo a reproductive cycle that includes the production of sexual spores (Fig. 7.6). A sexual spore contains one nucleus that has set of chromosomes – just half of the total set of the fungal-cell chromosomes – they are haploid. Some spores contain two or more nuclei (Joseph et al. 2013). When a spore germinates, it eventually develops into a mycelium that produces fruiting bodies with sexual spores – and so the reproductive cycle starts all over again (Fig. 7.7).

7.5.1 Asexual Reproduction

Asexual spores may be produced directly from the hypha in some fungi without the need for fruiting bodies (Taylor et al. 1992). The spores then germinate and produce additional mycelium, which spreads rapidly. This allows more rapid dispersal than sexual reproduction.

7.5.2 The Dikaryon Stage

There are two mating strains of hyphae which exist in the mycelium – the plus and the minus

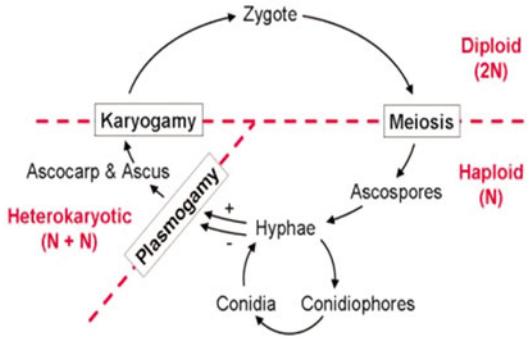


Fig. 7.8 Dikaryotic hyphae within the ascocarp produce *asci*

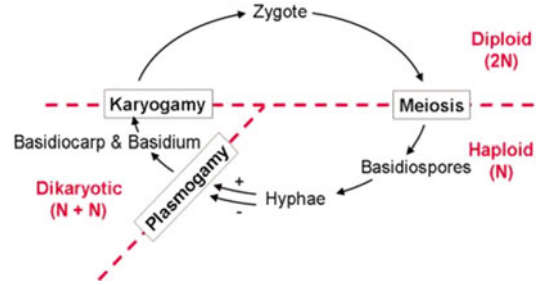


Fig. 7.9 Diagrammatic presentation of basidiospores and basidiocarp formation (https://b51ab7d9e5e1e7063dcb-70cee5c33cf7f4b7bad8.googledrive.com/host/0Bx6hk6AUBHxDc2d4TDJZTFIyMGs/files/Bio%20102/Bio%20102%20lectures/Fungi/fungi.htm#Club Fungi)

strain (Fig. 7.8). They both look the same but are different. Sexual reproduction occurs when the plus and minus strains fuse. Their nuclei will remain separate during the initial stages – this intermediate stage is called the *dikaryon*. Dikaryon means a pair of associated but unfused haploid nuclei of a fungus cell capable of participating in repeated cell division as separate entities before their eventual fusion – i.e., two nuclei, each with one half of the chromosome pairs, participating in cell division, but with nuclei not fusing yet, before the nuclei eventually fuse. With some species, the dikaryon stage may last for several years, while with others, it may be just for weeks. Eventually the two nuclei fuse and become one nucleus with the pairs of chromosomes joined up (two sets containing half the total chromosomes each), forming a diploid cell. The diploid cell then divides producing daughter cells with half the parent cells genetic material – this process is called *meiosis*. Usually four genetically unique haploid spores are produced, and the cycle restarts. This form of procreation using genetically different spores helps fungi adapt more effectively to novel diseases and environmental changes. If all the fungi were genetically identical, they could all be destroyed by a single disease or a significant environmental change.

7.5.3 Fragmentation

In some types of fungi, the hyphae fragment, and each fragment developing into a new separate organism. With the single cell of yeast, a bump forms on the cell which eventually breaks off and ultimately becomes a new yeast cell. Before karyogamy, the zygosporangium contains many haploid nuclei after fusion, i.e., karyogamy; it contains many diploid nuclei. Dikaryotic hyphae within the ascocarp produce *asci* (singular: *ascus*), sacs that are walled off from the rest of the hyphae. Nuclear fusion within an ascus will produce a diploid zygote. The zygote will undergo meiosis, followed by mitosis, to produce eight haploid *ascospores. Basidiospores* are produced on *basidia* within the basidiocarps (Fig. 7.9). In mushrooms, the basidia are located along the gills on the lower side of the cap.

The fungi imperfecti/imperfect fungi, also known as *Deuteromycota*, are fungi which do not fit into the commonly established taxonomic classifications of fungi that are based on biological species concepts or morphological characteristics of sexual structures. Their sexual form of reproduction has never been observed, hence the name "imperfect fungi." Only their asexual reproduction is known, meaning that this group of fungi reproduces asexually by spores.

7.5.4 Heterothalism (Sexual incompatibility)

For a long time, it was thought that a spore or an individual derived from any spore is totipotent (bisexual) (Robertson et al. 1988). In mucoraceous species, an interaction of two thalli is

required for zygospore (heterothallic) that leads for heterothallism (Mathieu and Sven 2009). The mycelium of homothallic species is bisexual, and heterothallic species is unisexual. Most fungi are now classified as follows:

(a) Hermaphroditic (male and female on the same thallus)
(b) Dioecious (male and female on different thalli)
(c) Sexually undifferentiated (male and female organs are morphologically similar)

When mycelium is sexually self-fertile and two mating types of genes "A" and "a" are in the same thallus, it is called "homothallic." When each thallus is sexually self-fertile and mating type genes "A" and "a" are in different thalli, it is called "heterothallic." When two nuclei of opposite mating types "A" and "a" are incorporated in the same spore, it is called as "pseudo (secondary) homothallic."

Whitehouse (1949) defined the heterothallism in two ways: (1) morphological heterothallism, two interacting thalli produce morphologically dissimilar male and female sex organs, and (2) physiological heterothallism, interacting thalli differ in mating types and are entirely independent of morphological differences between male and female.

Heterothallism in Mucorales (*Mucor*, *Rhizopus*) was noticed, and trisporic acid B and C were found to be responsible for inducing zygospore formation in (+) and (−) strains.

Heterothallism in Ascomycotina (*Neurospora*, *Sordaria*) was with eight ascospores, where four are (+) (gene A) and four are (−) (gene a), and formation of dikaryons was with A+a.

In heterothallism of Basidiomycotina, 25 % of organism should have a single-gene controlled homokaryon compatibility. "A" and many alleles of this gene exist in the population like $A_1 + A_2$; $A_1 + A_3$ are compatible, and hyphal fusion results in the formation of dikaryon, where homokaryons with the same "A" alleles are incompatible, i.e., bipolar. While A and B with two alleles at each locus and both of which have many alleles, A1, A2, A3, A4,….An. The tendency of having A_1B_1, A_2B_2, A_2B_1, and A_2B_2 is called "tetra polar."

Bipolar differs in genetic makeup; a single spore carries only one allele.

"A" locus and A1, A2, A3, and A4 alleles

	A_1	A_2	A_3	A_4
A_1	−	+	+	+
A_2	+	−	+	+
A_3	+	+	−	+
A_4	+	+	+	−

Tetra polar where the alleles are at two loci and these two loci are on different chromosomes

	A_1B_1	A_1B_2	A_2B_1	A_2B_2
A_1B_1	−	FL	B	+
A_1B_2	FL	−	+	B
A_2B_1	B	+	−	FL
A_2B_2	+	B	FL	−

- Here, when two thalli with common "A" and "B" alleles are mated through plasmogamy, heterokaryons are formed; fruits never occur.
- Common "A" alleles and dissimilar "B" alleles result in poor growth, irregular branches, absence of clamp connections, and disrupted nuclear distribution which is called FL (flat reaction).
- Common "B" alleles and dissimilar "A" alleles result B (barrage reaction). It causes cessation of growth.

7.6 Parasexual Cycle

In Parasexual cycle means where the plasmogamy, karyogamy, and haploidization take place in a sequence but not at specified points in the life cycle (Raper 1966). It was reported in *Aspergillus nidulans* which is the imperfect state of *Emericella nidulans*. It is very common in deuteromycetes and rare in other fungi. The results of this process, i.e., recombination of hereditary properties, are similar to those achieved by meiosis (sexual reproduction). The progress of parasexual cycle can be explained in six steps:

Step 1 – Anastomosis
Step 2 – Unlike nuclei fuse heterokaryon
Step 3 – Diploid conidia
Step 4 – Mitotic crossing over in diploid nuclei
Step 5 – Haploidization through aneuploidy
Step 6 – Sorting out of new haploid nuclei through conidia

I. *Formation of heterokaryon*: The heterokaryosis means the coexistence of genetically different nuclei with cytoplasmic continuity with one another.

   Heterokaryons will form by (a) fusion (anastomosis) of vegetative cells, (b) inclusion of dissimilar genetic nuclei in a single spore, (c) mutation of one or more nuclei of a homokaryotic mycelium, and (d) fusion of some of the nuclei and their subsequent multiplication.

II. *Formation of diploid nuclei*: When mycelium becomes heterokaryotic, fusion of the few haploid nuclei may take place. Fusion may be between like (homozygous diploid) or unlike (heterozygous diploid) genetic constitution.

III. *Multiplication of the diploid nuclei and mitotic crossing over*: The most important event in parasexual cycle is the mitotic recombinations, which bring out new combinations of genetic material. More genetically different nuclei are formed after chromosome segregation.

IV. *Occasional haploidization through aneuploidy*: The diploid nuclei give rise to genetically different haploid nuclei by gradual loss of chromosomes by a series of typical and irregularly occurring successive mitotic divisions, i.e., via aneuploidy.

$$2n+1 = \text{tetrasomics}$$
$$2n-1 = \text{monosoics.}$$

The important applications of parasexual cycle are:

1. To provide missing links in the imperfect fungi
2. To compare segregation process in higher organisms
3. For genetic analysis
4. For strain improvement
5. To develop strategies in control of pathogenicity in crop plants

7.6.1 Sex Hormones in Fungi

The participation of hormones in sexual reproduction of fungi was proposed by Graham et al. (1993). The hormone is a diffusible substance playing a specific role during the sexual reproduction. The important sex hormones reported in fungi are sirenin, antheridiol, trisporic acid, yeast α-factor, and zearalenone (Fig. 7.10).

Sirenin is reported in *Allomyces* belonging to chytridiomycetes for the fusion between uniflagellate motile "male" and "female" gametes. Sirenin is a bicyclic sesquiterpene diol (Fig. 7.10) with $C_{15}H_{25}O_2$ molecular formula and 236 as molecular weight. It is active at very low concentrations, less than 10^{-10} g/ml but it has no effect on the diploid gametes.

Antheridiol and oogoniol were recorded in *Achlya* by Raper (1966), the female filament secreted hormone A which induced antheridiol on the male. Hormone A is believed to be a mixture of four hormones, two secreted by the female hyphae (A^2 and A) and the two produced by the male hyphae (A^1 and A^3). The oogonial initials produce hormone C which directs the growth of antheridia to the oogonia and hormone D secreted by the antheridia which control the cleavage of the oogonium into oospores.

Trisporic acid hormone was discovered in *Rhizopus nigricans*. The two incompatible strains could not be distinguished morphologically and labeled as (+) and (−). The (+) and (−) mycelia of *Mucor mucedo* when grown together in a liquid culture accumulate substances in the medium which induce zygospore in both mating types of the same fungus. It is known that each (+) and (−) strain produces precursor molecules such as B and Y carotene that the compatible strain converts to trisporic acid.

Saccharomyces cerevisiae forms haploid single cells which are of two genetically determined mating types ("a" and "α"). When two such cells are in proximity, normally budding is inhibited, and the cells elongate toward each other. α cells produce a diffusible chemical substance which reduces the formation of copulatory processes. The "a" cells under the influence of the α -factor stop growth and budding.

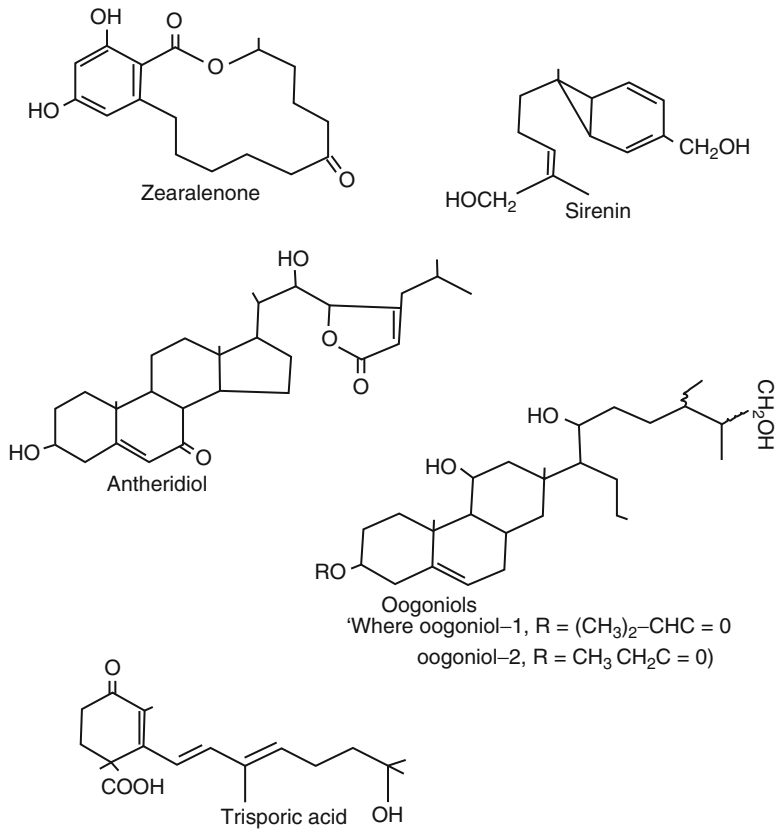


Fig. 7.10 Showing structural formula of chemically characterized sex hormones in fungi (*Source*: *An Introduction to Mycology* by Meharotra and Aneja (1990))

Most fungi reproduce by producing spores characteristic of the group they belong (ascospores ascomycetes; basidiospores – basidiomycetes. Production of spores is in enormous and may be trillions.

7.7 Fungal Classification

The major divisions are based upon the reproductive structures and similarities in life cycle. The classification of fungi has long been a subject of controversy. For a long time, the study of fungi (Mycology) was subdivision of botany; Whittaker (1969) introduced the Five-Kingdom taxonomy, granting fungi equal status with animals and plants. The Five-Kingdom taxonomy included Animalia (animal kingdom), Plantae (plant kingdom), Fungi (fungi kingdom), Protista (types of eukaryotic organisms; containing complex structures enclosed within membranes), and Monera (types of microscopic single-celled organisms whose genetic material is loose in the cell, instead of being held in the cell's nucleus). Today, the main criteria for fungal classification are the type of spores and fruiting bodies it produces (Kendrick 1971).

The traditional taxonomic scheme used by mycologists classifies the fungi into four divisions, based primarily on variations in sexual reproduction.

1. Zygomycota (zygomycetes)
2. Ascomycota (sac fungi)
3. Basidiomycota (club fungi)
4. Deuteromycota (fungi imperfecti)

A comprehensive phytogenetic classification of the kingdom Fungi was proposed by Ainsworth (1973)with reference to recent molecular phylogenetic analysis and with input from diverse members of the fungal taxonomic communities.

The classification includes 195 taxa, down to the level of order, of which 16 are described or validated. The outline of the Ainsworth (1973) classification is as follows:

Outline classification of Fungi as proposed by Ainsworth (1973).

Myxomycota **(wall-less organisms)**

A. *Acrasiomycetes* (cellular slime molds)
   Amoeboid organisms that aggregate to form a fungus-like fruiting body.
B. *Hydromyxomycetes* (net slime molds)
   Spindle-shaped cells that migrate within a tubular network of extra cellular polysaccharide.
C. *Myxomycetes* (true slime molds)
   Multinucleate protoplasmic mass (plasmodium) that engulfs food particles.
D. *Plasmodiophoromycetes* (endoparasitic slime molds)
   Small plasmodia parasites in cells of algae, fungi, and higher plants.

Eumycota **(true, walled fungi)**

A. *Mastigomycotina* produces flagellate asexual spores (zoospores).
   1. *Chytridiomycetes*: unicellular or primitive chains of cells, sometimes attached to food base by tapering rhizoids. Zoospores have a single posterior whiplash flagellum.
   2. *Oomycetes*: mycelia, aseptate zoospores have two flagella, an anteriorly directed tinsel type and posteriorly directed whiplash type.
B. *Zygomycotina*: Usually mycelia, aseptate, nonmotile asexual spores formed in a sporangium.
   1. *Zygomycetes*: Usually saprophytic.
   2. *Trichomycetes*: Usually parasitic in guts of arthropods.
C. *Ascomycotina*: Septate mycelium or yeasts, asexual spores not formed in sporangium, sexual spores formed in as ascus.
   1. *Hemiascomycetes*: Yeast or mycelia, ascus is not enclosed in a fruiting body (ascocarp).
   2. *Euascomycetes*: Mycelia, asci enclosed in an ascocarp.
D. *Deuteromycotina*: Septate mycelium or yeasts. Asexual spores not formed in sporangium. Sexual reproduction absent, rare, or unknown.
   1. Blastomycetes: Typically yeasts.
   2. Hyphomycetes: Mycelial, asexual spores (conidia) formed on simple hyphae or conidiophores.
   3. Coelomycetes: Mycelial, asexual spores formed from conidiophores in flask-shaped structure (pycnidium) or on pad tissue (acervulus).
E. *Basidiomycotina*: Septate mycelium or yeasts, asexual spores absent or as in ascomycotina, sexual spores formed on a basidium.
   1. *Teliomycetes*: No special fruiting body (basidiocarp) to enclose the basidia, parasite on higher plants (rusts and smuts)
   2. *Hymenomycetes*: Basidiocarp is a toad stool or bracket on which the basidia are exposed.
   3. *Gastromycetes*: Various basidiocarps, enclosing the basidia.

Phylogenetic classification of asexually reproducing fungi now commonly uses molecular system (Fig. 7.11). Phylogenetic trees constructed from comparative analyses of DNA sequences, such as RNA, or multigene phylogenies may be used to infer relationships between asexually reproducing fungi and their sexually reproducing counterparts (David et al. 2013). With these methods, many asexually reproducing fungi have now been placed in the Tree of Life. However, because phylogenetic methods require sufficient quantities of biological materials that are from pure (i.e., uncontaminated) fungal cultures, for many asexual species, their exact relationship with other fungal species has yet to be determined.

7.8 Fossil Record of Fungi

In contrast to plants and animals, the early fossil record of the fungi is meager. Factors that likely contribute to the underrepresentation of fungal species among fossils include the nature of fungal-fruiting bodies, which are soft, fleshy, and easily degradable tissues and the microscopic

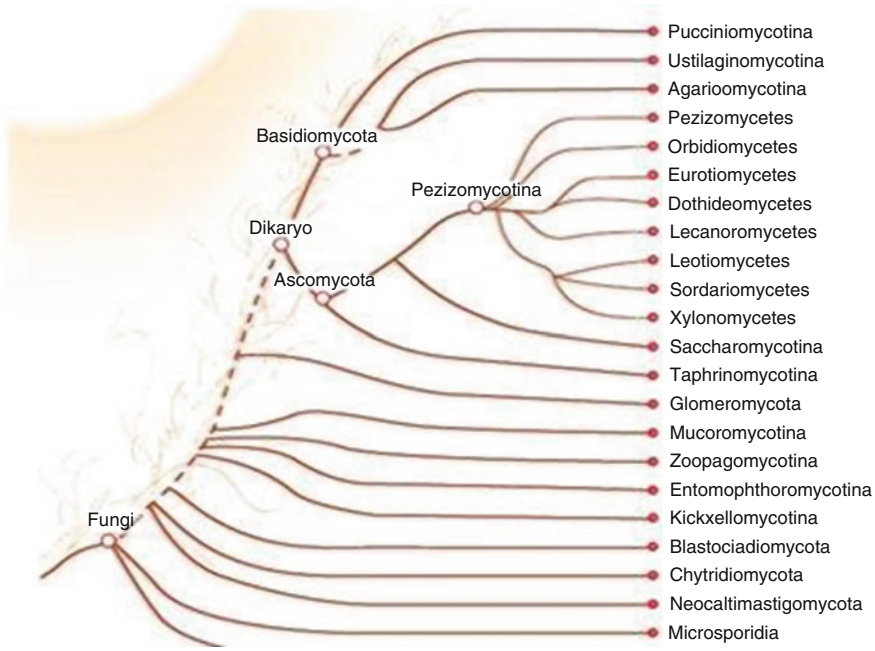


Fig. 7.11 Phylogenetic tree of fungi (*Source*: http://genome.jgi.doe.gov/programs/fungi/index.jsf)

dimensions of most fungal structures, which therefore are not readily evident (Krings et al. 2013). Fungal fossils are difficult to distinguish from those of other microbes and are most easily identified when they resemble extant fungi. Often recovered from a permineralized plant or animal host, these samples are typically studied by making thin-section preparations that can be examined with light microscopy or transmission electron microscopy. Researchers study compression fossils by dissolving the surrounding matrix with acid and then using light or scanning electron microscope to examine surface details. The earliest fossils possessing features typical of fungi date to the Proterozoic eon, some 1,430 million years ago (Ma); these multicellular benthic organisms had filamentous structures with septa and were capable of anastomosis. More recent studies estimate the arrival of fungal organisms at about 760–1,060 Ma on the basis of comparisons of the rate of evolution in closely related groups. For much of the Paleozoic Era (542–251 Ma), the fungi appear to have been aquatic and consisted of organisms similar to the extant chytrids in having flagellum-bearing spores. The evolutionary adaptation from an aquatic to a terrestrial lifestyle necessitated a diversification of ecological strategies for obtaining nutrients, including parasitism, saprobism, and the development of mutualistic relationships such as mycorrhiza and lichenization. Recent studies suggest that the ancestral ecological state of the Ascomycota was saprobism, and that independent lichenization events have occurred multiple times. It is presumed that the fungi colonized the land during the Cambrian (542–488.3 Ma), long before land plants. Fossilized hyphae and spores recovered from the Ordovician of Wisconsin (460 Ma) resemble modern-day Glomerales and existed at a time when the land flora likely consisted of only nonvascular bryophyte-like plants. At about this same time, approximately 400 Ma, the Ascomycota and Basidiomycota diverged, and all modern classes of fungi were present by the Late Carboniferous (Pennsylvanian, 318.1–299 Ma). Two amber-preserved specimens provide evidence that the earliest known mushroom-forming fungi (the extinct species *Archaeomarasmius leggetti*) appeared during the mid-Cretaceous, 90 Ma.

Some time after the Permian–Triassic extinction event (251.4 Ma), a fungal spike (originally thought to be an extraordinary abundance of fungal spores in sediments) formed, suggesting that fungi were the dominant life form at this time, representing nearly 100 % of the available fossil record for this period.

7.9 Genetics of Fungi

Genetic research has provided important knowledge about genes, heredity, genetic mechanisms, metabolism, physiology, and development in fungi and in higher organisms in general, because in certain respects, the fungal life cycle and cellular attributes are ideally suited to both Mendelian and molecular genetic analysis (Finchman and Day 1971). Fungal nuclei are predominantly haploid; that is, they contain only one set of chromosomes. This characteristic is useful in the study of mutations, which are usually recessive and therefore masked in diploid organisms. Mutational dissection is an important technique for the study of biological processes, and the use of haploid organisms conveniently allows for the immediate expression of mutant genes. Reproduction in fungi can be asexual, sexual, or parasexual. Asexual reproduction involves mitotic nuclear division during the growth of hyphae, cell division, or the production of asexual spores. Sexual reproduction is based on meiotic nuclear divisions fairly typical of eukaryotes in general. In ascomycetes and basidiomycetes, the spores, containing nuclei that are the four products of a single meiosis, remain together in a group called a tetrad. The isolation and testing of the phenotypes of cultures arising from the members of a tetrad (tetrad analysis) permit the study of the genetic events occurring in individual meioses; this possibility is offered by virtually no other eukaryotic group. In other groups, genetic analysis is limited to products recovered randomly from different meioses. Since a great deal of genetic analysis is based on meiosis, fungal tetrads have proved to be pivotal in shaping current ideas on this key process of eukaryotic biology.

Three different kinds of reproduction occurring in fungi, each of which provides opportunities for genetic analysis because their preparation in large numbers is simple, fungal cells are useful in the study of rare events (such as mutations and recombinations) with frequencies as little as one in a million or less. In such cases, selective procedures must be used to identify cells derived from the rare events. The concepts and techniques of fungal asexual and parasexual genetics have been applied to the genetic manipulation of cultured cells of higher eukaryotes such as humans and green plants. However, the techniques remain much easier to perform with fungi. The fact that each enzyme is coded by its own specific gene was first recognized in fungi and was of paramount importance because it showed how the many chemical reactions that take place in a living cell could be controlled by the genetic apparatus. A surprising development in the molecular biology of eukaryotes was the discovery of transposons, pieces of DNA that can move to new locations in the chromosomes. Although transposons were once known only in bacteria, they are now recognized in many eukaryotes. The transposons found in fungi mobilize by either of two processes: one type via a ribonucleic acid (RNA) intermediate that is subsequently reverse-transcribed to DNA, and the other type via DNA directly. In either case, a DNA copy of the transposed segment is inserted into the new site and may contain, in addition to the transposon itself, segments of contagious DNA mobilized from the original chromosomal site. Because of the rearrangements which transposons may produce, they have been important in the evolution of the eukaryotic genome.

Fungal genetics is a comparatively young discipline which is correlated with the beginning of human civilization. It was at first almost exclusively devoted to fundamental research and came only during the last two decades into close relation to biotechnology, when it appeared meaningful to apply chromosomal genetics in a concerted manner to improve the production or transformation capacities of industrial and agriculturally important fungi. A landmark in the young relationship between fungal genetics and

biotechnology was the discovery of fungal plasmids and their relationship to mitochondrial DNA. This allowed incorporating also the fungi in the concept of genetic engineering, thus making fungal genetics part of the new biology and therewith giving applied mycology a new aspect.

7.10 Economic Uses of Fungi

Many fungi are useful to humans and have been exploited both industrially and commercially (Shakuntala et al. 2009). Societies have utilized fungi for centuries in a wide variety of ways by capitalizing on the metabolism and metabolites (chemicals made from metabolism) produced. Fungal enzymes are also used to make food more edible or desirable by removing, adding, or modifying components such as vitamins, nutritional elements, colors, and flavors. In the various fields of agriculture, medicine, environmental biology, biotechnology, research, and development, fungi provide novel and important products and applications (Lene 2010). Their extraordinary usefulness has provided us with numerous advantageous products and will undoubtedly afford us with additional medicines, foodstuffs, enzymes, amenities, and other valuable items in the future. The important uses of fungi are discussed below:

7.10.1 Fungi in Medicine

Some fungi are known to produce organic substances which inhibit the growth of certain other microorganisms; these are called antibiotics. The production of antibiotics by fungi was first discovered by Alexander Fleming in 1929. He discovered penicillin, the wonder antibiotic, which is produced by *Penicillium notatum*. Fumagillin from *Aspergillus fumigatus* inhibits certain phages and amoebae. Griseofulvin, another antibiotic from *Penicillium griseofulvum*, is used against the skin diseases such as ring worm and athlete's foot. This antibiotic interferes with the wall formation of the disease-causing fungi. The compound accumulates in the skin and hair when taken orally and so it is effective against skin diseases. A mixture of alkaloids from *Claviceps purpurea* (causal organism of ergot of rye) is highly poisonous. This is used to control bleeding during childbirth. Clavacin, a substance extracted from *Clavatia*, prevents stomach tumors.

7.10.2 Fungi in Food and Baking Industries

Yeasts are used in both the brewing and baking industries to convert sugars into carbon dioxide and ethyl alcohol (Moore and Chiu 2001). Alcohol is the main product in brewing and wine-making industry, whereas carbon dioxide is a valuable product in baking industry. An enzyme complex called zymase is secreted by yeast. *Saccharomyces cerevisiae* is the yeast commonly employed. This enzyme complex converts sugars into alcohol and carbon dioxide. Instead of sugars, starch is used as a substrate in the production of industrial alcohol. In this case, other fungi such a *Mucor* and *Rhizopus* are used initially for the conversion of starch into sugars; yeasts are generally used in the second stage, i.e., in the conversion of sugars to alcohol and carbon dioxide. The peoples of Asia have developed a wide variety of interesting fermented foods, sauces and drinks, using fungi. Other examples and the applicable fungi include koji (*Aspergillus*); miso, soybean paste (*Aspergillus*); sufu, Chinese cheese (*Rhizopus*), nyufu or fuyu, bean cake or bean cheese (*Rhizopus*); shoyu or soy sauce (*Aspergillus*, *Saccharomyces*); and tempeh (*Rhizopus*). Another way in which fungi are used industrially in the food industry is in cheese production. Various cheeses are inoculated with *Penicillium roqueforti* to impart a strong and pungent flavor in the resultant cheeses. Several mushrooms (*Agaricus bisporus*) puffballs, morels (*Morchella* spp.), and truffles are edible, and they are grown commercially in several western countries; in north India, they are becoming popular and slowly expanding to the south. These fungi are used in the preparation of different types of food stuffs, e.g., pizza, and these are rich in proteins and vitamins.

7.10.3 Fungal Enzymes

The enzymes, such as digestin, *taka* diastase, polyzyme, which are used for dextrinization of starch and desizing of textiles, are produced by *Aspergillus*. The enzymes, amylase and invertase, are extracted from *Aspergillus* and *Saccharomyces*, respectively.

7.10.4 Organic Acids from Fungi

Several organic acids such as oxalic acid, citric acid, gluconic acid, gallic acid, and fumaric acid are produced commercially as fermentation products of *Aspergillus* and *Penicillium*, *Aspergillus niger*, *A. glaucus*, *A. clavatus*, and *Citronyces citricus* have been recommended for preparation of citric acid. Many fungi also produce gluconic acid and lactic acid.

7.10.5 Gibberellins from Fungi

Gibberellin is used to accelerate the growth of several horticultural and some commercial fruit crops. This is produced by a fungus called *Gibberella fujikuroi*.

7.10.6 Vitamins and Fungi

The dried yeast or yeast extract which is rich in vitamin B complex is being sold in the market. Some molds and yeasts are also used in the synthesis of ergosterol, which is *a* precursor to vitamin D.

7.10.7 Fungi in Agriculture

Fungi play both positive and negative roles in agriculture. The harmful activities are more than the useful activities. Some of the saprophytic fungi in the soil decompose the dead material of animals and plants. The enzymes secreted by these fungi convert the fats, carbohydrates, and nitrogen compounds of the dead animals and plants into simpler compounds such as carbon dioxide, ammonia, hydrogen sulfide, water, and some other nutrients in a form available to green plants. Some of them will be in the soil to form humus, and the remaining go into air where they can be used up as raw material for food synthesis. By liberating carbon dioxide, these fungi participate in maintaining the never-ending cycle of carbon in nature. The carbon dioxide is very important for green plants in the preparation of food materials by photosynthesis. Some fungi are in symbiotic association with the roots of certain plants. Satisfactory growth of the plant can be observed only when the specific fungal partner is present inside the roots of the plants. This type of association of a fungus and plant is called mycorrhiza. A few fungi (e.g., *Dactylaria*) are known to destroy the nematodes. These predatory fungi produce mycelial loops. When the nematodes pass through, these loops get tightened up to catch the nematodes. Then the fungus sends special hyphae into the nematodes to absorb the nutrients from them. *Pythium* is known to cause damping off disease in the seedlings of certain crops. Certain fungi such as *Trichoderma* and *Gliocladium* are known to inhibit the growth of *Pythium* in soil. In this way, certain fungi serve to suppress the growth of disease-causing fungi. Several fungi are known to cause severe crop losses by causing diseases. About 20,000 diseases of crop plants are known to be caused by fungi. The fungal diseases of crop plants have become a major problem for the agricultural farmers. The scientists are seriously trying to develop special techniques and chemicals to control these diseases. Some of the economically important diseases of crop plants are given in Table 7.1.

7.10.8 Fungi in Industries

A number of applications of fungi are involved in the alteration of plant cell walls in many industries. Fungi are able to break down plant cell walls by the production of a wide variety of enzymes. Enzymes are used to treat and modify fibers, particularly during textile processing and

Table 7.1 Some of the economically important diseases of crop plants

	Disease	Fungi	Crop plans
1.	Rice blast	*Pyricularia oryzae*	Paddy, wheat, rye, barley, pearl millets
2.	Botrytis	*Botrytis cinerea*	Grapes, strawberry
3.	Rust	*Puccinia graminis*	Wheat
4.	Fusarium head blight	*Fusarium graminearum*	Wheat, corn, barley
5.	Fusarium wilt	*Fusarium oxysporum*	Tomato, tobacco, legumes, cucurbits
6.	Powdery mildews	*Blumeria graminis*	Grapes
7.	Septoria leaf blotch	*Mycosphaerella graminicola*	Wheat
8.	Anthracnose	*Colletotrichum graminicola*	Maize, wheat, onion, garlic, tomato
9.	Smut	*Ustilagomaydis*	Maize
10.	Flax rust	*Melampsora lini*	Flax
11.	Soya bean rust	*Phakopsora pachyrhizi*	Soya bean
12.	Color/root rot	*Rhizoctonia solani*	Beans

in caring for textiles. Fungal enzymes, catalases, are used to treat cotton fibers and prepare them for the dyeing processes. By degrading surface fibers, many enzymes, including some cellulases and xylanases, are used to finish fabrics, help in the tanning of leathers, or give jeans a stone-washed effect. Stone-washed jeans are placed in a large vat containing the fungus *Trichoderma*, which produces enzymes (cellulases) that partially digest the cotton fibers of the jeans to add softness and produce the stone-washed look. The natural enzyme supplement Beano™ contains the enzyme (α-galactosidase) from *Aspergillus terreus* used for digestive discomfort. The pulp and paper industry benefits from the enzyme production capabilities of certain fungi to soften wood fibers and provide alternatives to chemical bleaching. The basidiomycetes, *Trametes* and *Phanerochaete*, are used for lignin biodegradation and *Bjerkandera* is used for hardwood cellulose biobleaching by producing the enzymes peroxidase and xylanase. Certain fungi are the primary source for xylanases, which are used industrially to breakdown xylan, the second most abundant polysaccharide in nature. Enzymes are a sustainable alternative to the use of harsh chemicals in industry. Because enzymes work under moderate conditions, such as warm temperatures and neutral pH, they reduce energy consumption by eliminating the need to maintain extreme environments, as required by many chemically catalyzed reactions. Reducing energy consumption leads to decreased greenhouse gas emissions. Enzymes also reduce water consumption and chemical waste production during manufacturing processes. Because enzymes react to specific situations and minimize the production of by-products, they offer minimal risk to humans, wildlife, and the environment. Enzymes are both economically and environmentally beneficial because they are safely inactivated and create little or no waste; rather than being discarded, end-product enzymatic material may be treated and used as fertilizer. Enzyme research using fungi has been very active and promising in recent years. For example, the enzyme laccase produced from different fungi was used to make paper. This process led to a 30 % reduction in energy consumption, a 50 % reduction in chemical product usage, and a greater resistance to tearing.

7.10.9 Applications of Fungal Enzymes

Fungi thrive in harsh environments shunned by higher organisms. Fungi can exploit marginal living conditions in large part because they produce unusual enzymes capable of performing chemically difficult reactions. These fungal enzymes can convert wood, plastic, paints, and jet fuel, among other materials, into nutrients. Some of these enzymes have already been harnessed in pulp and paper processing (biopulping and biobleaching) and in the synthesis of fine

chemicals (biocatalysis). Using enzyme permits these industrial processes to be performed under milder conditions, using less energy, and producing fewer toxic by-products. In addition, many fungal enzymes are capable of breaking down a broad range of complex compounds, making them potentially useful for destruction of persistent pollutants (bioremediation and biodegradation).

7.11 Miscellaneous Uses of Fungi

7.11.1 Plastic Manufacture

Certain fungi like *Odium lactis* is widely used in plastic industry.

7.11.2 Control of Insect Pest

Many fungi like *Ascherroni adeyroides*, *Isaria ferinosa*, and *Mbusa sepulchralis* help in controlling the infection by insect pests of the plants.

7.11.3 Phytohormone/Auxins

Many growth-promoting substances like Gibberellins are synthesized from the fungi like *Fusarium moniliforme* and *Dematium pullulans*.

7.11.4 Nutrition of Plant

Many members of Phycomycetes, Ascomycetes, Basidiomycetes and fungi imperfecti are involved in the formation of mycorrhizae which are of fundamental importance in nutrition of trees like *Cycas*, *Zamia*, and *Pinus*.

7.11.5 Destruction of Organic Waste

Saprophytic fungi (vegetative vultures) decompose plant and animal remains by acting as natural scavengers. Carbon dioxide released in the process is used by green plants.

7.11.6 Nematophagous Fungi

Nematodes cause diseases to crop plants like root knot (*Meloidogyne* sp.) disease or root cyst (*Heterodera* sp. and *Globodera* sp.) diseases. Fungi have evolved a range of mechanisms to attack nematodes, which can be grouped into three broad categories:

1. Predation
2. Endoparasatic
3. Egg and cyst parasites

1. *Predatory fungi*: These fungi are distributed in Zygomycotina, Ascomycotina, and Basidiomycotina. The common examples are *Acaulapage*, *Stylopage*, *Gamsylella*, *Dactylolline*, *Arthrobotrys*, *Nematoctonus*, and *Pleurotus*. The mode of parasitism in there fungi is adhesive, nets, constricting rings, and poisonous knobs.
2. *Endoparasatic fungi*: These fungi are distributed in Oomycota, Chytridiomycota, and Ascomycota. The common examples are *Haploglous*, *Myzocyticum*, *Nematophthora*, *Catenaria*, *Harposporium*, and *Hirusutella*. The mode of parasitism in these fungi is through germ cells, encysting zoospores, and ingestion.
3. *Egg and cyst parasites*: These fungi are distributed in Zygomycota and Ascomycota. The common examples are *Rhapalomyces*, *Pochonia chlamydosporia*, and *Paecilomyces lilacinus*. The mode of parasitism is through hyphal colonization of eggs and cysts.

7.12 Model Fungi

Lower eukaryotes, like yeast and filamentous fungi, are attractive organisms to study fundamental processes of the eukaryotic cell. Relative to higher eukaryotes, yeast has the advantage of easy cultivation on simple-defined media with short generation times and easy accessibility toward molecular and classical genetics. The entire genome sequence of a growing number of yeast and fungal species is available, which enables to extensively apply modern system biology approaches. The fact that fungi are more

related to animals than to plants emphasizes the value of these organisms as favorable models for human cells.

Some of the examples for widely studied model fungi are as follows:

- *Ashbya gossypii*, cotton pathogen, subject of genetics studies (polarity, cell cycle), *Aspergillus nidulans*, mold *subject* of genetics studies.
- *Coprinus cinereus*, *HYPERLINK* "http://en.wikipedia.org/wiki/Mushroom" *mushroom* (*genetic* studies of mushroom development, genetic studies of meiosis).
- *Cryptococcus neoformans*, opportunistic human pathogen.
- *Cunninghamella elegans* is a fungal model of mammalian drug metabolism. *Neurospora crassa* – orange bread mold (genetic studies of meiosis, metabolic regulation, and *circadian rhythm*).
- *Saccharomyces cerevisiae*, *baker's* yeast *or* budding yeast (used in brewing and baking) *Schizophyllum commune* – model for mushroom formation.
- *Schizosaccharomyces pombe*, *fission* yeast (cell cycle, cell polarity, RNAi, centromere structure and function, transcription).
- *Ustilago maydis*, dimorphic yeast and plant pathogen of maize (dimorphism, plant pathogen, transcription).

References

Ainsworth GC (1973) Introduction and keys to higher taxa. In: Ainsworth GC, Sparrow FK, Sussman AS (eds) The fungi. An advanced treatise IVB: a taxonomic review with keys. Academic, New York, pp 1–7

Alexopolous CJ, Mims CW, Blackwell M (1996) Introductory mycology. Wiley, New York

Barnett JA, Payne RW, Yarrow D (2000) Yeasts: characteristics and identification. Cambridge University Press, Cambridge

Burnett JH (1976) Fundamentals of mycology. Edward Arnold, London

David S, John H, Taylor W (2013) Fungal systematics: is a new age of enlightenment at hand? Nat Rev Microbiol 11:129–133

Finchman JRS, Day PR (1971) Fungal genetics. Blackwell Scientific, Oxford

Gander JE (1974) Fungal cell wall glycoproteins and peptido-polysaccharides. Annu Rev Microbiol 28:103–120

Graham W, Gooday D, Adams J (1993) Sex hormones and fungi. Adv Microb Physiol 34:69–145

Hawksworth DL, Sutton BC, Ainsworth GC (1983) Ainsworth and Bisby's dictionary of the fungi. 7 Commonwealth Mycological Institute, Kew, Survey

Jennings DH (1995) The physiology of fungal nutrition. Cambridge University Press, Cambridge

Joseph H, Sheng S, Timothy JY (2013) Evolution of fungal sexual reproduction. Mycologia 105:1–27

Kendrick WB (1971) Taxonomy of fungi imperfecti. Toronto University Press, Toronto

Krings M, Taylor TN, Dotzler N (2013) Fossil evidences of the zygomycetous fungi. Rev Article Persoonia 30:1–10

Lene L (2010) The importance of fungi for a more sustainable future on our planet. Br Mycol Soc Fungal Biol Rev 24:90–92

Mathieu P, Sven JS (2009) Fungal incompatibility: evolutionary origin in pathogen defense? Hypothesis Bioessays 31:1201–1210

Moore D, Chiu SW (2001) Fungal products as food. In: SB Pointing, Hyde KD (eds) Bio-exploitation of filamentous fungi. Fungal Diversity Press, Hong Kong, pp 223–251

Raper JR (1966) Genetics of sexuality in higher fungi. Ronald Press Co, New York

Robertson SK, Bond DJ, Read ND (1988) Homothallism and heterothallism in Sordaria brevicollis. Mycol Res 102:1215–1223

Shakuntala G, Samudra PB, Deepak V, Sudeshna C, Soumya M, Suman K (2009) Fungal biotechnology in food and feed processing. Rev Food Res Int 42:577–587

Taylor JW, Jacobson DJ, Fishe MC (1992) The evolution of sexual fungi: reproduction, speciation and classification. Annu Rev Phytopathol 37:197–246

Webster JH (1970) Introduction to fungi. Cambridge University Press, Cambridge/London/New York

Whittaker RH (1969) New concepts of kingdoms of organism. Science 163:150–160

Arbuscular Mycorrhizal Fungi: The Nature's Gift for Sustenance of Plant Wealth

8

C. Manoharachary and I.K. Kunwar

Abstract

Mycorrhizal fungi exist in diversified soils supporting varied plant communities in different climatic zones with diverse soil conditions. Seventy to 80 % plants including bryophytes, primary vascular plants, aquatic plants, and xerophytes possess mycorrhizae. Ectomycorrhizae are mostly associated with gymnosperms and woody plants, while 80 % plant groups are mainly colonized by arbuscular mycorrhizal fungi. The present account presents a methodical review of mycorrhizal types and in-depth analysis of arbuscular mycorrhizal fungi and their role as benefactor for plant growth.

Keywords

AM fungi • Diversity • Ecology • Methodology • Nutrition • Plant growth • Taxonomy • Xerophytes

8.1 Introduction

There are several symbiotic groups, phosphorus solubilizers, mycorrhizae, plant growth promoters, and other such beneficial important microorganisms reported from diversified soils and substrates. Dynamic microbial systems contribute to the sustainability in agriculture, forestry, and their management. The term mycorrhiza (fungus root) was coined by Frank (1885), and Harley and Smith (1983) defined mycorrhiza as an association between fungal hyphae and roots of higher plants concerned with absorption and transport of mineral substances from the soil. Brundrett (2004) stated mycorrhiza as a symbiotic association for one or both partners between a fungus and a root of living plant that is primarily responsible for nutrient transfer. Harley and Smith (1983) divided mycorrhizae into the following.

C. Manoharachary (✉) • I.K. Kunwar
Department of Botany, Osmania University, Hyderabad 500 007, Telangana, India
e-mail: cmchary@rediffmail.com

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_8,

8.1.1 Ectomycorrhiza

Ectomycorrhizae are characterized by a fungal sheath or mantle that encloses the root, forming a Hartig net, which is a plexus of fungal hyphae between epidermal and cortical cells. These roots are generally short, swollen, dichotomously branched, with distinctive colors, and form common association with forest trees and shrubs in subarctic and temperate regions. The host plants that show ectomycorrhizal association belong to the families Pinaceae, Fagaceae, Betulaceae, and Myrtaceae, and fungi belong to Basidiomycotina with representatives from 25 families. Few Ascomycotina and two species of Zygomycotina also form ectomycorrhizae. Many ectomycorrhizal fungi can be cultured in laboratory media, for example, *Boletus edulis*, *Lactarius deliciosus*, *Laccaria laccata*, *Pisolithus tinctorius*, *Paxillus luteolus*, and *Rhizopogon roseolus*.

8.1.2 Ectendomycorrhiza

Ectendomycorrhiza are nothing but ectomycorrhiza only but also display morphological characteristics of the endomycorrhiza. The association appears to be an intermediate type. These types are found in *Pinus* and *Larix* species.

8.1.3 Arbuscular Mycorrhiza

Arbuscular mycorrhizae are the common mycorrhizal associations and have a widespread distribution throughout the plant kingdom that forms mutualistic relationship with most of the vascular plants. Families that fail to show arbuscular mycorrhiza include Betulaceae, Chenopodiaceae, Commelinaceae, Cruciferae, Cyperaceae, Ericaceae, Fumariaceae, Pinaceae, Polygonaceae, and Urticaceae. The arbuscular mycorrhizal (AM) fungal partner belongs to Glomeromycota. The fungus forms vesicles within or between cortical cells that act as storage or reproductive organs. Arbuscules are formed within the cortical cells, and these provide a large surface area of contact between host and fungus. The genera, which form AM fungal association, are *Acaulospora*, *Ambispora*, *Aracheospora*, *Diversispora*, *Entrophospora*, *Geosiphon*, *Gigaspora*, *Glomus*, *Intraspora*, *Kuklospora*, *Otospora*, *Pacispora*, *Paraglomus*, and *Scutellospora* (Schenck and Perez 1990; Schüβler et al. 2001; Oehl and Sieverding 2004; Sieverding and Oehl 2006; Walker et al. 2007a).

8.1.4 Orchid Mycorrhiza

Orchidaceous plants are dependent on mycorrhiza, and the fungal partner commonly is the species of *Ascomycotina* or *Basidiomycotina*. Orchid embryos normally become infested with mycorrhizal fungi, and thin-walled hyphae enter the protocorm through the epidermis and anastomose repeatedly with the cortical cells to form pelotons or hyphal coils, which provide a large surface area of contact.

8.1.5 Ericoid Mycorrhiza

Mycorrhizal association involves mostly ascomycetous fungi and sometimes basidiomycetous fungi with members of Ericales mostly growing in acidic soils. These fungi are present in the young cortical cells of the host, with branched and coiled vegetative mycelia, but never penetrate the stele and without vesicles and Hartig net. These fungi form hyphal coils that are intracellular. Culturing of these fungi in laboratory medium is rare.

8.1.6 Monotropoid Mycorrhiza

It is associated with the members of Monotropaceae, which lack chlorophyll and is totally dependent on the mycorrhizal fungi for supply of carbon. The fungal partner is a member of *Basidiomycetes*. The hyphae of the sheath ramify in the surrounding humus and a Hartig net-like structure is also formed. Fungal pegs achieve close contact with cortical cells of the host.

8.1.7 Arbutoid Mycorrhiza

Arbutoid mycorrhizal associations are associated with Arbutoideae and Pyrolaceae. The fungal partners belong to *Basidiomycetes*. Roots which are long have very sparse infections and the intercellular hyphae form a Hartig net. Some short roots have a thicker outer sheath, and a well-developed Hartig net between the outer cortical cells besides the fungus also penetrates and forms coils within the cells of the outer cortex.

Morphological, anatomical, histochemical, and biochemical tools and molecular and genetical tools are used for identification of mycorrhizal fungi, besides exploring the structural and regulatory genes in both fungi and host plants that allow mycorrhiza formation.

8.2 Benefits of AM Fungi

Major land area in India shows clear evidence of soil degradation due to salinity, alkalinity, soil erosion, water logging, and so on, which in turn affects the country's productive resource base. The soils in India have low available phosphorus and much of the phosphorus is in mineral state. The fundamental problem that the country is facing today is the rapidly increasing pressure population on the limited resources of land. In order to meet the pressure of population, it is essential to efficiently manage the agricultural inputs for sustaining high crop productivity on long-term basis, with minimum damage to environment. Mycorrhizae are used as biofertilizers in order to reduce the cost and harm rendered by different agrochemicals. Mycorrhizal fungi help crops, forest plants, and others to acquire mineral nutrients from the soil, especially immobile elements such as phosphorus, zinc, and copper and mobile elements such as sulfur, calcium, potassium, iron, magnesium, manganese, chlorine, bromine, and nitrogen. Mycorrhizae also help in soil particle aggregation. Economically important plants have been found to be mycorrhizal. It is also known that Glomalean fungi existed 400 Ma ago and helped in the colonization of land by primitive plants establishing the fact that primary land plant establishment was also due to mycorrhiza.

The symbiotic association of mycorrhizal fungi appears to have evolved as a survival mechanism for fungi and higher plants, including their adaptation to extreme conditions. Mycorrhizae also offer primary biological defense to host plants against biotic and abiotic stress.

Mycorrhizae help in increased uptake of nutrient and water by improving absorptive area and translocation of elements to host tissues and their accumulation. The unique ability of mycorrhiza increases the uptake of phosphorus by plants as these fungi have the potential for utilization as a substitute for phosphatic fertilizers. These fungi improve host nutrition by increasing the delivery of soluble phosphorus and other minerals to roots and plants. The inorganic phosphorus gets solubilized by a number of microbes, fungi, actinomycetes, cyanobacteria, and others. Ectomycorrhizal fungi enter the F and H horizon of forest soil floor followed by mobilization of minerals in these zones. Later absorption occurs before they reach subsoil system. AM fungi are known to degrade complex minerals and inorganic substances in soil and make these essential elements available to the plants. Mycorrhizal association also offers resistance in host plants to drought, plant pathogens, and adverse conditions and helps in the production of growth hormones like auxins, gibberellins, and growth regulators such as Vitamin B. In addition to that mycorrhizal fungi also contribute to organic matter turnover and nutrient cycling in ecosystems, soil aggregation, soil stabilization, and increased soil fertility. Mycorrhizae being symbiotic live hand in hand with other living organisms and as eco-friendly organisms.

8.2.1 Morphological Diversity in Arbuscular Mycorrhizal Fungi

Morphological characters are used in identification and classification of AM fungi. Hyphal characters, arbuscules, vesicles, auxiliary cells, stalk of the spore, spores, sporocarps,

subcellular structures, wall layers, size, ornamentation, shapes, and related parameters have been used.

8.2.2 Ecological Diversity in Arbuscular Mycorrhizal Fungi

Ecological data provides useful information regarding their diversity, biogeographical distribution, dispersal patterns, and competitive interactions by member organisms in plant communities and soil microorganisms. The biogeographical distribution, dispersal patterns, and competitive interactions of AM fungi with plant communities and soil microorganisms are as follows.

8.2.2.1 Biogeographical Distribution

There is more or less uniform distribution of AM fungi in different ecological zones though some may predominate in certain areas with broad ecological range (Onguene and Kuyper 2001). AM fungi are known to be present in the top 15–30 cm of soil, and their numbers decrease below the top 15 cm of soil (Redhead 1977). AM fungi distribution is as per climatic and edaphic factors. For example, *Gigaspora* and *Scutellospora* seem to be common in tropical soil, whereas *Acaulospora* species favor soils of pH below 5 (Abbott and Robson 1977).

8.2.2.2 Dispersal Patterns

Majorities of the AM fungal species are present in most of the continents of the world. The dispersal of AM fungi is usually by spread from one living root to another through AM propagules, like mycelia and spores, which can also be moved by biotic and abiotic agents. Dispersal of spores over greater distances is also dependent upon passive dispersal by wind and water, especially in arid environment. The dispersal by animals occurs through ingestion and egestion of spores.

8.2.2.3 Interactions with Plant Communities

AM fungi are geographically ubiquitous and are usually associated with crop plants, horticulture, pastures, and tropical forests. About 80–90 % of vascular plants establish symbiotic relationship with AM fungi (Kendrick and Berch 1985).

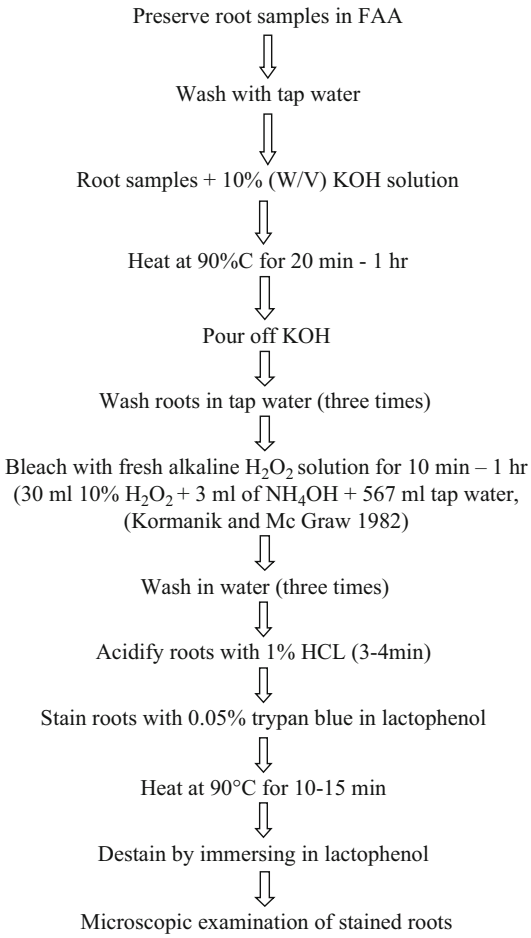
The presence and root colonization by AM fungi have been reported from an exceptionally wide range of plants. Colonization has been reported in other plant parts, for example, in leaves of Salvinia (Bagyaraj et al. 1979); in senescent leaves of *Fumaria hygrometrica* (Park and Linderman 1980); in decaying peanut leaves; in scales of *Colocasia antiquorum*, *Elettaria cardamomum*, *Musa paradisiaca*, and *Sanseviera trifasciata*; in garlic; and in ginger (Kunwar and Manoharachary 1998, 1999).

The interactions of AM fungi with plants bring about certain changes in the host physiology which include increased production of cytokinins degradation by compounds produced by the fungus or plant as a result of the interaction. The presence of two gibberellin-like substances in culture extracts of *Glomus mosseae* and increased nitrate reductase activity have also been reported in mycorrhiza-inoculated plants.

8.2.3 Methodology

The morphological formations, structures produced by AM fungi within the host roots, consist of an internal hyphal system connected to the external hyphal network through the initial entry points, intracellular arbuscules, and vesicles. Anatomical features of the mycorrhizal roots can be examined by their proper clearing and staining (Phillips and Hayman 1970). Fine terminal feeder roots are to be collected to observe root colonization. A representative root sample is obtained, and roots are cut into 1 cm pieces and stored in FAA (formalin acetic acid-50 % ethanol) (5:5:90, v/v/v) solution.

The steps involved in clearing and staining of mycorrhizal roots are as follows (Phillips and Hayman 1970).



Clearing and staining of mycorrhizal roots (Adapted from Phillips and Hayman 1970)

Microscopic observation of stained roots in lactophenol under a good dissecting microscope is necessary for rapid assessment of root colonization. AM fungal structures are to be observed under a compound microscope by mounting and gently squashing the randomly selected root segments in glycerol or lactophenol or polyvinyl alcohol resin lactophenol.

Quantification of root colonization has to be done by detecting the presence or absence of colonization by the hyphae, arbuscules, vesicles, and internal spores and rated positive or negative on per sample or per plant basis. Root segments colonized are counted and expressed as a percentage of total root segments in the sample. Root segments may be observed in Petri dishes or on slides. For slides ten root segments/slide are mounted from around 30 to 100 randomly collected and stained root segments per sample and observed (Giovannetti and Mosse 1980).

$$\% \text{ Colonization} = \frac{\text{Number of colonized segments}}{\text{Total number of segments examined}} \times 100$$

The propagules of AM fungi comprising spores, sporocarps, and hyphal bits can be extracted from soil using modified wet-sieving and decanting technique (Gerdernann and Nicolson 1963). One hundred grams air dried soil is taken and mixed with 1,000 ml water; stirred, heavier particles are allowed to settle for few minutes. Liquid is poured through a coarse sieve (500–850 μm) to remove large pieces of organic matter. The liquid which passes through this sieve is collected, and the sieve is washed with a stream of water so that all small particles pass through. The liquid collected is allowed to settle for 0.5–1 h. The aqueous suspension is passed through a series of sieves stacked one over the other in ascending order (50, 125, 250, 400 μm). The material retained on the sieves is washed so that all colloidal material passes through the sieves. The residues are transferred separately to beakers with a fine jet of water directed at both sides.

The contents of the various beakers are filtered separately by passing them through a single layer of very closely woven white synthetic cloth (lint free). The debris, spores, and sporocarps are retained on the cloth mesh. Petri plate is marked into 1 cm^2 with the help of water proof marker. This is done to facilitate in the counting of spores, sporocarps, etc. The cloth mesh with the spores, sporocarps, debris, etc. is kept on the marked Petri plate along with little water and observed under a stereobinocular dissecting microscope at 20 % and 40 % magnification. The arbuscular mycorrhizal fungal propagules existing as spores and sporocarps are counted by scanning the sieving from each mesh, and the total arbuscular mycorrhizal fungal propagules/100 g. of soil is calculated. The spores and sporocarps are picked under stereomicroscope with the help of a flattened needle tip or Pasteur pipette and mounted on a slide containing a drop of lactophenol. The coverslip is sealed with DPX mountant.

8.2.4 Morphotaxonomy of AM Fungi

The AM fungi produce hyphae, vesicles, and arbuscules inside the root cortex, while the spores and sporocarps along with hyphae are produced outside the root tissues (Fig. 8.1). Various criteria are used for the identification of AM fungi, viz., hyphal characters, auxiliary cells, subtending hypha, spore/sporocarp ontogeny, color, morphology, germination, germinating shield, spore wall, and biochemical, molecular, and immunological characters.

8.2.5 Classification

Arbuscular mycorrhizal fungal taxonomy has been basic and is the subject of much debate. Earlier the identification of AM fungal species was mainly based on morphological criteria and spore characteristics. Dangeard (1900) was first to name AM fungi, and Peyronel (1924) had recognized the AM fungi as *Endogone* species. Thaxter (1922) described all the species known of that time. Mosse (1953) was the first to demonstrate experimentally that *Endogone* species could produce vesicles and arbuscules and was successful in producing typical structures of arbuscular mycorrhizae using *Endogone* species as inoculum. Berch (1986), Hall (1983), Schenck and Perez (1990), and Trappe and Schenck (1982) have compiled additional information on the taxonomy of AM fungi. Gerdemann and Trappe (1974) described AM fungi based on different types of spore walls. Hall (1983) listed 115 characteristics that can be used for separating AM fungal species. Walker (1983) suggested for standardized terminology to describe the wall layers. Different monographs have been used to illustrate the interrelation of these spore walls. Morton (1989) emphasized that morphological characters are important in identifying endomycorrhizal fungi besides elaborating their evolutionary relationships. Almeida (1989) has proposed critical evaluation of AM fungi and discussed scientific names in Endogonales. Berch (1986) has detailed out the taxonomy, specificity, fossil record, and phylogeny of Endogonaceae. Bonfante-Fasolo (1984) proposed that anatomy and morphology of AM fungi are important. Morton (1990) used arbuscules as the only character that unites Glomineae and Gigasporineae under Glomales. Brundrett and Kendrick (1990) have revealed critical differences in arbuscular structures of *Gigaspora* and *Glomus*. Various types of walls (Walker 1983) form a stable and reliable criterion in the identification and classification of fungi.

Arbuscular mycorrhizal fungi have been placed in Zygomycotina as the cell walls of this group of fungi possess chitin or chitosan, coenocytic hyphae, aplanospore, or chlamydospore formation and nature of nuclei being similar to zygomycetous fungi.

8.2.6 Molecular Approach

Modern classification of AM fungi is based on molecular data. The classification of arbuscular mycorrhizal fungi presented here is that of Schüβler et al. (2001) with emendations of Oehl and Sieverding (2004), Sieverding and Oehl (2006), Spain et al. (2006), Walker et al. (2007a, b), and Palenzuela et al. (2008).

Glomeromycota C. Walker & Schüβler
Glomeromycetes Cavalier-Smith
Archaeosporales C. Walker & Schüβler
Ambisporaceae C. Walker, Vestberg & Schüβler
Ambispora Spain, Oehl & Sieverd.
Archaeosporaceae J.B. Morton & D. Redecker emend. Oehl & Sieverd.
Archaeospora J.B. Morton & D. Redecker
Intraspora Oehl & Sieverd.
Geosiphonaceae Engler. & E. Gilg emend. Schüβler
Geosiphon (Kutz.) F. Wettst.
Diversisporales C. Walker & Schüβler
Acaulosporaceae J.B. Morton & Benny
Acaulospora Gerd. & Trappe emend. S.M. Berch
Kuklospora Oehl & Sieverd.
Diversisporaceae C. Walker & Schüβler
Diversispora C. Walker & Schüβler
Otospora Oehl. J. Palenzuela & N. Ferrol
Entrophosporaceae Oehl & Sieverd.

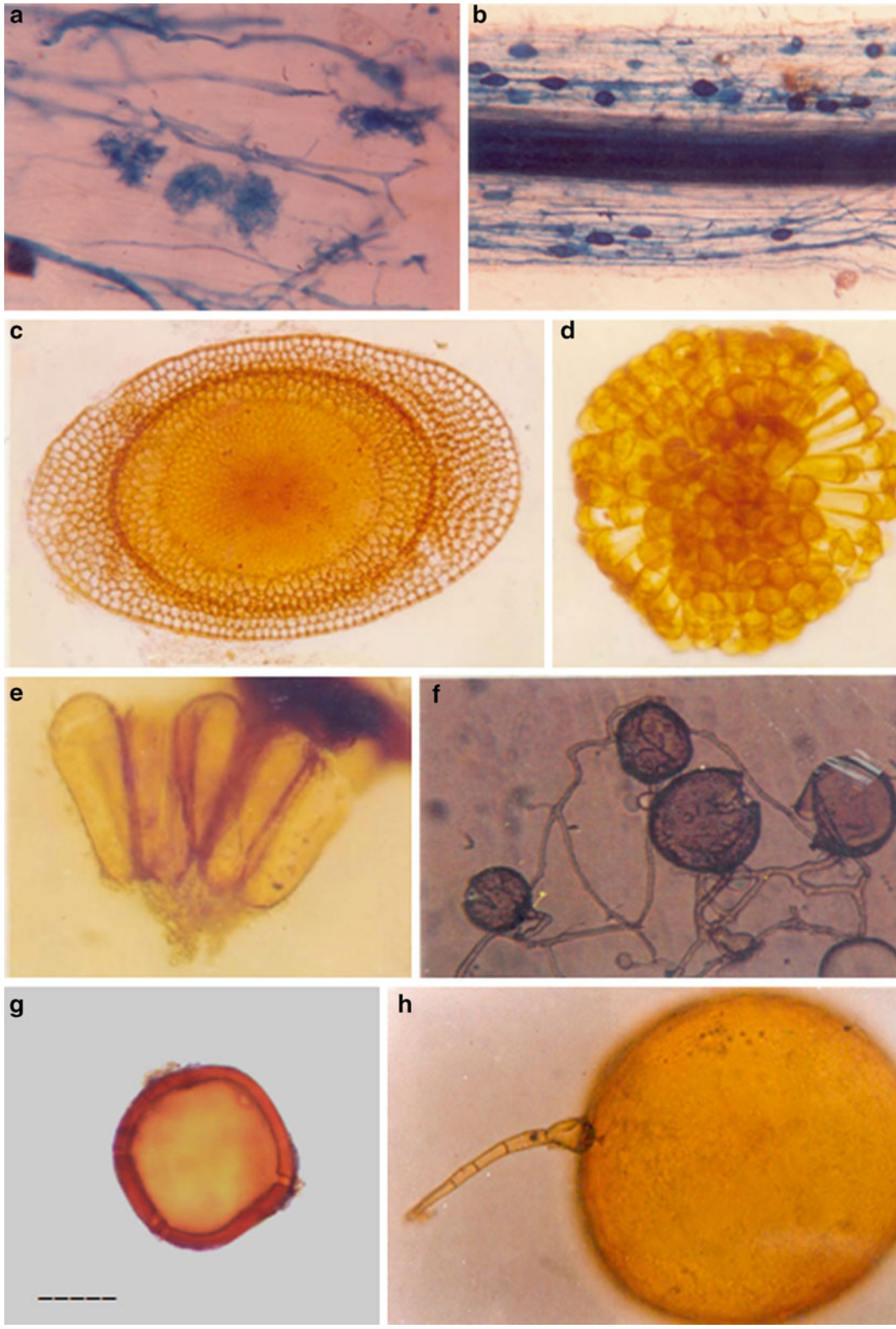


Fig. 8.1 Arbuscular mycorrhizal fungi. (**a**) Arbuscules in the root tissue. (**b**) Vesicles in root tissue. (**c**, **e–h**) Spores. (**c**) *Entrophospora* sp. (**d**) Sporocarp of *Sclerocystis microcarpus*. (**e**) *Glomus sinuosum*. (**f**) *Glomus versiforme*. (**g**) *Acaulospora mellea*. (**h**) *Scutellospora* sp. Bar. (**c**, **g**) 30 µm; (**d**, **h**) 90 µm; (**e**) 55 µm; (**f**) 100 µm

Entrophospora R.N. Ames & R.W. Schneid. Emend. Oehl & Sieverd.
Gigasporaceae J.B. Morton & Benny
Gigaspora Gerd. & Trappe emend. C. Walker & F.E. Sanders
Scutellospora C. Walker & F.E. Sanders
Pacisporaceae C. Walker, Blaszk., Schüβler & Schwarzott
Pacispora Oehl & Sieverd.
Glomerales J.B. Morton & Benny
Glomeraceae Piroz. & Dalpe
Glomus Tul. & C. Tul.
Paraglomerales C. Walker & Schüβler
Paraglomaceae J.B. Morton & D. Redecker
Paraglomus J.B. Morton & D. Redecker

As of now the number of described species of arbuscular mycorrhizal fungi is around 200. As per Morton et al. (1994), the number of species of this group of fungi may go up 300 as an approximate estimate.

8.2.7 Phosphorus Nutrition

Mineral nutrients are essential for plant growth. The forest soils and unproductive soils are deficient in phosphorus and other minerals. Such soils harbor more AM fungi than cultivated soils which are rich in phosphorus and other nutrients. Much of the phosphorus is in non-soluble state. Generally in between the zones of soluble phosphorus and non-soluble phosphorus, a "P" depletion zone is there. In order to obtain more phosphorus, plants must bypass these depletion zones by further root activity elsewhere in the soil. The surface area of plant's root determines the amount of "P" that gets solubilized by other microbes and gets mobilized by AM fungal hyphae as ultimately polyphosphate granules are released into host tissue through arbuscules.

8.2.8 AM Fungi and Plant Growth

Application of AM fungi as bioinoculants in plants enhances the plant growth responses (Gianinazzi-Pearson et al. 1981). The positive effect of mycorrhizae on plant growth can be related to higher efficiency in nutrient acquisition especially the "P" (Bolan 1991). The AM fungal inoculated plants show more height, biomass, and preflowering, higher yields and productivity in crop plants and other plants. AM fungi can aggregate soil particles and bind them through its intensively growing mycelium. The utilization of AM fungi in plant growth and yield is associated with their capability to reduce or prevent plant disease development.

Diversity of AM fungi is a bioindicator of environmental quality and impact of anthropogenic activity. AM fungi also contribute to soil stabilization and soil fertility. AM fungi can be inoculated into wastelands, degraded sites, polluted soils, and mine soils so as to convert them into green pastures indicating that AM fungi are good bioremediation agents.

The culture of AM fungi through aeroponics is a cost-effective technology. It is important to develop and improve strategies of inoculum production so as to produce AM fungal inoculum as commercialized product in order to serve the purpose of biofertilizer on par with chemical fertilizers. Genetic manipulation, Ri-plasmid-transformed root cultures, root organ culture, protoplast fusion, and other such modern inoculum production technologies may offer suitable promise for commercial production of AM inoculum. Efficiency of indigenous AM fungal strain along with soil factors and genotypic response of host cultivar are the possible factors playing an important role. Experimental documentation pertaining to plant growth and AM fungal symbiosis in cereals, legumes, soybean, peanut, forest seeding establishment, and soil stabilization is of immense importance (Manoharachary and Ranganayaki 2003).

8.3 AM Fungus Inoculum Production

AM fungi are obligate symbionts, and there are difficulties in inoculum production. In order to exploit the potential of AM fungi as biofertilizer, it is necessary to translate the technology to field conditions. Bagyaraj and Manjunath (1980) have

studied the role of AM fungi under unsterile soils using indigenous efficient strains of AM fungi. Mosse (1981) had evidenced improving plant growth under field conditions. In view of this, there is a great potential for AM fungi as biofertilizer subject to their efficient multiplication and mass production of inoculums besides the commercialization (Manoharachary 2000). The utility of mycorrhizae is documented, and such technology can be used for the development of sustainable systems of agriculture (Jeffries and Dodd 1991). AM fungi are multiplied in the form of soil pot culture and root organ culture and also through hydroponics, nutrient film technique, and other technologies. Carrier materials like vermiculate, sawdust, lignite, clay, and others have been identified. Some efforts to develop bulk inoculum have proved unsuccessful as these fungi have failed to grow in axenic culture. Slurries, gels, pellets, and multi-seeded pellets are used in the preparation and formulation of inoculum. AM fungal infection has been achieved using root organ culture and aeroponic system. Mycova and mycogrow and few others are the commercialized products sold in some world markets (Manoharachary 2000).

8.3.1 AM Fungi and Medicinal Plants

Seventy to 80 % of world population is dependent on drugs synthesized from medicinal plants, microbes, and fungi. In view of the above, there has been increasing demand for medicinal plants, their cultivation both in the field and under laboratory conditions. One of the issues that are faced by medicinal plants is the unstable quality of the products. The arbuscular mycorrhizal symbiosis aspects, secondary metabolism, production of active ingredients, and quality of herbal medicines have to be taken into consideration.

Around 50 research papers have been published on the secondary metabolites of medicinal plants associated with AM fungi. Few examples are given in Table 8.1; fungi help to increase total yield while maintaining quality: The AM symbiosis not only helps in plant growth but also offers disease resistance and quality of nutrients, thus becoming natural benefactors sought after in the herbal industry.

Table 8.1 Medicinal plants investigated for AM fungi

Species	Medicinal use
Datura stramonium L.	Treatment of cough
Salvia officinalis L.	For treatment of infections in mouth and throat
Coleus forskohlii Briq.	For treatment of cardiac diseases and glaucoma
Aloe barbadensis Mill.	Antibacterial and anti-inflammatory
Allium sativum L.	For arteriosclerosis, BP, and cardiovascular and immune system
Tagetes erecta L.	Diminishing inflammation of vocal cord, cough
Citrus aurantium L.	Promoting digestion
Ocimum basilicum L.	Treating trauma
Mentha arvensis L.	Treating cough and headache
Catharanthus roseus (L.) G. Don	Anticancer effects

AM symbiosis promotes absorption of N and K and other mineral nutrients, thus contributing both to yield and quality of medicinal material. The most important challenge in understanding the role of AM fungi is changes in the concentration of phytochemicals in plant tissues (Toussaint 2007).

8.3.2 AM Fungi and Xerophytes

Xerophytes are the plants adapted for life and growth with a limited water supply and poor soil conditions. They possess the morphological, anatomical, and physiological modification which makes the plant cope up with environmental water deficit and tolerate abiotic and biotic stress (Oppenheimer 1960). Ephemeral annual succulents and non-succulent perennials are the categories included under xerophytes.

AM fungi are associated with xerophytic plants growing in arid regions (Khan 1974), and these plants build up resistance to water stress

and poor soil conditions (Table 8.2). Xerophytic plants along with AM fungal association are known as pioneer colonizers of sand dunes, industrial waste lands, disturbed lands, and other such habitat. AM fungi have potential in the reclamation of disturbed land, arid land, and unproductive land. Khan (1988) has demonstrated the occurrence and distribution of AM fungi and their role on growth of plants growing in arid zones including observation of increased growth of grasses and other indigenous flora on dump sites. Indigenous AM fungal isolates were used in the colonization of indigenous flora in the disturbed sites.

Table 8.2 AM fungi associated with some xerophytes

AM fungus	Xerophyte
Acaulospora laevis Gerd. & Trappe	*Opuntia nigricans* (Haw.) Haw.
Acaulospora nicolsonii C. Walker, L.E. Reed & F.E. Sanders	*Calotropis procera* R. Br.
Acaulospora scrobiculata Trappe	*Caralluma fimbriata* Wall
Entrophospora infrequens (I.R. Hall) R.N. Ames & R.W. Schneider	*Chlorophytum tuberosum* Baker
Glomus albidum C. Walker & L.H. Rhodes	*Argemone mexicana* Link
Glomus caledonium (T.H. Nicolson & Gerd.) Trappe & Gerd.	*Agave americana* Linn.
Glomus fasciculatum (Thaxt.) Gerd. & Trappe	*Opuntia nigricans* (Haw.) Haw., *Tribulus terrestris* Linn.
Glomus microcarpum Tul. & C. Tul.	*Agave americana* Linn.
Glomus mosseae (T.H. Nicolson & Gerd.) Gerd. & Trappe	*Tribulus terrestris* Linn.
Gigaspora rosea T.H. Nicolson & N.C. Schenck	*Agave americana* Linn.
Sclerocystis coremioides Berk. & Broome	*Scilla indica* (Roxb.) Baker
Sclerocystis pachycaulis C.G. Wu & Z.C. Chen	*Aristolochia bracteata* Retz.
Scutellospora fulgida Koske & C. Walker	*Argemone mexicana* Linn.

8.3.3 Arbuscular Mycorrhizae: Ecology

AM fungal propagules get disturbed due to soil degradation and erosion. Soil disturbances including agricultural practices are responsible for the destruction of AM fungal network. AM fungi are known to bear water stress, drought, etc. Salt stress is known to influence occurrence, distribution, and effectiveness of AM fungi.

AM fungi can alleviate the toxicity of certain metals, and AM fungal colonization protects plant from heavy metal toxicity. AM fungi are involved in the formation of stable soil aggregation, a process crucial for soil conservation.

8.3.4 AM Fungi: Soil–Plant Interaction

Soil–plant interface seems to be a favorable medium for microbes to establish communication and interaction not only with each other but also with their host plants. This microecological niche called rhizosphere is inhabited by AM fungi. In soil–plant interface, AM fungi serve the purpose of ecosystem services which include biofertilization, bioregulation, and other functions through their beneficial effects in ecosystem. The AM fungi existing in such ecosystems, their increase, and their diversity are known to enhance host diversity and productivity. These are influenced by the availability of phosphorus and other soil factors; besides stress conditions like submerged condition possibly affect the plant nutrition adversely though AM fungi are present. AM fungal colonization also varies not only with host type but also with soil conditions. Metabolites like glomalin and other phytohormone production too vary with soil ecological conditions and host type. However, many studies have shown that there is no host preference for AM fungi. It is a known fact that metabolites secreted by bacteria, actinomycetes, and other fungi have their own impact in root region on AM fungi which may be positive or negative. Root exudates of diversified host plants vary from host to host, and this variation also has got impact on the role of

AM fungi in plant growth and productivity. Mycorrhizae are very important in bioremediation, wasteland reclamation, and ecological restoration. Mycorrhizae increase the absorption capacity of roots and bring out morphological and physiological changes in plants. Mycorrhizal roots explore more soil area for nutrients and mobilize them to different parts of plant besides bringing out changes in the nutrient storage and their utilization as per age of the plant and prevailing soil conditions.

8.3.5 Mycorrhizal Benefits

Mycorrhizal fungi are a heterogeneous group of symbiotic soil fungi that colonize the roots of about 240,000 plant species in all terrestrial ecosystems. About 6,000 species within the Zygo-, Asco-, and Basidiomycotina have been recorded as mycorrhizal. It is now known through molecular techniques that inter- and intraspecific genetic diversity exists. The diversity has significant ecological consequences.

Individual populations vary in their potential range of host species and their ability to colonize different host genotypes and promote plant growth. Adaptation to abiotic factors, pH levels, toxic levels of heavy metals, their dissemination in the ecosystems, symbiosis, and other parameters affect their differentiation at the root surface. AM fungi successfully and effectively transport orthophosphate besides nitrogen, zinc, and other nutrients. Mycorrhizal fungi are crucial component of the rhizosphere, where they work at the soil–plant interface. The knowledge and scientific data of interactions between mycorrhizal fungi and rhizosphere microbes and their multiple interactions offers new tools for the development of biofertilizer to be used for sustainable agriculture.

Molecular approaches will clarify at least some of the unsolved questions of mycorrhizae. Mycorrhizal symbiosis among plants growing in natural ecosystem is the norm. The plants which do not form mycorrhizae appear to have a secondary adaptation to ecosystem where mineral resources are abundant but the time for growth is limited. AM fungi are the universal compensators needed to accomplish the mission of sustainable agriculture.

Hyphal web of AM fungi within the soil is a vital component of the soil ecosystem and is the functional organ for the uptake and translocation of nutrients to from mycorrhizae. P, C, N, and other mineral nutrients can be transported from remote sources in the soil by AM hyphae. Mycorrhizae also act as bridges between plants. Mycorrhizal symbioses influence the relative abilities of plants to compete for limiting nutrients.

8.3.6 Controversies

Spores of AM fungi are produced singly or in sporocarps in soil or roots. Sporocarps have spores characteristically organized into loose or compact structures. In compact sporocarps pyridium may be of loosely interwoven fungal hyphae or compact, thick, and rigid. In some cases the sporocarp may be without pyridium. Formation of sporocarps has been reported in *Glomus* and *Sclerocystis*. Exceptionally few species of *Acaulospora* may possess sporocarps. Almeida and Schenck (1990) have reported that sporocarps of some species of *Sclerocystis* resemble that of *Glomus* except in *Sclerocystis coremioides* Berk & Broome. Further they have suggested the transfer of all other species of *Sclerocystis* to *Glomus*. The studies of the authors indicate that the transfer of several species of *Sclerocystis* to *Glomus* will create confusion and stress the need for developmental studies as *Sclerocystis* spp. form spores in compact sporocarps. The authors have observed that the hyphae of central plexus in *Sclerocystis* spp. develop radially and branch dichotomously. The ultimate branches enlarge into clavate or variously shaped spores. The development of spores may be holothallic or holoblastic. Further the apical portion of the spores in most of the species earlier included in *Sclerocystis* is very much thickened. The above ontogenic features have been observed in *Sclerocystis pakistanica* Iqbal & Bushra, *S. microcarpus* Iqbal & Bushra, and *S. sinuosa*

Gerd. & Bakshi. However, the authors have observed loose sporocarp and single-stalked or pedicellate spores in *Sclerocystis rubiformis* Gerd. & Trappe, and spore ontogeny was similar to *Glomus* sp.

Almeida's (1989) modification of the genus name *Scutellospora* Walker & Sanders to *Scutellispora* was found to be unacceptable by Walker (1995) though Morton (1990) and Morton and Benny (1990) accepted Almeida's alteration without critical analysis. This acceptance was erroneous and should not be taken as lending credence to the change. Therefore, it is suggested that ICBN has to be followed very carefully while proposing the names, change of names, classification, and other taxonomic interpretations.

In recent times Melbourne code has been proposed (2012) which is effective from 2013. According to this code: (1) There is no necessity of Latin diagnosis. (2) One fungus needs to have one name – perfect stage. (3) Fungus needs to be deposited/registered in MycoBank. (4) Molecular data has to be provided. In view of this, since none of the genera/species in Glomeromycota have any perfect stage, this may lead to its merger in Zygomycota after ascertaining the molecular base. Schüβler et al. (2001) and others have added molecular data strengths. Still controversies and phylogenetic systematics of AM fungi remain to be an unsolved problem and need scientific debate.

8.3.7 Terminology

Daft and Nicolson (1974) used the term vesicular arbuscular mycorrhizal (VAM) fungi for endogones or endomycorrhizal fungi. Various observations revealed that *Scutellospora* and *Gigaspora* form mycorrhiza with arbuscules only; vesicles are not formed. Walker (1995) has proposed that VAM fungus is not the proper term to be used as vesicles are absent in some arbuscular mycorrhizal fungi. In 1993 a group of mycorrhizists who met in Spain discussed all the aspects of nomenclature including terminology and adopted the usage of AM fungi instead of VAM, since AM fungi is more accurate and scientific. Therefore, it is suggested that no confusion should be created in the future and usage of confusing terminology be avoided.

Acknowledgments CM is grateful to NASI, India, for financial support.

References

Abbott LK, Robson AD (1977) The distribution and abundance of vesicular-arbuscular endophytes in some western Australian soils. Aust J Bot 25:515–522

Almeida RT (1989) Scientific names in the Endogonales, Zygomycotina. Mycotaxon 36:147–159

Almeida RT, Schenck NC (1990) A revision of the genus *Sclerocystis* (Glomaceae, Glomales). Mycologia 8:703–714

Bagyaraj DJ, Manjunath A (1980) Selection of a suitable host for mass production of VA mycorrhizal inoculum. Plant Soil 55:495–498

Bagyaraj DJ, Manjunath A, Patil RB (1979) Occurrence of vesicular arbuscular mycorrhizas in some tropical aquatic plants. Trans Br Mycol Soc 72:164–167

Berch SM (1986) Endogonaceae-taxonomy, specificity, fossil record, phylogeny. Front Appl Microbiol 2:161–188

Bolan NS (1991) A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant Soil 134:189–207

Bonfante-Fasolo P (1984) Anatomy and morphology of VA mycorrhizae. In: Powell CL, Bagyaraj DJ (eds) VA mycorrhiza. CRC Press, Florida

Brundrett MC (2004) Diversity and classification of mycorrhizal associations. Biol Rev 79:473–495

Brundrett M, Kendrick B (1990) The roots and mycorrhizas of herbaceous woodland plants. II structural aspects of morphology. New Phytol 114:469–479

Daft MJ, Nicolson TH (1974) Arbuscular mycorrhizae in plants colonizing coal wastes in Scotland. New Phytol 73:1129–1138

Dangeard PA (1900) Le *Rhizophagus populinus* Dangeard. Botaniste 7:285–291

Frank AB (1885) Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. Ber. Deutsch. Bot Ges 3:128–145

Gerdemann JW, Trappe JM (1974) The endogonaceae in the Pacific Northwest. Mycol Mem 5:1–76

Gerdernann JW, Nicolson TH (1963) Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc 46:235–244

Gianinazzi-Pearson V, Morandi D, Dexheimer J, Gianinazzi S (1981) Ultrastructural and ultracytochemical features of a *Glomus tenuis* mycorrhiza. New Phytol 88:633–639

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Hall IR (1983) A summary of the features of endogonaceous taxa. Technical report 8, 3rd edn. Invermay Agricultural Research Centre, Mosgiel

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic, London

Jeffries P, Dodd JC (1991) The use of mycorrhizal inoculants in forestry and agriculture. In: Arora DK, Rai B, Mukerji KG, Knudsen GR (eds) Handbook of applied mycology, vol 1, Soil and Plants. Marcel Dekker, New York

Kendrick WB, Berch SM (1985) Mycorrhizae: applications in agriculture and forestry. In: Robinson CW, Howell JA (eds) Comprehensive biotechnology and bioengineering: the principles, applications, and relations of biotechnology and industry, agriculture, and medicine, vol 4. Paragon Press, London

Khan AG (1974) The occurrence of mycorrhizas in halophytes and xerophytes, and of endospores in adjacent soils. J Gen Microb 81:7–14

Khan AG (1988) Inoculum density of *Glomus mosseae* and growth of onion plants in unsterilized bituminous coal spoil. Soil Biol Biochem 20:749–753

Kormanik PP, McGraw AC (1982) Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: Schenck NC (ed) Methods and principles of mycorrhizal research. American Phytopathological Society, St. Paul, Minnesota

Kunwar IK, Manoharachary C (1998) Colonization by arbuscular mycorrhizal and mycophyllous fungi in roots and scale leaves of garlic. J Ind Bot Soc 77:201–203

Kunwar IK, Manoharachary C (1999) Colonization of the roots, rhizomes and scale like leaves of ginger by arbuscular mycorrhizal fungi. In: Manoharachary C (ed) Proceedings of national symposium on fungi in diversified habitats. Osmania University, Hyderabad

Manoharachary C (2000) Role of vesicular arbuscular mycorrhizal fungi in biotechnology. In: Maheswari DK, Dubey RC, Prasad G, Navneet (eds) Microbes: agriculture, industry and environment. Bishen Singh Mahendrapal Singh, Dehradun

Manoharachary C, Ranganayaki N (2003) AM fungal biotechnology in the new millennium. In: Trivedi PC (ed) Glimpses in plant sciences and biotechnology. Pointer Publishers, Jaipur

Morton JB (1989) Mycorrhizal fungi slide set: morphological characters important in identifying endomycorrhizal fungi in the Zygomycetes. Audio-visual-electronic publication 2. Agric and Forestry Exper Station. West Virginia University, Morgantown

Morton JB (1990) Evolutionary relationships among arbuscular mycorrhizal fungi in the endogonaceae. Mycologia 82:192–207

Morton JB, Benney GL (1990) Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): new order, Glomales, two new suborders, Glomineae and Gigasporineae and two new families, Acaulosporaceae and Gigasporaceae with emendation of Glomaceae. Mycotaxon 37:471–481

Morton JB, Bentivenga SP, Bever JD (1994) Discovery, measurement, and interpretation of diversity in arbuscular endomycorrhizal fungi (Glomales, Zygomycetes). Can J Bot 73:S25–S32

Mosse B (1953) Fructifications associated with mycorrhizal strawberry roots. Nature 171:974

Mosse B (1981) Vesicular arbuscular mycorrhiza research for tropical agriculture. Res Bull 194:5–81, Hawaii Institute of Tropical Agriculture and Human Resources

Oehl F, Sieverding E (2004) *Pacispora*, a new vesicular arbuscular mycorrhizal fungal genus in the Glomeromycetes. J Appl Bot 78:72–82

Onguene NA, Kuyper TW (2001) Mycorrhizal associations in the rain forest of South Cameroon. For Ecol Manage 140:277–287

Oppenheimer HR (1960) Adaptation to drought: xerophytism. In: Plant water relationships in arid semi-arid conditions. Review of Research UNESCO, Paris

Palenzuela J, Ferrol N, Boller T, Azcon-Aguilar C, Oehl F (2008) *Otospora bareai*, a new fungal species in the Glomeromycetes from a dolomitic shrubland in the Natural Park of Sierra de Baza (Granada, Spain). Mycologia 100:296–305

Park JL, Linderman RG (1980) Association of vesicular arbuscular fungi with the moss *Fumaria hygrometrica*. Can J Bot 58:1898–1904

Peyronel B (1924) Specie de *Endogone* protructria di micorize endotrofiche. Bolletins Mensile Della R Stazione Patologia Vegetals Roma 5:73–75

Phillips JM, Hayman DS (1970) Improved procedure for clearing root and staining parasitic and VAM fungi for rapid assessment of infection. Trans Brit Mycol Soc 55:158–161

Redhead JF (1977) Endotrophic mycorrhizas in Nigeria: species of the endogonaceae and their distribution. Trans Br Mycol Soc 69:275–280

Schenck NC, Perez Y (1990) Manual for the identification of VA mycorrhizal fungi. Synergistic Publications, Gainesville

Schüβler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1421

Sieverding E, Oehl F (2006) Revision of *Entrophospora* and description of *Kuklospora* and *Intraspora*, two new genera in the arbuscular mycorrhizal Glomeromycetes. J Appl Bot Food Qual 80:69–81

Spain JL, Sieverding E, Oehl F (2006) *Appendicispora*: a new genus in the arbuscular mycorrhiza-forming Glomeromycetes, with a discussion of the genus *Archaeospora*. Mycotaxon 97:163–182

Thaxter R (1922) A revision of the endogonaceae. Proc Am Acad Arts Sci 57:291–351

Toussaint JP (2007) Investigating physiological changes in the aerial parts of AM plants: what do we know and where should we be heading? Mycorrhiza 17:349–353

Trappe JM, Schenck NC (1982) Taxonomy of fungi forming endomycorrhizae. In: Schenck NC (ed) Methods and principles of mycorrhizal research. American Phytopathology Society, St Paul

Walker C (1983) Taxonomic concepts in the endogonaceae: spore wall characteristics in species descriptions. Mycotaxon 18:443–455

Walker C (1995) AM or VAM: what's in a word? In: Varma A, Hock B (eds) Mycorrhiza structure, function, molecular biology and biotechnology. Springer, Berlin

Walker C, Vestberg M, Demircik F, Stockinger H, Saito M, Sawaki H, Nishmura I, Schüβler A (2007a) Molecular phylogeny and new taxa in the Archaeosporales (Glomeromycota): *Ambispora fennica* gen. sp. Nov., Ambisporaceae fam. Nov., and emendation of *Archaeospora* and Archaeosporaceae. Mycol Res 111:137–153

Walker C, Vestberg M, Schüβler A (2007b) Nomenclatural clarifications in Glomeromycota. Mycol Res 111:253–255

Diversity and Applications of Mushrooms

9

S.M. Reddy

Abstract

Mushrooms which are earliest known fleshy fungi are widely distributed in tropical temperate regions alike. They are attractive in colour, design and shape and intimately associated with human civilization. They are ubiquitous saprophytes as well as symbiotic in nature. Though many are edible and have medicinal properties, some are deadly poisonous and are called toadstools. Taxonomically they represent ascomycotina and basidiomycotina. They can be active and produce variety of metabolites exhibiting wide-spectrum biological activities including antimicrobial, haematological, antioxidant, anti-inflammatory, hepatoprotective, antitumour, etc., receiving increasing attention of pharmacologists and medical practitioners. Some mushrooms produce hallucinogens and are consumed on festive occasions. Mushrooms have received greater attention as food for healthy life. They being fibrous in nature, low lipid and sugar content mushrooms are a recommended food for diabetes and heart patients. In spite of all these good attributes, very few mushrooms have been studied and still many more await study. In spite of these facts, only about 20 mushrooms are being cultivated. Therefore, there is not only an urgent need for survey of these fungi in unexplored and non-accusable regions but also a need to develop methods for their cultivation in an economical way. Further efforts to increase shelf life and processing different edible mushrooms need to be taken up.

Keywords

Diversity and applications of mushrooms • Cultivation

S.M. Reddy (✉)
Department of Botany and Microbiology, Kakatiya University, Warangal 506 009, Telangana, India
e-mail: profsmreddy@yahoo.com

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_9,

9.1 Introduction

Mushrooms have been recorded as fossils, their record dating back to the Silurian period (408–438 million years). Their occurrence increased during the Pennsylvanian period (286–320 million years). The earliest record of interface between a fungus and human beings is in the form of burnt remains of the bracket of the polyporaceous fungus. *Fomes fomentarius* was recorded near hearth stones and iron pyrites in excavations dating back to 9,000 BC, following the termination of the Ice Age. As such, mycelial network increase in size becomes visually apparent, and indeed, in some cases the mycelia form large complicated structures exemplified by the large fruit bodies known colloquially as mushroom.

Mushroom finds place in the prehistory of Aryans who came down from the Northwest through Afghanistan and occupied the historic Indus Valley. These comers to Indus Valley were related to the people of Iranian plateau and probably responsible for the ruin of early Indus Valley civilization. The ancients of India, Iran and China used certain mushrooms in their ritualistic performance. A ritual drink having an intoxicating property was obtained from hallucinogenic mushrooms. It is believed that the Rigvedic rhymes in the IX Mandala dedicated to SOMA and its juice SOMARASA were obtained from a mushroom *Amanita muscaria*.

The word mushroom has been used in a wide variety of ways at different times and in different countries (Fig. 9.1a–9.1j). A broad use of the term mushroom includes all larger fungi or all fungi with stalks and caps or all large fleshy fungi (Bahl 1984). A more restricted use include just those large fungi that are edible and/or of medicinal value. The term mushroom is broadly defined as a macro fungus with a distinct fruiting body which can be either epigeous (above ground) or hypogenous (underground) and large enough to be seen with the naked eye and to be picked by hand. In India cultivation of mushrooms was started from 1943 (Thomas et al. 1943). World production of different edible mushrooms is summarized in Table 9.1.

9.2 Taxonomy of Mushrooms

The taxonomy of mushrooms is a fascinating field. Morphological characters of fruiting body, spore production and spore colour prove to be useful. Proper identification of the mushroom species involved is important not only for taxonomic importance but also for their edibility. Kim et al. (1993) discussed various aspects of mushroom biology and their identification. Some of the methods and characters used in the identification are discussed below (Singh and Singh 2005).

Mushroom keys given by different mycologists, field guides and expert guidance and collection of samples give some basic information about the mushrooms. Macroscopic physical structures and microscopic characteristics will also be of some help. The consistency (rubbery, droops, battle, woody, tough, corky or spongy), cap (plane depressed, campanulate, convex, umbranate, conicles), stipe (bulbus, club shape, tapered (Fig. 9.3) and base, annulus (presence of ring, type of ring, skirt-like, fragile), and volva (Fig. 9.2) are useful in mushroom identifications.

Gills (type and arrangement of gills), pores setae and cystidia (Fig. 9.3) and spores (Fig. 9.4) are some of the macro- and micromorphological features that help in the classification of mushroom. On the cap there may be warts (universal veil remnants), an edge (margin) and many other structures that are helpful in the identification of mushrooms. Size varies with place and age, shape varies considerably (bell shaped, club shaped, pear shaped, lobed, bracket, nest, crust-like, phallic shaped, star shaped and pestle shaped), and smell and taste are helpful criteria in the identification of mushrooms. Some chemical tests like potassium hydroxide and Meltzer's reagent are also useful in the identification of mushrooms. Similarly the altitude of the mushroom-growing

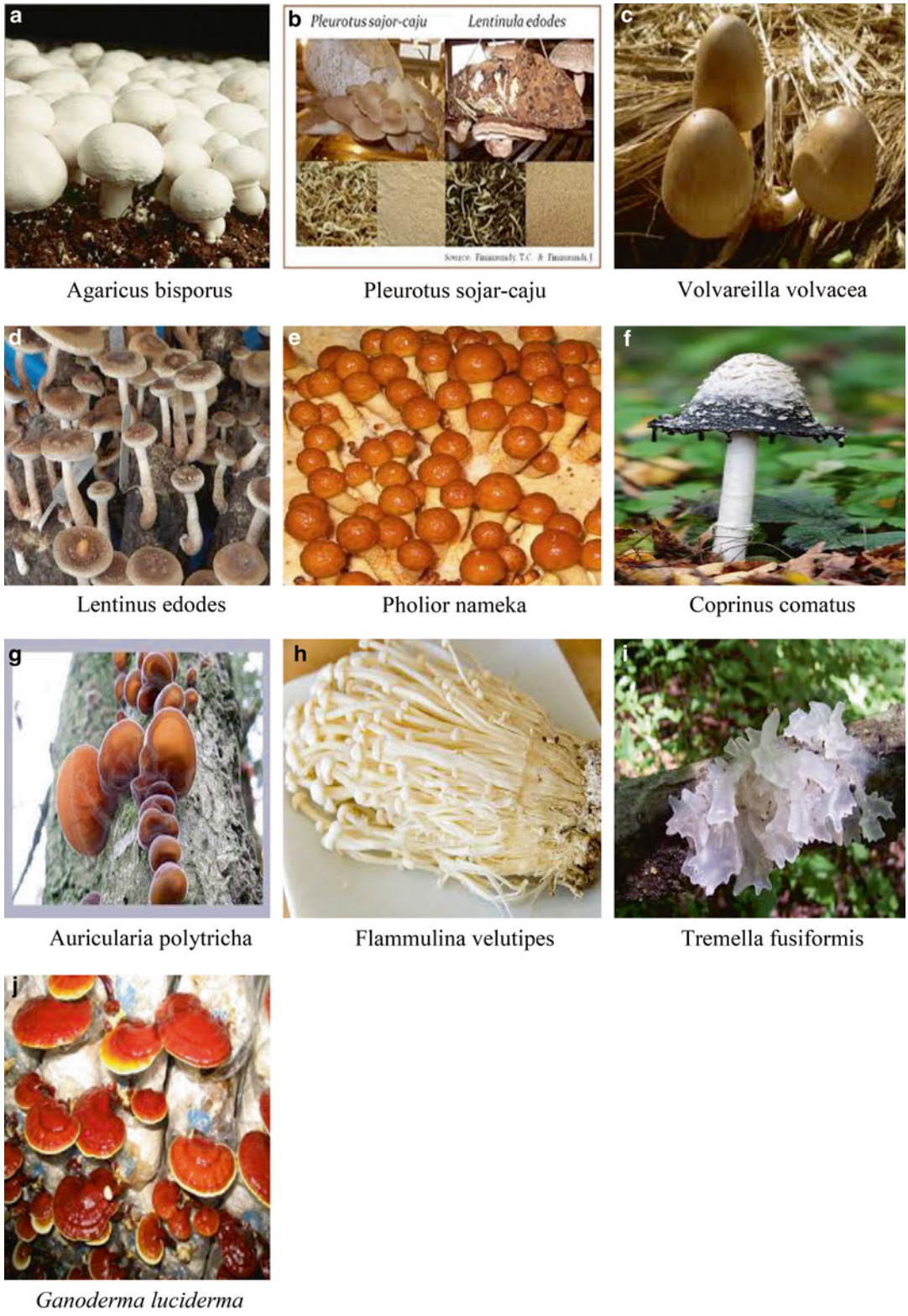


Fig. 9.1 (**a**–**j**) Different edible mushrooms

Table 9.1 World production of mushroom

Common name	Contribution (in %)	Botanical name	Purpose	Substrate	Production (in 10^3 tonnes)
Button mushroom	37.8	*Agaricus bisporus A. bitorquis* (Fig. 9.1a)	Food	Straw	1,424
Oyster mushroom	24.2	*Pleurotus sajor-caju* (Fig. 9.1b)	Food	Straw	909
Straw mushroom	5.5	*Volvariella volvacea* (Fig. 9.1c)	Food	Straw	207
Shiitake mushroom	10.4	*Lentinus edodes* (Fig. 9.1d)	Food and shii and medicine	Oak tree	393
Nameko	1.4	*Pholiota nameko* (Fig. 9.1e)	Food	Sawdust	53
Ink cap	–	*Coprinus comatus* (Fig. 9.1f)	Food	Straw	–
Wood ear or Jew's ear	10.6	*Auricularia polytricha* (Fig. 9.1g)	Food	Wood and other species	400
Winter mushroom	3.8	*Flammulina velutipes* (Fig. 9.1h)	Food	Wood	143
Silver ear	2.8	*Tremella fuciformis* (Fig. 9.1i)	Medicine	Wood	105
Bracket	–	*Ganoderma luciderma* (Fig. 9.1j)	Medicine	Wood	–

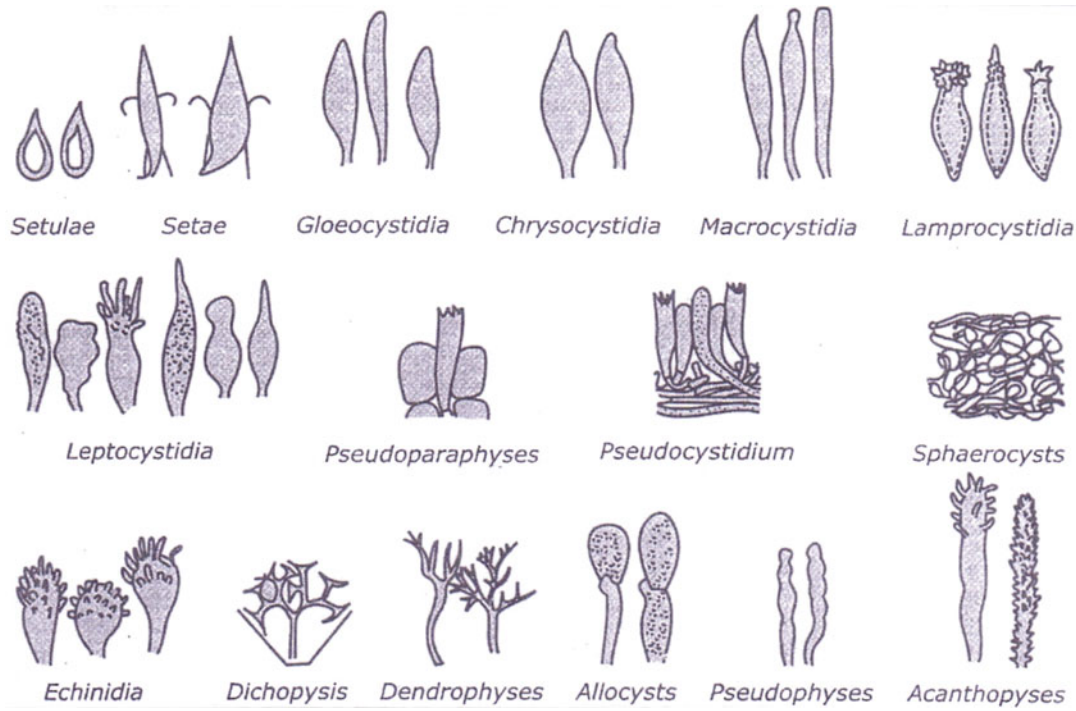


Fig. 9.2 Setae and cystidea of mushrooms

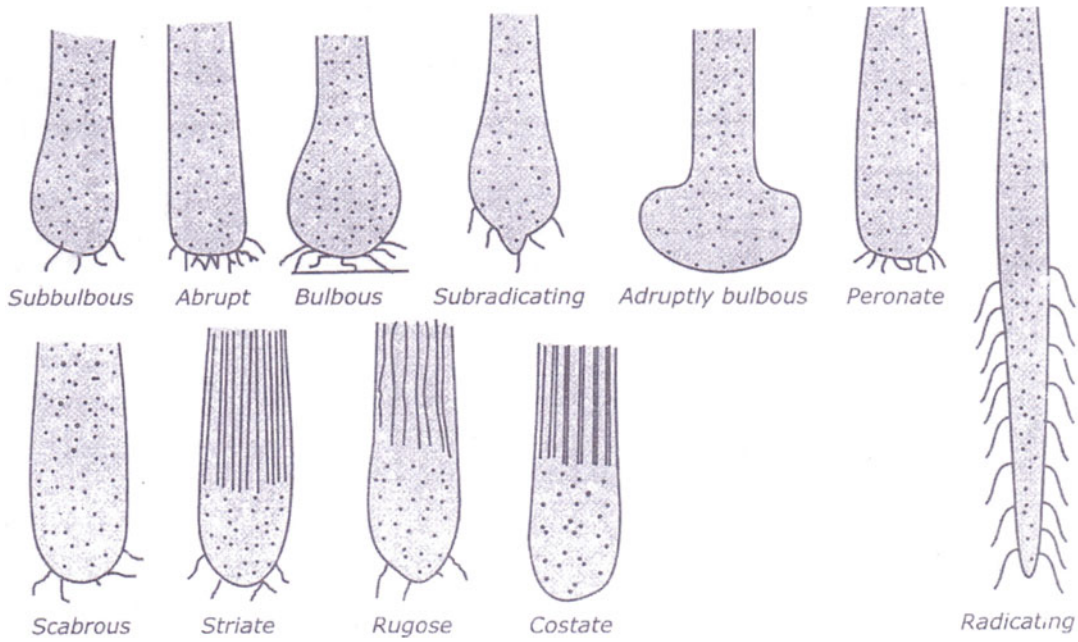


Fig. 9.3 Stipe of mushrooms

area, yield of type of latex and season of their growth are also helpful criteria in the identification of mushrooms. The spore print can be obtained by placing cut fruit body on white paper and covered with a bell jar overnight. The next day, one can get spore print (Fig. 9.5) which reveals spore colour and arrangement of gills and is useful in the identification of the genus. The shape and attachment of basidiocarp to the substratum is also equally useful in the identification (Fig. 9.6a, b).

9.3 Classification of Mushrooms

9.3.1 Categories of Mushroom Based on Common Belief and Use

Certainly this approach of classifying mushrooms is not absolute. Many kinds of mushrooms are not only edible but also possess tonic and medicinal qualities (Hawksworth 1990).

1. Edible mushrooms that are fleshy fall in the edible mushroom category, e.g. *Agaricus bisporus*.
2. Mushrooms with medicinal applications are medicinal mushrooms, e.g. *Ganoderma lucidum* (Stamets 1993).
3. Poisonous mushrooms are named as toad stools (poisonous mushrooms) because they contain toxic poison, e.g. toadstools (*Amanita phalloides*).
4. Miscellaneous category includes a large number of mushrooms whose properties remain less well defined. They are tentatively grouped as 'other mushrooms'.

9.3.2 Classified According to Their Growth Characteristics (Table 9.2)

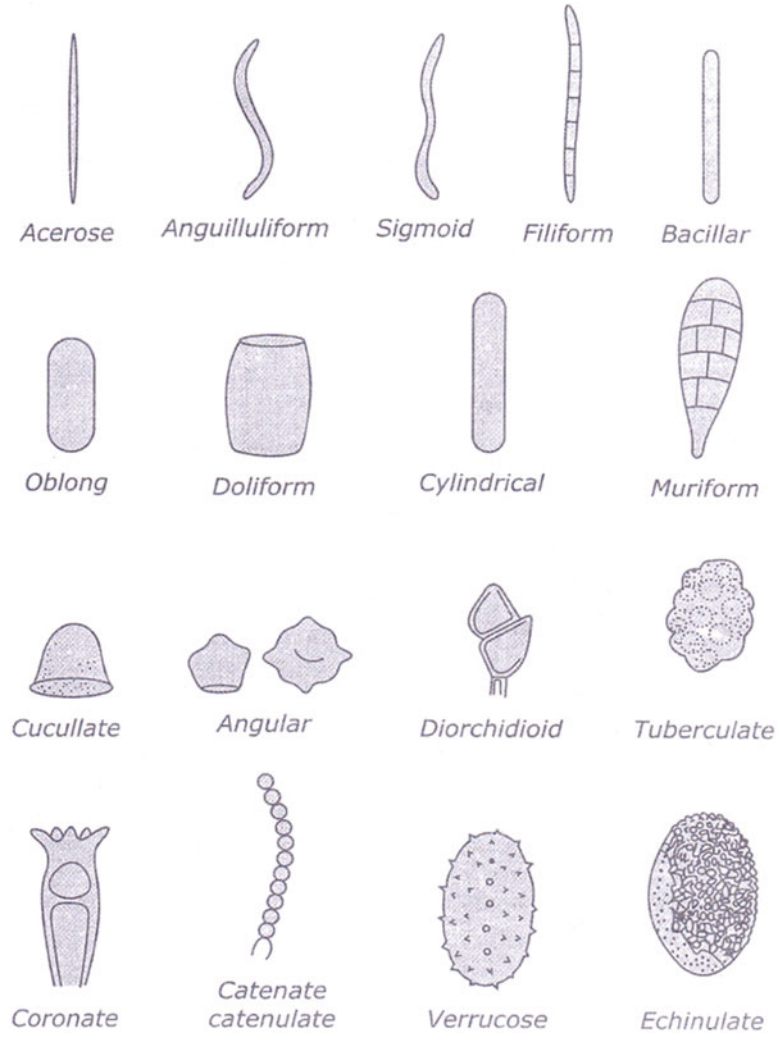


Fig. 9.4 Spores of mushrooms

Fig. 9.5 Spore print

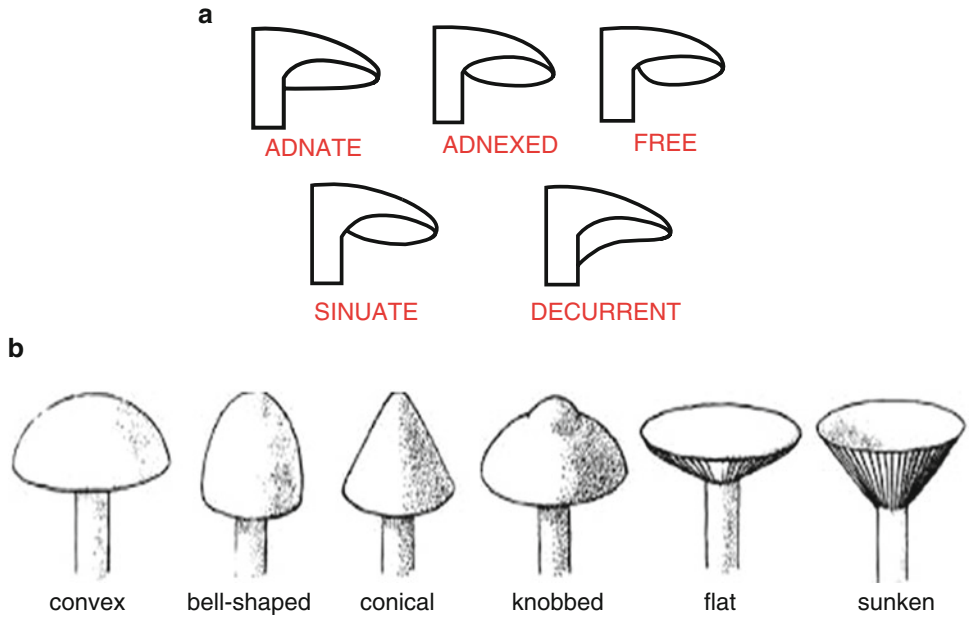
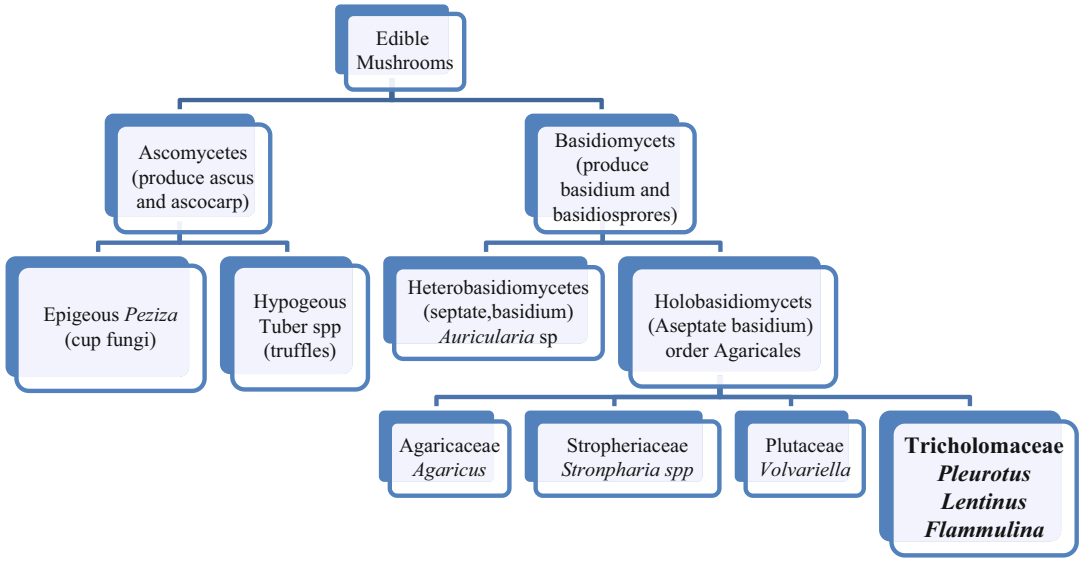


Fig. 9.6 (**a**) Attachment of gills to the stem of mushroom (**b**) Shapes of cap of mushrooms

Table 9.2 Classification of mushrooms based on substratum and mode of growth

Type of mushrooms	Examples	Remarks
A. Lignicolous		
(i) Parasitic mushrooms	*Polyporus squamosus*, *Fomes annosus*, *Armillaria mellea* and *Ganoderma lucidum* etc.	Mushroom attacks a living host plant, usually a tree, and eventually kills it
(ii) Mycorrhizal mushrooms (symbiotic)	Fungi form beneficial association with higher plants	Roots may be ecto-mycorrhizal or endo-mycorrhizal depending upon the nature of association
	Boletus, *Lactarius* truffles, *Cantharellus*, *Amanita* etc.	
	Tricholoma, *Tuber*, *Morchella*	
B. Humicolous		
Saprophytic mushrooms	*Lentinus edodes*, *Pleurotus* spp.	They obtain their food by decomposition of dead organic matter such as dead trees, stumps, old roots, grass, straw, compost, etc. Hence they are found in habitats rich in rotting vegetation
	Agaricus bisporus, Portobello, *Ganoderma lucidum*, Maitake, paddy straw, etc.	
C. Coprophillous (Inhabiting dung)	*Agaricus* sp., *Coprinus* sp.	Dung

9.3.3 Classified on the Basis of Fungal Fruit Bodies



On the basis of their edibility, mushrooms can be classified into two groups:

1. Edible mushrooms (mushrooms)
2. Non-edible mushrooms (toadstools)

9.4 Applications

For many years, mankind has benefited from green plants as a source of food, drugs and herbal remedies, but mushrooms are now beginning to receive much deserved attention for their very real health-giving qualities. It is their flavours and texture for which they have long been devoured by the mankind. Laessoe et al. (1996) have discussed excellently developments in mushrooms biology.

9.4.1 Nutritional Status

Some mushrooms are of good nutritional value, while others have medicinal value as dietary supplement, and still there are some that have both of these properties. Mushrooms can be considered as a functional food (medical food or nutritional food). Mushrooms may be consumed for their palatability and nutritional value. Mushrooms have been equated to 'vegetable beef stock' by some workers; while extreme some authors consider them to be devoid of any nutritional value or of minor nutritional significance (Dhar 1997).

On the other hand, Barros et al. (2008) consider that in addition to their high-quality protein, mushrooms are relatively good source of fat, phosphorus, iron, vitamins and ergosterol. Fruiting bodies are low in calories, carbohydrates, calcium and lipid (between 0.6 and 3.1 %). Unsaturated fatty acids are essential and significant to our diet and health, constituted at least 70 % of total fatty acid content.

Mushrooms, rich in nutrients, are being used as nutraceuticals. They are considered to provide strength to warriors in battles as believed by the Greeks, while the Chinese feel that they are health food and treat them as elixir of life. Romans consider them as God-given food, while the Pharaoh of Egypt and some European tribes consider mushrooms as a delicacy. Mexican Indians eat them during festive occasions as hallucinogens. They are food of fibrous nature, low in fat and rich in proteins and vitamins and considered to be a preferred food for diabetes and heart disease patients. Similarly mushrooms are rich in all essential vitamins and contain full complement of

mineral composition. The nutritive value is superior to egg, meat and pulses. In the nutritional index (NI), it stands fourth, while in the essential amino acid index (EAI) and protein efficiency ratio (PER), it stands third. Thus, these mushrooms proved to be quality food (Cochran 1978).

Recipes such as mushroom puree, mushroom paneer, mushroom pulao and mushroom omelette and mushroom soup which are for delicacy and taste are eaten. In view of rich nutrients, each kg of mushroom is said to be equal to 0.5 kg meat, 2 ½ egg, 1.5 kg potato, 0.5 kg soybean and 1.0 kg pulses.

The desirability of a food product is not necessarily correlated with its nutritional value, but appearance, taste and aroma are often important in stimulating the appetite and determining preference. Thus, in addition to nutritional value, edible mushrooms possess unique characteristics in terms of colour, taste, aroma and texture, which make them attractive for human consumption.

9.4.2 Nutritional Analysis

Early civilization by trial and error built up a practical knowledge of those suitable to eat and those to be avoided, for example, poisonous or even psychotropic. However, in the orient several thousand years ago, there was recognition that many edible and certain non-edible mushrooms could have valuable health benefits. The general profile of nutritional status of mushrooms is summarized in Table 9.3.

9.4.3 Medicinal Status

Mushrooms have long been considered to have different medicinal properties depending on the stage and environment of its growth. These have been used in folk medicine throughout the world since ancient times. The early herbalists were more interested in the medicinal properties of mushrooms than in their basic value as source of food. Molitoris (1994) discussed the role of mushrooms in medicine as a food source and for religious purposes. Out of 15,000 species of mushrooms in the world, around 700 are known to have medicinal properties and almost hundreds of species have been tested for cultivation. However, it has been estimated that there are about 1,800 species of mushrooms that have potential medicinal attributes. Thus, mushrooms have vast prospects as source of medicine (Sporke 2001). These have been investigated in the last decade both in in vivo and in vitro model systems. Many bioactive substances with immunomodulating effect have been isolated. These include polysaccharides of high molecular weight, glycoproteins (lectins), triterpenoids and fungal immunomodulatory proteins. Many mushrooms contain biologically active polysaccharides, which have antitumour and immunostimulating properties. Fruiting body and submerged mycelium are the source of these bioactive compounds. Majority (77.3 %) of compounds are extracted from fruiting bodies by hot water treatment or with organic solvent at different temperatures (Findlay 1982).

Although bioactive polysaccharides are widespread among mushrooms, different species can produce a variety of polysaccharides having distinct properties. For instance, the protein-bound polysaccharide PSK, obtained from the cultured mycelium of *Coriolus versicolor* CM-101 strain, is composed of 62 % polysaccharides and 38 % protein. The main component of the carbohydrate moiety is glucose with galactose, mannose, xylose and fructose as minor components, whereas polysaccharide peptides (PSP) of strain COV-1 contained 90 % polysaccharides and 10 % peptides in submerged mycelium. The polysaccharides and polysaccharide–protein complexes from mushrooms are able to stimulate the nonspecific immunity and exert antimicrobial and antitumour activity through the stimulation of the host's defence (Ng 1998). These biomolecules activate effector cells like macrophages, T lymphocytes and NK cells to secrete cytokines like TNF-α, IFN-γ, IL-1β, etc., which are antiproliferative and induce apoptosis. Mizuno et al. (1995) have elucidated the structure of polysaccharides isolated from *A. blazei* mainly as β-glucan protein in the fruiting body, glucomannan protein in the mycelium and mannan protein in the filtrate (Jong and Donovick 1998).

Administration of exopolymer obtained from the submerged culture of *Lentinus edodes* reduced the plasma glucose level and increased plasma insulin and lowered the plasma cholesterol and

Table 9.3 Nutrition values of mushrooms

Composition	Remarks	Range (%) on dry weight basis
Protein	High content of protein supports nutritional importance of mushrooms as a food source	19–39 %
Carbohydrate	Pentose, methylpentose, hexose, as well as disaccharides, amino sugar, sugar alcohol and sugar acid are major constituents of carbohydrates	46.6–81.8 %
Fibre	High-fibre diet reduces insulin requirement and stabilizes blood glucose profile, possibly by decreasing the rate of glucose absorption and delaying gastric emptying	4–27.6 %
Fat	It is an important constituent and has a defined role, and presence of fatty acids in mushroom is responsible for the growth and reproduction	1.18–8.39 %
Moisture	Moisture content of mushrooms is affected significantly by factors such as metabolic water, temperature and relative humidity of the environment	80–95 %
Vitamins	Mushrooms are a good source of several vitamins including thiamine, riboflavin, niacin, biotin and ascorbic acid	Varies with strain
Essential amino acids	Mushrooms contain, in addition to the common amino acids and amides, the less common amino acids and related nitrogenous compounds such as methionine sulphoxides, β-alanine, cystic acid, hydroxyl-prolines, aminoadipic acid, phosphoserine, cystathionine, cadaverine, creatinine, citrulline, ornithine, glucosamine and ethanolamine	Varies with strain
	Highest content of macroelements like potassium (K) followed by phosphorus (P), sodium (Na), calcium (Ca) and magnesium (Mg)	
Macro elements		Varies with strain
Minerals	Copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Ma) and cadmium (Cd) make up the minor mineral elements	
Ash	Mushroom ashes contain Na, K, P, Ca, Mg, etc.	6–13 %
Energy	Mushrooms are a good source of energy	Fresh mushrooms provide 120 k calories

triglyceride levels. Much of the knowledge on medicinal properties of mushroom comes from the literature of Far East, where mushrooms such as *G. lucidum*, *L. edodes*, *C. versicolor*, *Tremella fuciformis*, *Grifola frondosa*, *Schizophyllum commune*, *Cordyceps sinensis*, *C. sobolifera*, etc., are collected, cultivated and used for thousands of years (Willard 1990). During the past two decades, several pharmacologically active substances have been isolated and identified. Polysaccharide krestin (PSK) from *C. versicolor* mycelia containing (1→4), (1→3) or (1→4), (1→6)-β-glucans, lentinan, high-molecular-weight (1→3)-β-D-glucans from *L. edodes* fruiting bodies and mycelium and LEM-a protein-bound polysaccharide derived only from the mycelium are reported to have anticancer activity. These are reported to enhance the immune system rather than attack the cancer cells directly (Kurashige et al. 1997). Such compounds are increasingly being used in Japan as adjuvant to support immune function in cancer patients during radio- and chemotherapy and can prolong survival period in some types of cancer. Schizophyllan, a high-molecular-weight β-D glucan obtained from *S. commune* culture filtrates, has considerable anticancer activity in xenograph and, in clinical practice, also has high antitumour activity. Protein bound to low-molecular-weight polysaccharide (EA6) was isolated from *Flammulina velutipes* exhibited antitumor activity.

Ganoderma lucidum has the longest historical usage for medicinal purpose. Extracts of *Ganoderma* and its product are not only popular in China, Japan and Korea but also in North America, Asia and several other parts of the world. It attracted international attention for its

antitumour (Oikawa et al. 1993), immunomodulatory, cardiovascular, respiratory, antihepatotoxic and antinociceptive (active against pain) activity. The major compounds with significant pharmacological activities are to be ganoderic acid, triterpenes and polysaccharides. *G. luteum*, *G. tsugae*, *G. applanatum*, *G. austral*, *G.capense*, *G. tropicum*, etc., are other species of *Ganoderma* which are being exploited for medical purpose. It is interesting to note that during the last three decades, more than 150 carcinostatic polysaccharides have been isolated from these mushrooms (Jong et al. 1983). Therefore, *G. lucidum* products with different triterpenes and polysaccharides or combinations of these are most likely to yield different pharmacological formulations. The exopolymer of *G. lucidum* has exhibited hypoglycemic, hypolipidemic and enhanced immunomodulating activities of glucose in the experimental animals. Medical efficacies of *Ganoderma lucidum* are listed in Table 11.6.

Phellinus linteus, a parasite on living deciduous trees known as 'song gen' in Chinese medicine and 'meshimakobu' in Japanese, is reported to inhibit tumour proliferation which was attributed to β-D-glucans.

Poria cocos, a mycorrhizal fungus sometimes referred to as *Wolfiporia cocos*, 'Indian bread', which occur throughout the year, is very important medicinally. Polysaccharides – spachyman and pachymaran – from this mushroom exhibit strong anticancer and immunomodulatory activities. Tetracyclic triterpenes of these mushrooms have considerable immunostimulating and antiviral activities.

Auricularia auricula-judae, widely known as Jew's ear, wooden ear or tree ear, a facultative parasite growing on trunks of many broadleaved trees or on dead wood, has a gelatinous, elastic rubber texture and is used as a food and medicine since ancient times. It is known to produce immune toxins and anticoagulants and lower cholesterol (Stamets 1993). Extracts of *Auricularia* prevent egg implantation in animals, terminating early and mid-pregnancy, and it is particularly useful for stopping pain and bleeding, generally haemorrhoids and excessive uterine bleeding.

Hericium erinaceus, an edible mushroom occurring widely in Japan, China and few parts of Southeast Asia, grows on standing and decayed broadleaf trees such as oak, walnut and beech and causes heart rot of standing tree. It is known as the hedgehog in the west, as monkey head fungus in Asian countries and as Shishigashida in China (head of lion). The polysaccharides from this mushroom have cytostatic effect on gastric, oesophageal, hepatic and skin cancers. Hot water extract of several *Hericium* spp. was used in the 11th Asia Sports Festival (1990) as a sports drink named Houtou and is believed to have contributed to the remarkable performance of Chinese players. Medicinally important constituents of this fungus are β-D-glucans, ergosterol (provitamin D) and cyathane derivatives (nerve growth stimulators) (Chen 1992).

Grifola frondosa, large fan-shaped mushrooms that often fuse together in masses at the base of trees, cause extensive decay. It is often called 'hen of the woods' or 'sheep's head,' and its very famous name is maitake, which means 'dancing nymph'. It has a delicious taste and an excellent aroma and has been used for improving spleen and stomach ailments, calming nerves and treating haemorrhoids in China (Onno et al. 1993). The key active constituent of this mushroom is acidic polysaccharide (glucuronoxylomannan).

Cordyceps sinensis and *C. sobolifera*, also known as caterpillar fungus or Tochukaso, parasites of larvae of Lepidoptera, have high value in Chinese medicine for many centuries (Birks 1991). Anticancer polysaccharides have been isolated from several of *Cordyceps*, and some of them have shown hypoglycaemic activity as well. Its extract is also used to treat fatigue and improve motor function. The major chemical, pharmacological and toxicological studies on *C. sinensis* showed that the main activities of the fungus are oxygen free radical scavenging (Findlay 1982). The key constituents are galactomannans, cordycepin and sterols.

Agaricus is also a medicinally important mushroom, and the polysaccharide–protein complex of *Agaricus blazei* is active against a variety of xenographs. The key active constituents of this mushroom are β-(1, 3)-D-glucan, β-(1, 4)-glucan, β-(1, 6)-D-glucan and proteoglycans.

1. *Haematological effects*: Lectins are glycoproteins with specific binding site for sugars. Lectin isolated from *A. campestris* is a tetramer and its haemagglutination reaction is not inhibited by sugar but is inhibited by a sonic suspension of red cell ghosts. The lectin purified from *Flammulina velutipes* is mitogenic to mouse spleen lymphocytes in addition to its haemagglutination activities (Liu et al. 1993). This lectin does not contain carbohydrate, half-cystine, methionine or histidine. Volvatoxin A, a lectin isolated from *V. volvacea*, is shown to reduce haemolytic activity towards group 'O' red blood cells (Hsue 1993). Another lectin isolated from *V. volvacea* showed moderate haemagglutination towards group 'O' red blood cells. Pleurotolysin is a lectin obtained from *Pleurotus ostreatus* and is used as a haemolytic agent for mammalian red blood cells (Opletal 1993).
2. *Antioxidant and anti-inflammatory activity*: The ability of mushroom-derived preparations (MDPs) to prevent oxidative damage to cellular DNA has been evaluated using the single-cell gel electrophoresis ('comet'). MDPs obtained from fruiting bodies of nine common mushrooms were able to protect oxidative DNA damage. Cold-water extraction of *Agaricus bisporus* (Ab-cold) and hot water extract of *G. lucidum*. Methanol and aqueous extracts of *G. lucidum* are reported to have marked free radical scavenging activity. Similarly some edible mushrooms are reported to contain biologically active compounds which can protect cellular DNA from oxidative damage. Such protective compounds have possible commercial value as dietary supplements for offsetting adverse biological effects associated with coronary heart disease, cancer and age-related neurodegenerative disease. They might also facilitate the development of treatment for the repair of indiscriminate cellular DNA damage that occurs during certain types of chemotherapy and radiotherapy.
3. *Hepatoprotective effects*: Fruiting bodies of some wild mushrooms have long been a major factor in folk medicine for treatment of chronic hepatitis. Ganoderic acids R and S were isolated from cultured mycelia of *G. lucidum* and shown to have strong antihepatotoxic activity in galactosamine-induced cytotoxic test with primary-cultured rat hepatocytes (Hirotomi et al. 1985). Another hepatoprotective compound, ganosporeric acid, was isolated from the ether-soluble fraction of the spores of *G. lucidum*. A polysaccharide fraction from *L. edodes* showed liver-protective action in animals together with improved liver function and an enhanced production of antibodies to hepatitis B. Positive results have been also obtained in treating chronic persistent hepatitis and viral hepatitis B patients.

There have been other medical reports relating to marked improvement in patients suffering from cirrhosis of the liver and chronic hepatitis B with extracts of *Dendropolyporus umbellatus*, *Schizophyllan commune*, *T. versicolor* and *P. cocos*.

A highly significant cause of death in most developed countries is coronary artery disease (CAD). The main risk factors are hypercholesterolaemia and dyslipoproteinaemia, disturbance in blood platelet binding, high blood pressure and diabetes. The initial step in the prevention and treatment of CAD and hypercholesterolaemia is the modification of the nutritional regime with a diet low in fat and saturated fatty acids and rich in crude fibres. Mushrooms like *Pleurotus*, *Lentinus*, *Auricularia*, *Grifola*, *Agaricus* and *Termitomyces* are important in the diet because of their high fibre content, sterols, proteins, microelements and low calorific value to prevent cardiovascular disease. When diet control is not successful, the next step is drug therapy. Attempts made in such direction to identify inhibitors of cholesterol biosynthetic pathways could not give clear evidence. Later the compound had been identified as HMG-CoA reductase which catalyses the reduction of HMG-CoA into mevalonate and is used for the treatment of hypocholesterolaemia. Several species of *Pleurotus*, viz. *P. ostreatus*, *P. saca* and *P. sapidus*, *G. frondosa* and *Auricularia auricula-judae* have been recommended by

many workers as a natural cholesterol-lowering substance within the human diet. Antilipemic effects of biomolecules from *Tremella fuciformis* and *T. aurantia* have been shown to lower plasma cholesterol levels, while antihypercholesterolaemic agents have been isolated from fruit bodies of *T. aurantia. Lentinus* spp. are reported to lower down the blood pressure and free cholesterol in plasma in young women and people older than 60 years of age.

4. *Antimicrobial activity*: Antimicrobial drugs have long been used for prophylactic and therapeutic purpose. Unfortunately the recent increase in the occurrences of drug-resistant bacterial strains is creating serious concern. Consequently, the antimicrobial activities of various antitumour polysaccharides from medicinal mushrooms are being evaluated for their clinical efficacy which is likely to mobilize the body's humoral immunity towards viral, bacterial, fungal and protozoal infections resistant to current antibiotics. Different substances of higher basidiomycetes are reported to be effective against different viral, bacterial and parasitic infections, including AIDS. Cochran (1978) was the first to report the antiviral substance in mushroom. Aqueous extract of *Lentinus edodes* is reported to be antivirally active against influenza A/SW15 virus infection. The antiviral influenza activity was mediated by the induction of interferon on the host. Phenol fraction of the mushroom extract was capable of conferring the antiviral activity. The RNA fraction of the mushroom extract induced interferon since double-stranded RNA (ds-RNA) has been documented as capable of inducing interferon. Interferon-inducing activity is due to the ds-RNA from the spore extract of *L. edodes*. The origin of this ds-RNA is reported to be derived from the mycophages attached to the spore and the fruiting body.

Many cancer and AIDS patients die to opportunistic infection and immunodysfunction; the spectrum of mycoses and mycetes observed in AIDS is summarized in Table 11.5. It is extremely important to protect AIDS patients from these infections. Lentinan from *L. edodes*, when used in combination with azidothymidine (AZT), suppressed the surface expression of human immunodeficiency virus (HIV) on T-cell than did AZT alone. Lentinan and sulphated lentinan exhibited a potent anti-HIV activity, resulting in inhibition of viral replication and cell fusion (Chihara et al. 1970). They further suggested that AIDS therapy must include a strategy to enhance the immune system. *Lentinus edodes* mycelial extract (LEM) is also useful in the treatment of AIDS (Table 9.4). It has been shown to inhibit HIV infection of cultured human T-cell and it potentates the effect of AZT against viral replication in vitro.

Table 9.4 Spectrum of mycoses and mycetes associated with AIDS

Mycoses	Causative organism/saprophytes	Main target issues
Dermatophytoses	Anthropophilic dermatophytes: *Trichophyton rubrum*, *Epidermophyton floccosum* and others	Skin and appendages
Cardidoses	*Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, *C. krusei* and other species	Oral cavity, skin, vagina and oesophagus
Torulopsidoses	*Torulopsis glabrata*, *T. candida*	Intestinal tract parasitic
Trichosporosis	*Trichosporon cutaneum*	Systemic mainly brain
Trichosporosis	*Trichosporon cutaneum*	Brain (lungs, skin)
Coccidioidomycosis	*Coccidioides immitis*	Lungs, brain
Aspergillosis	*Aspergillus fumigates*, *A. flavus*, *A. nidulans*, *A. glaucus*, *A. terreus*, etc.	Respiratory tract sinuses, intestinal tract brain, liver, kidney
Blastomycosis	*Blastomyces dermatitidis*	Lungs, skin, bone
Sporotrichosis	*Sporothrix brasiliensis*	Skin, lymph vessels, brain

Tanabe-Tochikura et al. (1990) reported that extract of *L. edodes* activated macrophages and stimulated the production of interleukin-1.

Water-soluble lignins EP3 and EPs-4 from *L. edodes* mycelium have antiviral and immunomodulating properties. Water-soluble extracts of mycelium known as JLA and JLS-1B have the ability to block the release of herpes simplex virus type 1 in animals. JLS-1B, consisting of 65–75 % lignin, 15–30 % polysaccharides and 10–20 % protein, has inhibited the herpes virus both in vitro and in vivo. (1) antiviral activity in mice against VSV (vesicular stomatitis virus), encephalitis and adenovirus type 12 was reported; (2) stimulated nonspecific resistance against respiratory viral infection in mice; (3) complete protection against LD 75 challenge close of virulent mouse influenza; (4) enhanced broncho-alveolar macrophage activity; (5) increased resistance against parasites *Schistosoma japonicum* and *S. mansoni*; (6) exhibited activity against *Mycobacterium tuberculosis* bacilli resistant to antituberculosis drugs, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Candida albicans*, *Saccharomyces cerevisiae* and *Escherichia coli*; and (7) the chloroform and ethylacetate extracts of the dried were active against *Streptococcus mutants* and *Prevotella intermedia* and (8) increased host resistance to infections with the potentially lethal *Listeria monocytogenes*. Antibacterial polyacetylene compounds, centinamycin A and B, have also been identified in shiitake mushroom. Eritadenine, a compound that affects cholesterol metabolism, also possesses antiviral properties.

Water extract of *L. edodes* has promoted the growth of colon-inhabiting beneficial lactic acid bacteria *Lactobacillus brevis* and *Bifidobacterium breve*. The effective factor in the extract is considered to be the disaccharide sugar trehalose. *L. edodes* extract improved the beneficial intestinal flora of the gut and reduced the effects of certain bacterial adverse enzymes such as β-glucosidase, β-glucuronidase and tryptophanase as well as colon cancer (Morigawa et al. 1986).

Protein fraction of shiitake mushroom fruiting bodies prevented infection of plants with tobacco mosaic virus (TMV). The binding of the virus to the plant cell was inhibited by FBP. *Armillaria mellea* showed antibiotic action against *Staphylococcus aureus*, *Bacillus cereus* and *B. subtilis*. Armillaric acid, isolated from *A. mellea*, inhibits Gram-positive bacteria and yeasts.

Sulphated schizophyllan polysaccharide of *S. commune* displayed strong anti-HIV activity, while the antitumour effect was reduced or lost. Protective effects against *Pseudomonas aeruginosa*, *S. aureus*, *Staphylococcus* sp., *E. coli* and *K. pneumonia* have also been reported.

An alcoholic extract of *Dendropolyporus umbellatus* inhibited *S. aureus* and *E. coli*. Species of *Trametes* contained cariolin, an antibiotic that has been shown to inhibit Gram-positive bacteria and *Trichomonas vaginalis*. Sesquiterpenes velleral and isovelleral isolated from *Lactarius vellereus* have strong antibacterial (against *E. coli*) and antifungal (*Candida utilis*) activities. *Ganoderma* spp. (*G. lucidum*, *G. applanatum*, *G. oregonense*) are strong antibacterials against *Staphylococcus aureus*. Mushrooms which have medicinal properties are précised in Table 9.5.

The combination of different mushrooms (*Amanita agglutinate*, *Boletus edulis*, *Lactarius insulsus*, *L. picinus*, *L. piperatus*, *L. vellereus*, *Lenzites betulina*, *Panus conchatus*, *Pulveroboletus raventis*, *Russula alutacea*, *R. devisifdia*, *R. foetens*, *R. integra*, *R. nigricans and Thelephora vialis*) is used in the treatment of numb hands and feet and problems with veins, tendons and limb tetany.

9.5 Mushroom Cultivation

Mushroom cultivation is carried out in following steps. The selection of quality mushroom is an important event. It should show good quality in respect of growth conditions, taste, aroma, fruiting characters and resistance to different diseases and pests and has good keeping quality. The mushroom cultivation is carried out partly in the laboratory under aseptic conditions and other in mushroom house (Chang and Miles 1989).

1. *Spawn production*: Good spawn production from monoculture is one of the methods of

Table 9.5 Mushrooms useful in the treatment of different health problems of man

Name of mushroom	Product	Medical application
Amanita muscaria	Muscarine	Diabetes and night sweats
Auricularia auriculata	Soothing drink	Sore throat, antitumour inflamed eyes
A. polytricha	Black tree ear	Anticoagulant, against Ehrlich carcinoma and sarcoma –180 Demulcent
Battarrea phalloides	–	Poullice for swelling and sores
Beauveria bassiana	Tablets made from fungus	
Bovista pila	Puff ballis	Diabetes, epilepsy, enuresis paralysis
B. plumbea	Puff batis	Stops bleeding
Calvatia craniiformis	Clavicin	Hemostat
C. cyathiformis	Clavicin	Hemostat, nose bleeding
C. gigantea	Clavicin	Antitumour, hemostat, active against influenza, anesthesia
C. utriformis	Clavicin	
Coprinus atramentarius	Coprine	Disulphirams (inhibits acetaldehyde dehydrogenase)
C. comatus	–	Antibiotic and anti neoplastic
Cordyceps sinensis	Insect colours	Invigorating tonic, chronic coughing anaemia and asthma
Coriolus versicolor	Polysaccharide	Stimulates gamma interferon, interleukin-2 and T-cell proliferation
Elaphomyces granulatus	–	Increase breast milk
Flammulina velutipes	Flammulin (polysaccharide)	Anticancer
Fomes fomentarius	–	Cauterize wounds, bruises, broken limbs, liver problem
F. officinalis	Agaricum	Asthma, dysentery, kidney diseases, sores and stomach disorders, purgative
Fungus sambuci	–	Conjunctivitis
Ganoderma lucidum	Agaricum acrid resin	Antineoplastic, immunomodulatory, lowers cholesterol, anticoagulation virility
G. tsugae	Water-soluble polysaccharides	Antineoplastic
Grifola frondosa	Grifolan	Anti-HIV, antitumour
Hericium erinaceus	Powder of full body	Ulcers, cancer, inflammation tumours of alimentary system
Hirneola auricula-judae	Holds water like sponge	Throat infections, astringent
Hypholoma sublateritium	–	Antitumour
Hypsizygus tessulatus	–	Antitumour
Lentinula edodes, *H. ulmarius*	Lentinan	Anticancer, antiviral, hypolipidaemia
Lycoperdon perlatum		Band acid, bleeding
Mutinus caninus		Stimulates sex power
Morganella subincarnata	–	Cures for headache
Pleurotus citrinopileatus		Neoplastic activity, pulmonary oedema, antibacterial
Pleurotus ostreatus	Serine protease	Antineoplastic, hypocholesterol immunomodulatory effect
P. florida		
P. sajor-caju	Protein containing	Antineoplastic, blood pressure
P. tuber-regium	Polysaccharide sclerotia	Fever, high BP, stomach pain, constipation, smallpox
Polyporus officinalis		Bleeding, central health chaplains with sweats, antiviral

production. It should be fresh, fast growing and free from insects, moulds and mites.

2. *Preparation of substrate/beds*: Preparation of bed is another event. Substrate preparation varies with the mushroom. Either short-term period or long-term period of substrate preparation can be adapted. Finally it should be finely decomposed and should support the growth of mushroom well.
3. *Spawning and spawn running*: Healthy spawn should be spread on the bed in any one of the methods of spawning and allowed to grow. Sufficient moisture is to be maintained.
4. *Cropping*: Once budding appears, one should be ready to harvest. The bud grows to full fledged mushroom just boomed mushroom should be harvested. Care should be taken not to break mushroom mycelium.
5. *Canning*: Mushrooms as for as possible are to be consumed freshly. If needed, they may be stored for a few days after canning as detailed in latter part. The details of each stage are depicted in Figs. 9.7 and 9.8.

9.5.1 Mushroom Cultivation in India

There are three major types of mushrooms cultivated in India. They are (1) button mushroom (European button mushroom), (2) oyster mushroom and (3) paddy straw mushroom. European mushroom is more specific in its temperature requirement and requires costly equipment and more investment. The two other types of mushrooms can be very easily cultivated in India. The mushroom cultivation proved to be profitable both at industrial scale as well as part time for unemployed youth and household wives (Suman and Sharma 2005). The economics of mushrooms cultivation is given in Table 9.6.

9.5.2 Oyster Mushroom (*Pleurotus sajor-caju*)

Oyster mushroom is a lignocellulolytic fungus and grows in nature in the temperate and tropical regions, mainly on dead and decaying wooden logs

Location	Tissue culture	Substrate		
Climate	Spore culture	Vigorous growth		
Raw materials	(a) Without mating for homothallic species	Free contamination	Establishment of mycelium	Concept of composing
Acceptability	(b) Mating with compatible isolates for heterothallic species	Avoid use of senescent and degenerate spawn	Environmental requirements	Microbial activity
	Mixed culture	Good survival of storage	(a) Temperature	Softening of substrate for case of colonization
	Preservation		(b) Light	Physical characteristics
			(c) Aeration (O_2, CO_2)	Chemical components
			(d) pH	Aeration
			(e) Moisture	Water content
			Casing	
			Watering and care	

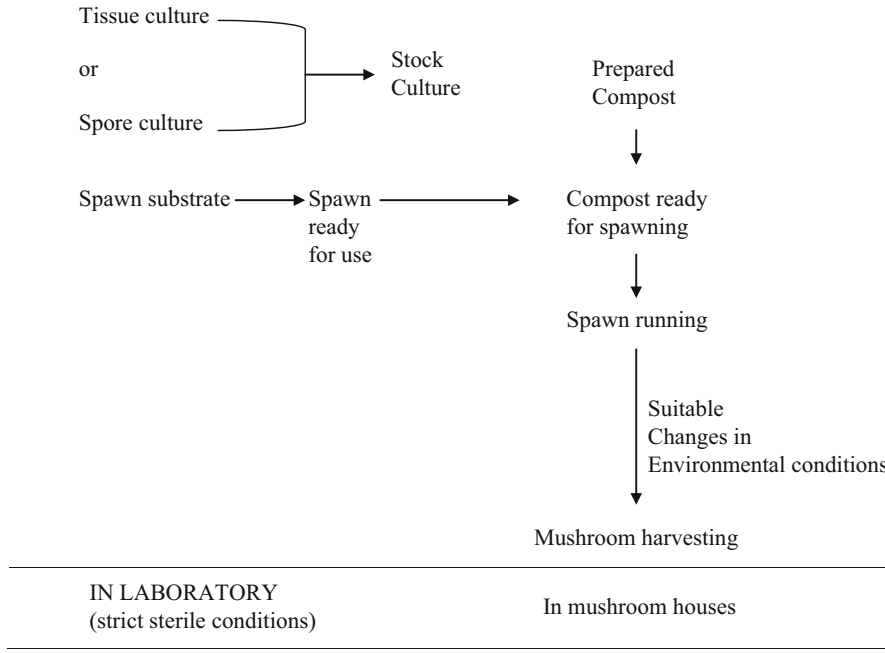


Fig. 9.7 Different phases of Mushroom cultivation

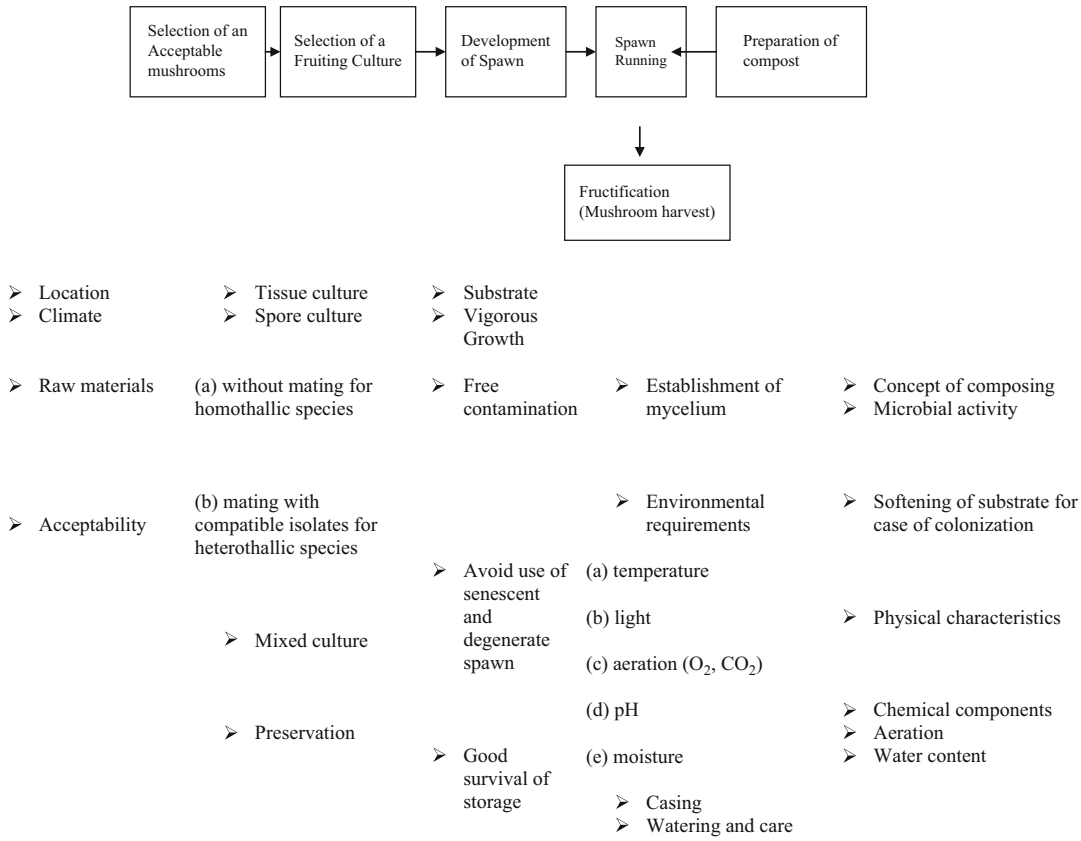


Fig. 9.8 Major steps in Mushroom cultivation

Table 9.6 Economics of mushroom cultivation

A. Non-recurring expenditure		B. Recurring expenditure	
1. Drums with heating coil	4,000	1. Paddy straw 16 tonnes	38,000
2. Chaff cutters	6,000	2. Pesticides and fungicides	2,400
3. Wooden table	2,000	3. Spawn bottles	10,000
4. Wire mesh frame	1,000	4. Polythene bags	5,000
5. Bamboo racks	4,000	5. Labourer (Wages)	38,000
6. Sprayers and buckets	4,000	6. Electricity and water	7,000
7. Drums for soaking straw	4,000	7. Miscellaneous	2,000
		8. Rent for building	8,000
	25,000		55,000
Interest 15 % on 12,500	3,750		
Depreciation 20 %	5,000		
	8,750	Say:	120,000
Anticipated yield at 30 % of 16 tonnes		4,800 kg	
Sale	Rs. 30/kg	144,000	
Total expenditure 178,000 + 8,750 = 178,000		120,000	
Net income: Rs. 144,000 – 190,000 =		24,000	

or sometimes on decaying organic matter. Oyster mushroom cultivation was first started in Germany by Flack in 1917 by inoculating tree stumps and wood logs. Later Block et al. (1958) used a mixture of oatmeal and sawdust for its cultivation. In India, Bano and Srivastava (1962) for the first time used paddy straw for growing *P. flabellatus*. The first domesticated species under *Pleurotus* was *P. ostreatus*. But later, *P. sajor-caju* has gained much importance as a commercial species in major parts of the country. Rangaswamy et al. (1975) reported successful cultivation of *P. sajor-caju* in Tamil Nadu using different waste materials like paddy straw, sawdust and wood shavings. Commercial cultivation of *P. ostreatus* on sawdust and wood shaving was standardized by Dhar (1996) in Kashmir. Various other substrates were used by Jandaik and Kapoor (1974) for growing *P. sajor-caju*.

The cultivation of oyster mushroom in India needs to be widely popularized because it can be cultivated round the year in the tropical and subtropical regions. Moreover, with the increase in agricultural production, more and more agroresidues are available which can be properly recycled, and there is a growing demand for its consumption all round the world.

Pleurotus sp. is one of the easiest mushrooms to grow and does not require complicated substrate preparation technique. The substrate preparation is less time consuming and labour intensive.

It grows well on different types of lignocellulosic materials, converting the materials into digestible and protein-rich substances suitable for animal feeds. *Pleurotus* spp. may be produced maximally on cotton waste, corncobs, sugarcane bagasse and other agroindustrial by-products. The substrate used in each region depends upon the availability of agricultural waste.

The oyster mushroom fruit bodies can be easily sun-dried and stored for future use. The shelf life of fresh mushrooms may be extended by refrigeration at 1–4 °C on perforated polythene bags for 7–10 days. It also yields more and one can harvest a minimum of 500 kg fresh oyster mushroom for one tonne of dry wheat or paddy straw in a short time. It tolerates higher concentration of carbon dioxide which acts as protection cover against competitor moulds.

Steam Pasteurization

The growers having bulk pasteurization or peak-heating room can employ this technique. The prewetted straw is either placed in wooden trays or piled into a heap in the pasteurization room. It is subjected to steam treatment at 75–80 °C for 2 h, cooled down to room temperature within 24 h and spawned.

Table 9.7 Dominant thermophilic and thermotolerant microorganisms involved in composting process

Species dominant at		
	Start of establishment stage	End of maturation stage
Bacteria	*Bacillus subtilis*	*B. stearothermophilus*
	Flavobacterium sp.	*Pseudomonas* sp.
Actinomycetes	*Streptomyces thermovulgaris*	*S. thermorulgaris*
		S. rectus
		Streptomyces sp.
Fungi	*Mucor pusillus*	*Humicola griseus*
	Aspergillus fumigatus	*Torula thermophila*
	Humicola lanuginosa	

Pre-wetted straw is made into a pile of 3–4′ height and 5′ width and kept as such for 2–3 days. Due to microbial fermentation, the temperature of the heap rises in the centre. After cooling, it is spawned. In some of the Southeast Asian countries, modified fermentation technique is also in use. In this, ammonium sulphate or urea is added to wheat or paddy straw at 0.5–1 % and lime 1 %; 10 % chicken or horse manure can be used in place of nitrogen fertilizers. After wetting all those ingredients, it is made into a triangular heap of 75–90 cm. It is kept as such for 2 days after which turning is done and 1 % superphosphate and 0.5 % lime are added. After cooling, this mixture can be used as such for spawning (Vijay 1990). There are several edible varieties of *Pleurotus*, namely, *P. ostreatus*, *P. florida*, *P. sajor-caju*, etc., which are well known for their delicacy and flavour. These species are being grown on commercial scale in various countries. However, in India *P. sajor-caju* and *P. florid*a are most popular for commercial cultivation.

Substrate Preparation

Oyster mushroom can be grown on various substrates, viz., wheat/paddy straw, maize stalks, maize cobs, cotton waste, wooden logs, sawdust and vegetable plant residues. Since paddy straw is easily available throughout the year in most parts of the country, it is widely used. One should always prefer to use fresh and well-dried paddy straw from which compost is prepared. Both bacteria and fungi of mesophilic and thermophilic nature are involved in compost preparation (Table 9.7). Major steps in mushroom cultivation are given in Fig. 9.7.

Soaking

Paddy straw chopped into 3–5 cm pieces is soaked in fresh water for 8–24 h. Old, broken and rotten straw and stagnant water should never be used. Wet substrate is spread on wire mesh to drain off excess moisture.

Pasteurization

Water is boiled in a wide-mouth container such as a tub or drum. The wet substrate is filled in gunny bags or basket and closed. The filled bag/basket is dipped in hot water (80–85 °C) for about 30–60 min, and to avoid floating, it is pressed with the help of a wooden piece. After pasteurization, excess of hot water should be drained off in a container so that it can be used for other sets also. Care should be taken to maintain boiling water temperature 80–85 °C, for all sets to achieve pasteurization. Pasteurized substrate is kept inside the chamber where bag filling and spawning are to be done. Once it cools down to room temperature, it is filled in bags. The moisture content should be 70 %.

Spawning

Polythene bags (35 × 50 cm, 150 gauge) or polypropylene bags (35 × 50 cm, 100 gauge) may be used for its cultivation. Two percent spawn on the basis of wheat straw, i.e. one 500 ml bottle spawn (200–250 g) for 10^{-12} kg wheat straw, is used. Spawning can be done by one of the methods.

(a) *Surface spawning*: Bags are opened, 2 % spawn is broadcasted on top and a little ruffling is done to mix it in top layer (2–5 cm thick), and they are quickly closed.
(b) *Layer spawning*: Substrate is filled and gently pressed at a depth of 8–10 cm and spawn is broadcasted above it. Similarly second and third layers are put and simultaneously spawned and bags are closed. This is more suitable when pasteurized straw is filled in bags.
(c) *Through spawning*: Pasteurized straw is mixed with 2 % spawn and filled in bags. It is gently pressed and closed for spawn running. This will not be convenient for sterilized polypropylene bags.
(d) *Spot spawning*: In this method bags are filled to capacity and compost surface levelled. Holes 2.5–5 cm deep are made using a finger at about 8–12 cm apart in rows. A spoon full of spawn (5 g) is introduced in the cavities which are later covered with compost.

Spawned bags should be stacked in racks in a neat and clean place and in a closed position. The temperature of 25 ± 5 °C and relative humidity of 70–85 % should be maintained by spraying water twice a day on walls and floor. At this stage, fresh air requirement is minimal. It takes 20–25 days when bags will be fully covered with white mycelium. Earthen pots, wooden treys, bamboo baskets and gunny bags are also used for growing this mushroom. Different materials like chicken manure, oatmeal and arhar dhal powder are added to compost to increase the yield.

Cropping, Picking and Packing

Once bags are fully covered with mycelium, they are transferred to cropping room and polythene/polypropylene covers are removed. The open blocks are kept in racks about 20 cm apart. Rack should be 60 cm wide and there should be a gap of 50–60 cm between two shelves. There should be a 60–75 cm gap between two rows of racks for working. It grows in a temperature range of 20–33 °C (optimum 25 ± 2 °C). Relative humidity of 80–85 % is maintained by spraying water twice a day, but watering on blocks should be avoided for the first 2–3 days. After 2–3 days, light mist spray of water is given on blocks. This is the time when small pinheads appear. Once pinheads are of 2–3 cm size, a little heavier watering is done on blocks and further watering is stopped to allow them to grow. This helps to avoid bacterial rotting. However, the optimum relative humidity must be maintained inside the cropping room. Once mushrooms are 6–8 cm in size, they are plucked. Mushrooms should not be allowed to produce spores as it results in poor quality of mushrooms. Care must be taken after harvesting the first crop and to initiate second flush. Similarly, the third and fourth flushes will appear in 8–10 days interval. However, almost 80 % of the crops will be over in the first and second flushes. Hence, many growers take only two flushes. Care must be taken that the beds do not become too wet which may otherwise cause rotting.

Mushrooms should be harvested before watering and their lower portion is cleaned with dry cloth. Mushrooms are packed in perforated (five to six small holes) polythene or polypropylene bags. It should be sent to the market while fresh. It can also be sun-dried by keeping fresh mushrooms in the sun for 2 days. Even polythene sheet may be spread to about 30–40 cm above the mushrooms for quicker dehydration in the sun. It can be mechanically dried at 40–45 °C. The dried product can be packed in polythene bags for marketing. Dried mushrooms should be soaked in water for 10 min before use. Details of oyster mushroom cultivation are presented in a flowchart (Fig. 9.9).

9.5.3 White Button Mushroom or European Mushroom (*Agaricus bisporus*)

Agaricus bisporus (large) is popularly known as white button mushroom or European mushroom and extensively cultivated throughout the world and contributes about more than 40 % of the world production of mushrooms. For the first time in 1650, the melon growers of Paris observed spontaneous appearance of *A. bisporus* on used compost of melon crops. The indoor cultivation of *A. bisporus* in caves was started in France and Holland around 1810. Cultivation of this mushroom in India is of recent origin, and it started in

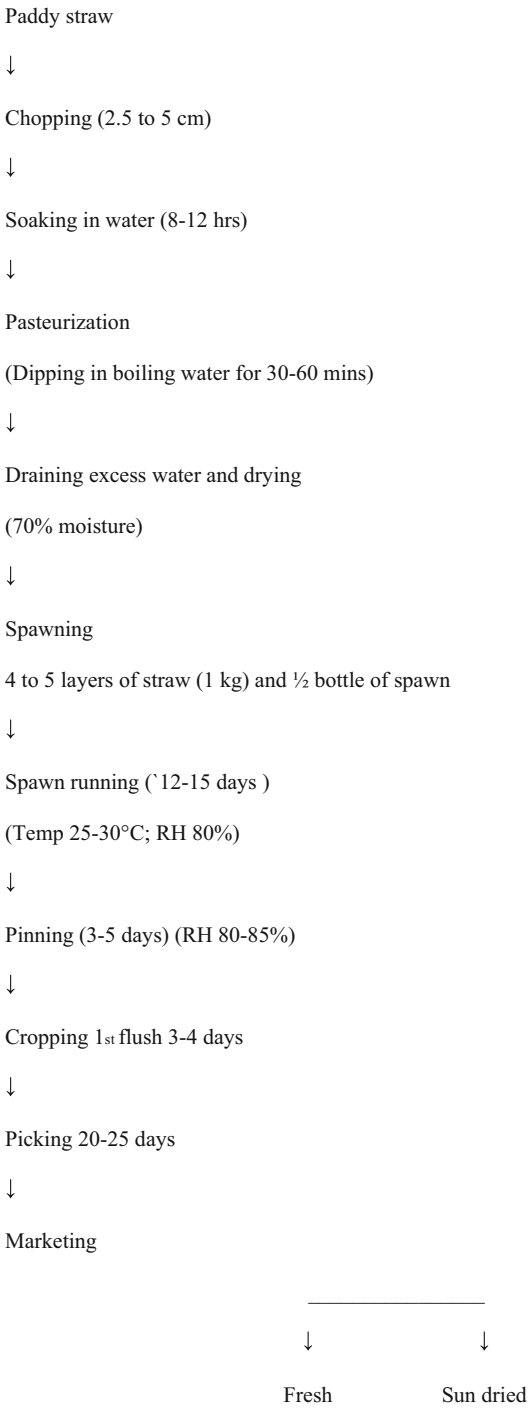


Fig. 9.9 Flowchart of cultivation of oyster mushroom

1961 at Solan by the Department of Agriculture, Himachal Pradesh, in collaboration with the Indian Council of Agriculture Research, New Delhi. Since then, a number of organizations including the National Centre for Mushroom Research and Training, Chambaghat, Solan (HP), started to work on this mushroom. For growth, *A. bisporus* requires two different temperatures: 22–28 °C for spawn run or vegetative growth and 12–18 °C for fruit body formation. Besides specific temperature and proper humidity (85–95 % RH), enough ventilation during fructification is a limitation for its cultivation. Mushroom production includes the following steps.

Spawn Production

The spawn is generally produced in milk or saline bottles. The entire process of spawn preparation should be aseptic from the beginning to the end. The rice straw cuttings, cotton wastes, cotton seed hulls, rice hulls, sorghum grains, rye grains, etc., are generally used either singly or in combination for spawn production. The method of spawn production from jowar grains is explained here.

Jowar grain is boiled in an equal volume of clean soft water till the entire water is absorbed by the grain. Later calcium carbonate at the rate of 20 g/kg of grain is added to the cooked material and the mixture is filled in clean, dry, empty saline bottles up to three-fourths full. The mouth of the bottle is plugged with non-absorbent cotton. The cotton plug and a part of the bottle neck are covered with a clean 10 cm square paper bit and tied with twin or rubber band. The bottles are sterilized in a pressure cooker or autoclaved at 15 lb pressure for 1.5 h. After cooling, a small quantity of the mushroom culture should be inoculated to the bottle in an inoculation chamber. On incubation at 25 °C for about 2 weeks, the fungus covers the grain in the bottle. This is called spawn. It requires slightly higher temperature for cropping. White button mushroom cultivation includes the following steps (Fig. 9.10).

Compost Preparation

In olden days composts made of horse dung and cow dung were used. But nowadays synthetic compost made out of straw of grain crops is widely used. In South India locally available substrates like paddy straw, ragi straw, maize straw,

horse dung and chicken manure are used. Paddy straw or mixture of paddy and maize straw forms a good compost. Two compost formulae and the method of compost preparation are given below (long method).

Formula I		Formula II	
Paddy straw	1,000 kg	Paddy straw	500 kg
Ammonium sulphate	30 kg	Maize stalk	500 kg
Superphosphate	10 kg	Ammonium sulphate	30 kg
Urea	13 kg	Superphosphate	10 kg
Rice bran	150 kg	Urea	13 kg
Cotton seed	20 kg	Rice bran	150 kg
Chalk powder	33 kg	Molasses	40 kg
Gypsum	40 kg	Cotton seed	20 kg
		Chalk powder	33 kg

Paddy straw watered for 2 days to have moisture content of 75–77 % is mixed with fertilizers and stacked in a heap of 1.65−1.8 × 1.65−1.8 m or required length and turned on the 6th, 10th, 13th, 16th, 19th, 22nd, 25th and 26th day. On the 10th and 13th days, chalk powder and gypsum are added, respectively. On the 26th day the substrate compost will be ready. It is filled into trays, boxes or shelves to make the so-called beds and transferred into the room of pasteurization. Live steam generated from a water boiler is introduced to compost for 2 h so as to have air temperature of 60–62 °C. Maintain this temperature for 2 h and then introduce a gentle steam of fresh air to lower the temperature for the next 6–8 h. The objective of pasteurization of compost is to keep away the insects and pests in the substrate and spores of contaminating microorganisms and bring the temperature of compost uniformly to 50–55 °C which promotes decomposition of the substrates by thermophilic microorganisms. Through this final adjustment, a more selective medium favouring the growth of the mushroom is accomplished. Carelessness at this stage may lead to crop failure. There are two methods for preparing mushroom compost: the long and short methods.

Long Method of Composting (LMC)

The long method takes about 3–4 weeks and does not utilize the pasteurization facilities, whereas composing by short method takes 14–18 days and utilizes pasteurization facility. This is completely an outdoor process. The compost should be prepared on a cleaned, cemented floor at a higher level to prevent the run-off water from collecting near the heap. It may be done under a shade whose sides are open. The floor of the low-cost composting platform should be simply cemented/brick layered with a low-cost roofing of high-density polythene fixed on iron tubular structure. Under no circumstances should the compost stack be left uncovered with polythene sheet to prevent entry of rainwater. Otherwise it will set in anaerobic fermentation in the compost pile and disrupt the normal composting process. If composting is done inside the room, the room should be well ventilated. For a medium-sized farm producing 20 tonnes of compost in one lot, a platform of 100′×40′×15′ is sufficient. It needs to have a gentle slope working towards a guddy or pit and is provided with a dewatering pump and nose. Besides composting yard, there should be a provision to store raw materials.

As hygiene is very important in every step of mushroom cultivation, the first step in the compost preparation is to clean the composting yard properly and wash it with percent formalin 24 h in advance of operation. The wheat straw is spread not more than 9–12 in. deep, drenched for 24 h with a liberal spray of water and frequently turned by forks till it absorbs sufficient moisture. At this stage the water content is 75 %. One tonne of dry straw will require about 5,000 l of water to bring it into saturation. Care should be

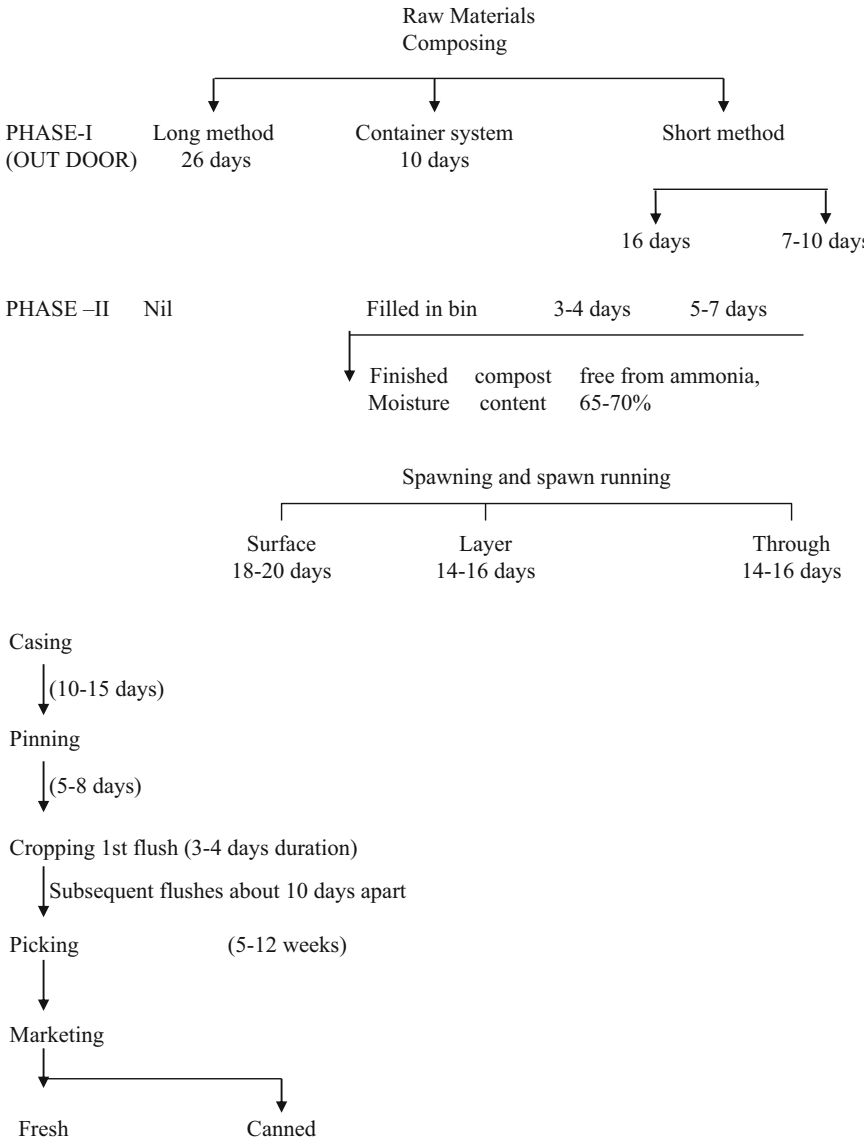


Fig. 9.10 Flowchart of white button mushroom cultivation

taken that each and every portion of the straw absorbs required quantity of water. However, during summer and when the composting is done in the open, additional watering of the pile may be required since top and side layers may dry due to the sun and wind. Wetted straw may be collected and made into a smaller heap and kept as such for 24 h. Except gypsum and pesticides, all other ingredients are mixed together and moistened by spraying water on them, and a small heap is made. Later on it is covered with moist gunny bags. This day is called minus 1, while the following day when the stack is prepared is called zero day.

Pre-wetted straw and fertilizer heaps are thoroughly mixed with the forks. The mixture is filled in a rectangular block (mould) made of wood or iron. It has three boards: one end board of size 5.5′×5′ and two side boards each 6′ long and 5′ high and held together 5′ apart with

clamps. The end board is placed on the smaller size of the rectangular side boards. After stack preparation, the boards are removed. The side boards are detached from the end board and are moved lengthwise, and again the mixed ingredients are put in the mould. In this way a long pile of the compost can be made. The straw should be firmly but not compactly compressed into the mould. The dimensions of the heap can be adjusted according to the size of straw and air temperature. The principle is that the longer the straw, the bigger the heap. If composting is done in cooler months or hills where the temperature ranges between 7 and 20 °C, the size of the stack should be 5′×5′×5′. Otherwise a smaller heap would be unable to retain heat and moisture and the composting would be unsatisfactory and unproductive. During hot weather in the plains, particularly in tropical and subtropical regions, the temperature differences between the inside of the compost and the surrounding air is too small to produce chimney effect necessary for the compost ventilation. Improper ventilation may result in anaerobic conditions in the centre of the pile.

Short Method of Composting

This is the widely used method all over the world in which composting period is reduced from 28 days to 16–18 days and is superior to long-method compost. Short method of composting is carried out in two phases.

Phase I (Outdoor composting): In India, formulations based on wheat straw and chicken manure are widely used. Like long method of composting, this method starts with wetting of the mixture of wheat straw and chicken manure till they absorb sufficient moisture. Leached water is regularly resprayed over the ingredients. From such material, low stack is prepared, which is trampled hard to encourage anaerobic fermentation. After 2 days the stack is broken open and sufficient water is added especially to dry patches, and again an anaerobic stack is prepared.

- Day 0 (aerobic stack): The stack is opened, full quantity of urea is added and a higher stack is prepared (5′×5× length) as in long method of compost. Water is added to dry patches, if any.
- Day 2: First turning.
- Day 4: Second turning.
- Day 6: Third turning. Required quantity of gypsum is added.
- Day 8: The compost is ready for pasteurization.

In phase I the ingredients are allowed to decompose outside under uncontrolled conditions. At this stage the manure is partially decomposed.

Phase II: *Indoor composting*: In peak-heating rooms or bulk pasteurization tunnels.

In India, compost formulations and short method of composting were standardized by Shandilya (2000). Tunney (1980) first introduced the bulk pasteurization system in India. Indoor composting has two main purposes:

1. *Conditioning*: In which ammonia is converted into microbial protein
2. *Pasteurization*: Killing of microorganisms which are competitors to make the substrate suitable only for *Agaricus bisporus*

Steam pasteurization is done in a well-insulated room designed for the purpose. This can either be done in trays (peak heating) or in a tunnel (bulk pasteurization). In both the cases, construction of an insulated room is involved where walls, roof, ducts which carry steam and doors are insulated by insulating material. A boiler is required to produce the steam, while a blower is needed to blow air. Ventilation and recirculation of air and ammonia is required for proper aerobic conditions and maintaining the temperature for proper development of thermophilic microflora (conditioning). Ammonia is recirculated for its conversion into microbial proteins.

Spawning

The spawn is spread over the surface and covered with a thin layer of compost.

Spawn Running

The growth of active mycelium in the beds is called spawn running. Spawn running requires 15–20 days at 90–96 % RH and 25 ± 2 °C temperature.

Casing

After spawn running, the beds are covered by casing material (thin layer of pasteurized) and incubation is continued for another 8–10 days, i.e. till pinheads start appearing. At this stage the temperature is lowered to 16 ± 2 °C and fresh air is introduced. Watering of the beds is to be carried out as and when required.

Cropping

The crop starts producing mushrooms on the third week after casing which continues for 10–12 weeks. Mushrooms are picked by griping the cap and twisting when they are in button stage. Mushrooms or fruit bodies appear in rhythmic cycles which are called flushes or breaks. Generally, a large and more number of mushrooms are produced in the first four flushes. After the fourth flush, a smaller and less number of mushrooms are produced. Hence, beds are usually removed after the fourth flush. The spent out bed material can be used as a manure.

9.5.4 Paddy Straw Mushroom (*Volvariella volvacea*)

The paddy straw mushroom relatively grows fast on paddy straw, hence the name. Though its cultivation started in China during the eighteenth century, its cultivation in India began only in 1960 in Coimbatore, Tamil Nadu. Three species of this genus, *V. esculenta*, *V. volvacea* and *V. diplasia*, are being cultivated in India.

It is used as a highly priced delicacy in China and Southeast Asia. Around 1932–1935, the straw mushroom was introduced into the Philippines, Malaysia and other Southeast Asian countries by overseas Chinese (Chang 1982). Since then its cultivation has been carried out in various countries outside this region where suitable tropical conditions favourable for its growth persist. In India Su and Seth (1940) have first cultivated *Volvariella diplasia*. The scientific cultivation of paddy straw mushroom was first attempted by Thomas et al. (1943) in Coimbatore, and since then its cultivation has been taken over at other places also. There is a steady increase in the world production every year with China as the top producer followed by Thailand, Indonesia and Vietnam (Table 9.8).

Paddy straw mushroom cultivation is précised below:

1. Chop the paddy straw and soak in water for 24 h.
2. Cut waste paper into small pieces and soak for them for some period.
3. Decant water after 24 h.
4. Mix thoroughly the chopped straw, paper pulp and spawn.
5. Fill the mixture in polythene bags.
6. Puncture the bags with needle to facilitate the exchange of air and drain excess water.
7. Tie the mouth of polyethylene bag and keep them on wooden rock at 35–40 °C.
8. Maintain the humidity of the contents to about 80–90 °C by sprinkling water regularly.
9. At the end of 10–15 days, small pinheads of mushrooms appear which grow into mature mushroom within 2–3 days.
10. Harvest mushroom while they are tender before they sporulate.

This mushroom grows at a relatively high temperature (30–35 °C). It does not grow at temperatures below 15 °C and above 45 °C and there is no mushroom production below 20 °C. More people in Southeast Asian countries prefer straw mushroom over white button mushroom because

Table 9.8 Estimated world production of paddy straw mushroom in 1991

Country	Production (MT)	Percentage
Mainland China	150,000	58.6
Thailand	63,000	24.6
Indonesia	35,000	13.7
Vietnam	3,500	1.4
Taiwan	3,000	1.2
Philippines	8,000	0.3
India	400	0.2
Others	400	0.2
Total	256,000	100.0

of its easy cultivation with a cropping period of 3 weeks.

Advantages of growing this mushroom are:

1. This mushroom can be cultivated in places where temperature is relatively high (30–40 °C).
2. Its cultivation technology is simple and of low cost.
3. Paddy straw mushroom is popularly grown on paddy straw, but other substrates like cotton waste, dried banana leaves, sugarcane, oil palm pericarp waste, jute waste and other cellulosic waste can be also used for its cultivation.
4. This is a fast-growing mushroom and takes about 10 days from spawning to harvesting.
5. *Volvariella* sp. is quite suitable for cultivation in rural areas of India.

However, some disadvantages of cultivation of this mushroom are:

1. These mushrooms are highly perishable and cannot be refrigerated for more than 1–2 days.
2. Though 13 species of *Volvariella* have been recorded from India, only 3 species (*V. volvacea*; *V. diplasia* and *V. esculenta*) are being cultivated. The pileus of *V. diplasia* has light-grey tinge with smaller spores and whitish fruiting body than *V. volvacea*. The pileus of *V. volvacea* has pinkish tinge. *V. esculenta* and *V. bakeri* are considered to be synonyms of *V. volvacea*.

The most common substratum on which the straw mushroom is grown is the paddy straw and cotton waste, but it can also be grown on diverse substrates, which include guinea grass, sugarcane bagasse, dried banana leaves, oil palm bunch waste, oil palm pericarp waste, water hyacinth, wood waste, pineapple waste, straw of paddy, wheat, barley and oat and sawdust.

Mushroom can produce a range of extracellular hydrolytic and oxidative enzymes including lignocellulolytic enzymes. The primary function of the former is to break down the macromolecular polymer into more readily assimilated low-molecular-weight carbohydrates. The cellulolytic and hemicellulolytic enzymes produced by mushrooms can degrade respective polysaccharides into glucose and xylose, which support the growth of mushrooms. Cellulose is the most suitable carbon source.

Several techniques have been used in the cultivation of *Volvariella volvacea* which thrives in the temperature range of 30–35 °C and RH of 75–85 %. In recent years straw mushroom cultivation has been commercialized on cotton waste compost.

There are several techniques for cultivation of straw mushroom, and the choice of cultivation technologies is mainly dependent on the availability of substrates and the amount of resources available. While a more sophisticated indoor technology is recommended for the industrial-scale production of mushroom, most of other technologies are of low cost and appropriate for rural development, especially when production is established at the community level.

In India this mushroom is entirely cultivated on paddy straw during the summer months (April–August) to raise at least 4 crops in the plains of North India and 10–12 crops throughout the year in South India with little warming in winter months.

Indoor Cultivation

Paddy straw beds are prepared with straw bundles (1 kg each). Paddy straw for bundle preparation should be clean, fresh, dried and uncrumpled. Bundles are prepared using whole straw, tied at two places to make them from loose ends and trimmed. The bundles are soaked in clean fresh water for 16–24 h and removed the following day on a slope for draining the extra water. The straw can also be soaked in hot water at 80 °C for 2 h to prevent various diseases and pests during spawn run. These bundles are placed on bamboo or wooden frame or on slightly raised platform in regular heaps in the growing room. Each bed consists of 22 bundles arranged in four layers of five bundles in a criss-cross fashion with two loose bundles at the top with 5–7-day-old grain spawn broadcast (1.5 % of dry straw) between the layers. The spawn is placed 3–4 in. from the periphery into a strip of 4–6 in. The top layer is spawned all over, and the loose straw trimmed from bundles may be used to cover it.

If the temperature goes more than 38 °C, the side cover should be lifted and the bed should be watered with sprayer to reduce temperature. Beds are watered regularly till the end of the crop season by sprinkling water once or twice a day. Watering depends upon humidity of the air. Mostly no watering is required for the first 3 or 4 days. Small beds were found to be more economical than the large-sized beds. Similarly small bundles give more yield per kilogram paddy straw than large bundles.

Recently it was found that hollow interior (triangular, squarish, rectangular type) beds have always given better yield than the traditional bed. These beds have homogenous moisture level, ambient temperature and fresh air between layers of straw. These conditions favour better proliferation of mycelia and hence improve fruiting.

Outdoor Cultivation

Outdoor cultivation still appears to be most popular among the small farmers of Asia due to its simplicity and low cost. Farmers engaged in straw mushroom cultivation used bundled paddy straw and spawn brought from spawn producers. The bundles of paddy straw are arranged on a raised bamboo platform under a shady place to protect them from direct sunlight. In order to protect them from winds and heavy rains, the beds are covered with polythene sheet, straw mulches or straw mats, but these are removed immediately after the rain. Instead of rice straw, bundled dried banana leaves or water hyacinth may also be used. Mushroom beds should be made in east-west direction to provide more uniform sunlight and temperature. Watering is done to each bed frequently according to air humidity and continued till the end of the crop period. After 10–12 days of spawning, the pinhead comes out and the mushroom is ready for picking after 3–5 days.

Box-Type Portable Beds

For easier management of pest and diseases in the production of *Volvariella* mushroom, open portable wooden boxes are very useful. Good spawn run usually takes 5–7 days. The pinheads begin to form along the open edges of the boxes in about 10 days.

The Modern Cotton Waste Method

This method is being extensively used in Hong Kong for commercial production since 1973 on cotton waste. This technology is nowadays also used in Thailand, Indonesia, Singapore, Vietnam, Malaysia and the Philippines for the commercial-scale production of this mushroom in controlled indoor conditions. Chang (1982) described the indoor cultivation technology of *Volvariella* on cotton waste.

Preparation of the Compost

Different grades of cotton wastes (droppings, cardflies, cutter flies and dust/oily sweeping) are mixed and put inside a wooden frame (90 × 90 × 30 cm). Water and 2.5 % limestone and other materials, e.g. chicken manure (5 %), can be added gradually. The compost is stamped on to enhance absorption of water and mixing of the components. Large pieces of cotton waste are torn into small parts at this stage. When the frame is completely filled with moistened cotton wastes, it is pulled up so that another layer or compost can be put within it. One pile of compost consists of four to six such layers and is about 70–90 cm high. Then the pile is covered with a plastic sheet and allowed to ferment. After 2 days, the fermenting composts are turned and thoroughly mixed by means of a mechanical stirrer and supplemented with 5 % of rice bran and of course water when needed. The mixed supplemented compost is then piled up and covered with plastic sheets again. Fermentation is continued for another 2 days.

Filling the Beds

It takes about 4 days to prepare the compost after which it can be made into beds. One standard plastic mushroom house contains altogether ten beds with 146.5 m^2 bed area. The layer of compost is about 10 cm thick. The mushroom house is constructed with bamboo/wooden frame (Ho 1978) and is lined inside with 0.4 mm film of polyethylene plastic sheet and outside with a 1.27 cm layer of polyethylene sheet for insula-

tion. It is usually equipped with a 0.25–0.5 hp electric blower, a polyethylene air duct for ventilation and four windows, two on each long side of the house, which can be opened when needed to adjust temperature, humidity and ventilation during the hot summer months.

Pasteurization

After the beds are filled, live steam is introduced into the mushroom house. Within 2 h, the air temperature gradually rises to 60–62 °C. This temperature is maintained for 2 h, then cooled down to 52 °C by the introduction of gentle stream of fresh air. A temperature of 50–52 °C is maintained for 8 h with a continued air supply. The stream valves are then closed and the temperature is allowed to drop gradually to 34–36 °C for spawning. This latter step takes about 12–16 h depending on the outdoor temperature.

Spawning

The amount of spawn used is 1.4 % of the dry weight of the compost or 0.4 % of the wet weight. Full growth is achieved within 3–4 days depending upon compost quality and temperature. The pure-culture spawn is taken out of the container and placed in a tray for easy handling. It is then pressed and broken into small pieces which are inserted into the compost which has been scooped out to a depth of 2–2.5 cm at intervals of 12–15 cm. The inserted spawn is then covered with the displaced compost. The beds are covered with thin plastic sheets. The temperature of the room is maintained at 32–34 °C during the period of spawn running.

Spawn Running

During spawn running, no water or light is needed, but a little ventilation is provided. Three days later, white light will be introduced into the room by means of fluorescent lamps and more ventilation will be given. Under good composting and pasteurization conditions, unidentified species of actinomycetes and *Humicola fuscoatra* usually develop in and on the beds with mycelia of *Volvariella* during the spawn-running period. There are convincing observations that *H. fuscoatra* can stimulate the growth of straw mushroom mycelium and, at the same time, prevent the growth of harmful fungi. After removal of all plastic sheets and sprinkling the beds with water on the fourth day, growth of actinomycetes and *Humicola* will be retorted, but *Volvariella* will continue to grow. On the fifth day after spawning, primordial fruit bodies usually appear on the surface of the beds.

Cropping and Harvesting

Under controlled conditions, tiny fruit bodies of the straw mushroom usually appear on the surface of the cotton waste compost on the fifth-day spawn inoculation. It usually takes about 4 days for these tiny bodies to develop to the stage of harvesting. The paddy straw mushroom should be harvested at the button or egg stage. After the universal veil has been split open to allow the cap to become visible, the mushroom is in the egg stage. The first flush usually lasts for 4–5 days. Four days later, a second flush usually will start, and it can last for another 3–4 days. The yield of the second flush is about 10 % of the first one. Therefore, in practice, the straw mushroom grown on cotton waste compost under indoor conditions is harvested for only one flush. A mushroom house can have two crops of the mushroom every month or at least three crops every 2 months.

9.6 Pests and Diseases of Mushrooms

The mushrooms are subjected to infection by viruses, bacteria and moulds; besides they are eaten by insects, nematodes and rodents. Some of major agents which are responsible for damage at pre- and post-harvest and unless suitable control measures are taken, a huge crop loss will occur and the mushroom cultivation becomes uneconomical. Some of the diseases and pests and control measure to be adapted are listed.

Pests

Different insects and nematodes grow on mushroom beds and will be responsible for yield loss.

Even rats eat away fruit bodies along with grains and other things, causing yield loss.

Insects

(i)	Springtails	*Lepidocyrtus cyaneus*
(ii)	Sciarid flies	*Lycoriella solani*
(iii)	Phorid flies	*Megaselia halterata*
(iv)	Coccids	*Heteropeza phagmae*
		Mycophila brunnesi
(v)	Mites	*Rhizoglyphus phylloxerae*

Nematodes

Dactylenthus myceliophagus affect mycelial growth

Rats

Rodents damage the mushroom beds and consume grain spawn

Control Measures of Pests

1. Maintain cleanliness.
2. Prevent compost and straw from coming in contact with soil.
3. Treat the tools with 2 % formalin.
4. Spray dichlorvos (Nuvan) at the rate of 0.6 ml/l.
5. Spray Dicofol (0.01 %) to control mites.
6. Bait and kill rats.

Diseases of Mushrooms

Different parasites grow on mushrooms. They grow in beds and cause characteristic diseases and are responsible for considerable loss to fruit bodies during harvest (Bano et al. 1975).

A. *Fungal diseases*		
(i)	Dry bubble	*Verticillium malthousia*
(ii)	Wet bubble	*Mycogone perniciosa*
B. *Bacterial diseases*		
(i)	Bacterial pit	*Pseudomonas* sp.
(ii)	Bacterial brown blotch	*Pseudomonas tolassii*
C. *Viral diseases*		
(i)	Elongated bend stipes	Seven types of viral particles are responsible for these diseases
(ii)	Disintegration of mycelium	

Weed Moulds and Competitors

The following moulds grow on growing mushrooms and will be responsible for yield loss.

(i)	False truffles	*Diehliomyces microsporus*
(ii)	White plaster mould	*Scopulariopsis fimicola*
(iii)	Ink cap	*Coprinus* sp.

9.7 Canning of Mushrooms

Mushrooms have good taste when cooked fresh. However, canning is required when consumers are located in far-off places. The following steps are involved in the canning process:

1. *Pre-cleaning*: Mushrooms are cleaned to remove foreign particles, soil, etc.
2. *Washing*: Mushrooms are washed in water.
3. *Blanching*: Mushrooms are blanched in hot water having 0.2 % citric acid for 3–5 min. This process results in loss of 30 % weight.
4. *Cooling*: Blanched mushrooms are cooled through continuous counterflow cooling system.
5. *Grading*: Mushrooms are graded according to size.
6. *Slicing*: Mushrooms are sliced for the required size.
7. *Filling*: The cans are filled with mushroom slices and weighed.
8. *Brining and exhausting*: Hot brine solution (salt 2 % + sugar 2 % + citric acid 0.3 %) is added and temperature is raised to 80 °C in the centre of the can to exhaust.
9. *Can sealing*: Cans are sealed with lid.
10. *Retorting*: The sealed cans are sterilized at 15 PSI for 15–20 min.
11. *Labelling and packing*: The cans are labelled and packed in cartons. Different sizes of cans are used as per requirements.

9.8 Future of Mushroom Cultivation

Increasing awareness of nutritive and medicinal value of mushrooms, a boost to mass cultivation of mushrooms resulted. Further cultivation of mushrooms helps to convert agro-wastes into human food. Their cultivation provides labour incentive and employment as they are fast growing and responsible for production of quality food. Mushrooms represent untapped source of nutraceuticals and valuable palatable food. Substrate/compost preparation is done with special reference to fermentation, strains of mushrooms from different geographical regions and their evaluation and use in breeding work. Breeding for high-yielding strains of species will be of immense value for *Agaricus* and *Pleurotus*, and other promising mushrooms for both cold and hot climates are of an urgent need. Improvements in prolongation of shelf life and canning and processing will also boost the prospects of mushroom cultivation. However, lack of awareness, shelf life maintenance of pure culture, unpredictable yield and shorter shelf life are some of the limitations in mushroom industry. Indifference of academicians, government and institutions alike adds to the constraints of mushroom cultivation. Developing sporeless or low spore-shedding *Pleurotus* and other mushrooms with desired traits will also help the mushroom industry. The methods of cultivation and other associated problems in cultivation of untapped mushrooms such as *Amanita*, *Agrocybe*, *Armillaria*, *Boletus*, *Cantherellas*, *Lactarius*, *Lepiota*, *Marasmius*, *Morchella*, *Peziza*, *Hydnum*, *Psalliota*, *Rhodopaxillus*, *Russula* and *Termitomyces* need to be taken up for cultivation and nutritive quality determination. Protoplast fusion technique in closely and distantly related species/genera in developing quality hybrid mushroom is also the need of the hour.

Acknowledgement Thanks are due to Head, Department of Botany and Microbiology for kind encouragements.

References

Bahl N (1984) Hand book on mushroom. Oxford and IBM Publishing Co, New Delhi, p 123

Bano Z, Srivastava HC (1962) Cultivation of Pleurotus species on paddy straw. Food Sci 12:361–365

Bano Z, Narasimha P, Singh NS (1975) Efficacy of some fungicides in controlling *Penicillium digitatum* Saccardo infection during the cultivation of *Pleurotus flabellatus* (Berk. & Br.) Sacc. Indian J Microbiol 15(2):68–74

Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira IC (2008) Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food Chem Toxicol 46:2742–2747

Birks AA (1991) Fungi in folk medicine. Mellvaninea 10:89–94

Block SS, Tso G, Han LN (1958) Experiments in the cultivation of *Pleurotus ostreatus*. Mushroom Sci 4:309–325

Chang ST (1982) Cultivation of Volvariella mushroom in South East Asia. In: Chang T, Quimio TH (eds) Tropical mushrooms, biological nature and cultivation methods. The Chinese University Press, Hong Kong, p 221

Chang ST, Miles PG (1989) Edible mushrooms and their cultivation. CRC Press, Inc, Boca Raton

Chen GL (1992) Studies on the cultivation and medical efficacy of Hericium erinaceus translation. The Edible Fungi Research Institute of the Shanghai Academy of Agricultural Science, China

Chihara G, Humuro J, Maeda YY, Arai Y, Kolera K (1970) Cancer Res 30:2776–2781

Cochran KW (1978) Medical effects. In: Chang ST, Hayes WA (eds) The biology and cultivation of edible mushroom. Academic press, New York, pp 137–168

Dhar BL (1996) In: Royse DJ (ed) Mushroom biology and mushroom products. Pennsylvania State University, USA, pp 251–257

Dhar BL (1997) In: Rai RD, Dhar BL, Verma RN (eds) Advances in mushroom biology and production. MSI, Solan, pp 369–378

Findlay WPK (1982) Fungi: folklore, fiction, and fact. Mad River Press, Eureka

Flack R (1917) Uber die waldkultir des Austernpilzes (Agaricus ostreatus) anf Laub holt stubben. Zeitschriff Forstund Jagdwesen 49:159–165

Hawksworth DL (1990) The fungal dimensions of biodiversity magnitude significance and conservation. Mycol Res 95:642–655

Hirotomi M, Furuya MT, Shrip M (1985) A ganoderic acid derivative, a highly oxygenic Lanostene type triter period from *Gonoderma lucidum*. Chem Pharm Bull (Tokyo) 34:3025–3028

Ho MS (1978) Cultivation of *Agaricus bisporus* in Asian countries growing in subtropical areas. In: Chang ST, Hayes WA (eds) Biology and cultivation of edible mushrooms. Academic, New York, pp 337–343

Hsue RY (1993) An overview on *Ganoderma* mushrooms. Wannian publishing company, Taichung, 141

Jandaik LL, Kapoor JN (1974) Mush Sci 9:667–672

Jong SC, Birmingham JM, Pai SH (1983) Immunomodulatory substances of fungal origin. J Immunol Immunopharmacol 11:115–122

Jong SC, Donovick R (1989) Antitumor and Antiviral substances from fungi. Adv Appl Microbiol 34:183–262

Kim BK, Kim HW, Choi EC (1993) Medicinanal efficacy of ganoderma lucidum xv ants hvact vites of ganoderma lucidum. In: Royse DJ (ed) Mushroom biology and mushroom products. Pennsylvania State University, USA, pp 187–194

Kurashige S, Akiizawa Y, Endo F (1997) Effects of *Leutinus edodes Grifola frondosa* and *Pleurotus ostreatus* administration on cancer outbreak and activities of macrophages and lymphocytes in mice treated with a carcinogen, N-butyl-N-butanol nitroso – amine *Immunopharmacol*. Immunotoxicology 19:175–183

Laessoe T, Delconte A, Lineoff G (1996) The mushroom book. D.K. Publishing Co, New York

Liu WK, Ng TB, Sze SF, Tsui KW (1993) Activation of peritoneal macrophages by polysccharopeptide from the mushroom *Coriolus* versicolor. Immunopharmacology 26:139–146

Mizuno T, Wang G, Zang J, Hawagishi H, Nishitoba T, Li J (1995) Reishi, Ganoderma lucidum and G.tsugae bioactive substances and medicinal effects. Food Rev Int 11:151–156

Molitoris HP (1994) Mushrooms in medicine. Folia Microbiologia (Praha) 39:91–98

Morigawa A, Ditabatake K, Fujinoto F, Kekawa N (1986) Angiotensin converting enzyme inhibitory triterpenes from Ganoderma lucidum. Pharm Bull 34:3025–3028

Ng TB (1998) A review of research on the protein bound polysaccharide (Polysaccharide peptide PSP) from the mushroom Coriolus versicolor. Gen Pharmavol 30:1–4

Oikawa S, Miyazakai T, Yadamae T (1993) Antitamer active polysaccharides from the Chinese, Ganoderma tsugae. Biosci Biotechnol Biochem 57:894–900

Onno N, Lino K, Takeyama, Suzuki T et al (1993) Antitumor active polysaccharides from Chinese mushroom Songshan lingzhi the fruiting body of Ganoderma tsugae. Biosci Biotechnol Biochem 57:894–900

Opletal L (1993) Phytotherauptic aspects of diseases of the circulatory system 2. The oyster mushroom and its potential. Cesk Farm 42:160–166

Rangaswamy G, Kandaswamy, Swamy KR (1975) Pleurotus sojar a protein rich nitrogen fruiting mushroom fungus. Curr Sci 44:403–404

Shandilya TR (2000) J Mycol Plant Pathology 30:285, 31: 57–59

Singh R, Singh UC (2005) Modern mushroom cultivation. Agrobios, Jodhpur, p 229

Sporke DA (2001) Fungi in folk medicine and society in food-borne diseases. In: Hui YH, Smith RV, Spoerke DG (eds) Hand book, vol 3. Marcel Dekker Inc, New York, pp 781–801

Stamets P (1993) Psilocybin mushroom of the world an identification guide. Ten Speed Press, Berkley

Su UT, Seth LN (1940) Cultivation of straw mushrooms. Indian Farming 1:332–333

Suman BC, Sharma VP (2005) Mushroom cultivation processing and uses. Agrobios, Jodhpur, p 343

Tanabe-Tochikura A, Tochikura TS, Yoshida O, Oki T, Yamamoto N (1990) Pradimicin A inhibition of human immunodeficiency virus: attenuation by mannan. Virology 176:467–473

Thomas KM, Rama Krishnan TS, Narsimhan IL (1943) Paddy straw mushroom. Madras Agric J 31:57–59

Tunney (1980) In: Biswas S, Datta M, Ngachan SV (eds) Mushrooms: a manual for cultivation. HI Learning Pvt Ltd, Raj Press, New Delhi

Vijay B (1990) Substrate preparation of Pleurotus cultivation with recent advances. In: Sharma SR, Dhar BD, Mehta KB (eds) Recent developments in the cultivation technology of edible mushrooms. Summer Institute NCMRT, Solan, Himachal Pradesh, pp 231–233

Willard T (1990) The reishi mushroom: herb of spiritual potency and medicinal wonder. Sylvan Press, Vancouver

Lichenology: Current Research in India

10

D.K. Upreti, Rajesh Bajpai, and S. Nayaka

Abstract

Lichens are one of the important constituents of the Indian flora. The vast topographical and climatic diversity has endowed it with a rich lichen flora, both in luxuriance and diversity. Despite intense effort in exploration and survey during the last five decades, our knowledge about lichens from different floristic regions of India is incomplete as many areas are still unexplored for their lichen wealth. The lichens are most valuable biomonitors to atmospheric pollution as they can be used as sensitive indicators to estimate the biological effects of pollutants by measuring changes at community or population level of an area. Lichen monitoring can be effective as an early warning system to detect environmental changes. The periodical monitoring and documentation of floristic data is necessary and useful for future biomonitoring and climate change studies. The lichens are peculiar organisms which produces unique secondary compounds mostly not known in other plant groups. Most of the secondary compounds produced by lichens have antibiotic properties. Recent research shows that our knowledge of lichen bioprospection is still very limited and exploration of lichens is likely to yield many more useful species for an unexpectedly wide variety of human needs and pursuits. There is a need for the more widespread use of such organism as bioprospection agents. The vast and diverse topographical area of the country exhibits rich diversity of lichens which provided a lot of scope to utilize these organisms for their potent biomolecules. The lichens may be a good source of unique phytochemicals; however, not much work has been done so far for their medicinal bioprospection and chemistry in India, probably due to their nonavailability in bulk and slow growth rate in nature. The culture of lichens will

D.K. Upreti (✉) • R. Bajpai • S. Nayaka
Lichenology Laboratory, Plant Diversity, Systematics and Herbarium Division, CSIR – National Botanical Research Institute, Lucknow 226 001, Uttar Pradesh, India
e-mail: upretidknbri@gmail.com

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_10,

definitely help to exploit the medicinal treasures of this plant. Further investigations on biological activity of lichens as well as fast isolation method of lichen metabolites are needed to attract other researches in search of novel compounds beneficial for humankind.

Keywords
Indian lichens • Biomonitoring • Biomarkers • Lichenometry • Bioprospection

10.1 Introduction

Among the plant groups, lichens are the most fascinating and widely distributed organisms on earth. The lichens are unique in having two microorganisms in a single plant, a phycobiont (alga) and a mycobiont (fungus), forming a thallus. By virtue of the peculiar structure and physiology, lichens have high tolerance to drought and cold and are able to grow in the diverse geographical regions from icy expanses to tropical and subtropical parts and from drier, hot deserts to moist, humid climate. Lichens grow on any substratum that provides a convenient foothold to them. This may be soil (terricolous), humus (humicolous), stones, rocks, brick (saxicolous), lime plaster (calcicolous), leaves (folicolous), tree trunk (corticolous), decaying wood (lignicolous) and other man-made substratum like iron pipes, asbestos sheet, lime or cement plaster and glass panes. Sometimes lichens also grow on some insects and animals. Lichens which are bigger in size and shape can be easily recognized as leaflike (foliose) and threadlike (fruticose) commonly called macrolichens, while taxa which form a crust over the substratum and are quite smaller in size are categorized under microlichens.

Based on the type of substrate, the corticolous (bark inhabiting) lichens exhibit their dominance followed by saxicolous (rock inhabiting) and terricolous (soil inhabiting) species in the country. Among the different altitudinal zones, the temperate regions exhibit the luxuriant growth of lichens followed by alpine and tropical regions.

Apart from altitudinal variation, the vegetation and forest types of higher plants also play an important role in determining the type of lichen flora of the region. Based on the forest type, six different lichen vegetation zones of the country are moist tropical evergreen forest, cold deserts in the Himalayas, South Peninsular region, mid-Eastern Indian and Peninsular plateau, dry and arid regions and Indo-Gangetic Plains of central India and coastal regions of India and Andaman and Nicobar Island. The cold deserts in the Himalayas exhibit some unique group of lichens having restricted distribution only in such habitats.

The Himalayan region in India is exhaustively explored for lichen wealth in the past and the lichen flora of different Himalayan states is well worked out. Most of the substrates exhibit dense growth of different species of lichens growing in close association, forming mixed patches, which are sometimes overlooked by the collectors during collection.

Approximately 20,000 species of lichens are known from the world and India represents more than 10 % of the species. It is estimated that at present, the Indian lichen flora comprises about 2,319 species under 305 genera and 74 families widely distributed in tropical, subtropical, temperate and alpine regions of India (Singh and Sinha 2010). The lichen family Parmeliaceae is the largest family in India comprised of 345 species followed by Graphidaceae, Thelotremataceae, Pyrenulaceae, Caliciaceae and Lecanoraceae represented with 279, 131, 123, 103 and 99 species, respectively. The largest lichen genus in India is *Graphis* which contains 111 species followed by genera like *Pyrenula*, *Lecanora* and *Caloplaca*, represented by 90, 83 and 65 species, respectively, while *Usnea* and *Porina* are represented by 60 species each (Singh and Sinha 2010).

In India, the corticolous (growing on bark) exhibit their dominance followed by terricolous (soil inhabiting) and saxicolous (rock inhabiting) lichens. Khare et al. (2009) recorded the occurrence of 65 lichen genera on soil from India under 22 terricolous families. The Cladoniaceae, Collemataceae, Peltigeraceae, Parmeliaceae, Sterocaulaceae, Physciaceae and Lobariaceae are the dominant families of soil lichens. The genera like *Cladonia*, *Collema*, *Peltigera*, *Leptogium*, *Lobaria* and *Stereocaulon* are the dominant soil lichens in India. Soil lichen communities exhibit their colonization mostly in temperate, higher temperate and alpine regions of India where tree vegetation is lacking. The moist evergreen forests have luxuriant growth of soil lichens than the dry deciduous forests.

In temperate regions of India, the corticolous lichen dominates over saxicolous and terricolous lichens. The ground flora under coniferous forest at lower temperate areas remains mostly dry and supports scanty to poor growth of soil lichens. The evergreen temperate forest and coniferous forest of upper temperate regions provide a moist shady environment suitable for species of *Lobaria*, *Peltigera*, *Stereocaulon* and *Cladonia* to colonize on soil among mosses. The common crustose soil lichen genera of the region are *Caloplaca*, *Diploschistes*, *Diplotomma* and *Pertusaria*.

Most of the alpine region in the Himalaya exhibit dominant growth of the terricolous communities of lichens. Fruticose species of lichen genera *Cladonia* such as *Cladonia rangiferina* and *Cladonia aggregata* grow luxuriantly in moist slope in alpine regions. The cold desert in the Himalaya also exhibits good growth of terricolous lichens. Out of 81 species of lichens recorded from the cold desert of Lahaul and Spiti area of Himachal Pradesh, 18 were soil inhabiting (Srivastava et al. 2004). *Cetraria* sp., *Bryoria himalayana*, *Hypogymnia hypotrypa*, *Lethariella cladonioides* and *Thamnolia vermicularis* are the most common terricolous lichen species in eastern Himalayan region of India.

Despite intense effort in exploration and survey during the last five decades, our knowledge about lichens from different floristic regions of India is incomplete as many areas are still unexplored for their lichen wealth. The lichens are most valuable biomonitors to atmospheric pollution as they can be used as sensitive indicators to estimate the biological effects of pollutants by measuring changes at community or population level of an area. Lichen monitoring can be effective as an early warning system to detect environmental changes. The periodical monitoring and documentation of floristic data is necessary and useful for future study (Garty 2001).

10.2 Current Researches in Indian Lichenology

Apart from diversity, the economic use of lichens is well known in the world and India from several decades. The lichens also have been well known as valuable plant resources in the ancient time and are still used as medicine, food, fodder, perfume, spices and dyes. Apart from extensive systematic studies carried out in the last five decades, recently the lichenological studies initiated in India are mostly related to biodeterioration, bioprospection, air pollution and mycobiont culture. The current development in the lichenological studies in India is discussed as follows.

10.2.1 Bioprospection of Lichens

10.2.1.1 Lichens as Food and Spices

The species of lichen genus *Umbilicaria* in Japan are eaten as salad called ‘Iwatake’. They are rich in carbohydrates and fats. Species of *Cladonia*, *Stereocaulon*, *Usnea* and *Ramalina* are mixed with flour and eaten, as they are considered as good source of carbohydrates. The lichen *Lecanora esculenta*, found in various parts of the world covering the soil, is gathered and powdered, and flour is used to prepare earth bread from it. *Cetraria islandica*, commonly known as ‘Iceland moss’, is used as human food. After collection and removal of certain bitter principles by allowing them to diffuse into cold water, the thallus is dried and the decoction of this dried thallus which forms a demulcent drink with milk is believed to be highly nutritious.

Parmelioid lichens available in large quantities in the market are used as food material and as condiment. In Sikkim, *Everniastrum cirrhatum*, a commonly growing lichen of that area, is eaten as a vegetable after boiling and frying it in fat. *Leptogium denticulatum*, a common foliose lichen, is used by the 'Adi' tribe of Arunachal Pradesh as food. The local 'Adi' people collect the lichen from soil, rock and tree trunk, wash it properly and boil it with water. The souped and boiled thallus which becomes jellylike after boiling is used as vegetable (Fig. 10.1).

In Indian markets, lichens are sold by the name of 'Chharilia', which consist of a mixture of two or three species of *Everniastrum*, with other foliose parmelioid lichen species which are used as spices. These lichens provide a special fragrance to meat, pulse and other important vegetables (Fig. 10.2).

In view of the high protein content and the interesting amino acid composition together with ergosterol and inorganic constituents of iron and calcium, *Dermatocarpon moulinsii* (20 % crude protein), *Lobaria isidiosa* (20 % crude protein), *Roccella montagnei* (14 % crude protein) and *Parmotrema tinctorum* (14 % crude protein) appear to have good food value.

10.2.1.2 Lichens as Fodder

Lichens are important food for animals in the arctic regions. During winter the reindeer and caribou supplement their normal diet of sedges and willow twigs with most common species of *Usnea* and *Cetraria*. Sheep in the Libyan deserts are reported to graze on the sub-foliose lichens *Lecanora esculenta*, which forms a thick loose crust on soil and rocks and is easily eaten by the sheep.

Fig. 10.1 *Leptogium denticulatum*, Lichen thallus used as food by Adi tribal in Arunanchal Pradesh

In alpine meadows, the commonly growing species of lichens are common source of food for the land snails and termites. Lichens provide a protective environment for a number of invertebrates. Lichenophagous insects, such as bark lice, springtails and moth caterpillars, possess mandibulate mouthparts, with which they bite off the lichen and chew it. Species of lichen genera *Ramalina*, *Parmelia* and *Usnea* on twigs of bushes are favoured by the musk deer during scarcity.

In south India, *Roccella montagnei*, luxuriantly growing on plants, is used as a common fodder for animals. Several new lichen species and varieties, especially of *Rhizocarpon*, have been described, which are actually no more than well-known species damaged by snails and mites.

10.2.1.3 Medicinal Bioprospection

Lichens were held in high regard by medicinal practitioners in medieval times, and their use has persisted to present times. In various pharmacopoeias, lichens are listed purely on the basis of their folklore medicinal use. Several species of lichens enjoyed a good position in ancient and traditional systems of Indian medicine like Ayurveda and Unani. Some of the species of lichen genera *Usnea*, *Parmelia*, *Umbilicaria*, *Heterodermia*, *Sticta*, *Lobaria* and *Cladonia* that are frequently used as traditional medicines are listed in Table 10.1.

10.2.1.4 Lichens in Perfume and Dyes

Some of the species of lichens are aromatic, such as *Evernia prunastri* and *E. furfuracea*, commonly known as oakmoss and tree moss, respectively, and these species are used commercially for production of aromatic resinoids. The resinoids are used extensively in perfumes, flavours and cosmetics as they have excellent odour fixatives and are universally employed in the blend-

Fig. 10.2 Grading of lichens for trading in various Indian markets

Table 10.1 Common species of Indian lichens used in medicines

Usnea longissima Ach.	*Stereocaulon himalayense* Awasthi et Lamb
Parmotrema sancti-angelii (Lynge) Hale	*Cladonia crispate* (Ach.) Floton
Peltigera polydactyla (Neck.) Hoffm.	*Umbilicaria esculenta*
Heterodermia diademata (Taylor) Awasthi	*Lasallia pensylvanica* (Hoffm.) Llano.
Cetraria sp.	*Lobaria orientalis* (Asahina) Yoshim.
Usnea rubescens Stirton	
Sticta gracilis (Müll. Arg.) Zahlbr.	

ing of perfumes. The lichen-yielding oakmoss and tree moss resinoids occur only in Central and Southern Europe and some parts of North Africa. The main producers of oakmoss are France, Czechoslovakia, Yugoslavia and Morocco.

In India, more than 35 species, mostly parmelioid lichens, are used for preparation of perfumes called '*Hina attar*' in Kannauj district of Uttar Pradesh, India. Before the discovery of coal tar dyes, lichens had considerable economic importance as dyestuffs. In Scotland and Scandinavia woollens and tweed are still dyed with native lichen dyestuffs. The dyeing substance of lichens is an orchil substance extracted from *Ochrolechia* and *Evernia* that dyes wool and tweeds in shades of purple, red or brown for many decades. A familiar acid-base indicator (litmus dye) in chemistry laboratories is derived from depside-containing lichen such as *Roccella montagnei*. It is related to orchil but represents a more complex of polymeric compounds with the 7-oxyphenoxazon chromophore and its anion. *Buellia subsoriroides*, a crustose lichen growing very commonly on rocks in Garhwal Himalayas, yield an orange dye, locally called 'maidi'. The herdsmen use it for colouring their fingertips and palms like '*Henna*', a well-known red-orange dye obtained from the leaves of *Lawsonia inermis*. The herdsmen along with their hands, when visiting the high temperate region, spit saliva on

the rock over lichen and start rubbing it with the help of small pieces of rough stone until a small amount of paste accumulates. The paste thus obtained is applied in the form of drops on the fingertips and palms to make designs. More than 60 species of macrolichens are proposed to have dyeing properties for fibres particularly silk.

Recently Shukla et al. (2014) screened out 11 species of lichens collected from Garhwal Himalaya and estimated for dye production using different methods like boiling water method (BWM), ammonia fermentation method (AFM) and dimethyl sulphoxide extraction method (DEM) and tested upon silk and cotton. The result indicated that parmelioid lichens are a potential source of natural dyes and provide brilliant colours in different solvents. AFM is the best method to get a wide range of colours such as pink, violet, orange, grey, brown and yellow. The *Parmotrema nilgherrensis* produced grey-brown dyes, *Evernia mesomorpha* produced pink-purple dyes and *Flavoparmelia caperata* and *Parmotrema tinctorum* produced brown colour dyes (Fig. 10.3).

10.2.1.5 Bioprospection of Lichens for Active Biomolecules

10.2.1.5.1 Mycobiont Culture

The Indian Himalaya and Western Ghats are huge reservoirs of most of the lichens especially parmelioid lichens; therefore, local people can use them in dyeing handicrafts and drugs after harvesting them. Since lichens are slow-growing organisms unable to provide large-scale biomass for commercial use, mycobiont culture and whole thallus culture of lichens are the only way to get good biomass and prevent the huge loss of lichen diversity from nature.

The spore discharge method for mycobiont culture is the most suitable and quick method of culture: The sterilized young perithecia/apothecia were incised under a dissection zoom microscope and were embedded with petroleum jelly to the inverted lids of the Petri plates containing plain agar medium. Care was taken to prevent the petroleum jelly from covering the epithecium/ostile of the ascomata for effective spore discharge on the agar medium. The plates were observed regularly for the germination of the discharged spores onto the medium, using an inverted microscope. Apart from ascospores' hymenial layer, thallus fragment, isidia and soredia are other methods used for culture of lichen mycobiont. It is well known that more than 1,000 secondary metabolites are known from lichens; these chemicals are produced through three pathways like acetate polymalonate pathway (APP), mevalonic acid pathway (MAP) and shikimic acid pathway (SAP) (Fig. 10.4).

10.2.1.5.2 Antimicrobial Activity

The chemicals produced by lichens have unique properties and most of them show promising results against various microbial strains. Srivastava (2013) screened out antimicrobial properties of some Indian lichens against six human pathogens such as *Staphylococcus aureus*, *S. faecalis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. The result showed that the Gram+ive and Gram–ive bacteria are inhibited in general. There was no antimicrobial activity of the extracts against *S. typhimurium*, but potent activity was noted against *P. aeruginosa* by some of the lichen extracts and some lichens also inhibited the growth of *Escherichia coli*. The extracts of *Usnea longissima* and *U. ghattensis* have a large zone of inhibition against *B. cereus*. The lichen species *Dermatocarpon vellereum* showed good result against *S. aureus*, *S. faecalis* and *P. aeruginosa*. Sharma et al. (2014) studied the effect of usnic acid and *Cladonia furcata* extract on gastroesophageal reflux disease (GERD) in rat and concluded that hydro-ethanolic extract of *C. furcata* scavenges the free radicals and possesses antioxidant activity. The result suggested that the antioxidant activity of the extract may be attributed to its antisecretory and justify the traditional ethnic usage of this herbs to treat GERD.

10.2.1.6 Pedogenic Significance of Lichens and Soil Development

The crustose form of lichens is the world's greatest pioneer. No organism other than a crustose lichen can maintain itself on a perfectly plain,

Fig. 10.3 Lichens and silk threads dyed with lichen dyes extracted through (**a**) ammonia fermentation method and (**b**) boiling water method. *a Evernia mesomorpha* Nyl, *b Everniastrum cirrhatum* (Fr.) Hale, *c Flavoparmelia caperata* (L.) Hale, *d Nephromopsis nephromoides* (Nyl.) Ahti & Randl, *e Parmotrema nilgherrensis* (Nyl.) Hale

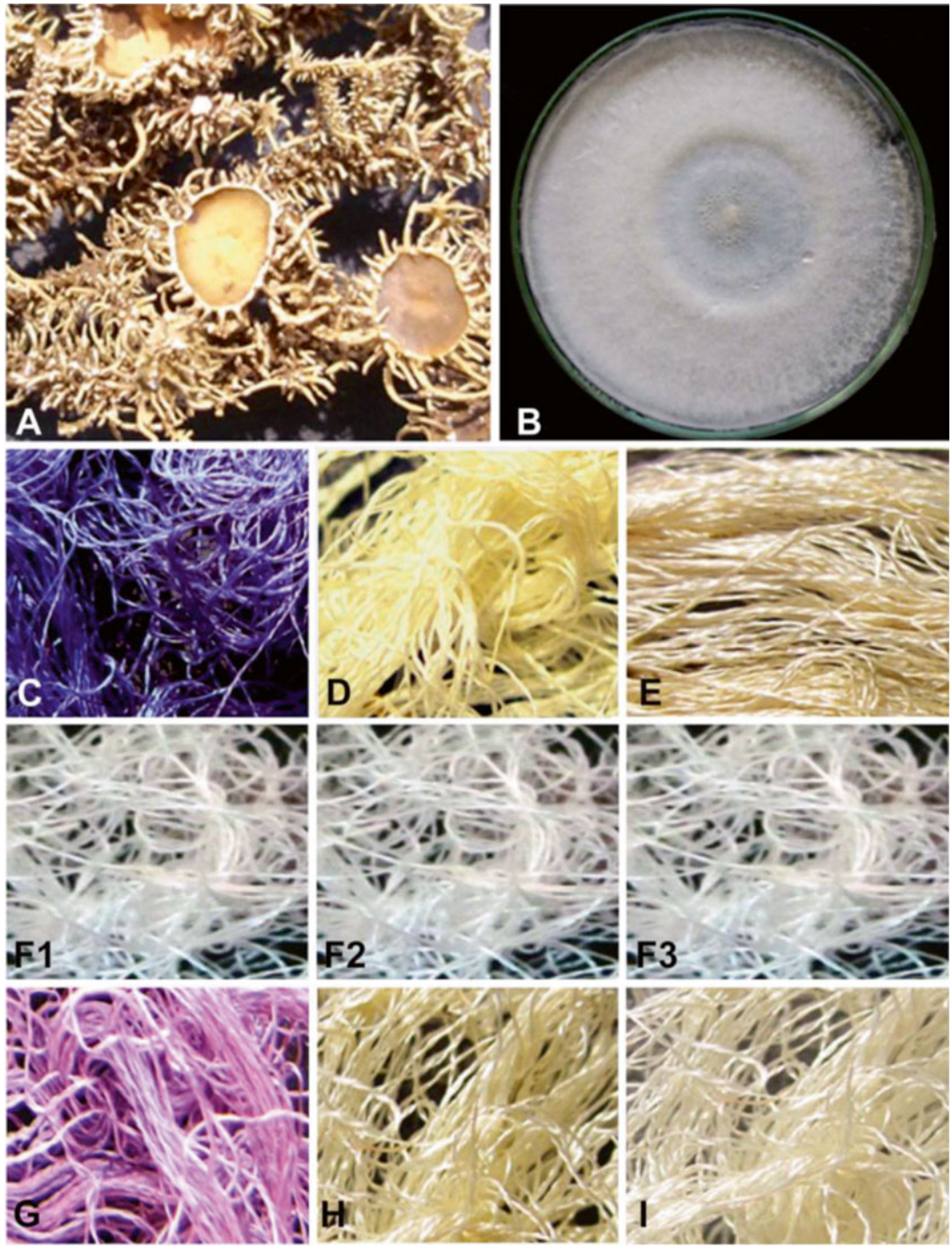


Fig. 10.4 Lichen mycobiont culture as source of natural dyes. (**a**) Natural thallus of *Usnea ghattensis*. (**b**) Mycobiont culture of *Usnea ghattensis*. (**c**) Silk thread dyed by ammonia method. (**d**) Silk thread dyed by cow urine method. (**e**) Silk thread dyed by boiling water method. ($\mathbf{f_1}$ $\mathbf{f_2}$ $\mathbf{f_3}$) Natural silk thread as control. (**g**) Silk thread dyed by mycobiont with ammonia. (**h**) Silk thread dyed by mycobiont with cow urine. (**i**) Silk thread dyed by mycobiont with boiling water

clean rock surface. Thus, after colonizing on a substratum, lichens accumulate several elements, frequently in large amounts. The accumulated N, P and sulphur elements can be used by mosses and higher plants which may replace lichens during soil development. The mixing of organic matter from the decay of the thallus mineral particles detached from the substratum and atmospheric dust trapped by the lichen thallus (due to their spongy nature) may produce a primitive soil.

Lichens are important agents in the biogeochemical and biochemical weathering of minerals and rocks and in a certain situation play an important role in plant succession. The substances execrated by the thalli of lichens are obviously too weak to alter the rocks by hydrogen ion exchange, but chelation could be an important mechanism in mineral breakdown (Bajpai and Upreti 2011). The lichen acids bind metal atoms of the substrates between their own molecules and form a metal complex. The metal complex is an unstable attachment and the metal is easily released in free form and the complex relents to its original form.

10.2.2 Climate Change and Pollution Monitoring

10.2.2.1 Lichenometry

Lichenometric technique is useful in dating moraine ridges on recent glacier forelands in alpine regions. The method was originally developed and used by Beschel (1961). Glaciers and their retreat are recognized as being among the most sensitive indicators of climate change, advancing substantially during climate cooling and retreating during climate warming. Lichens, due to their slow growth rate and uniform growth size, help in dating the exposure time of the sequences of the rock-forming glacier moraines due to retreat of the glacier, thus providing the approximate time of glacier retreat. The study is based on lichen size/age correlation and population distribution that involves the measurement of large specimens growing on large boulders that are supposed to be unaffected by the prevailing climatic conditions as well as human and animal interferences. Field studies of climate change impact in India can be conducted by initiating lichenometric studies in relation to climate change.

10.2.2.2 Climate Change

Lichens grow on a wide range of substrates, both natural and man-made, lack vascular system and absorb water and nutrients passively from their environment. Because of their peculiar nature, lichens are particularly sensitive to environmental factors such as temperature, water availability and air pollutants. Lichen community composition and changes in composition can provide information about changes in air quality, climate and biological processes.

The dependence of lichens for uptake of nutrients from the atmosphere makes them good indicators of environmental disturbance as they bioaccumulate airborne pollutants. Excessive levels of pollutants in the atmosphere, in particular sulphur dioxide (SO_2), can alter the physiology and morphology of sensitive species, ultimately killing them and changing lichen community structure. Once the lichen community composition of an area is available, the periodical re-measurement of lichens in an area will provide a trend. The trend analysis can indicate changes in lichen communities brought about by changes in climatic condition. Therefore, the use of historical data available on herbarium specimens, lichen community composition and remote sensing are important information that can be utilized in climate change. The different lichen bioindicator communities and their indication about the climatic condition of an area are listed in Table 10.2. The study will lead to the assessment of large-scale patterns of responses with broad species representation and is an important step towards understanding current and future importance of climate change on species performance and biodiversity (Fig. 10.5).

10.2.2.3 Air Pollution Monitoring

Lichens are one of the most valuable biomonitors of atmospheric pollution and can be used as sensitive indicators to estimate the biological effects of pollutants by measuring changes at community or population level and as accumulative

Table 10.2 Lichen communities and their indicators

Lichens community	Taxa	Indicates
Calcioid lichen community: pinhead lichens	Members of Calciales	Indicates old-growth forests
Alectoroid and usnioid lichens community	*Sulcaria*, *Bryoria*, *Ramalina* and *Usnea*	Indicates older forest with better air quality
Cyanophycean lichen community	Cyanolichens (*Leptogium*, *Peltigera*, *Sticta*)	Indicates forest ecosystem function and indicates forest age and continuity
Lobarian lichen community	*Lobaria*, *Pseudocyphellaria*, *Peltigera* and *Sticta*	Indicates species-rich old forest with long forest continuity
Xanthoparmelioid lichen community	*Xanthoparmelia*	Indicates landscapes with no accelerated erosion
Graphidioid and pyrenuloid lichen community	*Graphis*, *Opegrapha*, *Scarcographa*, *Phaeographis*, *Anthracothecium*, *Pyrenula*, *Lithothelium*, *Porina*	Indicates moist and evergreen forest
Lecanorioid lichen community	*Lecanora*, *Lecidella*, *Biatora*	Indicates well-illuminated environmental condition of the forest with considerable exposure of light and wind
Parmelioid lichen community	*Bulbothrix*, *Flavoparmelia*, *Parmotrema*, *Parmelia*, *Punctelia*	Indicates open thinned-out forest with more sunlight
Pertusorioid lichen community	*Pertusaria*	Indicates old tree forest with rough-barked trees
Lecideoid lichen community	*Lecidea*, *Protoblastenia*, *Haematomma*, *Bacidia*, *Buellia*, *Schadonia*	Indicates deciduous trees in sheltered and well-lit exposed sides
Leprarioid lichen community	*Chrysothrix*, *Cryptothecia*, *Lepararia*	Indicates moist and dry vertical slopes, rough-barked trees of moist and dry habitats. Species of *Chrysothrix* appears first after forest fire
Physcioid lichen community	*Physcia*, *Pyxine*, *Dirinaria*, *Heterodermia*, *Phaeophyscia*, *Rinodina*	Indicates as pollution-tolerant lichens and have ability to grow on varied substrates in both moist and dry habitats
Teloschistacean lichen community	*Caloplaca*, *Letroutia*, *Brigantiaea*, *Xanthoria*	Indicates both exposed and sheltered rocks. The dark-orange pigment present on the upper cortex of the thallus acts as a filter to protect the lichens from high UV radiation and indicate high UV radiation area
Lichinioid lichen community	*Phylliscum*, *Endocarpon*	Indicates presence of calcareous substrates in the habitats
Peltuloid lichen community	*Peltula*	Indicates a stable rock substratum

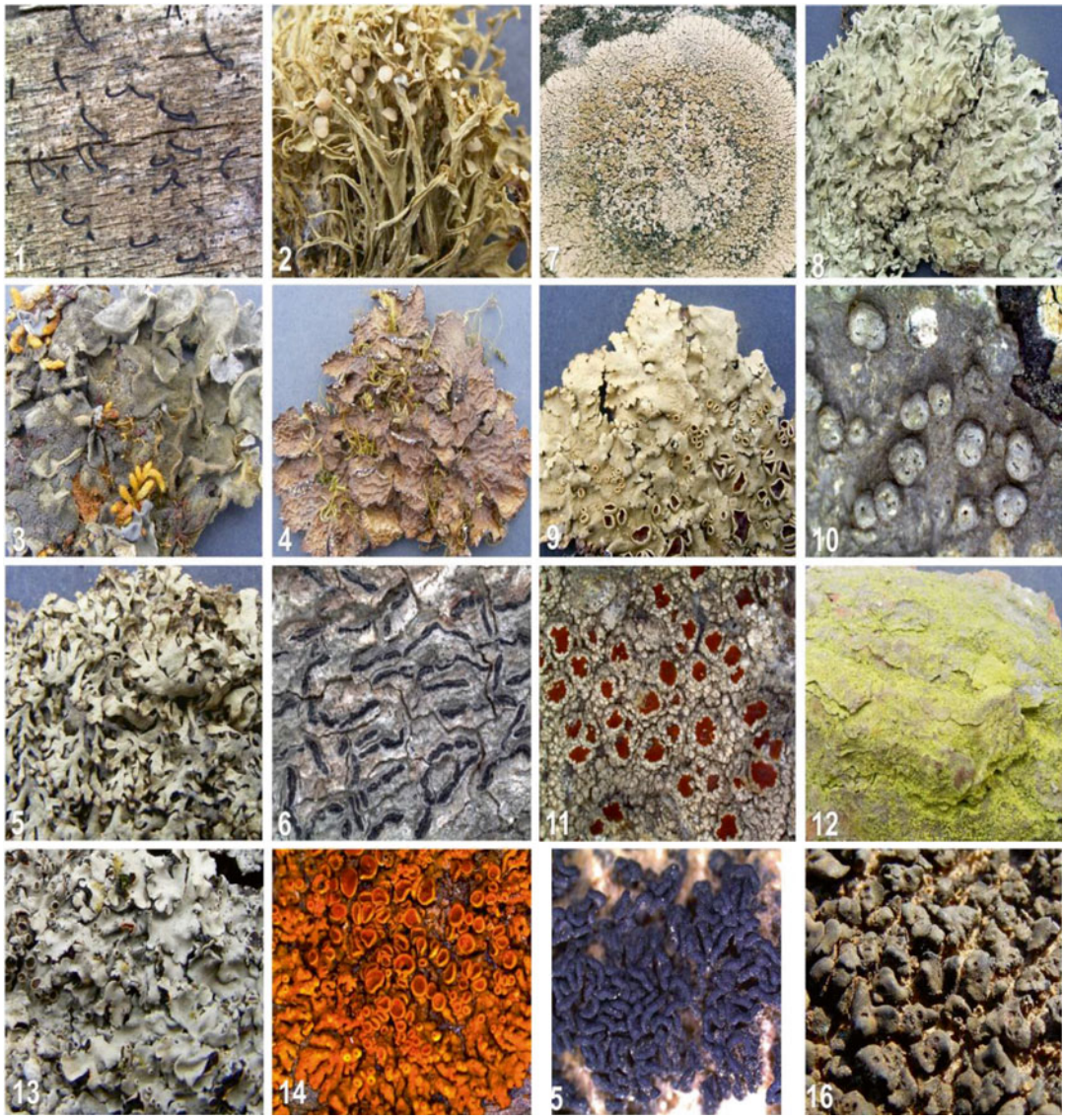


Fig. 10.5 Shows different Indian lichen bioindicator communities: (*1*) calcioid, (*2*) usnioid, (*3*) cyanophycean, (*4*) lobarian, (*5*) xanthoparmelioid, (*6*) graphidioid, (*7*) lecanorioid, (*8* and *9*) parmelioid, (*10*) pertusarioid, (*11*) lecanorioid, (*12*) leprarioid, (*13*) physcioid, (*14*) teloschistacean, (*15*) lichinioid, and (*16*) peltuloid

monitors of persistent pollutants. The high capability of lichens to accumulate air pollutants and their resistance to environmental stress and longevity are the major features that make them most suitable organisms for biomonitoring studies. Free diffusibility of lichen thalli due to lack of cuticle enables quick penetration of toxic compounds from the atmosphere to the photobiont layer. Thus, the response of lichens to the environmental pollution is more sensitive than the higher plants. Owing to their dependence on the atmosphere for nutrient supply and capacity to biomagnify accumulated environmental contaminants, lichens can provide details on the presence of persistent pollutants in the atmosphere and their biological effects (Garty 2001).

Lichens are excellent bioindicators of air pollution due to their sensitivity to acidic gases,

exhibit distinctly the incited damage in relation to morphological and/or physiological symptoms and are also excellent accumulators of pollutants. Broad geographical distribution, which allows documentation of the widespread pattern, perennial, slow growth rate uniform morphology and not shedding parts as in higher plants provide ability to cumulatively accumulate pollutants are the features which make lichens more suitable organisms for pollution monitoring.

All lichens are not equally sensitive to air pollutants. Rather, different lichen species exhibit differential sensitivity to specific air pollutants. As a consequence, lichens are well suited as biological indicators for monitoring environmental quality. During the last three decades, a number of studies devoted to assessing the effect of air pollution on lichens were carried out throughout the world (Garty et al. 2002). The mapping of lichen communities is one of the major areas of research to study the variation in lichen communities. The frequency of occurrence of certain species is related to specific air pollutants and in some cases to their concentrations. Apart from the distribution map, the morphological and anatomical changes in response to air pollutants further provide an assessment of the effect of environmental pollutants on living organism. The physiological reaction and changes in the lichen thallus due to air pollutants can also be measured and predict the environmental conditions of that particular area. The most common and easily available lichen species of a particular zone can be utilized for analysis to predict the environmental condition (Table 10.3).

The level of airborne pollutants arising from anthropogenic (point and line) sources such as power plants, smelters, automobiles, industry and agriculture can be easily monitored through lichens. The degradation of chlorophyll in the symbiotic photobiont is one of the most obvious signs of the damage that occurs in sensitive lichens. Heavy metals are known to interfere with chlorophyll synthesis either through the direct inhibition of an enzymatic step or through the induced deficiency of an essential nutrient. The species accumulate relatively high amounts of heavy metals and contain less chlorophyll, which clearly indicates that lichen chlorophyll contents interfere with the thallus metal contents (Loppi et al. 2000).

The lichen species express particular symptoms or response to indicate the changing environment (bioindicator); distribution or population which is studied over time and compared to some standard or baseline survey (biomonitor); species accumulating particular environmental substance within their fruiting bodies, thallus and rhizine (bioaccumulator); and physiological and biochemical changes in sensitive species caused by environmental pollutants (biomarkers).

The biomonitoring with lichens offers other advantages compared to instrumental methods such as low-cost, independence of power supply, easier sample handling and trace element determination methods. The perennial nature of lichens, absence of root or other special organs for uptake of nutrients and lack of cuticle enable them to absorb metals directly from the atmosphere and these characteristics make them ideal biomonitoring organisms (Seaward and Richardson 1989). The lichens are used as bioindicators and/or biomonitors in two ways: passive monitoring in situ and active monitoring, that is, transplant of lichens from one place to the other.

Table 10.3 Common biomonitoring species of lichens

Phytogeographical (altitudinal) zones	Bioindicator species
Tropical areas	*Dirinaria consimilis*, *Rinodina sophodes*, *Pyxine cocoes*, *Lepraria lobificans*, *Cryptothecia punctulata*
Subtropical areas	*Phaeophyscia hispidula*, *Pyxine subcinerea*, *Parmotrema praesorediosum*, *Parmelinella wallichiana*
Temperate areas	*Cladonia praetermissa*, *Heterodermia diademata*, *Candelaria concolor*, *Dermatocarpon vellereum*, *Usnea longissema*
Alpine areas	*Rhizocarpon geographicum*, *Aspicilia* sp., *Xanthoria elegans*, *X. fallax*

10.2.2.3.1 Lichens as Passive Biomonitors

Passive biomonitoring is the use of organisms, organism associations and parts of organisms which are a natural component of the ecosystem and appear there spontaneously. Lichen communities are currently used as indicator of forest ecosystem function in several contexts. Studies of lichens of particular forest type often have goals of monitoring effects of forest management practices and landscape context, including a variety of indirect human impacts on forest environment (Dettki and Esseen 1998).

10.2.2.3.1.1 Lichen Zone Mapping

Sernander (1926) recognized the disappearance of lichens from cities and conducted the systematic mapping and proposed there distinct zonations. The first zone is the 'lichen desert' which is found in the city centre, where the tree trunks were bare of lichens. The second zone is called 'struggle zone' which comprised of areas outside the city centre with tree trunks poorly colonized with lichens, followed by the 'normal zone' where lichen communities on the tree trunks were well established. Subsequently, the large number of similar city maps showed that these zonations were well correlated with the degree of pollution, the size of the urbanization area and the prevailing winds.

The distribution data of lichens collected from all the four directions of a particular area of Lucknow district provide an idea about the overall picture of the lichen distribution and the detailed distribution to segregate the area into four different zones (Fig. 10.6): Zone A, no lichen growth, an area in the centre of the city up

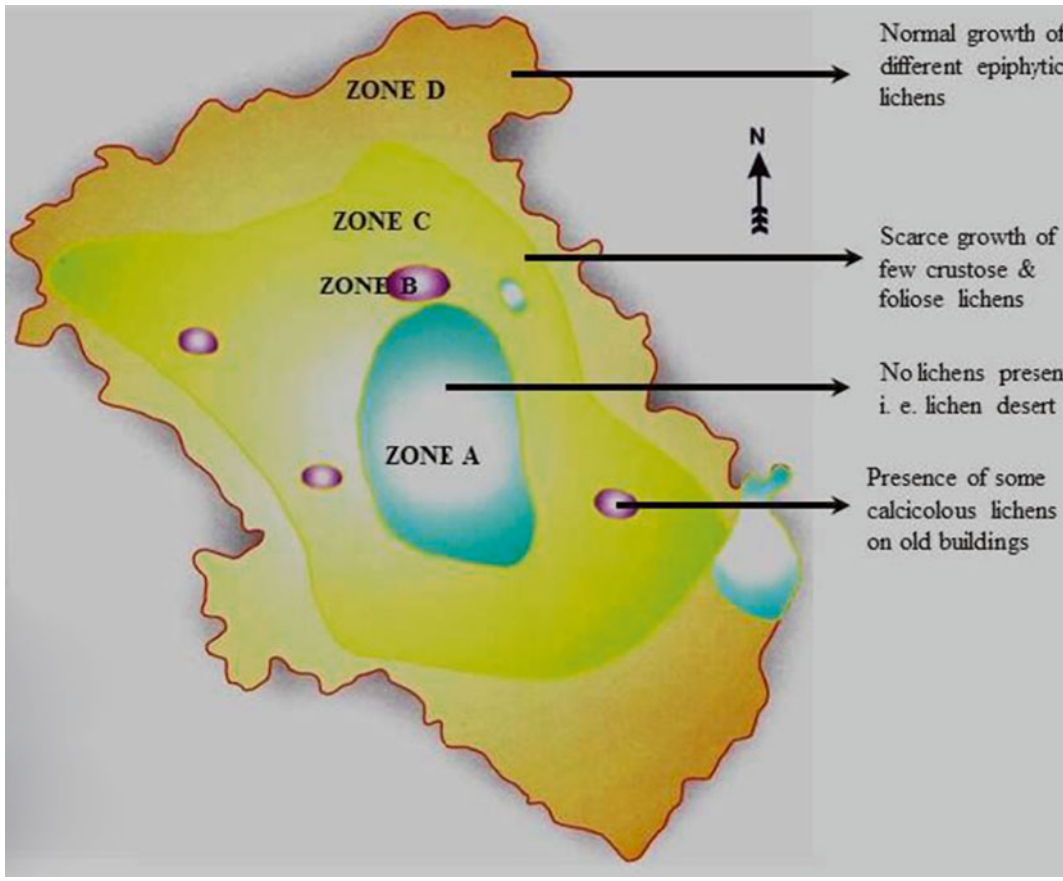


Fig. 10.6 Lichen zone mapping in Lucknow city

to 5 km all around; Zone B, presence of some calcicolous lichens, mostly the areas with old historical buildings; Zone C, scarce growth of few crustose and foliose lichens, areas with scattered mango trees; and Zone D, normal growth of different epiphytic lichen taxa together with some folicolous lichens.

10.2.2.3.2 Lichens as Active Biomonitors

Active biomonitoring studies include all methods which insert organisms under controlled conditions into the site to be monitored. The bioindicators are commonly grouped into accumulation indicators and response indicators. Accumulation indicators store pollutants without any evident changes in their metabolisms. Response indicators react with cell changes or visible symptoms of damage when taking up even small amounts of harmful substances.

In the active monitoring, such as areas where lichens are in scarcity, the transplant technique (active) was employed for determining the levels of pollutants in the area. The same size of thallus along with substratum is glued on cardboard of 20×20 cm and fixed vertically on exposed pole boundary wall of the same height at the selected (absence of lichen) sites. After transplant for a minimum of a week, those samples are taken from the transplant site to the laboratory for further analysis.

10.2.2.3.2.1 Accumulation Studies (Metals)

In India, the accumulation of metal (Al, Cd, Cu, Cr, Fe, Pb, Ni, Zn) pollutants in lichen thallus by passive as well as active principals is well known from different cities of the state of Uttar Pradesh (Faizabad, Lucknow, Kanpur and Raebareli district), Madhya Pradesh (Dhar, Katni and Rewa district), West Bengal (Hooghly and Nadia district), Maharashtra (Pune and Satara district) and Uttarakhand (Dehradun and Pauri district) (Shukla and Upreti 2007). The accumulation level of different metals decreases with increasing distance from the city centre. The metals Cr, Cu and Pb were more at the higher vertical position (20–25 ft), whereas other metals (Zn, Fe) accumulated maximum at lower vertical position (4–5 ft) (Bajpai et al. 2004).

The damage caused by the metallic pollutants in the lichen *Pyxine subcinerea* Stirton, by measurements of Chl a, Chl b, total Chl, carotenoid and protein and OD 435/415 ratio, significantly exhibits the changes in physiology. It was observed that Cu, Pb and Zn significantly affect the physiology of the lichen. Multiple correlation analyses revealed significant correlation (<0.001) among the Fe, Ni, Cu, Zn and Pb metals analyzed. Cd did not correlate with any other metals except Fe ($P<0.05$). Cu, Pb and Zn, the main constituents of vehicular emissions, had significant positive correlation ($P<0.001$) with protein content while the OD 435/415 ratio values decreased statistically ($P<0.001$) with increase in amount of Cu, Pb and Zn (Shukla and Upreti 2008, 2009).

Pyxine cocoes, a foliose lichen commonly growing on mango trees in tropical regions of India, is an excellent organism for determining the pollutants emitted from coal-based thermal power plant and accumulated in lichens after prolonged exposure. The diversity and distribution of lichens in and around such power plant act as a useful tool to measure the extent of pollution in the area. The distributions of heavy metals from power plant showed positive correlation with distance for all directions. The speed of wind and direction plays a major role in dispersion of the metals. The accumulation of Al, Cr, Fe, Pb and Zn in the thallus suppressed the concentration of pigments (chlorophyll a, chlorophyll b and total chlorophyll); however, it enhanced the level of protein. Further, the concentration of chlorophyll content in *P. cocoes* increased with decreasing distance from the power plant, while protein, carotenoid and phaeophytization exhibit a significant decrease.

The morphology, chemistry and anatomy of lichens play an important role in the accumulation of metals. Another common tropical lichen species *Phaeophyscia hispidula* belongs to the same lichen family (Physciaceae) as of *Pyxine* have distinct morphology and chemistry. A thick tuft of rhizinae (hairlike structure) on the lower surface of the thallus in *Phaeophyscia hispidula* acts as a metal reservoir and thus exhibits higher accumulation of most of the metals than *Pyxine*.

The crust-forming lichens attached tightly to the substrates through their whole lower surface have the highest accumulation of Al in the metal sequence, while the squamulose and foliose forms show Fe in the higher concentrations. The lichens have special affinity with iron, and they accumulate iron in greater amounts than other metals.

10.2.2.3.2.2 Accumulation Studies (PAHs)

Apart from inorganic metals, lichens are excellent indicators of polycyclic aromatic hydrocarbon compounds (PAHs) too. The PAHs accumulation studies in Indian lichens are initiated recently in the Himalayan region of Uttarakhand. The PAHs accumulation in lichens of different localities of Dehradun city and on the way to Badrinath is estimated recently. The first baseline data on the distribution and origin of polycyclic aromatic hydrocarbons (PAHs) in *Phaeophyscia hispidula* collected from nine different road crossings of Dehradun city and en route to Badrinath of Uttarakhand exhibit the presence of 13 types of PAHs: naphthalene (0.14–5.65 ppm), acenaphthylene (0.89–22.13 ppm), fluorene+acenaphthylene (0.07–3.38 ppm), phenanthrene (0.06–6.47 ppm), anthracene (0.01–0.38 ppm), fluoranthene (0.01–3.58 ppm), pyrene (0.13–14.46 ppm), benzo(a) anthracene+chrysene (0.01–0.13 ppm), benzo(k) fluoranthene (0.01–0.03 ppm), benzo(b)fluoranthene (0.02–0.09 ppm), benzo(a)pyrene (0.00–0.03 ppm), dibenzo(a,h)anthracene (0.17–0.31 ppm) and indino (1,2,3-cd ppm) pyrene+benzo(ghi)perylene (0.00–0.20 ppm) (Shukla and Upreti 2009).

The PAHs were of mixed origin, a major characteristic of urban environment. Significantly higher concentration of phenanthrene, pyrene and acenaphthylene indicates road traffic as major source of PAH pollution. The acetyl polymalonyl pathway in lichens results in biosynthesis of secondary metabolites of depsides and depsidones containing highly reactive –OH radicals (due to ortho effect). The depsides and depsidones easily provide their hydroxyl group for adduct formation. The higher accumulation of 2- and 3-ring PAH in lichens may be because most of the species contains depsides and depsidones with active –OH sites, which readily combine with PAH to form an adduct. *Phaeophyscia* and *Pyxine* have skyrin triterpine and lichenoxanthone (having hydroxyl group) which readily combine with most of the PAHs.

The growth form of lichens may also play a significant role in the accumulation of PAHs. The saxicolous, crustose and squamulose species growing on rocks mostly accumulated uniform concentrations of low-molecular-weight 2- and 3-ringed compounds. The higher vehicular activities or excess uses of wood and coal in a particular area are responsible for higher concentration of PAHs. The study establishes the utility of *Phaeophyscia hispidula* as an excellent biomonitoring organism in monitoring of PAHs from foot hill to sub-temperate area of Garhwal Himalaya (Shukla and Upreti 2008).

Selection of Suitable Lichen Species for Pollution Monitoring

The ability of lichens to uptake, translocate, metabolize and accumulate arsenic (As) and chromium (Cr) is one of the determining factors for phytotoxicity of these elements. Among the different growth forms, the leafy form (foliose) accumulates higher amounts of arsenic followed by the powdery (leprose) form. The squamulose (crust to leafy) and crustose (crust forming) forms accumulate lower concentration of arsenic that ranged between 0.46 ± 0.03 and 20.99 ± 0.58 μg g^{-1} DW, while the foliose and leprose lichens accumulate arsenic in the ranges of 10.98–51.95 and 28.63–51.20 μg g^{-1}DW respectively.

The cyanolichens (with blue-green photobiont) exhibit higher concentration of arsenic than the green photobiont-containing squamulose form. The active monitoring (transplant) of the same metalloid was also adopted to investigate the toxicity of excess arsenic on physiochemical process of foliose lichen *Pyxine cocoes*. The arsenic solution with concentrations of 10, 25, 50, 75, 100 and 200 μM were sprayed on lichen thallus for 45 days. The arsenic content in the thalli was then correlated with the pigment degradation, total protein concentration and the activi-

ties of antioxidant enzymes focusing on superoxide dismutase, catalase and ascorbate peroxidase. The resultant information was utilized to assess the suitability of *P. cocoes* as a biomarker against arsenic pollution in tropical environment (Fig. 10.7).

Chromium is a highly toxic nonessential metal that inhibits a variety of metabolic activities in plants. In order to investigate the physiological, chemical including amino acid profiles and genotypic changes due to Cr (+VI) stress, *Pyxine cocoes*, a known toxitolerant lichen species, were treated with different concentrations of chromium (0, 10, 25, 50, 75 and 100 μM) for 10, 20, 30 and 45 days. Results revealed that exposure to Cr (+VI) for 10, 20, 30 and 45 days under laboratory conditions causes significant decline in physiological processes with increasing metal stress. Amino acid profile at different concentrations on the 45th day too indicated stress as proline content significantly increased at 100 μM. Inter-simple sequence repeat (ISSR) and internal transcribed spacer (ITS) techniques were used to evaluate genotoxicity induced by chromium stress. Results showed that ISSR technique is more sensitive and reproducible to study polymorphism induced by environmental stress. ISSR profiles showed consistent increase in appearance and disappearance of bands with increasing concentration of the chromium.

The amino acid profile shows significant increase in concentration from control to 100 mM that ranges from 3.61 to 34.32. Among the different amino acids, proline shows significant increase in concentration. Proline plays a vital role in cellular homeostasis, including redox

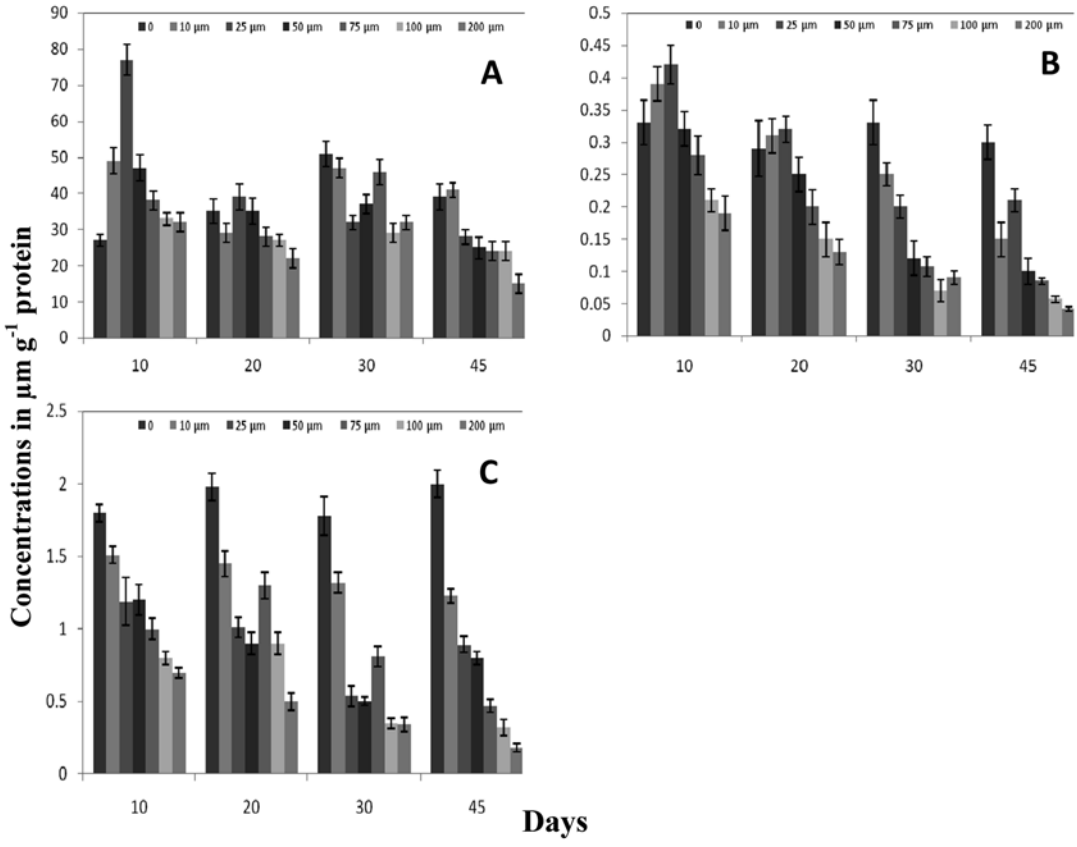


Fig. 10.7 Comparison between days and various concentration of arsenic on enzymatic activities of *P. cocoes*: (**a**) superoxide dismutase, (**b**) ascorbate peroxidase, and (**c**) catalase

balance and energy status. Proline can act as a signalling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress. The linear correlation of proline content with other biochemical parameters reveals that proline is negatively correlated with the physiological parameters, APX and catalase activity, while it has a significant positive correlation with metal content, EC, carotenoid and protein content and SOD activity.

PCR-based DNA fingerprinting methods provide an efficient tool for the investigation of mutational changes, especially point changes. ISSR-PCR analysis not only helps to find out the mutational effects of heavy metals in relation to many different environments but is also especially useful for pollution studies, as it can compare polluted and nonpolluted samples at the same time and in relatively short periods. An attempt has been made to test the applicability of ISSR-PCR technique in observing mutagenic changes due to Cr stress.

ISSR analysis showed that there were detectable DNA band variations when the lichens were exposed to chromium stress. The clear correlation between chromium stress and percentage of DNA polymorphism supports the effectiveness of ISSR analysis for investigating environmental toxicity. Changes in ISSR profiles were detected in an unspecific form by appearance and/or disappearance of bands and variations in the amplification intensity. The DNA damage due to chromium stress (or in general for hazard identification in risk assessment studies) can be illustrated by the presence of any abnormalities in the band profiles that would be enough to identify a genotoxic effect (Fig. 10.8).

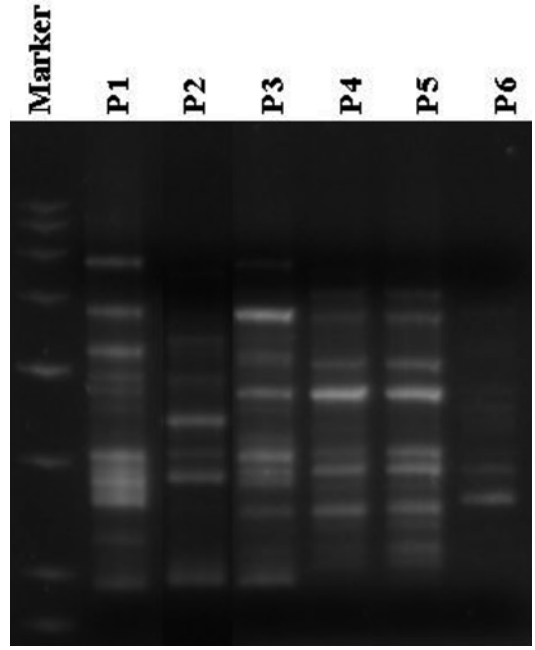


Fig. 10.8 Gel image of Cr treated lichen samples with Primer 861

10.2.2.3.3 Biomarkers

Pollutants cause damage to living organisms in different ways. Damage can occur at all levels of biological organization, from the components of individual cells to ecosystems. Traditionally, the rate of accumulation of contaminants, geographical distribution or morphological modifications have been studied in 'indicator' species. However, it is now realized that the impact of pollutants can be measured more quickly by testing their effects on certain physiological processes termed as 'biomarkers'. Lagadic et al. (1997) define a biomarker as 'an observable and/or measurable change at a molecular, biochemical, cellular, physiological or behavioural level, which reveals the present or past exposure of an individual to a chemical polluting substance'.

An ideal biomarker should be easy to measure and produce distinctive symptoms that are not confused with those caused by other environmental stresses. When properly used, biomarkers can 'forecast' impending harmful effects. Ideally, an environmental survey based on biomarkers can be used as a warning by early detection of the effects of pollutants and by detecting pollution below the dose that causes irreversible damage. Results from such survey can be used to argue for a more intensive survey of the particular ecosystem. Several parameters are best used in lichens for suitability as biomarkers are well known.

10.3 Conclusion

For a long time, lichens have been known as plant resources for various purposes and still they are the most important sources of dyes, medicines, food, fodder and perfumes. In recent years their use as a means of monitoring gaseous pollutants and extraction of HIV antigen product from these plants will definitely attract the attention of research workers to conduct more intensive researches in this branch, i.e. lichenology. It can thus be inferred that there is a vast treasure of medicines hidden in this small group of plants and proper study may unfold a vast fund of new information leading to discovery of some potential lichen species.

Acknowledgements The authors are thankful to the Director, CSIR-National Botanical Research Institute, Lucknow, for facilities and encouragements under in-house project OLP-083. RB is thankful to the Department of Science and Technology (DST-SERB), New Delhi, for award of Young Scientist fellowship (SR/FTP/ES-30/2013).

References

Bajpai R, Upreti DK (2011) Lichens a potential organism for sustainable agriculture. Environews 17(4):4–6

Bajpai R, Upreti DK, Mishra SK (2004) Pollution monitoring with the help of lichen transplant technique at some residential sites of Lucknow. J Environ Bio 25(2):191–195

Beschel R (1961) Dating rock surface by lichen growth its application to glaciology and physiography. In: Rassch GO (ed) Geology of the arctic, vol 1. University of Toronto Press, Toronto, pp 1044–1062

Dettki H, Esseen PA (1998) Epiphytic macrolichens in managed and natural forest landscapes: a comparison at two spatial scales. Ecography 21:613–624

Garty J (2001) Biomonitoring atmospheric heavy metals with lichens: theory and application. Crit Rev Plant Sci 20(4):309–371

Garty J, Tomer S, Levin T, Lehr H (2002) Lichens as biomonitors around coal fired power station in Israel. Environ Res 91:186–198

Khare R, Upreti DK, Nayaka S, Gupta RK (2009) Diversity of soil lichens in India. In: Gupta RK, Kumar M, Vyas D (eds) Soil microflora. Daya Publishing House, New Delhi, pp 64–75

Lagadic L, Caquet T, Amiard JC, Ramade F (1997) Biomarqueurs en écotoxicologie: aspects fondamentaux. Edition Masson 33:53–97

Loppi S, Putorti E, Pirintsos SA, Dominicis VD (2000) Accumulation of heavy metals in epiphytic lichens near municipal waste incinerator (central Italy). Environ Moni Ass 61:361–371

Seaward MRD, Richardson DHS (1989) Atmospheric sources of metal pollution and effect on vegetation. In: Shaw AJ (ed) Heavy metal tolerance in plants: evolutionary aspects. CRC Press, Boca Raton, pp 75–92

Sernander R (1926) Stockholms Natur. Almguist and Wiksella, Uppsala

Sharma J, Gupta SS, Kumar BP, Upreti DK, Khare R, Rao CV (2014) Effect of usnic acid and Cladonia furcata extract on gastroesophageal reflux disease in rat. Intern J Exp Pharm 4(1):55–60

Shukla V, Upreti DK (2007) Heavy metal accumulation in *Phaeophyscia hispidula* en route to Badrinath, Uttaranchal, India. Environ Monit Ass 131:365–369

Shukla V, Upreti DK (2008) Effect of metallic pollutants on the physiology of lichen *Pyxine subcinerea* in Garhwal Himalayas. Environ Monit Ass 141:237–243

Shukla V, Upreti DK (2009) Polycyclic aromatic hydrocarbon accumulation in lichen *P. hispidula* of DehraDun city, Garhwal Himalayas. Environ Monit Ass 149:1–7

Shukla P, Upreti DK, Nayaka S, Tiwari P (2014) Natural dyes from Himalayan lichens. Indian J Tradit Knowl 13(1):195–201

Singh KP, Sinha GP (2010) Indian lichens: an annotated checklist. Botanical Survey of India (MoEF), Kolkata

Srivastava R, Yadav V, Upreti DK, Sharma N (2004) Lichen flora of Bilaspur, Hamirpur & Una district of Himachal Pradesh. Phytotaxonomy 4:11–18

Srivastava P (2013) Screening of antimicrobial properties of some Indian lichens against human pathogens. PhD thesis, Dr RML Avadh University, Uttar Pradesh

Microbial Symbionts of Plants

11

N. Thajuddin, G. Muralitharan, D. Dhanasekaran, and M.H. Muhammad Ilyas

Abstract

Plants in nature always grow with soil microorganisms, and some become intimately associated with plants to form mutualistic symbiosis. Examples of such symbiotic microorganisms include mycorrhizal fungi, cyanobacteria, and N_2-fixing prokaryotes, especially rhizobia. Looser symbiotic associations involve bacteria and soil microfauna within the rhizosphere. Their metabolic activities increase nutrient availability. All of these symbioses may affect rates of growth and eventually reproduction of plants compared with growth in the absence of such associations. A symbiotic association is therefore a potential selection pressure that can influence the evolutionary success of vascular plants and hence the composition of plant communities. Application of associative bacteria for sustainable agriculture holds immense potential. These bacteria are known to enhance growth and yield of plants by fixing atmospheric nitrogen, solubilization of phosphate, production of phytohormones and siderophores, possession of antagonistic activity, as well as reducing the level of stress ethylene in host plants. This review provides examples of associations and interactions between microorganisms and plants. The cyanobacterial association with various plants such as bryophyte, pteridophyte, gymnosperm, and angiosperm was illustrated. It also describes the actinorhizae, *Frankia* and *Rhizobium*, interaction with plants and their applications.

Keywords

Symbiosis • Cyanobacteria • Actinorhizae • *Frankia* • *Rhizobium* • *Anthoceros* • *Azolla* • *Cycas* • Cyanolichen

N. Thajuddin (✉) • G. Muralitharan • D. Dhanasekaran
Department of Microbiology, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, India
e-mail: nthaju2002@yahoo.com

M.H. Muhammad Ilyas
PG & Research Department of Botany, Jamal Mohamed College, Tiruchirappalli 620 020, Tamil Nadu, India

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_11,

11.1 Introduction

A large number of microorganisms are commonly found in the soil including bacteria, fungi, actinobacteria, algae, cyanobacteria, and protozoa. Of these, bacteria are by far the most common type of soil organism as we know, grow quite rapidly, and have the ability to utilize a wide range of substances as their carbon or nitrogen sources. Bacteria can be found in soil, are bound to the surface of the soil particles, and are found in soil aggregates, but a number of these soil bacteria interact specifically with the roots of plants. The concentration of bacteria (per gram of soil) around the plant roots (rhizosphere) is greater than the bacterial concentration that is found in the rest of the soil (Montesinos 2003). This would indicate that there is a higher level of nutrient found in the zone around the roots that can be used to support bacterial growth and metabolism.

The interaction between bacteria and the roots of plants may be beneficial, harmful, or neutral for plants. Collectively phytopathogens (including harmful bacteria) can reduce crop yields by 25–100 %, which is an enormous loss of productivity and loss of income to farmers. Bacteria that stimulate plant growth represent an enormous opportunity for agriculture. Plant growth-promoting bacteria are of two general types: those that form a symbiotic relationship with the plant and those that are free-living in the soil but are found near or even in the roots of plants. Plant growth-promoting bacteria can directly stimulate plant growth in several ways. They can fix atmospheric nitrogen that is used by the plant, synthesize siderophores that solubilize and sequester iron from the soil and provide it to the plant, synthesize several different phytohormones that enhance various stages of plant growth, solubilize minerals such as phosphorus that are used by the plant, and synthesize enzymes that can modulate plant hormone levels (Buée et al. 2009).

Some beneficial bacteria live in association with eukaryotic host plant by symbiotic association and fix nitrogen. These include bacteria such as *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium* (collectively rhizobia) in legume symbiosis, *Frankia* in actinorhizal symbiosis, and cyanobacteria in plant symbiosis. Present review aims to highlight various plant symbiotic microorganisms and updates of bryophytes, angiosperm, and pteridophyte and gymnosperm interactions with cyanobacteria, *Frankia*, and *Rhizobia* and also their applications in plant growth improvement.

11.2 Cyanobacterial Associations

Cyanobacteria form a variety of associations, habitually symbiotic in nature with plants, lichens, sponges, and fungi (Thajuddin and Subramanian 2005). Their associations aid the host either by providing fixed nitrogen or carbon and in turn gain nutrients for their growth apart from using the host as a substratum for growth. Symbiotic associations are an important component of the ecology of many cyanobacterial lineages and include interactions with plants (Rai et al. 2000), fungi (Rai 1990), animals (Wilkinson 1992), and eukaryotic algae (Janson 2002; Murakami et al. 2004). The cyanobacterial genus *Nostoc* presents an interesting case for studying host specialization because of the wide number of symbiotic associations formed by members of this genus. In addition to its role as a photosynthetic partner (photobiont) of a wide range of lichenized fungi (Tschermak-Woess 1988), *Nostoc* also forms symbiotic associations with a number of different plants, including bryophytes (Adams 2002), cycads (Costa and Lindblad 2002; Thajuddin et al. 2010), the flowering plant *Gunnera* (Bergman 2002), and possibly the fern *Azolla* (Plazinski et al. 1990; Peters and Meeks 1989; Baker et al. 2003; Kannan et al. 2014), as well as the non-lichen fungus *Geosiphon pyriforme* (Kluge et al. 2002). Apart from having symbiotic associations with other organisms, cyanobacteria were also found to be associated with inanimate substances like rocks in extreme environments. A study on microbial mats collected from Antarctic land reports the presence of diverse cyanobacterial species dominated by the members of the genera, viz., *Phormidium*, *Nostoc*, *Chroococcus*, *Oscillatoria*, *Calothrix*,

Lyngbya, and *Plectonema*, highlighting the ability of cyanobacteria to thrive at extreme environments in association with abiotic substances (Singh et al. 2008).

11.3 Marine Cyanobacterial Symbiosis

In the marine environment, symbioses are known to occur between cyanobacteria and sponges, ascidians (sea squirts) and echiuroid worms in the benthos and diatoms, and dinoflagellates and a protozoan among the plankton (Carpenter 2002). Cyanobacteria have been found in cells of the sub-epidermal connective tissue of two marine echiuroid worms, *Ikedosoma gogoshimense* and *Bonellia fuliginosa* (Rai 1990). In the Didemnidae family of sea squirts, five genera form associations with either of the two cyanobacterial genera, *Synechocystis* and *Prochloron*. *Tridemnum miniatum*, a colonial ascidian, is found to harbor the photosymbiotic prokaryote *Prochloron* sp. (Hirose et al. 2006). Metagenomic analysis reveals 7 % cyanobacteria of the microbial community is associated with the coral *Porites astreoides* (Wegley et al. 2007).

11.4 Bryophytes

Bryophytes are small, nonvascular land plants encompassing the liverworts (Hepaticae), the hornworts (Anthocerotae), and the mosses (Musci), a relatively small number of which are able to form epiphytic or endophytic associations with cyanobacteria (Adams 2002; Meeks 2003). The cyanobacterial symbionts are often filamentous and fix N_2 in specialized cells known as heterocysts, enabling them to provide the host with fixed nitrogen and, in the case of non-photosynthetic hosts, with fixed carbon. The symbionts are usually *Nostoc* spp. that gain entry to the host by means of specialized motile filaments known as hormogonia. The host plant releases chemical signals that stimulate hormogonia formation by chemo-attraction and guide the hormogonia to the point of entry into the plant tissue. Inside the symbiotic cavity, host signals inhibit further hormogonial formation and stimulate heterocyst development and dinitrogen fixation. The cyanobionts undergo morphological and physiological changes, including reduced growth rate and CO_2 fixation and enhanced N_2 fixation, and release to the plant much of the dinitrogen fixed (Adams and Duggan 2008). In the case of the mosses, the cyanobacteria grow mostly epiphytically (Solheim and Zielke 2002; Gentili et al. 2005), the exception being two *Sphagnum* species in which the cyanobacteria occupy water-filled, hyaline cells, which are thought to provide some protection from the acidic bog environment (Solheim and Zielke 2002).

Similar protection for cyanobacteria on the moss leaf surface is provided by the secretion of alkaline substances (Belnap 2001). These nitrogen-fixing cyanobacterial associations with mosses often supply most of the combined nitrogen in local ecosystems in the Arctic, the Antarctic, and boreal forest regions (Zielke et al. 2002, 2005). This may be particularly significant in boreal forests where the feather moss *Pleurozium schreberi* (with its epiphytic cyanobacteria) can provide 80 % of the ground cover (DeLuca et al. 2002, 2007; Zackrisson et al. 2004; Gentili et al. 2005).

Cyanobacterial associations with liverworts are rare, being found in only four of the >340 liverwort genera, two of the associations (*Marchantia* and *Porella*) being epiphytic and two (*Blasia* and *Cavicularia*) endophytic (Meeks 1990). By contrast, in the hornworts, of which there are presently 13 genera described (Duff et al. 2007), endophytic associations are ubiquitous (Renzaglia et al. 2007). In nature, liverworts and hornworts exist as a flattened gametophyte thallus of a few centimeters in length, and symbiotic colonies are visible as small, dark spots. The endophytic associations with hornworts, such as *Anthoceros* (Fig. 11.1) and *Phaeoceros*, and liverworts, such as *Blasia*, are particularly suited to laboratory experimentation because the host plant can be freed of its symbiotic cyanobacteria and grown in shaken liquid culture and the symbiosis reestablished with the original or with

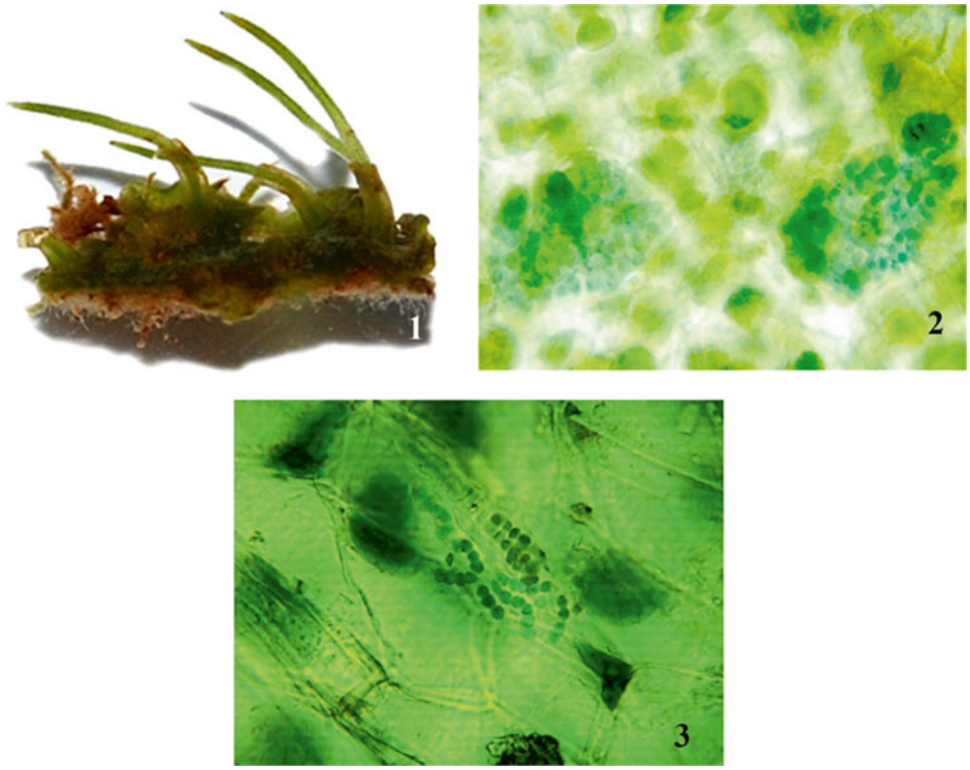


Fig. 11.1 (*1*) *Anthoceros* thallus with sporophyte; (*2*) CS of *Anthoceros* thallus with *Nostoc* colonies; (*3*) closer view of *Nostoc* filaments in the CS of *Anthoceros* thallus

novel cyanobacteria (Meeks 1998, 2003; Adams 2002).

11.5 Pteridophytes

Cyanobacterial associations with pteridophytes are limited to the genus *Azolla* in the family Azollaceae. *Azolla* is a small floating aquatic fern with a worldwide distribution ranging from tropical to warm temperate regions. It has been exploited for many years as a source of nitrogen for agriculture and is extensively used as a green manure and biofertilizer for rice (Watanabe and Roger 1984; Ladha et al. 2000; Van Hove and Lejeune 2002). The nitrogen-fixing cyanobacteria are hosted in a highly specialized cavity located on the dorsal lobe of the leaves (Peters and Mayne 1974; Zheng et al. 2009). An envelope lines the cavity where cyanobacterial filaments are localized in the periphery within a mucilaginous matrix surrounding a gaseous central region. Morphological analysis of the leaf cavity established that a pore remains open during leaf development, even when the leaf is mature, thus permitting gas exchanges (Veys et al. 1999). Throughout its life cycle, the symbiont remains associated with its host and is automatically transmitted from generation to generation, including during sexual reproduction (Calvert et al. 1985; Peters and Meeks 1989). So far, there are no confirmed reports of successful *in vitro* cultivation of the cyanobiont that belongs to the order Nostocales, making *Azolla* symbiosis the only known permanent symbiosis among cyanobacteria–plant associations (Lechno-Yossef and Nierzwicki-Bauer 2002; Pabby et al. 2003). Besides the cyanobiont, it has been shown that minor cyanobacterial and bacterial species coexist in the cavity (Gebhardt and Nierzwicki-Bauer 1991). Besides *Anabaena azollae*, there are other cyanobacteria like *Westiellopsis* sp., *Nostoc* sp.,

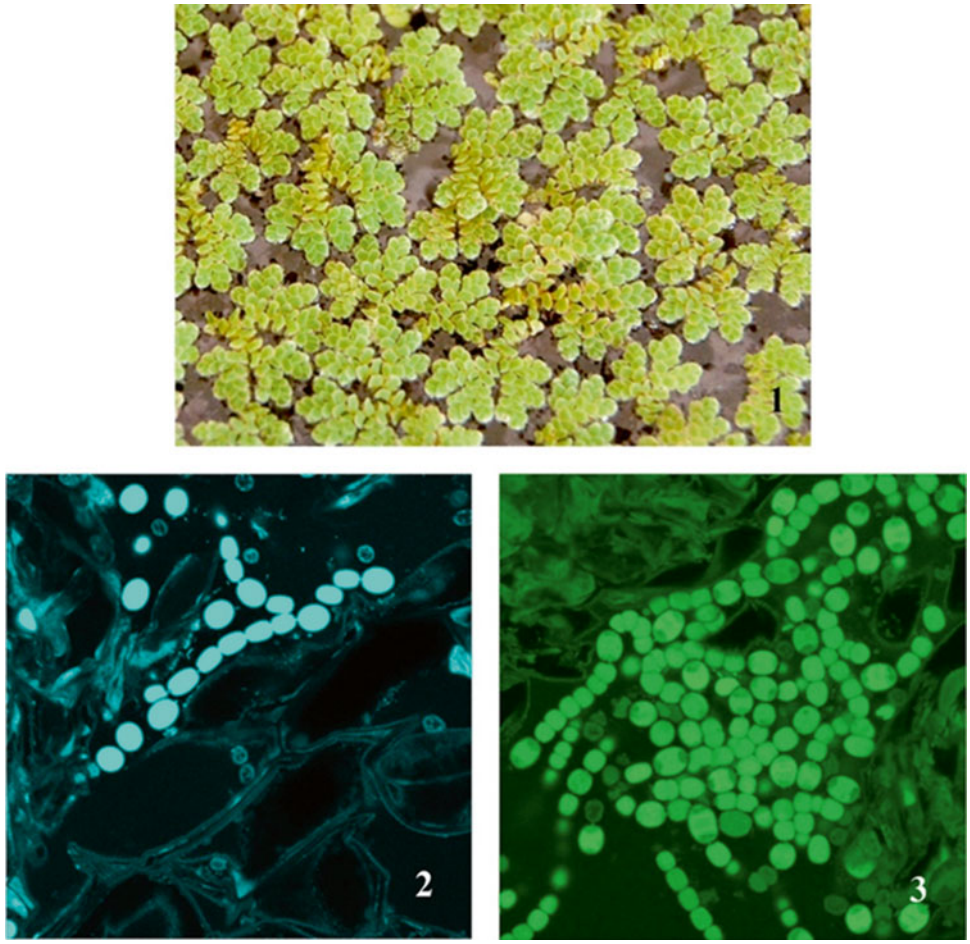


Fig. 11.2 (*1*) *Azolla filiculoides*; (*2*) confocal view of *Anabaena azollae* in the *Azolla* thallus; (*3*) confocal view of *Nostoc* sp. in the *Azolla* thallus

and *Anabaena variabilis* which were found to be associated with *Azolla filiculoides* (Fig. 11.2) (Thajuddin et al. 2007; Kannan et al. 2014).

11.6 Gymnosperms

Cycads are the only known gymnosperms that have the ability to develop a nitrogen-fixing symbiosis through an intimate association with cyanobacteria. Cycads include approximately 156 species in nine genera that grow naturally in tropical and subtropical regions, and all of them possess a symbiotic cyanobacterium. Cyanobacteria are hosted in specialized coralloid roots that arise from the lateral roots and are formed by the plant before being invaded by the cyanobacteria (Costa and Lindblad 2002). Filamentous cyanobacteria are located intercellularly in a zone filled with mucilage and comprise a large number of elongated cycad cells that interconnect two adjacent cortical layers in the coralloid roots. These cells may contribute to the transfer of metabolites between the symbionts and the host. In transverse root sections, cyanobacteria are visible as a green ring. *Nostoc* sp. is the most common cyanobiont in Cycadaceae, although *Calothrix* has occasionally been reported (Costa et al. 1999; Thajuddin et al. 2010).

The cycad family Zamiaceae contains three genera *Bowenia*, *Lepidozamia*, and *Macrozamia* (Hill and Osborne 2001). *Nostoc* spp. are the

most commonly found cyanobionts, although a species of *Calothrix* (Grobbelaar et al. 1987) has also been reported in *Encephalartos hildebrandtii* and *Anabaena* endosymbionts have been reported in *Cycas revoluta* (Obukowicz et al. 1981; Zhu 1982). Increased heterocyst frequencies were observed in the roots of *Zamia skinneri* from 16 to 86 % over the length of the coralloid root (Lindblad et al. 1985). Different strains of *Nostoc* cyanobacteria have been identified in host cycads from different geographical areas. Zimmerman and Rosen (1992) collected aseptic soil samples surrounding the coralloid roots of a cultivated population of *Z. integrifolia* and a *Dioon* plant and plated them on nitrogen-free BG11 medium. The filamentous soil cyanobacteria surrounding the cycad root system consisted of *Oscillatoria* and *Calothrix* spp. and a *Nostoc* sp. This *Nostoc* isolate was unable to colonize coralloid roots in infection studies, despite being the predominant *Nostoc* sp. in the soil. Lotti and associates (1996) discovered some diversity in cultured *Nostoc* isolates from cycad plants of the genera *Cycas*, *Encephalartos*, *Macrozamia*, *Lepidozamia*, and *Dioon* from different botanical gardens and greenhouses around Italy. Thajuddin et al. (2010) and Praveenkumar et al. (2007) reported the presence of *Calothrix* sp. and several *Nostoc* sp. and *Anabaena* sp. in symbiotic association with cycads such as *Cycas circinalis*, *C. rumphii*, and *C. revoluta* (Figs. 11.3 and 11.4).

Planktonic uncultured nitrogen-fixing cyanobacterium (UCYN-A) has a symbiotic association with a unicellular algae prymnesiophyte, closely related to calcifying taxa present in the fossil record. The partnership is mutualistic, because the prymnesiophyte receives fixed N in exchange for transferring fixed carbon to UCYN-A (Thompson et al. 2012).

11.7 Angiosperm

Gunnera is the only angiosperm that lives in a symbiotic organization with *Nostoc* (Khamar et al. 2010; Rasmussen et al. 1994; Svenning et al. 2005). The *Gunnera–Nostoc* relationship is endosymbiotic however facultative, in that neither partner needs the other for survival (Zimmerman and Bergman 1990). The *Nostoc–Gunnera* symbiosis exhibits unique features compared to other cyanobacteria–plant symbioses; the cyanobacterium infects specialized gland organs (mucilage secreting) located on the stems of the host, and once it has passed into the interior of the gland, the cyanobacterium also enters the *Gunnera* cells where it starts to differentiate the highest frequency of heterocysts (the N_2-fixing cells) recorded in any cyanobacterial population (Bergman et al. 1992). *Gunnera* may benefit its *Nostoc* symbionts by providing reduced carbon. Although free-living *Nostoc* species can support N_2 fixation through photosynthesis, under symbiotic conditions, they rely on photosynthate from the host plant (Khamar et al. 2010).

11.8 Lichens

Symbioses between cyanobacteria and lichen-forming fungi occur worldwide in a wide range of terrestrial environments, ranging from tropical rainforests to hot and cold deserts. Lichens are complex organisms involving a symbiotic relationship between a photobiont (green alga or a cyanobacterium or both) and mycobiont. Lichens appear in morphologically distinct forms such as crustose, foliose, or fruticose. The identity and taxonomic affiliation of lichenized cyanobacteria are generally less clearly understood than that of lichenized fungi. Majority of lichen-forming fungi, belonging to Ascomycota, are associated with green algae known as "phycolichens," and over 1,500 species of lichen-forming fungi have cyanobacteria as primary or accessory photosynthetic partners referred to as "cyanolichens" (Rikkinen 2002). Cyanolichens are highly specialized, stable symbioses between heterotrophic fungi (mycobionts), mainly Ascomycota, and photosynthetic diazotrophic cyanobacteria (cyanobionts).

Quraishi (1928) and Chopra (1934) were the pioneer lichenologists in India who studied the Himalayan lichens. D. D. Awasthi, "father of Indian lichenology," started the study of lichens

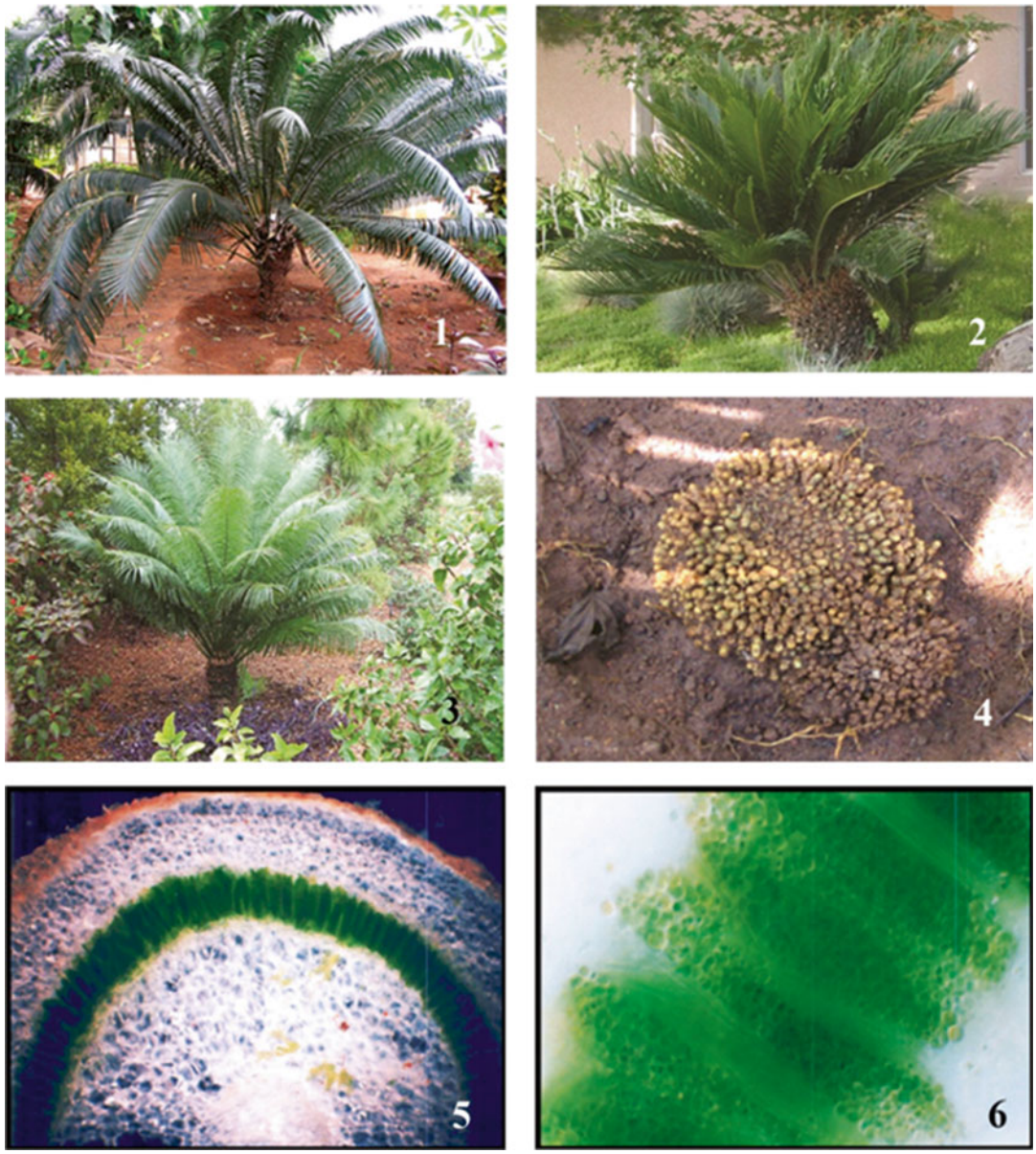


Fig. 11.3 (*1*) *Cycas rumphii*, (*2*) *Cycas revoluta*, (*3*) *Cycas circinalis*, (*4*) coralloid roots of *C. rumphii*, (*5*) coralloid root – CS Showing cyanobacterial zone; (*6*) closer view of cyanobacterial zone in the coralloid root

in a systematic way (Awasthi 1965, 1975, 1988, 1991). His work on lichens of Western Ghats was first published in 1957 (Awasthi 1957), which reported new species, like *Parmelia* (*Hypogymnia*) *pseudobitteriana* (from Kodaikanal, Tamil Nadu). The school of lichenology at Lucknow University contributed a lot to the lichens of Nilgiri and Palni Hills (Singh 1984). The lichenological investigation in Western Ghats was strengthened during the 1960s with the setting up of the school of lichenology at Agharkar Research Institute (Maharashtra Association for Cultivation of Science), Pune. Lichenology laboratory of the National Botanical Research Institute, Lucknow, and Botanical Survey of India have compiled

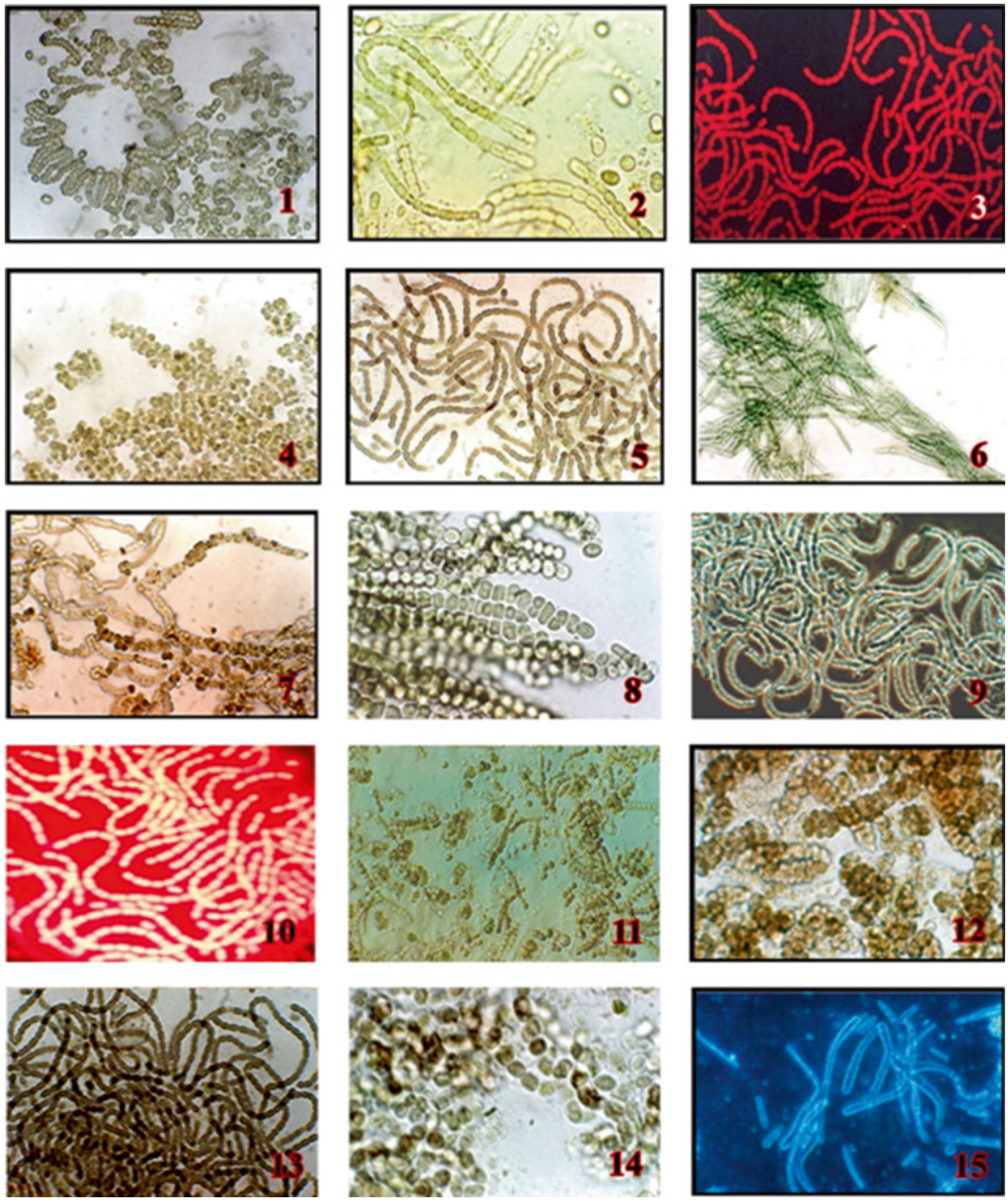


Fig. 11.4 Diversity of symbiotic cyanobacteria in the coralloid root of *Cycas rumphii*. (*1–8*), (*11*), (*12* and *14*), different strains of *Nostoc*; (*9*), (*10* and *13*), different strains of *Anabaena*; (*15*) *Calothrix* sp. (Thajuddin et al. 2010)

vast information on the lichens of Western Ghats. Singh (1980) consolidated the lichenological investigation between 1966 and 1977. Upreti (1995, 1997) and Upreti and Nayaka (2000, 2003) updated the developments in Indian lichenology. Notable work has also been carried out on the diversity and distribution of lichens in south Indian habitats (Hariharan 1991; Hariharan et al. 2003; Shyamkumar 2007; Shyamkumar et al. 2009, 2011b).

Symbiotic genotypes of *Nostoc* (Nostocales, Cyanobacteria) occur in a wide variety of cyanolichens, either as the primary photobionts in bipartite lichens or as accessory photobionts together with green algae in tripartite lichens. *Nostoc* symbionts are especially common in Peltigeralean lichens (*Peltigerales*, Ascomycota), and, for example, all species of *Collema*, *Leptogium*, *Nephroma*, *Peltigera*, *Pseudocyphellaria*, and *Sticta* associate with *Nostoc* (Rikkinen 2002, 2009). Species of *Peltigera* and *Nephroma* are common and sometimes abundant in many temperate and cool regions of the world. Species of *Peltigera* can be grouped into eight monophyletic sections: *Polydactylon*, *Chloropeltigera*, *Peltidea*, *Horizontales*, *Peltigera*, *Retifoveata*, *Phlebia*, and *Hydrothyriae*, with the last three groups being monotypic (Miadlikowska and Lutzoni 2000). Mycobionts of cyanolichens tend to associate with a limited number of closely related *Nostoc* genotypes or genotype groups. Symbiotic strains of *Nostoc* group into two distinct phylogenetic lineages which associate with different groups of lichen-forming fungi. One group is found in bipartite species of *Nephroma*, *Sticta*, and *Pseudocyphellaria*, while the other group associates with many species of *Peltigera* (Rikkinen 2013).

Shyamkumar (2007) and Shyamkumar et al. (2009, 2011a) recorded that a total 77 species belonging to 32 genera and 21 families of lichens from Yercaud and Kolli hills. As many as 55 and 48 species of lichens were recorded in Yercaud and Kolli hills, respectively. Of the 77 species of lichens recorded, only 5 were cyanolichens (*Leptogium javanicum*, *L. milligranum*, *L. chloromelum*, *Collema auriforme*, and *C. rugosum*) recorded in both Yercaud and Kolli hills, and 28 species of lichens were for the first time reported in Yercaud (Fig. 11.5). Physiological (pH, temperature, light, salinity, and heavy metals) and biochemical (fatty acid analysis) properties of lichen (*Collema auriforme*) and symbiotic cyanobacterial isolates from cyanolichens were studied (Shyamkumar and Thajuddin, 2009a, b; Shyamkumar et al. 2009, 2011b). Symbiotic association of cyanobacteria with lichens was proved to exhibit antimicrobial properties as well. The lichen *Collema auriforme* found associated with the cyanobacteria *Aphanocapsa* sp. and *Nostoc* sp. exhibited significant antimicrobial activity against bacterial pathogens such as *E. coli*, *Klebsiella* sp., and *Staphylococcus* sp. (Shyamkumar et al. 2010). A few free-living strains of *Nostoc* are known to produce microcystins in aquatic environments (Sivonen et al. 1990), and also symbiotic *Nostoc* strains from lichens and cycads were shown to encode this pathway and produce microcystins and nodularins (Oksanen et al. 2004; Kaasalainen et al. 2009, 2012; Gehringer et al. 2012). Kaasalainen et al. (2012) found microcystins in 45 lichen specimens representing several species of *Peltigera*, *Nephroma*, and *Sticta* and one specimen of *Lobaria* (*Peltigerales*). However, microcystins are not produced in all cyanolichens and appear to be more common in some genera than others. The lichen extract showed activity against three test pathogenic bacteria, and no antibacterial activity was found using the cyanobacterial extracts. Molecular studies, namely, 16S rRNA amplification and sequencing, secondary structure prediction, restriction enzyme mapping of 16S rRNA, and RAPD studies were carried out for the symbiotic cyanobacterial isolates such as *Aphanocapsa* sp. NTK28, *Nostoc* sp. NTK28, and *Nostoc* sp. NTY30 (Shyamkumar 2007). The 16S rRNA gene sequences were deposited in GenBank [Accession numbers (NCBI, EMBL, and DDBJ) DQ 513318, DQ 513319, and DQ 513320] and studied for their taxonomy and phylogeny. Similarly, 31 species of 18 genera belonging to 6 families of cyanobacteria were recorded as epiphytic forms on lichens. Several cyanobacterial genera *Chroococcus*, *Gloeocapsa*, *Synechococcus*, *Microcystis*, *Aphanocapsa*, *Dermocarpa*, *Myxosarcina*, *Xenococcus*, *Oscillatoria*, *Phormidium*, *Lyngbya*, *Nostoc*, *Anabaena*, *Plectonema*, *Scytonema*, and *Microchaete* were reported to be in association with lichens growing together as epiphytes, and three taxa, namely, *Nostoc* sp., *Plectonema* sp., and *Aphanocapsa* sp. were found most frequently and predominantly growing on lichens (Shyamkumar et al. 2013).

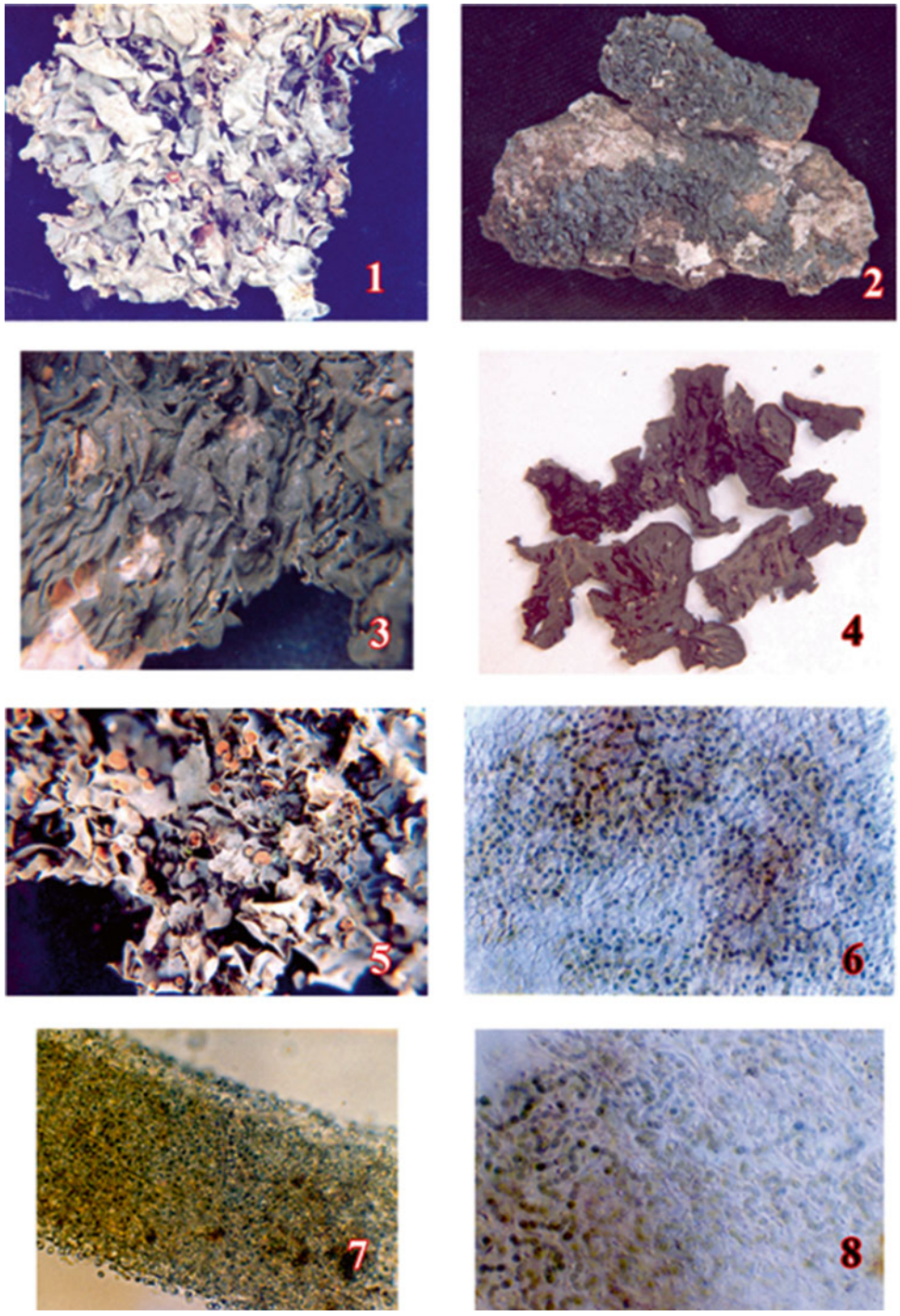


Fig. 11.5 Cyanolichens (*1*) *Leptogium chloromelum*, (*2*) *Leptogium milligranum*, (*3*) *Collema auriforme*, (*4*) *Collema rugosum*, (*5*) *Leptogium javanicum*, (*6*) CS of *Leptogium milligranum*, (*7*) CS of *Collema auriforme*, (*8*) CS of *Collema rugosum*

Fig. 11.6 Endophytic cyanobacterium *Richelia intracellularis* residing in *Rhizosolenia* (diatom)

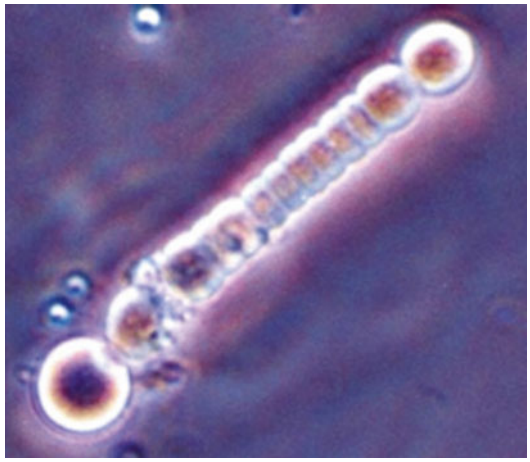


Fig. 11.7 *Richelia intracellularis* filament with terminal heterocyst

11.9 Diatom

The relationship between the diatoms and cyanobacteria is assumed to be symbiotic, and it has frequently been reported from different seas (Deyoe et al. 1992; Villareal 1994; Carpenter and Janson 2000). Gomez et al. (2005) reported the occurrence of a large number of *Richelia–Chaetoceros* consortia in western Pacific Ocean. *Hemiaulus hauckii–Richelia* blooms are very common in different parts of the world. *Richelia* associated with *Rhizosolenia clevei* Osten. is present in the surface waters. Sundström (1984) recorded the cyanobacterium *Richelia intracellularis* as endosymbionts in the diatom *Rhizosolenia clevei*. Thajuddin (1991) and Thajuddin and Subramanian (1992, 1994) reported the frequent occurrence of symbiotic cyanobacterium *Richelia intracellularis* within *Rhizosolenia* from Bay of Bengal, southeast coast of India (Figs. 11.6 and 11.7).

Villareal (1990, 1992) recorded the widespread occurrence of *Hemiaulus*–cyanobacterial symbioses in Southwest North Atlantic Ocean. Heinbokel (1986) studied the occurrence of *Richelia intracellularis* with diatoms *Hemiaulus hauckii* and *Hemiaulus membranaceus* off Hawaii. In the Baltic Sea, Snoejis and Murasi (2004) recorded the diatom communities found to occur in nitrogen-fixing cyanobacterium *Rivularia atra*. They reported these communities for the first time; among these, the dominant forms of pennate genera include *Amphora*, *Berkeleya*, *Cymbella*, *Entomoneis*, *Epithemia*, *Mastogloia*, etc. The main advantage of this symbiotic condition includes protection against grazing, protection against physical disturbances, and suitable substratum for mobility. Brehm et al. (2003) published a short communication on the symbiosis of cyanobacteria and diatom with bacteria. They observed in the case of filamentous cyanobacterium *Phormidium* together with benthic diatom *Navicula* in culture condition. In cultures, they found that *Phormidium* filaments tightly intertwined with each other and formed a surface of spheres, trapping the diatoms inside. The presence of *Richelia intracellularis* epiphytically associated with *Chaetoceros compressus* is restricted to Indian Ocean and western Pacific Ocean. The common occurrence of *Richelia intracellularis* as an endosymbiont is ubiquitous in all warm seas and substantial inputs of nitrogen. A novel symbiotic condition of *Hyalodiscus laevis* with *Oscillatoria* sp. has been observed in samples collected soon after the monsoon in the Bay of Bengal, India (Prema and Anand 2012).

11.10 Sponges

Sponges (phylum Porifera), the most ancient multicellular filter feeder animals, host a wide range of symbiotic microorganisms that have been largely represented by both heterotrophic

and photosynthetic bacteria (Wilkinson 1978; Lee et al. 2001). Cyanobacteria, the photosynthetic symbionts, are common among temperate and tropical coral reef sponges (Usher 2008) apart from zooxanthellae and filamentous algae (Carballo and Vila 2004). Symbiotic cyanobacteria provide a range of specialized services for the host's survival and growth, including photosynthesis, nitrogen fixation (Wilkinson and Fay 1979), UV protection (Proteau et al. 1993; Shick and Dunlap 2002), and antifeedants (Cox et al. 2005). Cyanobionts contribute up to 80 % of sponge's carbon budget (Cheshire et al. 1997) through photosynthesis or phagocytosis and digestion of symbiotic microbes (Maldonado and Young 1998). Coral reef sponges have been reported to be colonized by cyanobacterial symbionts belonging to the genera *Synechocystis* (Larkum et al. 1988), *Aphanocapsa* (Feldmann 1935; Usher et al. 2004), and *Anabaena* (Larkum 1999) and the species *Oscillatoria spongeliae* (Berthol et al. 1982; Thacker and Starnes 2003; Ridley et al 2005). The most common sponge cyanobiont, *Synechococcus spongiarum*, has a generalist to specialist association pattern across distantly related host species despite their geographical isolation by distance (Erwin and Thacker 2008), which has been hypothesized to be the result of selective enrichment by the host (Hentschel et al. 2002).

Epibiotic marine microbes are of recent interest as they were shown to produce novel metabolites and enzymes with unique properties. Marine microbes are believed to produce novel metabolites with bioactive potential so as to flourish in the highly competitive environment. Though actinomycetes from terrestrial soil were known for decades for their bioactive potential, *Brachybacterium paraconglomeratum*, an actinomycete found in association with marine sponge *Dendrilla nigra*, was reported to produce industrially important glycolipid biosurfactant (Kiran et al. 2014), and *Streptomyces* sp. has been reported with anticancer properties (Ravikumar et al. 2010). Kiran et al. (2010) reported that the marine fungi *Aspergillus* sp. MSF1 isolated from a marine sponge *Dendrilla nigra* produce rhamnolipid biosurfactant which showed potential activity against the pathogenic yeast *Candida albicans* and Gram-negative bacteria.

11.11 Actinorhizal Associations

Two types of root nodule symbioses between nitrogen-fixing soil bacteria and higher plants are known: rhizobial symbioses with ca. 80 % of all members of the legume family as well as one nonlegume genus, *Parapsonia*, and actinorhizal symbioses represented by a diverse group of more than 200 species from 8 different families, collectively called actinorhizal plants, and actinobacteria of the genus *Frankia* are symbiotically associated with these plants. In both cases, the microsymbionts induce the formation of special organs, the root nodules on the roots of their host plants, and fix nitrogen while being stably intracellularly accommodated in nodule cells and being supplied with carbon sources by the plant (Pawlowski and Demchenko 2012). The *Frankia*–plant symbiosis is widespread in nature, providing fixed nitrogen to nearly 200 known species of plants collectively distributed on every continent, except Antarctica, and in most climate zones (Benson and Silvester 1993). Actinorhizal plants are pioneer species that add nitrogen and organic material to nutrient-poor or new soils. Some species are grown commercially for timber and windbreaks, such as *Alnus* (alder) and *Casuarina* trees. When infected with *Frankia*, root nodules that appear as repeatedly branching truncated lateral roots are induced.

Frankia

Frankia is a filamentous bacteria with slow and pleomorphic growth. When grown on most media, frankiae are characterized by three structural forms, hyphae, sporangia, and vesicles. The hyphae are septate and often tightly interwoven in culture; in all strains studied, they produce either terminal or intercalary multilocular sporangia. Vesicles are typically formed under nitrogen-free or nitrogen-poor conditions (Tjepkema et al. 1980; Zhang and Benson 1992) and are the sites of nitrogenase. A striking feature

of the vesicles is the surrounding envelope which consists of multiple layers of bacterial steroid lipids, hopanoids (Berry et al. 1993), which presumably assist in the regulation of oxygen tension near dinitrogenase. In symbiosis, the host plant obviously plays a significant role in modifying *Frankia* morphology since there is a large variation in the presence and absence of sporangia and in the size, shape, or presence of vesicles. In liquid media, an exponentially growing culture forms spherical or ellipsoidal mycelial colonies, and in non-exponential or overgrown cultures, only a huge uniform mycelium is observed. In solid media, *Frankia* appears as colonies formed by branched and dispersed mycelia. NIR (nitrogen-reducing) vesicles and sporangia generally are seen in centers of colonies that correspond to the oldest hyphae due to peripheral growth in the hyphae tips.

Actinobacterial genus *Frankia* (Becking 1970) comprises of heterotrophic Gram-positive bacteria having a high G+C content between 66 and 77 mol%. *Frankia* is characterized for growing as a septated mycelium that differentiates into hyphae, NIR vesicles, and multilocular sporangia and for its ability to fix atmospheric N_2 both in free-living state (*in vitro*) and in symbiosis (*in planta*) with actinorhizal plants distributed in eight families of angiosperms (Benson and Silvester 1993; Dommergues et al. 1999).

All *Frankia* strains, including *Casuarina* isolates, are able to form NIR vesicles *in vitro* and *in planta*; however, no recognizable NIR vesicles have been observed in *Casuarina* nodules. The morphology of symbiotic NIR vesicles in root nodules is host plant dependent, forming either spherical or septate vesicles (*Alnus*, *Elaeagnus*), pear-shaped and nonseptated vesicles (*Ceanothus*), or club-shaped vesicles (*Coriaria*, *Comptonia*, *Datisca*) (Torrey 1985). Because the same strain can exhibit a different morphology in different host species, it has been suggested that the differentiation of the endosymbiont is under host plant control.

Frankia are heterotrophic, aerobic, and sometimes microaerophilic, are slow growers, and have an optimal temperature 25–33 °C (Lechevalier and Lechevalier 1990). It can be cultured in the presence of very high oxygen and still retain nitrogenase activity. The vesicles of culture grown in high concentration of oxygen bear more layers of wall material, indicating that the microorganism is morphologically as well as physiologically responsive to its environment. They are able to utilize a variety of carbon sources relatively well on small molecular weight compounds such as propionate, malate, pyruvate, and succinate, and particularly cluster 1 strains can grow on Tween 80. In contrast, growth on sugars is poor. Glycogen and trehalose have been identified as major storage compounds in *Frankia* with their level correlated negatively with the energy demanding N_2 fixation activity (Lopez et al. 1984). Several *Frankia* degrade cellulose and some have pectinase and proteinase activity.

Chemotaxonomic studies have revealed that *Frankia* has a type III cell wall containing meso-diaminopimelic acid, alanine, glutamic acid, muramic acid, and glucosamine (Lechevalier and Lechevalier 1990). Whole-cell sugars include variable amounts of fucose, ribose, xylose, madurose, mannose, galactose, glucose, rhamnose, and 2-*O*-methyl-D-mannose (Lechevalier and Lechevalier 1990; Mort et al. 1983). Phospholipid analysis places *Frankia* strains into group PI, containing phosphatidylinositol, phosphatidylinositol mannosides, and diphosphatidylglycerol; nitrogen-containing phospholipids are absent (Lechevalier and Lechevalier 1990). The discovery of hopanoids in some *Frankia* strains may provide an additional chemotaxonomic marker in the near future (Berry et al. 1991). In the few strains that have been analyzed, a menaquinone pattern consisting mainly of MK9(H4) (menaquinone with nine isoprene units, four of which are hydrogenated) with lesser amounts of MK9(H6) and MK9(H8) was found (Lechevalier et al. 1987).

11.11.1 Actinorhizal Plants

The plants able to establish a symbiotic association with *Frankia*, referred to as actinorhizal species, are perennial trees or shrubs (Tjepkema and Torrey 1979). To date, about 194 species and 24 genera of such plants have been identified (Table 11.1).

Table 11.1 Actinorhizal plants

Subclass	Order	Family	#nod/#gen	Genus	Nodulated species (number)	Habitat
Hamamelidae	Fagales	Betulaceae	1/6	*Alnus*	47	Bogs, riparian
		Casuarinaceae	4/4	*Allocasuarina*	54	Sand dunes, saline, desert
		Myricaceae	2/3	*Casuarina*	16	Bogs, ocean dunes
				Ceuthostoma	2	
				Gymnostoma	18	
				Comptonia	1	
				Myrica	28	
Rosidae	Rosales	Elaeagnaceae	3/3	*Elaeagnus*	38	Poor soils, disturbed sites
		Rhamnaceae	7/55	*Hippophae*	2	Chaparral, upland
		Rosaceae	5/100	*Shepherdia*	2	Semiarid soils, sand gravelly soil
				Ceanothus	31	
				Colletia	4	
				Discaria	5	
				Kentrothamnus	1	
				Retanilla	2	
				Talguenea	1	
				Trevoa	2	
				Cercocarpus	4	
				Chamaebatia	1	
				Cowania	1	
				Dryas	1	
				Purshia	5	
Magnoliidae	Cucurbitales	Coriariaceae	1/1	*Coriaria*	5	Gravel, poor soils
Dilleniidae		Datiscaceae	1/1	*Datisca*	2	Gravel streams

Contrary to rhizobial symbiosis that concerns a single family (Leguminosae) and the genus *Parasponia* of the Ulmaceae, actinorhizal species are taxonomically diverse and distributed in eight families and seven orders of angiosperms (Baker and Schwintzer 1990; Benson and Silvester 1993; Swensen 1996). These plants have in common a predilection to grow in marginally fertile soils, and they often serve as pioneer species early in successional plant community development.

Representatives can be found in most climatic zones, and they inhabit a variety of ecosystems including arctic tundra (*Dryas* species), coastal dunes (*Casuarina*, *Hippophae*, *Myrica*, and *Elaeagnus* species), riparian (*Alnus* and *Myrica* sp.), glacial till (*Alnus* and *Dryas* species), forest (*Alnus*, *Casuarina*, *Coriaria*, and *Shepherdia* sp.), chaparral and xeric (*Casuarina*, *Purshia*, *Ceanothus*, *Cercocarpus*, *Comptonia*, and *Cowania* sp.), and alpine (*Alnus* sp.). The input of fixed nitrogen by these plants can be considerable, especially in colder temperate areas where indigenous legumes are absent or rare (Silvester 1976). Pollen distributions in marine sediment cores have recorded the past roles played by actinorhizal plants in colonizing deglaciated soils during periods of major climatic change (Heusser and Shackleton 1979). In the postglacial period of the early Holocene (10,000 to 8,000 B.P.), *Alnus* became the dominant plant in North America and Europe, eventually accounting for about 40 % of the tree pollen in postglacial Britain and North America (Silvester 1976). Because they often thrive on marginal soils, actinorhizal plants have current and potential applications in reclaiming and conditioning the soil, producing timber and pulp, and acting as nurse, windbreak, ornamental, and fuelwood plants (Dawson 1990; Diem and Dommergues 1990). Globally, they have potential for integrating into schemes for addressing issues of pyrodenitrification (Crutzen and Andreae 1990) and reforestation (Anonymous 1984; Diem and Dommergues 1990).

11.11.2 Transgenic Actinorhizal Plants

Genetic engineering of Casuarinaceae plants has been developed at the Institut de Recherche pour le Developpement (IRD) at Montpellier, France, during the last years (Franche et al. 1994, 1998, 1999; Laplaze et al. 2000a, b, c). The potential for these techniques for introducing agronomically useful genetic characteristics, such as resistance to insects or fungi, has been discussed by Franche et al. (1998). Transgenic *Casuarina* plants are proving useful for exploring the regulation of plant genes and proteins involved in the symbiotic process with *Frankia* (Franche et al. 1998) as discussed in the section above "Genetics of *Frankia*." Three strategies of gene transfer have been developed for *Casuarina glauca*:

1. A transient expression system based on the bombardment of cotyledons by accelerated microprojectiles. This method allows evaluation of the expression of new gene constructs in *Casuarina* within 2 days.
2. A quick method by using *Agrobacterium rhizogenes* to obtain transgenic roots and nodules. This allows the analysis of nodulated roots within 2 months.
3. Regeneration of transgenic plants via disarmed strain of *Agrobacterium tumefaciens*. This technique opens the way for genetic improvement of *C. glauca* (Franche et al. 1999).

11.11.2.1 Actinorhizal Nodule-Specific Genes and Proteins

During the development of nodules in legumes, several plant genes involved in nodulation either are specifically expressed in root nodules or acquire enhanced levels in the symbiotic tissue. These nodule-specific proteins have been named nodulins (Legocki and Verma 1980; Schultze and Kondorosi 1998) and are involved in the early nodulation process (infection and development of the nodular structure) and in the late nodulation process (nodular function). The putative

actinorhizal nodule-specific proteins have been named actinorhizal nodulins or actinorhizins, parallel to nodulins from rhizobial symbiosis (Tremblay et al. 1986).

Screening of a nodule cDNA library from *Alnus glutinosa* allowed isolation of a sequence representing the mRNA of nodule-specific cysteine proteinase that appeared to be differentially enhanced in the nodule. The role of this proteinase is unknown, but it has been suggested that it could be part of a defense response to *Frankia* (Goetting-Minesky and Mullin 1994). Nevertheless, it may also participate in degrading unnecessary proteins to provide amino acids for the new, nodule-specific proteins needed. The *ag12* is another gene encoding a serine protease of the subtilisin family that is early and strongly induced in nodule cortical cells of *Alnus glutinosa* upon infection by *Frankia* (Ribeiro et al. 1995). Recently, Laplaze et al. (2000b) identified a gene-dominated *cg12* for its homology to gene *ag12*. The deduced amino acid sequences for the *ag12*/*cg12* proteins showed the presence of a putative signal peptide, suggesting an extracellular location for these proteases; probably they are involved in protein processing in the early stages of infection of plant cell material surrounding *Frankia*. Transgenic Casuarinaceae plants have proven quite valuable in providing new and interesting information on the plant side of symbiotic interaction. These results have been recently reviewed by Laplaze et al. (2000c).

11.11.2.2 Phytohormones in Nodulation

The involvement of phytohormones in legume nodule induction has been well examined. The application of Nod factors blocks auxin transport which leads to local auxin accumulation (reviewed by Ferguson and Mathesius 2003) and also leads to local cytokinin accumulation (Murray et al. 2007). Cytokinin signaling is essential for eliciting legume nodule organogenesis (reviewed by Oldroyd and Downie 2008). However, while auxin levels are increased in nodule primordia compared to roots, in mature nodules, auxin levels decrease (Fedorova et al. 2000). In actinorhizal symbioses, some *Frankia* strains have been shown to produce the auxin analogue phenoxyacetic acid (PAA; Hammad et al. 2003; Perrine-Walker et al. 2010) as well as auxin itself (Perrine-Walker et al. 2010).

The distribution of auxin exporters and importers in nodules leads to further accumulation of auxin in infected cells (Peret et al. 2007), and auxin levels are strongly enhanced in mature nodules compared to roots. However, no evidence for involvement of cytokinin signaling in nodule induction has been shown thus far. A homologue of the histidine kinase involved in nodule induction (LHK1/CRE1; Murray et al. 2007) was found to be expressed in the roots and nodules of *C. glauca* but not in *A. glutinosa* (Hocher et al. 2011). Hence, phytohormone effects seem to differ in both symbioses with regard to auxin which seems to play a larger role in actinorhizal than in legume symbioses. This is not surprising in that the hormone balance in legume roots seems to differ from that in other plants. Only in legumes can root nodule primordia be induced in the root cortex instead of in the pericycle like lateral root primordia. So, it would not be surprising if the phytohormone involvement in the induction of nodules on nonlegumes was different from that on legumes and that auxin would play a major role in inducing the formation of an organ primordium in the root pericycle. Previously, it has been suggested that in inducing legume nodules, rhizobia might have exploited a program evolved for the development of root storage organs (Joshi et al. 1993). An involvement of cytokinin in the induction of storage organs would, therefore, not be surprising (Arnholdt-Schmitt 1999). It is interesting that legumes as well as Betulaceae, Myricaceae, Casuarinaceae, and Elaeagnaceae can form cluster roots, which are defined as a densely packed group of determinate rootlets which develop, grow, cease growth, undergo an exudative burst, and absorb phosphate in a synchronous way. Cluster roots exude large quantities of malate and citrate during phosphate deficiency, increasing the availability of mineral-bound phosphate (Gilbert et al. 2000). Thus, the ability to form cluster roots and the ability to form root nodules are often correlated (Skene 1998; Shane and

Lambers 2005). Natural and synthetic auxins can induce some aspects of cluster root development (Laskowski et al. 1995; Gilbert et al. 2000), and the importance of auxin for inducible root responses to nutrient stress has been established (Landsberg 1996). In other words, the roots of legumes and four out of eight actinorhizal families have a pericycle that can easily be stimulated in response to nutrient stress and can form rootlets that exudate malate.

11.11.2.3 Application of Actinorhizal Symbioses

The *Frankia* nodules of actinorhizal plants are capable of fixing N_2. In this symbiosis, *Frankia* provides the plant with a source of N, and in return, the plant provides *Frankia* with a carbon source. Rates of N_2 fixation are highly variable and are dependent on the combinations of microsymbiont–actinorhizal plant, soil, and other environmental conditions. The contribution of fixed nitrogen of actinorhizal symbioses to ecosystems is similar to the rhizobial symbioses (Torrey 1978; Dawson 1986; Dommergues 1995). Field assays with actinorhizal plants had estimated that *Casuarina* fixed 288 kg N ha^{-1} a^{-1} (Gauthier et al. 1984), *Casuarina equisetifolia* 84.8 kg N ha^{-1} a^{-1} (Dommergues 1995), and alders between 40 and 200 kg N ha^{-1} a^{-1} (Silvester 1977).

In addition to their ability to fix atmospheric N_2, most actinorhizal species possess some essential traits which are not always found in legume trees.

1. They are able to survive in poor or wasted lands, even in cold climates.
2. They are tolerant or semitolerant to a range of toxic pollutants such as B, Cd, Pb, and Zn (Weeler and Miller 1990). Thus, they are good candidates for phytoremediation (Salt et al. 1995).
3. Some actinorhizal species are adaptable to environmental conditions that differ widely from those occurring in their native habitats (i.e., *Casuarina glauca*, *C. cunninghamiana*, *C. junghuhniana* Miq., and *Alnus glutinosa*).
4. They are fairly resistant to pests and major diseases.
5. Actinorhizal species are easily propagated by seed or through diverse methods of vegetative propagation.

11.11.3 Uses of Actinorhizal Plants

Actinorhizal trees with a major interest in agroforestry systems are *Alnus* spp., *Elaeagnus* spp., and *Casuarina* spp. In humid and cold climates, species of Betulaceae (*Alnus*, mainly red alder in North America and black alder in Europe) have been used to improve fertility and reclamation of industrial wastelands but have also become important trees as a resistant crop in areas infected with conifer root disease and as a valuable timber and pulpwood producer (Hibbs and Cromack 1990; Weeler and Miller 1990). *Alnus glutinosa* has been extensively used in the reclamation of mine spoils in Britain, and *Alnus*, *Elaeagnus*, *and Hippophae* have been widely used for land stabilization. Other examples including the use of *Alnus incana* to improve the fertility of degenerated forest soil have been discussed by Dommergues (1997). Species of *Myricaceae* have been proposed for improvement of waterlogged soils (Baker and Schwintzer 1990).

Ironically, nitrogen being an essential plant nutrient is most commonly the deficient element in soils. Moreover, cultivated soils containing appropriate amounts of combined nitrogen are prone to lose it either by erosion, removal by crops, denitrification, NH_3 volatilization, or leaching. The nitrogen balance must be improved or restored by appropriate management practices (mainly by fertilization and recycling of agricultural wastes) and by exploiting the N_2 fixation process. A detailed discussion of this aspect has been published (Dommergues 1997). Because of its input of nitrogenous substances, the actinorhizal symbiosis is an important factor in the nitrogen balance of forests, rangelands, deserts, and wetlands worldwide. Currently actinorhizal plants have been used in the following ways: (a) as a primary crop for timber and pulpwood (*Alnus*, *Casuarina*); (b) as interplanted plants

(*Elaeagnus*, *Casuarina*) for other more valuable species; (c) as shelterbelts along deserts and coastlines (*Casuarina*); (d) as plantings for environmental protection, land reclamation, and amenity planting (*Elaeagnus*, *Shepherdia*, *Purshia*); and (e) as windbreaks for protection of farmlands, fruit trees, and horticultural crops (*Casuarina*) (NRC 1984; Subba Rao and Rodriguez-Barruecos 1993).

11.12 Rhizobial Associations

Rhizobium is a genus of Gram-negative soil bacteria that fix nitrogen. *Rhizobium* forms an endosymbiotic nitrogen-fixing association with roots of legumes and *Parasponia*. The bacteria colonize plant cells within root nodules; here the bacteria convert atmospheric nitrogen to ammonia and then provide organic nitrogenous compounds such as glutamine or ureides to the plant. The plant provides the bacteria with organic compounds made by photosynthesis (Sawada et al. 2003).

Rhizobium forms a symbiotic relationship with certain plants such as legumes. The *Rhizobium* fixes nitrogen from the air into ammonia, which acts as a natural fertilizer for the plants. Current research is being conducted by Agricultural Research Service microbiologists to discover a way to utilize rhizobial biological nitrogen fixation. This research involves the genetic mapping of various *Rhizobium* species with its respective symbiotic plant species, like alfalfa or soybean. The goal of this research is to increase the plants' productivity without using fertilizers (Radeva et al. 2001).

11.12.1 Distinct Genera of Rhizobia

There are currently six phylogenetically distinct genera of rhizobia, namely, (1) *Allorhizobium* (*A. undicola* produces nodules on *Neptunia prostrata*), (2) *Azorhizobium* (*A. caulinodans* produce nodules on the aquatic legume *Sesbania rostrata*), (3) *Bradyrhizobium* (*B. japonicum and B. elkanii* are found in symbiosis with soybean. *B. lupini* is a symbiont of lupine), (4) *Mesorhizobium* (*M. loti* nodulates trefoils, *M. huakuii* nodulates *Astragalus*, *M. ciceri* and *M. mediterraneum* nodulate chickpea, and *M. tianshanense* are found in symbiosis with several legume species), (5) *Rhizobium* (*R. leguminosarum* nodulates vetch, *R. tropici* is a symbiont of beans and other hosts, *Rhizobium etli* nodulates both alfalfa and beans, *R. gallicum* can nodulate bean, *R. giardinii* nodulates *Leucaena*, *R. galegae* nodulates galega, and *R*. spp. NGR234 nodulate 112 genera of legumes and the nonlegume *Parasponia andersonii*), *and* (6) *Sinorhizobium* (*Sinorhizobium meliloti* nodulates alfalfa, medics, and sweetclover; *S. fredii* nodulates soybean; *S. saheli* and *S. terangae* nodulate roots of *Sesbania*, *Acacia*, *Leucaena leucocephala*, *and Neptunia prostrata*) (Russelle 2008).

11.12.2 Drought-Tolerant *Rhizobium*–Legume Symbiosis

Improved cultivars of plants for arid lands must have drought resistance mechanisms to enable them to grow and survive in areas with low moisture availability. In fact, *Rhizobium*–legume symbioses are currently the most important nitrogen-fixing systems, which may have the potential to increase N input in arid lands. The leguminous plants include species or varieties which are extremely well adapted to the drastic conditions of arid lands. Examples are *Medicago sativa*, *Arachis hypogaea*, *Cyamopsis tetragonoloba*, and *Melilotus* spp.; these legumes are known to be adapted to conditions prevailing in arid regions. In addition, a drought-tolerant cultivar of *Phaseolus vulgaris* has recently been identified (Ramos et al. 1999). The dry weight of this legume was not affected by water stress (50 and 30 % of field capacity), although the number and weight of nodules as well as N_2 fixation (acetylene reduction) were obviously reduced. However, these legume species require drought-tolerant rhizobia to form effective symbiosis under arid climates. Rhizobia with survival ability, which showed effective symbiotic characteristics

with their host legumes (e.g., *Prosopis* rhizobia) in desert soils and arid regions, were identified (Jenkins et al. 1987). Athar and Johnson (1996) reported that two mutant strains of *R. meliloti* were competitive with naturalized alfalfa rhizobia and were symbiotically effective under drought stress. These results suggest that nodulation, growth, and N_2 fixation in alfalfa can be improved by inoculating plants with competitive and drought-tolerant rhizobia. This could be an economically feasible way to increase alfalfa (*M. sativa*) production in water-limited environments. Rhizosphere-associated microbes have always attracted the interest of researchers because of their beneficial role to the host. A classical example for this is the bacterium *Rhizobium* sp. found associated with the root nodules of legumes where it fixes nitrogen for the plant. Similarly, there are other rhizobacterial associations like that of a rhizosphere-associated *Bacillus subtilis*, which protects the tomato plant from cucumber mosaic virus and its vector *Aphis gossypii* (Sudhakar et al. 2011).

Naturally occurring forage legumes (annuals and perennials) are well nodulated, and their root nodules are active in fixing N_2 (Zahran 1998). These legumes may be found in desert or in cultivated lands as wild plants. Recently, the suitability of *Rhizobium*-inoculated wild herb legumes for providing vegetation cover and improving soil fertility in unreclaimed lands was suggested (Jha et al. 1995). Isolation of effective rhizobia from wild legumes to inoculate other legume crops is a new strategy to improve the efficiency of the *Rhizobium*–legume symbiosis. The rhizobia of wild legumes may have better traits than the homologous rhizobia. *Rhizobium* strains from *Astragalus cicer* successfully nodulate *M. sativa* and *P. vulgaris* (Zhao et al. 1997). Rajalakshmi et al. (2013) isolated *Rhizobium* spp. associated with wild legumes found in coastal and forest areas of Andaman and Nicobar Islands and reported their salt tolerance and nitrogen-fixing properties. Praveenkumar et al. (2012) reported the multiple plant growth-promoting activities of *Bacillus* sp. isolated from rhizospheric soil of tomato (*Lycopersicon esculentum* L.). Endophytic bacteria of genera *Bacillus* and *Serratia* found associated with tomato (*Lycopersicon esculentum*) and chilli (*Capsicum annuum*) were reported to exhibit growth-promoting properties by producing indoleacetic acid, siderophores, etc. and also protect the host from invading pathogens by secreting antimicrobial metabolites (Amaresan et al. 2011a, b). Similarly, a vast diversity of endophytic and rhizospheric fungi has been identified from *Suaeda monica* and *Avicennia marina* in the mangrove forest of south India (Vijayalakshmi et al. 2014a, b).

11.12.3 Woody (Tree)–Legume Symbiosis

The use of leguminous trees for a variety of food, feed, and fuelwood purposes in semiarid regions has been reviewed (Felker et al. 1981). Trees of the genera *Acacia* and *Prosopis* are of central importance in the rural economy of many of the world's arid and semiarid areas. Species of both genera provide high-quality animal fodder, timber, fuelwood, charcoal, gums, and other products as well as contribute to soil stabilization and improvement through N_2 fixation (Fagg and Stewart 1994). Their particular value in arid zones lies in their extreme resistance to heat, drought, salinity, and alkalinity; they are better able to establish growth in disturbed areas of arid regions than are herb legumes (Postma et al. 1989). It has been reported that woody legumes, e.g., *Prosopis*, are well nodulated under drought stress; however, the value of many shrubby and woody legumes in arid areas probably lies in their extensive, deep root systems, in addition to their potential to fix N_2 (Jenkins et al. 1987). *Prosopis* forms a unique system of deep roots with significant tolerance to water stress (Nilsen et al. 1986). At many instances, bacteria and fungi grow as endophytes of higher animals and plants. Endophytic bacteria and fungi are of significant interest because of their ability to produce bioactive metabolites like antimicrobial, anticancer, and antiviral agents (Phongpaichit et al. 2006; Guo et al. 2008; Vaz et al. 2009).

11.13 Conclusions

Although a large number of recent studies and reviews have documented that plant can host a wide variety of microbes from nearly all bacterial lineages, we still understand little about the evolutionary, ecological, and physiological processes that control the abundance of microbes within the plant rhizosphere regions and the specificity of these associations. Future studies of plant–microbes interactions could manipulate the presence of microbial symbionts to better assess how cellular-level interactions influence these associations and host physiology. Future experiments should be designed to explicitly target different models of symbiont transmission, as different taxa of symbionts may be more likely to be transmitted by one mode or the other. The studies reviewed here demonstrate that not only do plants obtain carbon and nitrogen from their microbial symbionts, but also that there is likely to be a high degree of variability among host–symbiont interactions in the identity and amount of nutrients transferred. Our review has emphasized the need for additional experimental manipulations coupled with advanced laboratory techniques to determine whether potential microbial symbionts are active within host plants and whether symbionts of interest act as mutualists, commensals, or parasites. Cultured symbionts are needed for rigorous experimentation, and the development of novel methods to culture and transplant plant-associated microbes should be a high priority for our research community.

Acknowledgment The first author is thankful to the Department of Biotechnology (Govt. of India) for the constant funding support, and all authors are thankful to the Department of Science and Technology (Govt. of India) for confocal microscopy facility sponsored through PURSE grant to Bharathidasan University, Tiruchirappalli – 620024. Visiting Professorship provided to Prof. N. Thajuddin by King Saud University, Riyadh, Kingdom of Saudi Arabia and Deanship of Scientific research, College of Science, Research Center, King Saud University, Kingdom of Saudi Arabia has been thankfully acknowledged.

References

Adams DG (2002) Cyanobacteria in symbiosis with hornworts and liverworts. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer, Dordrecht, pp 117–135

Adams DG, Duggan PS (2008) Cyanobacteria-bryophyte symbioses. J Exp Bot 59:1047–1058

Amaresan N, Jayakumar V, Kumar K, Thajuddin N (2011a) Isolation and characterization of plant growth promoting endophytic bacteria and their effect on tomato (*Lycopersicon esculentum*) and chilli (*Capsicum annuum*) seedling growth. Ann Microbiol 62:805–810

Amaresan N, Jayakumar V, Kumar K, Thajuddin N (2011b) Endophytic bacteria from tomato and chilli, their diversity and antagonistic potential against *Ralstonia solanacearum*. Arch Phytopathol Pfl 45(3):344–355

Anonymous (1984) Casuarinas: nitrogen-fixing trees for adverse sites. National Academies Press, Washington, DC

Arnholdt-Schmitt B (1999) On the physiology of yield production in carrots-implications for breeding towards nutrient efficiency. Gartenbauwissenschaft 64:26–32

Athar M, Johnson DA (1996) Influence of drought on competition between selected *Rhizobium meliloti* strains and naturalized soil rhizobia in alfalfa. Plant Soil 184:231–241

Awasthi DD (1957) A new species of *Parmelia* from Kodaikanal, South India. Curr Sci 26:123–124

Awasthi DD (1965) Catalogue of the lichens from India, Nepal, Pakistan, and Ceylon. Verlag von J Cramer, Weinheim

Awasthi DD (1975) Lichen flora of pindari glacier valley. Geophytology 5:178–185

Awasthi DD (1988) A key to the macro lichens of India and Nepal. J Hattori Bot Lab 65:207–302

Awasthi DD (1991) A key to the microlichens of India, Nepal and Sri Lanka. Bibl Lichnenol 40:1–337

Baker DD, Schwintzer CR (1990) Introduction. In: Schwintzer CR, Tjepkema JD (eds) The biology of *Frankia* and actinorhizal plants. Academic, San Diego, pp 1–13

Baker JA, Entsch B, McKay DB (2003) The cyanobiont in an *Azolla* fern is neither *Anabaena* nor *Nostoc*. FEMS Microbiol Lett 229:43–47

Becking JH (1970) Frankiaceae, fam. nov. (Actinomycetales) with one new combination and six new species of the genus *Frankia* Brunchorst 1886. Int J Syst Bacteriol 20:201–220

Belnap J (2001) Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. In: Belnap J, Lange OL (eds) Biological soil crusts: structure, function, and management. Springer, New York, pp 241–261

Benson DR, Silvester WB (1993) Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants. Microbiol Rev 57:293–319

Bergman B (2002) *Nostoc-Gunnera* symbiosis. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer, Dordrecht, pp 207–232

Bergman B, Johansson C, Söderbäck E (1992) Tansley review No. 42: the *Nostoc-Gunnera* symbiosis. New Phytol 122(3):379–400

Berry AM, Moreau RA, Jones AD (1991) Bacteriohopanetetrol: abundant lipid in *Frankia* cells and in nitrogen fixing nodule tissue. Plant Physiol 95:111–115

Berry AM, Harriott OT, Moreau RA, Osman SF, Benson DR, Jones AD (1993) Hopanoid lipids compose the Frankia vesicle envelope, presumptive barrier of oxygen diffusion to nitrogenase. Proc Natl Acad Sci U S A 90(13):6091–6094

Berthol RJ, Borowitzka MA, Mackay MA (1982) The ultrastructure of *Oscillatoria spongeliae*, the blue-green algal endosymbiont of the sponge *Dysidea herbacea*. Phycologia 21:327–335

Brehm U, Krumbein WE, Palinska K (2003) Microbial spheres: a novel cyanobacterial – diatom symbiosis. Naturwissenschaften 90:136–140

Buee MD, Boer W, Martin F, van Overbeek L, Jurkevitch E (2009) The rhizosphere zoo: an overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. Plant Soil 321:189–212

Calvert HE, Pence MK, Peters GA (1985) Ultrastructural ontogeny of leaf trichomes in *Azolla* implies a functional role in metabolite exchange. Protoplasma 129:10–27

Carballo JL, Vila E (2004) Population dynamics of a mutualistic interaction between the sponge *Haliclona caerulea* and the red alga *Jania adherens*. Mar Ecol Prog Ser 279:93–104

Carpenter EJ (2002) Marine cyanobacterial symbioses. Biol Environ 102(B):15–18

Carpenter EJ, Janson S (2000) Intracellular cyanobacterial symbiont in the marine diatom *Climacodium frauenfeldianum*. J Phycol 36:540–544

Cheshire AC, Wilkinson CR, Seddon S, Westphalen G (1997) Bathymetric and seasonal changes in photosynthesis and respiration of the phototrophic sponge *Phyllospongia lamellosa* in comparison with respiration by the heterotrophic sponge *Ianthella basta* on Davies Reef, Great Barrier Reef. Mar Freshw Res 48:589–599

Chopra GL (1934) Lichens of the Himalayas, pt. I. Lichens of Darjeeling and Sikkim Himalayas. Punjab University, Lahore

Costa JL, Lindblad P (2002) Cyanobacteria in symbiosis with cycads. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer, Dordrecht, pp 195–205

Costa JL, Paulsrud P, Lindblad P (1999) Cyanobiont diversity within coralloid roots of selected cycad species. FEMS Microbiol Ecol 28:85–91

Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G et al (2005) Diverse taxa of cyanobacteria produce beta-N-methylamino-L-alanine, a neurotoxic amino acid. Proc Natl Acad Sci U S A 102:5074–5078

Crutzen PJ, Andreae MO (1990) Biomass burning in the tropics: impact on atmospheric chemistry and biogeochemical cycles. Science 250:1669–1678

Dawson JO (1986) Actinorhizal plants: their use in forestry and agriculture. Outlook Agric 15:202–208

Dawson JO (1990) Interactions among actinorhizal and associated plant species. In: Schwintzer CR, Tjepkema JD (eds) The biology of *Frankia* and actinorhizal plants. Academic, San Diego, pp 299–316

DeLuca TH, Zackrisson O, Nilsson MC, Sellstedt A (2002) Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature 419:917–920

DeLuca TH, Zackrisson O, Gentili F, Sellstedt A, Nilsson MC (2007) Ecosystem controls on nitrogen fixation in boreal feather moss communities. Oecologia 152:121–130

Deyoe HR, Lowe RL, Marks JL (1992) Effects of nitrogen and phosphorus on the endo symbiont load of *Rhopalodia gibba* and *Epithemia turgida* Bacillariophyceae. J Phycol 28:773–777

Diem HG, Dommergues YR (1990) Current and potential uses and management of casuarinaceae in the tropics and subtropics. In: Schwintzer CR, Tjepkema JD (eds) The biology of *Frankia* and actinorhizal plants. Academic, San Diego, pp 317–342

Dommergues YR (1995) Nitrogen fixation by trees in relation to soil nitrogen economy. Fertil Res 42:215–230

Dommergues YR (1997) Contribution of actinorhizal plants to tropical soils productivity and rehabilitation. Soil Biol Biochem 29:931–941

Dommergues Y, Duhoux E, Diem HG (1999) Les arbres fixateurs d'azote. ESPACES, Montpellier

Duff RJ, Villarreal JC, Cargill DC, Renzaglia KS (2007) Progress and challenges toward developing a phylogeny and classification of the hornworts. Bryologist 110:214–243

Erwin PM, Thacker RW (2008) Cryptic diversity of the symbiotic cyanobacterium *Synechococcus spongiarum* among sponge hosts. Mol Ecol 17:2937–2947

Fagg CW, Stewart JL (1994) The value of *Acacia* and *Prosopis* in arid and semi-arid environments. J Arid Environ 27:3–25

Fedorova EE, Zhiznevskaya GY, Kalibernaya ZV, Artemenko EN, Izmailov SF, Guskov AV (2000) IAA metabolism during development of symbiosis between *Phaseolus vulgaris* and *Rhizobium phaseoli*. Russ J Plant Physiol 47:203–206

Feldmann J (1935) Sur quelques cyanophycees vivant dans le tissu des eponges de banyules.Second volume jubilaire Tome 75, p. 331 a' 404.1er De'cembre 1933. Arch de zoologie expe'rimentale et ge'ne'rale 75:381

Felker P, Clark PR, Leag AE, Pratt PF (1981) Salinity tolerance of the tree legumes: mesquite (*Prosopis glandulosa* var. *torreyana*, *P. velutina* and *P. articulata*), algarrobo (*P. chilensis*), kiawa (*P. pallida*) and tamarufo (*P. amarugo*) grown in sand culture on nitrogen-free media. Plant Soil 61:311–317

Ferguson BJ, Mathesius U (2003) Signaling interactions during nodule development. J Plant Growth Regul 22:47–72

Franche C, Bougusz D, Le Van Q, Phelep M, Duhoux E (1994) Genetic transformation of trees in the *Casuarinaceae* family. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry. Springer, Berlin, pp 257–273

Franche C, Laplaze L, Duhoux E, Bogusz D (1998) Actinorhizal symbiosis: recent advances in plant molecular and genetic studies. Crit Rev Plant Sci 17:1–28

Franche C, N'Diaye A, Gobe C, Alloneau C, Bogusz D, Duhoux E (1999) Genetic transformation of *Allocasuarina verticillata*. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry. Springer, Berlin, pp 1–14

Gauthier DL, Frioni HG, Diem, Dommergues Y (1984) The *Colletia spinosissima* – *Frankia* symbiosis. Acta Oecol Oecol Plant 5:231–239

Gebhardt JS, Nierzwicki-Bauer SA (1991) Identification of a common cyanobacterial symbiont associated with *Azolla* spp. through molecular and morphological characterization of free-living and symbiotic cyanobacteria. Appl Environ Microbiol 57:2141–2146

Gehringer MM, Adler L, Roberts AA, Moffitt MC et al (2012) Nodularin, a cyanobacterial toxin, is synthesized in plants by symbiotic *Nostoc* sp. ISME J 6:1834–1847

Gentili F, Nilsson MC, Zackrisson O, DeLuca TH, Sellstedt A (2005) Physiological and molecular diversity of feather moss associative N_2-fixing cyanobacteria. J Exp Bot 56:3121–3127

Gilbert GA, Knight JD, Vance CP, Allan DL (2000) Proteoid root development of phosphorus deficient lupin is mimicked by auxin and phosphonate. Ann Bot 85:921–928

Goetting-Minesky MP, Mullin BC (1994) Differential gene expression in an actinorhizal symbiosis: evidence for a nodule- specific cysteine proteinase. Proc Natl Acad Sci U S A 91:9891–9895

Gomez F, Furuya K, Takeda S (2005) Distribution of the cyanobacterium *Richelia intracellularis* as an epiphyte of the diatom *Chaetoceros compressus* in the Western Pacific Ocean. J Plankton Res 27:323–330

Grobbelaar N, Scott WE, Hattingh W, Marshall J (1987) The identification of the coralloid endophytes of the southern African cycads and the ability of the isolates to fix dinitrogen. S Afr J Bot 53:111–118

Guo B, Wang Y, Sun X, Tang K (2008) Bioactive natural products from endophytes: a review. Appl Biochem Microbiol 44(2):136–142

Hammad Y, Nalin R, Marechal J, Fiasson K, Pepin R, Berry AM, Normand P, Domenach AM (2003) A possible role for phenyl acetic acid (PAA) on *Alnus glutinosa* nodulation by *Frankia*. Plant Soil 254:193–205

Hariharan GN (1991) The lichens of Shervaroy. PhD thesis, Bharathidasan University, Tiruchirappalli

Hariharan GN, Krishnamurthy KV, Upreti DK (2003) Lichens of Shervaroy hills of Eastern Ghats, India. Phytotaxonomy 3:1–23

Heinbokel JF (1986) Occurrence of *Richelia intracellularis* (cyanophyta) within the diatoms *Hemiaulus haukii* and *H. membranaceus* of Hawaii. J Phycol 22:399

Hentschel U, Hopke J, Horn M, Friedrich AB, Wagner M et al (2002) Molecular evidence for a uniform microbial community in sponges from different oceans. Appl Environ Microbiol 68:4431–4440

Heusser LE, Shackleton NJ (1979) Direct marine continental correlation: 150,000-year oxygen isotope-pollen record from the North Pacific. Science 204:837–838

Hibbs DE, Cromack K Jr (1990) Actinorhizal plants in Pacific Northwest forest. In: Schwintzer CR, Tjepkema JD (eds) The biology of Frankia and actinorhizal plants. Academic, San Diego, pp 343–363

Hill K, Osborne R (2001) Cycads of Australia. Kangaroo Press, Sydney, pp 63–112

Hirose E, Hirose M, Neilan BA (2006) Localization of symbiotic cyanobacteria in the colonial ascidian *Trididemnum miniatum* (Didemnidae, Ascidiacea). Zool Sci 23:435–442

Hocher V, Alloisio N, Auguy F, Fournier P et al (2011) Transcriptomics of actinorhizal symbioses reveals homologs of the whole common symbiotic signaling cascade. Plant Physiol 156:700–711

Janson S (2002) Cyanobacteria in symbiosis with diatoms. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer, Dordrecht, pp 1–10

Jenkins MB, Virginia RA, Jarrel WM (1987) Rhizobial ecology of the woody legume mesquite (*Prosopis glandulosa*) in the Sonoran desert. Appl Environ Microbiol 33:36–40

Jha PK, Nair S, Gopinathan MC, Babu CR (1995) Suitability of rhizobia inoculated wild legumes *Argyrolobium flaccidum*, *Astragalus graveolens*, *Indigofera gangetica* and *Lespedeza stenocarpa* in providing a vegetational cover in an unreclaimed limestone quarry. Plant Soil 177:139–149

Joshi PA, Caetano-Anolles G, Graham ET, Gresshoff PM (1993) Ultrastructure of transfer cells in spontaneous nodules of alfalfa (*Medicago sativa*). Protoplasma 172:64–76

Kaasalainen U, Jokela J, Fewer DP, Sivonen K, Rikkinen J (2009) Microcystin production in the tripartite cyanolichen *Peltigera leucophlebia*. Mol Plant Microbe Interact 22:695–702

Kaasalainen U, Fewer DP, Jokela J, Wahlsten M, Sivonen K, Rikkinen J (2012) Cyanobacteria produce a high variety of hepatotoxic peptides in lichen symbiosis. Proc Natl Acad Sci USA 109:5886–5891

Kannan K, Vijayan D, Ali DM, Praveenkumar R, Parveez AA, Thajuddin N (2014) Evidence-based analysis of novel symbiotic and epiphytic cyanobacteria associated with *Azolla* by cyto and molecular taxonomy. Biotechnology 13(20):46–53

Khamar HJ, Breathwaite EK, Prasse CE, Fraley ER et al (2010) Multiple roles of soluble sugars in the establishment of *Gunnera-Nostoc* endosymbiosis. Plant Physiol 154(3):1381–1389

Kiran GS, Thajuddin N, Hema TA, Idhayadhulla A, Surendar KR, Selvin J (2010) Optimization and characterization of rhamnolipid biosurfactant from sponge

associated marine fungi *Aspergillus* sp. MSF1. Desalination Water Treat 24:257–265
Kiran GS, Sabarathnam B, Thajuddin N, Selvin J (2014) Production of glycolipid biosurfactant from sponge-associated marine Actinobacterium *Brachybacterium paraconglomeratum* MSA21. J Surfactant Deterg 17:531–542
Kluge M, Mollenhauer D, Wolf E, Schuessler A (2002) The *Nostoc-Geosiphon* endocytobiosis. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer, Dordrecht, pp 19–30
Ladha JK, Dawe D, Ventura TS, Singh U, Ventura AW, Watanabe I (2000) Long-term effect of urea and green manure on rice yield and nitrogen balance under long-term green manure and mineral N application. Soil Sci Soc Am J 64:1993–2001
Landsberg EC (1996) Hormonal regulation of iron–stress response in sunflower roots: a morphological and cytological investigation. Protoplasma 194:69–80
Laplaze L, Duhoux E, Franche C, Frutz T, Svistoonoff S, Bisseling T, Bogusz D, Pawlowski K (2000a) *Casuarina glauca* prenodule cells display the same differentiation as the corresponding nodule cells. Mol Plant Microbe Interact 13:107–112
Laplaze L, Ribeiro A, Franche C, Duhoux E, Auguy F, Bogusz D, Pawlowski K (2000b) Characterization of a *Casuarina glauca* nodule-specific subtilisin-like protease gene, a homolog of *Alnus glutinosa* AG12. Mol Plant Microbe Interact 13:113–117
Laplaze L, Bon MC, Sy MO, Simouni A et al (2000c) Molecular biology of tropical nitrogen fixing trees in the Casuarinaceae family. In: Jain SM, Minocha SC (eds) Molecular biology of woody plants. Kluwer Academic Publishers, Dordrecht, pp 269–285
Larkum L (1999) The cyanobacteria of coral reefs. Marine cyanobacteria. In: Charpy L, Larkum AWD (eds) Bull I'Inst oceanograph Monaco Num special 19. Oceanographic Institute, France, pp 149–167
Larkum AWD, Kennedy IR, Muller WJ (1988) Nitrogen fixation on a coral reef. Mar Biol 98:143–155
Laskowski MJ, Williams ME, Nusbaum HC, Sussex IM (1995) Formation of lateral root meristems is a two stage process. Development 121:3303–3310
Lechevalier MP, Lechevalier HA (1990) Systematics, isolation, and culture of *Frankia*. In: Schwintzer CR, Tjepkema JD (eds) The biology of *Frankia* and actinorhizal plants. Academic, New York, pp 35–60
Lechevalier MP, Labeda DP, Ruan J (1987) Studies on *Frankia* sp. LLR02022 from *Casuarina cunninghamiana* and its mutant LLR02023. Physiol Plant 70:249–254
Lechno-Yossef S, Nierzwicki-Bauer SA (2002) *Azolla–Anabaena* symbiosis. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer, Dordrecht, pp 153–178
Lee YK, Lee JH, Lee HK (2001) Microbial symbiosis in marine sponges. J Microbiol 39:254–264
Legocki RP, Verma DP (1980) Identification of nodule-specific host proteins (nodulins) involved in the development of rhizobium-legume symbiosis. Cell 20:153–163
Lindblad P, Haselkorn R, Bergman B (1985) The cyanobacterium– *Zamia* symbiosis: C2H2-reduction and heterocyst frequency. Symbiosis 1:19–28
Lopez MF, Fontaine MS, Torrey JG (1984) Levels of trehalose and glycogen in *Frankia* sp. HFPArI3 (Actinomycetales). Can J Microbiol 30:746–752
Lotti F, Giovanetti L, Margheri MC, Ventura S, Materassi R (1996) Diversity of DNA methylation pattern and total DNA restriction pattern in symbiotic *Nostoc*. World Microbiol Biotechnol 12:38–42
Maldonado M, Young CM (1998) Limits on the bathymetric distribution of keratose sponges: a field test in deep water. Available: http://digital.csic.es/handle/10261/3243?mode=full&submit_simple=Show+full+item+record. Accessed 7 Aug 2012
Meeks JC (1990) Cyanobacterial-bryophyte associations. In: Rai AN (ed) Handbook of symbiotic cyanobacteria. CRC Press, Boca Raton, pp 43–63
Meeks JC (1998) Symbiosis between nitrogen-fixing cyanobacteria and plants. Bio Sci 48:266–276
Meeks JC (2003) Symbiotic interactions between *Nostoc punctiforme*, a multicellular cyanobacterium, and the hornwort Anthoceros punctatus. Symbiosis 35:55–71
Miadlikowska J, Lutzoni F (2000) Phylogenetic revision of the genus *Peltigera* (lichen-forming Ascomycota) based on morphological, chemical, and large subunit nuclear ribosomal DNA data. Int J Plant Sci 161:925–958
Montesinos E (2003) Plant associated microorganisms: a view from the scope of microbiology. Int Microbiol 6(4):221–223
Mort AP, Normand, Lalonde M (1983) 2-O-methyl-Dmannose, a key sugar in the taxonomy of *Frankia*. Can J Microbiol 29:993–1002
Murakami A, Miyashita H, Iseki M, Adachi K, Mimuro M (2004) Chlorophyll *d* in an epiphytic cyanobacterium of red algae. Science 303:1633
Murray JD, Karas BJ, Sato S, Tabata S, Amyot L, Szczyglowski K (2007) A cytokinin perception mutant colonized by *Rhizobium* in the absence of nodule organogenesis. Science 315:101–104
Nilsen ET, Virginia RA, Jarrall WH (1986) Water relations and growth characteristics of *Prosopis glandulosa* var. *torreyana* in a simulated phreatophytic environment. Am J Bot 73:430–433
NRC (1984) Casuarinas: nitrogen-fixing trees for adverse sites. National Research Council, Washington, DC
Obukowicz M, Schaller M, Kennedy GS (1981) Ultrastructure and phenolic histochemistry of the *Cycas revoluta–Anabaena* symbiosis. New Phytol 87:751–754
Oksanen I, Jokela J, Fewer DP, Wahlsten M, Rikkinen J, Sivonen K (2004) Discovery of rare and highly toxic microcystins from lichen-associated cyanobacterium *Nostoc* sp. strain IO-102-I. Appl Environ Microbiol 70:5756–5763
Oldroyd GE, Downie JA (2008) Coordinating nodule morphogenesis with rhizobial infection in legumes. Annu Rev Plant Biol 59:519–546
Pabby A, Prasanna R, Nayak S, Singh PK (2003) Physiological characterization of the cultured and freshly isolated endosymbionts from different species of *Azolla*. Plant Physiol Biochem 41:73–79

Pawlowski K, Demchenko KN (2012) The diversity of actinorhizal symbiosis. Protoplasma 249:967–979

Peret B, Swarup R, Jansen L, Devos G et al (2007) Auxin influx activity is associated with *Frankia* infection during actinorhizal nodule formation in *Casuarina glauca*. Plant Physiol 144:1852–1862

Perrine-Walker F, Doumas P, Lucas M, Vaissayre V et al (2010) Auxin carriers localization drives auxin accumulation in plant cells infected by *Frankia* in *Casuarina glauca* actinorhizal nodules. Plant Physiol 154:1372–1380

Peters GA, Mayne BC (1974) The *Azolla-Anabaena azollae* relationship. I. Initial characterization of the association. Plant Physiol 53:813–819

Peters GA, Meeks JC (1989) The *Azolla–Anabaena* symbiosis: basic biology. Ann Rev Plant Physiol Plant Mol Biol 40:193–210

Phongpaichit S, Rungjindamai N, Rukachaisirikul N, Sakayaroj J (2006) Antimicrobial activity in cultures of endophytic fungi isolated from *Garcinia* species. FEMS Immunol Med Microbiol 48(3):367–372

Plazinski J, Zheng Q, Taylor R, Croft L, Rolfe BG, Gunning BES (1990) DNA probes show genetic variation in cyanobacterial symbionts of the *Azolla* fern and a closer relationship to free-living *Nostoc* strains than to free living *Anabaena* strains. Appl Environ Microbiol 56:1263–1270

Postma J, Van Veen JA, Walter S (1989) Influence of different initial soil moisture contents on the distribution and population dynamics of introduced *Rhizobium leguminosarum* biovar. *trifolii*. Soil Biol Biochem 21:437–442

Praveenkumar R, Leo AM, Vijayan D, Muthukumar C, Thajuddin N (2007) Morphological diversity and phylogeny of cyanobionts from the coralloid roots of cycads. BDU J Sci Technol 1(2):169–176, in Tamil) & 177–184 (in English

Praveenkumar D, Anupama PD, Singh RK, Thenmozhi R, Nagasathya A, Thajuddin N, Paneerselvam A (2012) Performance studies of free-living tomato (*Lycopersicon exculentum* L.) rhizospheric *Bacillus* for their multiple plant growth promoting activity. J Soil Sci Environ Mgmt 3(6):142–153

Prema P, Anand N (2012) A novel diatom –cyanobacteria symbiosis. Phykos 42(1):72–75

Proteau PJ, Gerwick WH, Garcia-Pichel F, Castenholz R (1993) The structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of cyanobacteria. Experientia 49:825–829

Quraishi AA (1928) Lichens of Western Himalayas. Proceedings of 15th Indian Science Congress, Kolkatta, Abst 228

Radeva G, Jurgens G, Niemi M, Nick G, Suominen L, Lindström K (2001) Description of two biovars in the *Rhizobium galegae* species: biovar *orientalis* and biovar *officinalis*. Syst Appl Microbiol 24(2):192–205

Rai AN (1990) Cyanobacteria in symbiosis. In: Rai AN (ed) Handbook of symbiotic cyanobacteria. CRC Press, Boca Raton

Rai AN, Söderbäck E, Bergman B (2000) Cyanobacterium-plant symbioses. New Phytol 147:449–481

Rajalakshmi S, Thajuddin N, Jayakumar V (2013) Salt tolerance and nitrogen fixing properties of *Rhizobium* spp. associated with wild legumes found in coastal and forest areas of Andaman & Nicobar Islands. In: Balu A, Jayaraj RSC, Ragupathy A, Mohan V, Warrier RR, Raghunath TP, Krishnakumar N (eds) Forest health management. Institute of Forest Genetics and Tree Breeding, ICFRE, Coimbatore, pp 116–127

Ramos MLG, Gordon AJ, Minchin FR, Sprent JI, Parsons R (1999) Effect of water stress on nodule physiology and biochemistry of a drought tolerant cultivar of common bean (*Phaseolus vulgaris* L.). Ann Bot 83:57–63

Rasmussen U, Johansson C, Bergman B (1994) Early communication in the *Gunnera-Nostoc* symbiosis: plant-induced cell differentiation and protein synthesis in the cyanobacterium. Mol Plant Microbe Interact 7(6):696–702

Ravikumar S, Gnanadesigan M, Thajuddin N, Deepan Charavarthy VS, Beula Banerjee M (2010) Anticancer property of sponge associated Actinomycetes along Palk Strait. J Pharm Res 3(10):2415–2417

Renzaglia KS, Schuette S, Duff RJ, Ligrone R, Shaw AJ, Mishler BD, Duckett JG (2007) Bryophyte phylogeny: advancing the molecular and morphological frontiers. Bryologist 110:179–213

Ribeiro A, Akkermans ADL, Van Kammen A, Bisseling T, Pawlowski K (1995) A nodule-specific gene encoding a subtilisin-like protease is expressed in early stages of actinorhizal nodule development. Plant Cell 7:785–794

Ridley CP, John Faulkner D, Haygood MG (2005) Investigation of *Oscillatoria spongeliae*-dominated bacterial communities in four dictyoceratid sponges. Appl Environ Microbiol 71:7366–7375

Rikkinen J (2002) Cyanolichens: an evolutionary overview. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 31–72

Rikkinen J (2009) Relations between cyanobacterial symbionts in lichens and plants. In: Pawlowski K (ed) Prokaryotic symbionts in plants. Springer, Berlin, pp 265–270

Rikkinen J (2013) Molecular studies. London, UK, 6:3–32

Russelle MP (2008) Biological dinitrogen fixation in agriculture. In: Schepers JS, Raun WR (eds) Nitrogen in agricultural systems. American Society of Agronomy, Madison, USA, pp 281–359

Salt DE, Blaylock MJ, Kumar NPB, Dushenkov V, Ensley BD, Chet I, Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13:468–474

Sawada H, Kuykendall LD, Young JM (2003) Changing concepts in the systematics of bacterial nitrogen-fixing legume symbionts. J Gen Appl Microbiol 49(3): 155–179

Schultze DR, Kondorosi A (1998) Regulation of symbiotic root nodule development. Annu Rev Genet 32:33–57

Shane MW, Lambers H (2005) Cluster roots: a curiosity in context. Plant Soil 274:101–125

Shick JM, Dunlap WC (2002) Mycosporine-like amino acids and related gadusols?: Biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu Rev Physiol 64:223–262

Shyamkumar R (2007) Diversity and characterization of cyanobionts in the lichens of Yercaud and Kolli hills, Tamil Nadu, India. PhD thesis, Bharathidasan University, Tiruchirappalli

Shyamkumar R, Thajuddin N (2009a) Effect of salinity & pH on the growth of symbiotic cyanobacteria isolated from cyanolichens. Adv Biotechnol 8(11):35–38

Shyamkumar R, Thajuddin N (2009b) Influence of temperature and light intensity on growth of symbiotic cyanobacteria isolated from cyanolichens. Res Rev Biosci 3(4):179–182

Shyamkumar R, Thajuddin N, Upreti DK (2009) Additions to lichen flora of Yerkaud (Shervaroy Hills) Tamil Nadu, India. J Eco Taxon Bot 33(3): 673–676

Shyamkumar R, Thajuddin N, Venkateswari C (2010) Antibacterial activity of cyanolichen and symbiotic cyanobacteria against some selected microorganisms. Afr J Microbiol Res 4(13):1408–1411

Shyamkumar R, Al-Harbi NA, Thajuddin N (2011a) Macromolecular and fatty acid profile studies on symbiotic cyanobacterial isolates of Cyanolichens. J Med Plants Res 5(17):4188–4193

Shyamkumar R, Thajuddin N, Upreti DK (2011b) Diversity of Lichens in Kolli Hills of Tamil Nadu, India. Intl J Biodiver Conserv 3(2):36–39

Shyamkumar R, Al-Hemaid FMA, Al-Dhabi NA, Muthukumar C, Ajmal Ali M, Thajuddin N (2013) Distribution of epiphytic cyanobacteria on lichens from Eastern Ghats of Tamil Nadu, India. J Pure Appl Microbiol 7(1):505–514

Silvester WB (1976) Ecological and economic significance of the non-legume symbiosis. In: Newton WE, Nyman CJ (ed) Proceedings of the first international symposium on Nitrogen Fixation. Washington University Press, Seattle, USA, pp 489–506

Silvester WB (1977) Dinitrogen fixation by plants associations excluding legumes. In: Hardy R, Silvester W (eds) A treatise of dinitrogen fixation. Academic, New York, pp 141–190

Singh A (1980) Lichenology in Indian sub continent 1966–1977. EBIS, National Botanical Research Institute, Lucknow

Singh KP (1984) Synopsis of lichens from Palni Hills. IndiaBiol Mem 9(2):105–150

Singh SM, Singh P, Thajuddin N (2008) Biodiversity and distribution of cyanobacteria at Dronning Maud Land, East Antarctica. Acta Bot Malacitana 33:17–28

Sivonen K, Carmichael WW, Namikoshi M, Rinehart KL, Dahlem AM, Niemela SI (1990) Isolation and characterization of hepatotoxic microcystin homologs from the filamentous freshwater cyanobacterium *Nostoc* sp. strain 152. Appl Environ Microbiol 56:2650–2657

Skene KR (1998) Cluster roots: some ecological considerations. J Ecol 86:1060–1064

Snoejis P, Murasi LW (2004) Synopsis between the diatom and cyanobacterial colonies. Vie et Milieu 54(2–3):163–170

Solheim B, Zielke M (2002) Associations between cyanobacteria and mosses. In: Rai AN, Bergman B, Rasmussen (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 37–152

Subba Rao NS, Rodriguez-Barrueco C (1993) Symbiosis in nitrogen fixing trees. Oxford and IBH Publishing, New Delhi

Sudhakar N, Thajuddin N, Murugesan K (2011) Plant growth-promoting rhizobacterial mediated protection of tomato in the field against cucumber mosaic virus and its vector *Aphis gossypii*. Biocontrol Sci Technol 21(3):367–386

Sundström BG (1984) Observations on *Rhizosolenia clevei* Ostenfeld (Bacillariophyceae) and *Richelia intracellularis* Schmidt. Bot Mar 27:345–355

Svenning MM, Eriksson T, Ramussen U (2005) Phylogeny of symbiotic cyanobacteria within the genus *Nostoc* based on 16S rDNA sequence analyses. Arch Microbiol 183:19–26

Swensen SM (1996) The evolution of actinorhizal symbiosis: evidence for multiple origins of the symbiotic association. Am J Bot 83:1503–1512

Thacker RW, Starnes S (2003) Host specificity of the symbiotic cyanobacterium *Oscillatoria spongeliae* in marine sponges, *Dysidea* spp. Mar Biol 142: 643–648

Thajuddin N (1991) Marine cyanobacteria of the southern east coast of India – survey and ecobiological studies. PhD thesis, Bharathidasan University, Tiruchirappalli

Thajuddin N, Subramanian G (1992) Survey of cyanobacterial flora of the southern east coast of India. Bot Mar 35:305–314

Thajuddin N, Subramanian G (1994) Marine cyanobacterial flora of south India. In: Sharma TA, Saini SS, Trivedi ML, Sharma M (eds) Current researchers in plant sciences. Bisen Singh & Mahendra Pal Singh, Dehra Dun, pp 1–16

Thajuddin N, Subramanian G (2005) Cyanobacterial biodiversity and potential applications in biotechnology. Curr Sci 89:47–57

Thajuddin N, Nierzwicki-Bauer SA, Subramanian G (2007) Cyanobacterial biodiversity and recent trends in their taxonomy. BDU J Sci Technol 1:15–28

Thajuddin N, Muralitharan G, Sundaramoorthy G, Ramamoorthy R, Ramachandran S, Akbarsha MA, Gunasekaran M (2010) Morphological and genetic diversity of symbiotic cyanobacteria from cycads. J Basic Microbiol 50:254–265

Thompson AW, Foster RA, Krupke A, Carter BJ, Musat N, Vaulot D, Kuypers MMM, Zehr JP (2012) Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga. Science 337(6101): 1546–1550

Tjepkema JD, Torrey JG (1979) Symbiotic nitrogen fixation in actinomycete-nodulated plants. Bot Gaz 140:1–11

Tjepkema JD, Ormerod W, Torrey JG (1980) Vesicle formation and acetylene reduction activity in *Frankia* sp. CpI1 cultured in defined media. Nature 287:633–635

Torrey JG (1978) Nitrogen fixation by actinomycete-nodulated angiosperms. BioScience 28:586–592

Torrey JG (1985) The site of nitrogenase in *Frankia* in free-living culture and in symbiosis. In: Evans HJ, Bottomley PJ, Newton WE (eds) Nitrogen fixation research progress. Martinus Nijhoff, Dordrecht, pp 293–299

Tremblay FM, Perinet P, Lalonde M (1986) Tissue culture of *Alnus* spp. with regard to symbiosis. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry. Springer, Berlin, pp 87–100

Tschermak-Woess E (1988) The algal partner. In: Galun M (ed) CRC handbook of lichenology. CRC Press, Boca Raton, pp 39–92

Upreti DK (1995) Lichenology in India-1994. Br Lich Soc Bull 77:18

Upreti DK (1997) A review of lichenology in India during 1996. Br Lich Soc Bull 81:25–26

Upreti DK, Nayaka S (2000) Lichenology in India (1996–2000). Br Lich Soc Bull 87:66–67

Upreti DK, Nayaka S (2003) Review of lichenology in India during 2001–03. Br Lich Soc Bull 93:37–39

Usher KM (2008) The ecology and phylogeny of cyanobacterial symbionts in sponges. Mar Ecol 29:178–192

Usher KM, Fromont J, Sutton DC, Toze S (2004) The biogeography and phylogeny of unicellular cyanobacterial symbionts in sponges from Australia and the Mediterranean. Microb Ecol 48:167–177

Van Hove C, Lejeune A (2002) Applied aspects of *Azolla–Anabaena* symbiosis. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer, Dordrecht, pp 179–193

Vaz AB, Mota RC, Bomfim MR, Vieira ML, Zani CL, Rosa CA, Rosa LH (2009) Antimicrobial activity of endophytic fungi associated with Orchidaceae in Brazil. Can J Microbiol 55:1381–1391

Veys P, Waterkeyn L, Lejeune A, Van Hove C (1999) The pore of the leaf cavity of *Azolla*: morphology, cytochemistry and possible functions. Symbiosis 27:22–57

Vijayalakshmi S, Panneerselvam A, Vijayakumar N, Savery MA, Thajuddin N (2014a) Diversity of endophytic and rhizosphere soil fungi of *Suaeda monica* in Maravakadu mangrove forest. Int J Curr Res 6(3):5343–5345

Vijayalakshmi S, Panneerselvam A, Vijayakumar N, Savery MA, Thajuddin N (2014b) Diversity of endophytic and rhizosphere soil fungi of *Avicennia marina* in Maravakadu mangrove forest. IOSR J Phar Bio Sci 9(2):2319–3008

Villareal TA (1990) Laboratory culture and preliminary characterization of the nitrogen-fixing *Rhizosolenia-Richelia* symbiosis. Mar Ecol 11:117–132

Villareal TA (1992) Marine nitrogen-fixing diatom-cyanobacterial symbioses. In: Carpenter EJ, Capone DG, Reuter J (eds) Marine pelagic cyanobacteria. *Trichodesmium* and other diazotrophs. Kluwer Academic Press, Dordrecht, pp 163–175

Villareal TA (1994) Widespread occurrence of the *Hemiaulus*-cyanobacteria symbiosis in the southwest North Atlantic Ocean. Bull Mar Sci 54:1–7

Watanabe I, Roger PA (1984) Nitrogen fixation in wetland rice fields. In: Subba Rao NS (ed) Current developments in nitrogen fixation. Oxford IBH, New Delhi, pp 237–276

Weeler CT, Miller IM (1990) Current and potential uses of actinorhizal plants in Europe. In: Schwintzer CR, Tjepkema JD (eds) The biology of *Frankia* and actinorhizal plants. Academic, San Diego, pp 365–389

Wegley L, Edwards R, Rodriguez-Brito B, Liu H, Rohwer F (2007) Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*. Environ Micro 9:2707–2719

Wilkinson CR (1978) Microbial associations in sponges. I. Ecology, physiology and microbial populations of coral reef sponges. Mar Biol 49:161–167

Wilkinson CR (1992) Symbiotic interactions between marine sponges and algae. In: Reisser W (ed) Algae and symbiosis. Biopress, Bristol, pp 112–151

Wilkinson CR, Fay P (1979) Nitrogen fixation in coral reef sponges with symbiotic cyanobacteria. Nature 279:527–529

Zackrisson O, DeLuca TH, Nilsson MC, Sellstedt A, Berglund LM (2004) Nitrogen fixation increases with successional age in boreal forests. Ecology 85:3327–3334

Zahran HH (1998) Structure of root nodules and nitrogen fixation in Egyptian wild herb legumes. Biol Plant 41:575–585

Zhang X, Benson DR (1992) Utilization of amino acids by *Frankia* sp. strain CpI1. Arch Microbiol 158:256–261

Zhao Z, Williams SE, Schuman GE (1997) Renodulation and characterization of *Rhizobium* isolates from Cicer milkvetch (*Astragalus cicer* L.). Biol Fertil Soils 25:169–174

Zheng W, Rang L, Bergman B (2009) Structural characteristics of the cyanobacterium – *Azolla* symbioses. In: Pawlowski K (ed) Prokaryotic symbionts in plants. Springer, Dordrecht, pp 235–263

Zhu C (1982) Fine structure of blue-green algae and the cells lined along the endophyte cavity in the coralloid root of *Cycas*. Acta Bot Sin 24:109–114

Zielke M, Ekker AS, Olsen RA, Spjelkavik S, Solheim B (2002) The influence of abiotic factors on nitrogen fixation in different types of vegetation in the High arctic. Svalbard. Arct Antarct Alp Res 34:293–299

Zielke M, Solheim B, Spjelkavik S, Olsen RA (2005) Nitrogen fixation in the high arctic: role of vegetation and environmental conditions. Arct Antarct Alp Res 37:372–378

Zimmerman WJ, Bergman B (1990) The *Gunnera* symbiosis: DNA restriction fragment length polymorphism and protein comparisons of *Nostoc* symbionts. Microb Ecol 19:291–302

Zimmerman WJ, Rosen BH (1992) Cyanobiont diversity within and among cycads of one field site. Can J Microbiol 38:1324–1328

Phosphate-Solubilizing Microorganisms: A Critical Review

12

N. Kishore, Pavan K. Pindi, and S. Ram Reddy

Abstract

Nitrogen (N), phosphorus (P) and potassium (K) are the three important nutrients required by any plant for healthy growth. Among these, P stands as the second limiting nutrient next to nitrogen. Even though different forms of P are abundantly present in soil, its availability in plant-utilizable form is limited. This deficiency is usually compensated by adding chemical fertilizers. However, the chemical fertilizers are expensive and are not eco-friendly. Nonjudicious and irregular usage for a long time leads to decreased soil activity and soil microflora leading to imbalance in equilibrium. Usage of microorganisms to augment the P availability is the best alternative. Phosphate-solubilizing microorganisms (PSMs) when applied in appropriate numbers into the rhizosphere help the plant by supplementing P in plant-utilizable form by several mechanisms. In addition, few PSMs also possess added features as plant growth-promoting rhizobacteria (PGPR) and biocontrol agents conferring protection from phytopathogens. Improvement in soil characters by PSMs is an added advantage. Recent advances in technology paved the way for modifying PSMs with desired qualities. In spite of these, several areas in this area of research suffer different lacunae. Efforts are being made to discuss all major areas pertaining to PSMs in the present review.

N. Kishore • P.K. Pindi
Department of Microbiology, Palamuru University,
Mahabubnagar 509 001, Telangana, India

S. Ram Reddy (✉)
Department of Microbiology, Kakatiya University,
Warangal 506 009, Telangana, India
e-mail: sanditi.ramreddy@gmail.com

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_12,

Keywords
Microorganisms • Phosphate solubilization • Plant growth-promoting rhizobacteria • Crop protection • Phytopathogens

12.1 Introduction

Sustainable agriculture remains a stand-alone solution to scarcity of food and prevailing hunger. Statistical estimates of the Food and Agriculture Organization of the United Nations (FAO 2005) indicate that more than 923 million people face chronic hunger. Further this is expected to increase by 9.3 billion in 2050. In view of this, there is an urgent requirement for a revolution in agricultural productivity by bringing marginal and uncultivable lands (soils of low productivity) into the frontiers of agriculture. High yields in agriculture depend on healthy crops which in turn depend on plant health and soil fertility.

One of the major factors that deprive plant health is nutritional deficiency. Different metabolic processes of plants at any growth stage can be adversely affected by low or no availability of soil nutrients. Plants of different genotypes differ in their ability to uptake nutrients from soil by converting unavailable forms to assimilable form because of root surface area, root exudates and rhizosphere microflora (Sessitsch et al. 2013). Generally unavailability of nutrients is attributed to factors like low solubility, poor mobility or inherent low nutrient concentrations in different soils.

In soil bulk, plant essential nutrients are relatively high in total amounts but the concentration in soil solution (i.e. plant available form) of rhizosphere is not sufficient enough to meet the needs of healthy plant growth. Nutrients like phosphorus (P), potassium (K), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) have limited mobility/solubility in soils. These are transported into the roots by a slow process called diffusion. Nitrogen (N), phosphorus (P) and potassium (K) are among the main macronutrients required for plant growth. Among them, N is abundantly available from atmospheric sources and biologically/chemically available to plants.

Phosphorus/phosphate (P) plays an important role in life acting as a backbone in molecules like DNA (deoxyribonucleic acid), RNA (ribonucleic acid) and phospholipids of animal and plant cells. After nitrogen, P is the second major growth-limiting nutrient in agricultural production. Directly or indirectly P nutrition in plants affects root surface area, crop yield and quality, N fixation, seed formation, crop maturity, stalk and stem strength and resistance against plant pathogens. Most of the P acquired by the plant is used in various plant metabolisms. Phosphorus absorption occurs mainly during vegetative plant growth and a major part of it is translocated to fruits and seeds. Phosphorus is also essential for cell division, photosynthesis, sugar breakdown and nutrient uptake and transport. Phosphorus deficiency leads to retarded growth and dark-green coloration in plants.

Many soils contain high amounts of P; however, most of it is unavailable to plants for utilization because of adsorption, precipitation and conversion to organic form. Plants prefer P in water-soluble form, i.e. $(PO_4)^{3-}$, $(H_2PO_4)^{2-}$, $(HPO_4)^{2-}$. Concentrations of these forms of P are very low varying from 0.001 mg l^{-1} (poor soil) to 1 mg l^{-1} (highly fertile).

12.2 Forms of Soil P

Generally two forms of P exist in soils, namely, organic (Po) and inorganic (Pi), varying in terms of quality and quantity.

12.2.1 Organic P (Po)

More than 50 % of the total P is organic phosphorus. Chemically these are esters of orthophosphoric acid identified as inositol P, phospholipids and nucleic acids (Quiquam Poix and Mousain 2005). Inositol P (Ca-Mg salt of phytic acid) is the most abundant of total organic P ranging from 10 to 50 %. Water-insoluble fraction of total organic P includes phospholipids (1–5 %) (Dalal 1977) that is easily released and utilized by microorganisms in the soil. Nucleic acids (constituting 0.2–2.5 % of total organic P) are released from organism residues in two forms, viz. DNA and RNA, which are quickly broken down.

12.2.2 Inorganic P (Pi)

The natural source of P in soils is the mineral apatite, a calcium phosphate which is sparingly soluble. This mineral is found in very lower horizons of soil and is unavailable to plants. Mono- and dicalcium phosphates are simpler forms assimilable by the plants but their tendency to convert back to insoluble forms and their existence in extremely small quantities make them unavailable. Apart from apatite, P can also form minerals in combination with Fe (strengite) and Al (variscite) contributing little to the plant nutrition owing to their low solubility. Other common minerals of P in soil are represented in Table 12.1 in the order of decreasing solubility. Chemical dynamics of P and its constituents in soil were excellently reviewed (Jones and Oburger 2011).

Table 12.1 Different forms of phosphorus in soil

Mineral	Chemical formula
Strengite	$Fe\ PO_4 \cdot 2H_2O$
Variscite	$AlPO_4 \cdot 2H_2O$
Tricalcium P	$Ca_3(PO_4)_2$
Dicalcium P	$CaHPO_4$
Dicalcium P dihydrate	$CaHPO_4 \cdot 2H_20$
Fluorapatite	$Ca_5(PO_4)_5\ F$
Hydroxyapatite	$Ca_5(PO_4)_3\ OH$
Octacalcium P	$Ca_5\ H(PO_4)_3 \cdot 2\text{-}5H2O$

12.3 Availability of P to Plants

Phosphates that are soluble in water or 2 % citric acid solution are known as available forms which can easily be assimilated by plants. Most of the rock phosphate forms present in soil are insoluble except for very low quantities of P from sedimentary origin. Plant root exudates contain organic acids (citric, malic, etc.) that can dissolve insoluble phosphates and assimilate through diffusion.

Scientists from England (Rothamsted Experimental Station) developed single super phosphate (SSP/water-soluble P) and later on introduced diammonium phosphate (DAP), mono-ammonium phosphate and NPK mixtures. This agricultural chemicalization, though increased world food production, has invited new problems. Excessive and nonjudicious application of chemicals over a long period leads to destruction of soil properties and microbiota.

Several strategies are being adopted to enhance the availability of P in different soils. One such strategy is application of high dose of soluble P fertilizers (1,000 mg/kg), followed by small amounts of application in subsequent years. Most of the added P will be fixed and this may be released over several years. Acidic soils have high P fixation capacity. These soils when amended with chemical fertilizers, soluble P is quickly fixed. In tropical countries, strategies like

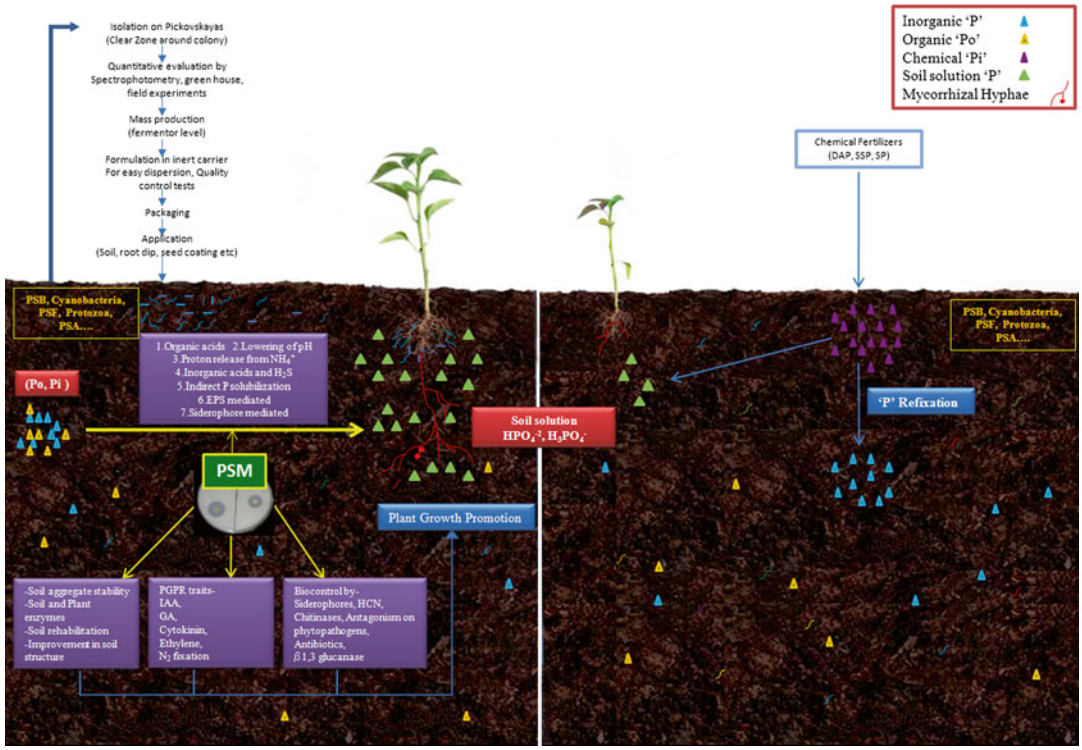


Fig. 12.1 Comparison of P-availability and plant growth promotion between soil fortified with PSM (*left hand side*) and soil amended with chemical P fertilizers (*right hand side*). *Arrows* in *left side of figure* indicate different mechanisms in which PSMs contribute to phosphate solubilization and plant growth promotion. Steps involved in isolation and mass multiplication of PSMs are shown as a flow chart (*top left of figure*). *Abbreviations*: *P* phosphorus, *Pi* inorganic P, *Po* organic phosphorus, *PSM* phosphate solubilizing microorganisms, *PSB* phosphate solubilizing bacteria, *PSA* phosphate solubilizing actinomycetes, *IAA* indole acetic acid, *GA* gibberellic acid, *HCN* hydrogen cyanide

amendment of soils with rock phosphate in combination with organic materials like farmyard manure, compost and green manures are shown to increase plant growth and crop yields. Added manures were shown to assist desorption of sorbed phosphorus (Pi). Some researchers even tried processing of rock phosphates by grinding, heat treatment and fusion with Na, Mg and Si which showed some satisfactory result (Redding et al. 2006).

The added chemical phosphorus cannot satisfy plant requirements owing to soil pH. Tropical and subtropical soils are predominantly acidic and often extremely P deficient. Below pH 5.5, soil cations Fe, Al and Mn lock up P and make it unavailable to plants. Calcium and magnesium ions precipitate P at a pH range above 7. Due to phosphate fixation, the use efficiency of added chemical P by plants is just 15 % in the first year and 1–2 % in subsequent years (Mark Evans 2012) (Fig. 12.1).

12.4 Phosphate-Solubilizing Microorganisms (PSMs)

The soil which acts as a substratum for plant growth is an ecological hot zone with many constant biological activities. It is a dynamic system regulating organic matter decomposition and availability of plant nutrients. Soil microorganisms are significantly responsible for major global biogeochemical cycles. Microorganisms in soil influence health of the soil directly or indirectly through their beneficial and detrimental activities. The major microbial activity is

confined to the aggregates with accumulated organic matter around the rhizosphere. The rhizosphere was defined in 1904 by Hiltner as being the volume of soil, influenced by the presence of living plant roots, whose extension may vary with soil type, plant species, age and other factors. Rhizospheric microorganisms are responsible for several activities like decomposition, nutrient mobilization and mineralization, storage and release of nutrients and water, N fixation and denitrification.

The concept of using pure cultures of soil microorganisms to increase P nutrition of plants through increased solubility of calcium phosphates is not new. Evidence of the involvement of microorganisms in solubilizing insoluble phosphates was shown as early as 1903. Since then several studies on solubilization of P by different kinds of microorganisms were extensively carried out. Phosphate-solubilizing microorganisms are ubiquitous in nature and vary from soil to soil in number (Lopez et al. 2012). Several microorganisms belonging to bacteria, fungi, actinomycetes, cyanobacteria and even protozoa were shown to be involved in making the soil P solution. The type and form of insoluble P present and type of soil determine the microbial community of PSB. Biodiversity of phosphate-solubilizing organisms in relation to plant host specificity and various rhizosphere soils was studied earlier. The capacity of bacterial isolates to solubilize phosphates depends upon the zone of their origin, those derived from the rhizoplane having the highest, the rhizosphere intermediate and non-rhizosphere soil the least capacity (Gurdeep Kaur and Sudhakara Reddy 2014).

Different properties of soil, viz. physical and chemical properties, organic matter and P content, determine the population of PSMs in soil. In general agricultural and range lands harbour high counts of PSMs. Most of the PSMs are effective in Ca-P containing calcareous soil compared to Fe-P and Al-P containing Alfisols. In total microbial population of soil, phosphate-solubilizing bacteria (PSB) constitute about 50 % and phosphate-solubilizing fungi (PSF) 0.1–0.5 %. In general, PSB outnumber PSF by 2–150-folds.

The interaction between microorganisms and soil constituents are vital to all terrestrial ecosystems. Plant and microbial ecosystems are often stressed in acquisition of P primarily due to low aqueous solubility of PO_4^{3-}. Plants obtain P from soil in the form of HPO_4^{-1} and $H_2PO_4^{-1}$. Thus, the ability of microorganisms to solubilize and mineralize P in soil is vital. Crop plants require approximately 10–100 kg P ha^{-1}.

In an average soil only 0.05–0.01 % of the total P present is available to plant because of its chemical fixation and low solubility. A survey of Indian soils revealed that 98 % of these soils need P fertilization either in the form of chemical or biological fertilizer. Phosphate availability in soil is greatly enhanced through microbial production of metabolites leading to lowering of pH and release of P from organic and inorganic complexes. In Russia a commercial biofertilizer by the name "phosphobacterin" containing *B. megaterium* var. *phosphaticum* was produced and widely used with yield increases of 5–10 % over controls. A commercial formulation of *Penicillium bilaii chalubuda* has also been registered in Canada (Jumpstart ®) as biological enhancer of plant nutrients now sold by Novozyme (Jumpstart 2012).

12.4.1 Phosphate-Solubilizing Bacteria (PSB)

Bacteria are well known for their ability to release bound phosphorus from different sources. PSB occur in moist soils and may represent up to 40 % of cultivable population. Both aerobic and anaerobic P-solubilizing strains of bacteria are found prevalent in considerable numbers in soil, in plant rhizospheres and even in marine environments (Syed and Damare 2013). Keeping this in view management strategies need to be formulated with introduction of native efficient strains suitable for sustainability (Zhaoa et al. 2014).

Common groups of bacteria involved in phosphate solubilization are *Bacillus*, *Pseudomonas*, *Azotobacter*, *Burkholderia* and *Rhizobium*. Even with a single bacterium species of single phylogenetic lineage, significant variations exist in terms of source of isolation (Zhaoa et al. 2014).

Phosphate solubilization in rhizobia accompanied with N fixation was reported earlier. About 60 % of *Bradyrhizobium* strains were reported to be capable of P solubilization (Hayat et al. 2010). Some strains of P-solubilizing rhizobia can colonize the roots like other plant growth-promoting rhizobacteria (PGPR) and increase the yield of legumes and nonlegumes.

Increase of plant growth in wheat by solubilization of inorganic phosphates from tricalcium phosphates (TCP) and Mussoorie rock phosphate (MRP) was observed using *Azotobacter chroococcum* (Kumar et al. 2001). Positive results were also observed in cotton and wheat varieties when inoculated with P-solubilizing *A. chroococcum* with greater NPK uptake (Narula et al. 2005).

Reports on phosphate solubilization by pseudomonads in general are scanty. It has been reported that 18 % of fluorescent pseudomonads were positive for solubilization of TCP as evident from visible dissolution halos on Pikovskaya's agar. Carbon and nitrogen sources are important parameters for active proliferation and production of organic and inorganic acids for P solubilization (Scervino et al. 2011). Fluorescent pseudomonads facilitated increase in root surface area and mineral phosphate solubilization leading to increased nutrient uptake and seedling biomass. Fluorescent pseudomonads and rhizobia were shown to solubilize organic and inorganic (Antoun 2012) phosphates. Several species of fluorescent pseudomonads such as *P. fluorescens* NJ-101, *P. fluorescens* EM85, *P. aeruginosa*, *Pseudomonas* sp., *P. chlororaphis*, *P. savastanoi*, *P. picketii*, *P. lulea* OK2, *P. rhizosphaerae* LMG 1640, *P. graminis* DSM 11363, *P. striata* and *P. corrugata* have been reported as efficient P solubilizers. Bacteria exhibiting solubilizing activity and also colonizing mycorrhizal hyphae may contribute indirectly to uptake P by mycorrhiza. Investigations in this regard have shown that these bacteria are found in hyphal mucilage, hyphoplane, in hyphal wall layers and even inside hyphae and spores (Gonzalez-Chavez et al. 2008). These bacteria apart from P solubilization may execute other functions simultaneously. Selection of bacteria based on a single trait may not be promising for inoculation technology (Praveen Kumar et al. 2012). Few bacteria studied for their P-solubilizing ability are listed below.

Gram-positive bacteria: *Bacillus brevis*, *B. cereus* var. *albolactis*, *B. circulans*, *B. coagulans*, *B. firmus*, *B. megaterium*, *B. megaterium* var. *phosphaticum*, *B. mesentricum*, *B. mycoides*, *B. polymyxa*, *B. pumilus*, *B. pulvifaciens*, *B. sphaericus*, *B. subtilis*, *Clostridium* sp., *B. licheniformis*, *B. amyloliquefaciens*, *A. atrophaeus*.

Gram-negative bacteria: *Acetobacter diazotrophicus*, *Achromobacter* sp., *Aerobacter aerogenes*, *Agrobacterium radiobacter*, *Agrobacterium* sp., *Alcaligenes* sp., *Arthrobacter mysorens*, *Bradyrhizobium* sp., *Brevibacterium* sp., *Burkholderia cepacia*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Enterobacter asburiae*, *Enterobacter cloacae*, *Escherichia freundii*, *Escherichia intermedia*, *Erwinia herbicola*, *Flavobacterium* sp., *Gluconobacter diazotrophicus*, *Micrococcus* sp., *Mycobacterium* sp., *Nitrosomonas* sp., *Pseudomonas calcis*, *P. cepacia*, *P. fluorescens*, *P. putida*, *P. rathonia*, *P. striata*, *P. syringae*, *Serratia marcescens*, *S. phosphaticum*, *Thiobacillus ferrooxidans*, *T. thiooxidans*, *Rahnella aquatilis*, *Rhizobium meliloti*, *Xanthomonas* sp., *Azotobacter chroococcum*, *Kluyvera ascorbata*, *Azospirillum brasilense*, *A. lipoferum*, *Acinetobacter calcoaceticus*.

12.4.2 Phosphate-Solubilizing Fungi (PSF)

Phosphate-solubilizing fungi are known for their ability to solubilize high amounts of bound P for plant growth promotion. Release of enzymes like acid and alkaline phosphatases, phytase and organic acids (citric, oxalate, gluconate, etc.) appears as strategies for dissolution of insoluble phosphates by PSF. Reports indicate that they are able to show 5–20 % increment in plant growth (Gunes et al. 2009). Out of the total fungal population, PSF constitute about 0.1–0.5 %. In general, the most commonly encountered genera of PSF are *Aspergillus* and *Penicillium*

(Reyes et al. 2002). A nematophagous fungus, *Arthrobotrys oligospora*, was also shown to solubilize phosphate in vitro and in vivo (Duponnois et al. 2006). Recently, aluminium and rock phosphate solubilization by a specific *Penicillium* sp. and yeast was studied by Xiao et al. (2013b) and Narsian et al. (2010), respectively. Unlike their bacterial counterparts, PSF were able to retain P-solubilization trait even after several subcultures. Moreover, they were able to solubilize more amount of bound P because of their ability to secrete more acids. Although bacteria have been used as commercial preparations to improve the plant growth, fungi seem to be a better option for the same purpose. A list of few fungi which were studied for P dissolution phenotype is given below.

PSF: *Achrothecium* sp., *Alternaria tenuis*, *Aspergillus aculeatus*, *A. awamori*, *A. carbonum*, *A. flavus*, *A. foetidus*, *A. fumigatus*, *A. japonicus*, *A. nidulans*, *A. nidulans* var. *acristatus*, *A. niger*, *A. rugulosus*, *A. terreus*, *A. wentii*, *Cephalosporium* sp., *Chaetomium globosum*, *Cladosporium herbarum*, *Cunninghamella* sp., *C. elegans*, *Curvularia lunata*, *Fusarium oxysporum*, *Helminthosporium* sp., *Humicola lanuginosa*, *H. inslens*, *Mortierella* sp., *Micromonospora* sp., *Mucor* sp., *Myrothecium roridum*, *Oidiodendron* sp., *Paecilomyces lilacinus*, *P. fusisporus*, *Penicillium aurantiogriseum*, *P. bilaji*, *P. digitatum*, *P. funiculosum*, *P. lilacinum*, *P. oxalicum*, *P. pinophilum*, *P. rubrum*, *P. rugulosum*, *P. simplicissimum*, *P. variabile*, *Phoma* sp., *Populospora mytilina*, *Pythium* sp., *Rhizoctonia solani*, *Rhizopus* sp., *Sclerotium rolfsii*, *Torulaspora globosa*, *Torula thermophila*, *Trichoderma harzianum*, *T. viridae*, *Schwanniomyces occidentalis*, *Emericella rugulosa*, *Penicillium camemberti*, *Colletotrichum* sp.

Yeast: *Yarrowia lipolytica*, *Schizosaccharomyces pombe*, *Pichia fermentas*

12.4.3 Phosphate-Solubilizing Actinomycetes (PSA)

Actinomycetes are widely distributed in nature and stand second to bacteria in terms of population in soil. They constitute 10–50 % of the total soil microflora depending on soil conditions. These bacteria with their ability to secrete secondary metabolites including antibacterial, antifungal, insecticidal and antihelminthic compounds are helping the plants to survive from ailments. They are also endowed with many other properties like production of phytohormones, siderophores, etc., through which they promote the healthy plant growth (Franco-Correa et al. 2010). Actinomycetes are isolated from various sources and screened for their efficiency to enhance P availability to plants. According to one study, 20 % of the total actinomycete population is PSA and that predominantly belong to genera *Streptomyces* and *Micromonospora* (Hamdali et al. 2008). Unlike fungi, actinomycetes cannot acidify the external medium though they release several organic anions in large quantities. Solubilization of P in these organisms is thought to be because of production of acid anions or by some other mechanisms (Hamdali et al. 2010). Field trials have shown increased plant growth but the reason for the increment is not clearly attributed to P solubilization or other beneficial effects. Owing to their ability to withstand extreme environments, these organisms are studied for enhancing P availability during municipal and animal waste composting (Chang and Yang 2009). Few actinomycetes listed below are known to have an active role in P solubilization.

Actinomyces sp., *Actinomyces coelicolor*, *Streptomyces* sp., *Streptomyces violascens*, *S. noboritoensis*, *S. cinereorectus*, *S. cinnabarinus*, *Microbacterium aurantiacum*, *M. kitamiense*, *Angustibacter luteus*, *Kocuria flava*, *Isoptericola hypogeus*, *Agromyces soli*, *Kocuria palustris*, *Microbacterium yannicii*, *Isoptericola variabilis*, *Nocardia* sp., *Streptoverticillium* sp., *Thermoactinomycetes* sp., *Micromonospora* sp.

12.4.4 Cyanobacteria P Solubilization

The ability of cyanobacteria to solubilize bound P became evident from the work of Kaushik (1995). These organisms apart from P

solubilization can extend many other benefits like N_2 fixation, production of growth-promoting hormones and many secondary metabolites. Inoculation of plants with P-solubilizing cyanobacteria has improved plant growth by increasing the availability of P and N nutrients.

Like bacteria, cyanobacteria are also known to mobilize bound phosphates. They were observed to solubilize $Ca_3 (PO_4)_2$, Fe PO_4, Al PO_4 and ($Ca_5 (PO_4)_3$.OH). They are also known to solubilize organic sources of phosphorus. Different strategies were thought to be involved in the release of bound P including production of organic acids, chelators, dissimilatory reduction and enzymatic solubilization or simultaneous action of one or more of these. Despite these proposals, it is still ambiguous with regard to definite operative method of P solubilization by cyanobacteria. In a recent study, *Westiellopsis prolifica* and *Anabaena variabilis* were shown solubilize TCP and MRP (Yandigeri et al. 2011). Further, it was demonstrated that P solubilization was due to the production and action of phthalic acid without any decrease in pH. Few examples of cyanobacteria studied for their ability to release bound P are listed below.

Anabaena, *Calothrix braunii*, *Tolypothrix*, *Scytonema*, *Hapalosiphon fontinalis*, *Nostoc* sp., *Scytonema cincinnatom*, *Tolypothrix tenuis*, *Tolypothrix ceylonica*, *Westiellopsis prolifica*, *Phormidium* sp.

12.4.5 Protozoa and Other Mesofauna P Solubilization

Protozoa live in soil at the expense of bacteria of the genera *Aerobacter*, *Agrobacterium*, *Bacillus*, *Escherichia*, *Micrococcus* and *Pseudomonas* by ingesting them into their protoplasm. Protozoa are abundant in the upper layer of the soil and their number is directly dependent on bacterial population. Owing to inadequate studies on soil protozoa, it is difficult to define their role in P cycling. They directly or indirectly influence the P cycling by regulating the number of bacteria in the soil. Even they can reduce the affectivity of the added PSB by grazing (Rosenberg et al. 2009). Apart from this, protozoa are known to increase P bioavailability by assimilating soluble minerals. But this line of research received less attention because of the hindrances in mass cultivation and negative impacts on dynamics of the food webs.

Interactions between P and soil mesofauna have recently been extensively reviewed (Chapuis-Lardy et al. 2011). Different mesofaunal populations exert both positive and negative impacts on P availability. Nematodes reduce the inoculated PSMs and thereby reduce the P availability. Earthworms play an important role in P nutrition by the method clearly not elucidated. It was shown that they enhanced ability of P solubilization by fungi (*A. awamori*) (Sreenivas and Narayanasamy 2009) and bacteria (*B. megaterium*) (Wan and Wong 2004) resulting in increased soluble P (organic and inorganic) in soil. In another study, earthworm casts and furrows were reported as sites for proliferation of PSMs and their activity (Mba 1997).

Although several PSMs occur in soil, usually their numbers are not high enough to compete with other bacteria commonly established in the rhizosphere. Thus, the amount of P liberated by them is generally not sufficient for substantial increase in in situ plant growth. Therefore, inoculation by a target microorganism at a much higher concentration than that normally found in soil is necessary to take advantage of the property of P solubilization for plant growth enhancement.

12.5 Mechanism of P Solubilization

Microorganisms, mainly residing in the milieu of rhizosphere, have the ability to influence the change of chemical environment by uptake and release of organic and inorganic ions. They can drastically influence the availability of P from organic (Po) and inorganic (Pi) phosphates. Dissolution and availability of Pi depends on the properties of inorganic minerals available. Broadly three main mechanisms are adopted by soil microorganisms for P solubilizations by (1) releasing mineral-dissolving substances like

organic acid anions, hydroxyl ions and CO2, siderophores and protons; (2) secreting extracellular enzymes (i.e. biochemical mineralization of Po); and (iii) substrate degradation (biological mineralization).

Phosphate immobilization and dissolution (i.e. P mineralization and solubilization) are two important processes deciding the fate of P in soil. Plants receive assimilable P if the rate of P solubilization (from minerals) and mineralization (bound organic P, disintegration of microbial biomass) exceeds P immobilization (by mineral formation, incorporation by uptake into microbial biomass). Phosphorus solution concentration in soil is affected by different processes of soil P cycle. Three different major processes described by Sims and Pierzynski (2005) that affect the solution P are (1) dissolution precipitation (mineral equilibria), (2) sorption desorption (interaction between P in solution and soil solid surfaces) and (3) mineralization immobilization (biologically mediated conversions of P between inorganic and organic forms). In all these processes of soil P cycle, microorganisms play an important role.

12.5.1 Solubilization of Inorganic Phosphates (Pi)

Many researchers have proposed different mechanisms responsible for release of P (in solution) from inorganic phosphates (Ca-P, Al-P, Fe-P, etc.). Some of them are (1) production of organic acids (chelation of P-bound cations), (2) production of inorganic acids, (3) H_2S production, (4) lowering of pH through release of protons, (5) proton release from NH_4^+ (assimilation/respiration), (6) P assimilation from liquid (indirect dissolution), (7) production of siderophores, (8) production of exopolysaccharides and (9) direct oxidation pathway.

12.5.1.1 Production of Organic Acids

Several studies have shown the ability of PSMs to solubilize insoluble phosphates in pure liquid culture medium, and this was often due to the excretion of different organic acids (Sharma et al. 2013). These organic acids are known to act through (1) lowering the pH, (2) enhancing chelation of cations bound to P, (3) forming complexes with P-associated metal ions and (4) competing with P for adsorption. Organic acids are produced by direct oxidation pathway (at the outer face of the cytochrome membrane) or mostly by oxidative respiration and fermentation of organic C sources. These acids can either dissolve P directly or chelate Fe, Al and Ca ions associated with P. Organic acids like gluconic acid, oxalic acid, citric acid, lactic acid, tartaric acid, aspartic acid, etc., were detected during the course of P-solubilization study using paper chromatography, TLC and HPLC. However, these studies lack correlation between acids produced and amounts of P liberated.

The chelating ability of organic acids also plays a significant role in making the P available from insoluble inorganic phosphates. In a study conducted by Kucey (1988), it was shown that addition of 0.05 M EDTA into the culture medium exerted the same effect shown by *Penicillium bilaji*. In another study, the addition of NaOH arrested P-solubilizing activity of *Rhizobium* which was thought to be associated with 2-ketogluconic acid. These studies emphasize that P solubilization by these organisms is due to their ability to reduce the pH of the medium.

12.5.1.2 Lowering of pH

P solubilization by acidification was well studied in many bacterial and fungal species. However, reports on P solubilization by alkalization are scanty. The excretion of organic acids by PSMs is associated with release of protons and lowering of pH (Maliha et al. 2004). Release of protons or hydroxide ions by PSMs can also influence soil solution pH.

Acidification and lowering of pH are not the only mechanisms involved in solubilization as reduction in pH does not always correlate with the amount of P solubilized. Correlating to this, HPLC analysis of culture filtrate from P-solubilizing *Pseudomonas* sp. did not reveal any organic acid formation. Parks et al. (1990) proposed an alternative mechanism of P solubili-

zation by proton excretion because of NH_4^+ assimilation.

12.5.1.3 Proton Release from NH_4^+ (Assimilation/Respiration)

The amount of protons released into the external medium influences the soil pH which in turn is influenced by the type of N sources used by microorganisms. Among different N sources, ammonium salts are reported to be the best followed by asparagine, sodium nitrate, potassium nitrate, urea and calcium nitrate. With NH_4^+ as sole N source, reduction in pH and amount of P solubilized was observed to be high compared to that of nitrate (NO_3^-). This is due to extrusion of protons to compensate NH_4^+ uptake (Sharan et al. 2008). This was in contradiction with the findings of Reyes et al. (1999), who reported decrease in P solubilization when higher concentration of NH_4^+ was supplied to the medium.

Ammonium-driven proton release may stand as sole mechanism for solubilization in some microorganisms. But in some organisms no significant relation could be accounted for pH change and P mobilized. This indicates existence of additional solubilization mechanisms. Also, proton release depends on different mechanisms and only partly on NH_4^+ assimilation as evidenced from the work of Park et al. (2009).

12.5.1.4 P Solubilization by Inorganic Acids and H_2S

Inorganic acids like HCl, H_2SO_4 and HNO_3 were reported to solubilize less amounts of Pi. *Enterobacter agglomerans* was shown solubilizing P from hydroxyapatite mediated by HCl. A study by Kim et al. (1997) using *E. agglomerans* and a genetically modified *E. coli* showed that incorporation of culture media with known acids like HCl, citric acid, oxalic acid and lactic acid increased P solubilization from hydroxyapatite although citric acid was able to solubilize more P than HCL. Production of acids like HNO_3 and H_2SO_4 and dissolution of phosphates were observed in bacterial genera *Nitrosomonas* and *Thiobacillus*.

Release of bound inorganic P by H_2S is another mechanism. Certain bacteria produce H_2S which reacts with ferric phosphate resulting in the formation of ferrous sulphate and P release. Microbial S oxidation is a mechanism increasing mineral phosphate solubility by production of H_2SO_4, nitrate and CO_2. However, these mechanisms are less accepted than P solubilization by organic acids.

12.5.1.5 Indirect P Dissolution

The sink theory proposed by Halvorson et al. (1990) states that rhizospheric microorganisms assimilate large amounts of P from soil solution by P uptake system. As a result equilibrium between insoluble and soluble P is disturbed. Consequently, sparingly soluble phosphates would then be dissolved indirectly. Phosphorus content in PSMs was similar to those observed in non-P-solubilizing microorganisms due to the fact that the P content of the organisms is correlated with decomposition of P containing organic substrates. Although some P released by PSMs will be used by plants and other soil organisms, where most of the part remains immobilized with the biomass. This P is released when cells die due to changes in environmental conditions, starvation or predation. Environmental changes such as drying-rewetting or freezing-thawing can result in sudden increase in available P due to the unusual high proportion of microbial cell lysis (Butterly et al. 2009).

12.5.1.6 Direct Oxidation Pathway

Goldstein (1995) suggested the essential role played by extracellular oxidation in soils where calcium phosphate provides a significant pool of unavailable mineral phosphorus. This was confirmed by the biochemical analysis of lowering pH and insoluble P solubilization by *Burkholderia cepacia* DA 23 (Song et al. 2008).

12.5.1.7 Exopolysaccharide (EPS)-Mediated P Release

The role of low molecular weight organic acids in the solubilization of mineral P is well documented. But the knowledge on the role of high molecular weight microbial exudates (nonenzymatic mucilage, EPS) on P solubilization is limited. EPS and biosurfactants are produced by

microorganisms largely in response to biofilm formation and stress. Microbial exopolysaccharides are polymers of carbohydrates (homo- or heteropolysaccharides) excreted by some bacteria and fungi on the outer side of their cell walls. Earlier studies have shown that the EPS have the ability to form complexes with metals in soil (order of affinity to form complexes $Al^{3+} > Cu^{2+} > Zn^{2+} > Fe^{3+} > Mg^{2+} > K^{+}$) (Ochoa- Loza et al. 2001) implicating their role of P solubilization in soil.

Microbial EPS, under pure culture studies, have shown to stimulate dissolution of TCP in synergy with organic anions. In support of this, four bacterial strains (PSB) – *Enterobacter* sp. (EnHy-401), *Arthrobacter* sp. (ArHy-505), *Azotobacter* sp. (AzHy-510) and *Enterobacter* sp. (EnHy-402) – were evaluated for their role of EPS in dissolution of insoluble phosphates (Yi et al. 2008). Further the rate of dissolution was showed dependent on microbial source and concentration of EPS.

12.5.1.8 Siderophore-Mediated P Release

Siderophores are iron (Fe)-chelating agents produced by almost all microorganisms when subjected to Fe deficiency. They are low molecular weight (<10,000 D) virtually ferric-specific ligands produced as scavenging agents in order to combat low iron stress. Siderophore production is not widely being investigated as a method for phosphate solubilization. In view of dominance of mineral dissolution by organic acids as a method for P solubilization, ligand exchange and its role in enhancing available P are not obvious. These ligands were extensively studied with respect to their ability to mobilize Fe.

Approximately, 500 different siderophores are known which are used by both plants and microorganisms. Several PSMs are also reported to produce siderophores (Collavino et al. 2010). Very few works have been carried out to evaluate siderophore production as a method of P solubilization. Reid et al. (1985) showed 13-fold increments in P diffusion when compared with water without knowledge about siderophore enhancing P solubilization. Here two siderophores (desferrioxamine-B, desferrichrome) and an iron-chelating agent EDDHA (ethylenediamine-N,N′-bis(2-hydroxyphenylacetic acid)) were compared to water for Fe and P diffusion using root simulating technique.

12.5.2 Solubilization of Organic P (Po)

Microorganisms adopt various mechanisms for making the available P from insoluble reserve in soil. Approximately, 4–90 % of the total soil P is organic P (Po). Generally, in order to solubilize this Po, microorganisms employ mechanisms involving secretion of several enzymes. These are either cell wall bound or freely excreted to the surrounding environment. Extracellular enzymes are thought to be more active inducing large changes in soil solution P concentration. However, experimental evidence of exo- and endoenzyme activity is still ambiguous.

The modes of action of enzymes vary with different types of enzymes secreted. They may involve in (1) dephosphorylation of esters and anhydrides of H_3PO_4 (Tabatabai 1994), in (2) release of P from phytate degradation (Singh et al. 2014) or by (3) cleaving C-P bond of organophosphates (Rodriguez et al. 2006).

Phosphatases or phosphohydrolases are a broad group of enzymes secreted by PSMs catalyzing dephosphorylation reaction. Among this, phosphomonoesterases (or phosphatases) are most abundant and well studied. Based on their pH optima, phosphomonoesterases are further classified as acid and alkaline phosphomonoesterases. Both of these phosphatases are produced by PSMs depending on external conditions (Jorquera et al. 2011). Acid phosphatases are typically predominant in acidic soils, while alkaline phosphatases are observed in neutral and alkaline soils (Singh and Reddy 2011).

Acid phosphatases are also secreted by plant roots apart from microorganisms. It is difficult to differentiate between root- and PSM-produced phosphatases (Richardson and Simpsom 2011). It was observed that phosphatases of microbial origin have more affinity towards Po compared to

those derived from plant roots. Laboratory studies evidenced that gross mineralization processes produced 1–4 mg P Kg^{-1}. However, it is not easy to distinguish between enzymatic (biochemical) and biological (microbial) mineralization. Lower concentration of divalent cations (Ca, Mg, Zn, Co) was observed to be acting as enzyme activators, whereas high concentration of several metals (Zn, Mn, Cu, Mn (II), Fe (II)), polyvalent anions (MoO_4^{2-}, AsO_4^{3-}) and orthophosphate (end product) were found to inhibit enzyme activities (Quiquampoix and Mousain 2005). Further, enzyme activities are not simply related to their release rate but are strongly influenced by soil properties.

Inositol phosphates are the most abundant of the total organic P in a soil. Microorganisms bring about degradation of phytate leading to the release of bound P. This process is assisted by secretion of enzyme phytase. Phytate is the major stored form of P in plant seed and pollen, although plants' ability to obtain P from phytate is limited. In an experiment it was shown that genetically transformed *Arabidopsis* plant with phytase gene (*Phy* A) could assimilate phytate with significant increment in growth and P nutrition (Richardson et al. 2001). Here the P content of the plant was equivalent to control plant supplemented with inorganic phosphates. This confirms the role of microorganisms in mineralization of phytate and thus increasing the P nutrition (Richardson and Simpson 2011). Yet in another study, Rodriguez et al. (2006) demonstrated the role of special enzyme phosphonotases and C-P lyases in cleavage of C-P bond of organophosphates in the availability of soluble P in soil. Besides this, phytase-producing isolates of *Advenella* sp. and *Cellulosimicrobium* sp. were recently reported as PGPR increasing P content in Indian mustard (Kumar et al. 2013; Singh et al. 2014).

Each P solubilizer may adopt one or more than one mechanisms to solubilize insoluble P. Any one single mechanism as the sole method responsible for P solubilization cannot be pointed out, although organic acid production and pH reduction were witnessed in most of the occasions. Recently, biochar addition was shown alluviating toxicity caused by fluoride produced during the course of P solubilization using *Aspergillus niger*, thereby increasing the solubilization efficiency (Mendes et al. 2014).

12.6 Effect of PSMs on Soil Properties

In addition to P solubilization, PSMs also provide multiple beneficial effects on many soil properties including soil structure, soil enzymes and their activities and soil microbial community (Vassileva et al. 2010) (Table 12.2).

12.6.1 PSM-Soil Aggregate Stability

Microorganisms affect the soil properties either mechanically or by excretion of polysaccharides

Table 12.2 Some PSMs studied for their ability to improve soil properties

S. No	Organism	Plant	Remarks	Reference
1	Cr-resistant PSB *Pseudomonas* sp., *Bacillus* sp.	*Brassica juncea*	Increased plant growth against Cr inhibition/IAA, siderophore	Rajkumar et al. (2006)
2	Antibiotic and heavy metal-resistant PSB *Burkholderia* sp.	Maize, tomato	Increased biomass/IAA, siderophore, ACC deaminase	Jiang et al. (2008)
3	Metal-resistant PSB *Pseudomonas* sp. (PsM6), *P. jesseni* (PjM15)	*Ricinus communis*	Reduced toxicity of metal to plants/ACC deaminase, IAA, growth, uptake of Ni, Cu, Zn	Raj kumar and Freitas (2008)
4	Cd-resistant PSB *P. aeruginosa*	Black gram (*Vigna mungo*)	Reduced toxicity of metal to plants/ACC deaminase, IAA	Ganesan (2008)

into the medium. Many investigators have suggested that production of polysaccharides by PSMs is responsible for soil structure improvement (Bearden and Petersen 2000). PSMs were shown to increase the soil aggregate stability, water-soluble C and carbohydrate C in rhizosphere.

12.6.2 PSM-Soil and Plant Enzymes

It is also interesting to note that phosphate solubilizers increased the enzyme content and activity in PSM-amended soils. They were shown to improve dehydrogenase, phosphatase, β-glucosidase (Medina et al. 2006) and antioxidant enzymes (ascorbate peroxidase (APOX), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT)) in plants grown in contaminated soils enriched with PSMs (Azcon et al. 2009b). Further, synergistic interaction between AM fungi and PSF was reported to improve enzyme activities in bulk soil at different salinities (Zhang et al. 2011). The increase of enzymatic activities in soils and plants confers increase in availability and acquisition of nutrients.

12.6.3 PSM-Soil Microbial Community

It is well known that AM fungi affect microbial community in both direct and indirect ways. Addition of PSMs enhanced the amount of biomarker fatty acids of all groups of microorganisms as a result of the increase in carbon sources, while the microbial community remained unaffected by inoculation with AM fungi alone. An increased AM fungal growth and activity was found in *A. niger* (a P solubilizer)-treated agrowaste (Medina et al. 2007). In another study, it was shown that *A. niger*-treated sugar beet/rock phosphate amendment is a suitable tool for increasing the bacterial community in the rhizosphere (Azcon et al. 2009a). This work emphasized on bacterial community profiles generated from DGGE of amplified soil DNA and clearly implies that application of PSMs seems to play a decisive role in improving microbial community structure and soil properties.

12.6.4 PSM-Soil Rehabilitation

Remediation of heavy metal-contaminated sites using phosphate amendments like soluble phosphate, insoluble P sources like rock phosphate (RP) is an interesting approach. Hydroxyapatite is shown to be a very efficient metal immobilizer. Through this approach, heavy metal stabilization was studied using hydroxyapatite, synthetic and natural apatites and rock phosphates. However, bioavailability of heavy metals in soil is affected by the presence of organic matter forming complexes and chelates of varying stability (Kiikila et al. 2002). In such cases, various microorganisms can mobilize metals through autotrophic and heterotrophic leaching, chelation and methylation. Acidification through organic acid production and siderophores can supply protons and metal-complexing anions leading to metal release (Gadd and Sayer 2000) (Table 12.2).

Medina et al. (2006) have demonstrated that *A. niger*-treated sugar beet and rock phosphate amendment improved growth and nutrition of white clover grown in heavy metal (Zn and Cd)-contaminated soil. Here microbially mineralized RP (simultaneously solubilized), sugar beet and fungal mycelium in combination with AM fungi (*G. mosseae*) increased the plant growth 28 times more than non-mycorrhizal control plants.

12.7 PSM Effect on Plant Growth Under Greenhouse and Field Conditions

New and novel solutions for plant growth enhancements are required to ease the burden imposed on environment and other resources. The major applications of bacteria for improved plant growth include agriculture, horticulture, forestry and environmental restoration. Certain cooperative microbial activities can be exploited as a low-input biotechnology and basis for a

strategy to help sustainable and environmentally friendly practices fundamental to stability and productivity of agricultural systems. Phosphate reserves are likely to be depleted in about 500–600 years at the present mining rates (7,100 million tons/annum). In India, 98 % of crop lands are deficient in available soil P and hence it imports two million tons of rock phosphates annually. Different P management practices enforced are costlier and practically not feasible.

The use of microorganisms to increase crop yield has been limited due to the variability and inconsistency of results between laboratory, greenhouse and field studies. Soil is an unpredictable environment and intended results are sometimes difficult to obtain. The bulk of literature available on the subject indicates that the number of failures equals the number of successful trails as pointed out by Goldstein and Krishnaraj (2007). Nevertheless, microbially mediated P management is an eco-friendly and commercially viable strategy for sustainable crop production. The use of PSMs can increase crop yields up to 70 % and at the same time reduce the usage of chemical P by 25 %.

There are several reports indicating substantial increase in plant growth resulting from single, dual or three-member association of beneficial inoculants in the rhizosphere. Such syntropic associations could be of greater practical value for plant growth under different agroecosystems. In agriculture and forestry, inoculation practices, mixing of two or more microbial species often has a more positive effect on plant growth than the use of a single bacterium (Kishore 2007; Yu et al. 2012).

12.7.1 Inoculation Effects of Single Species of PSMs

Numerous reports are available indicating the beneficial effects of PSMs when inoculated as a single agent. A published account on the overall growth and development in different crops with consistent P uptake and crop yield increase was compiled and reviewed by Lucy et al. (2004). In spite of many technological developments, the performance of microbial inoculants within the vicinity of rhizosphere cannot be precisely predicted. The success of microbial inoculants is mainly determined by their root-colonizing ability and survivability to harness considerable benefits in competition with indigenous resident microbes. The application of single species of PSMs and the positive effects on plant growth were reported in many studies. Phosphate solubilization trait in microorganisms like *Rhizobium*, *Azotobacter*, etc., which are natural nitrogen fixers confer added advantages (Kumar et al. 2001). Such organisms with additional traits of plant growth promotion create confusion among the researchers in determining the actual role of P solubilization in plant growth. However, increased P levels within the plant tissues were reported even in field experiments with acid-tolerant PSB.

The effectiveness of PSF in enhancing plant Pi uptake and growth was controlled by the type of soil, particularly by the Pi-sorption capacity of soil. PSF are studied individually and in combination with other fungi for increment in growth parameters of many plants (chick pea, maize, wheat, faba bean, lentil, rice, soybean). PSF in combination with RP was proved to be more effective in P availability and growth promotion. PSF alone or in combination with rock phosphate (Mussoorie, Telesmi, etc.) was found responsible for increase in growth, seed production, shoot height, seed weight, N,P accumulation, dry matter yield, root length and yield.

Application of rock phosphate alone did not significantly increase the growth and plant P uptake (Cabello et al. 2005). Mollisols usually exhibit a low Pi-fixation capacity; for this reason it is not surprising that inoculation with PSMs alone increased P uptake. The effectiveness of PSM inoculation alone to enhance plant Pi uptake in subtropical and tropical acidic soils is relatively low and variable. By contrast, the effectiveness of PSM inoculation to enhance plant Pi uptake of mycorrhizal plants grown in tropical or subtropical soils can be relatively higher compared to data reported in temperate soils. Most of the soils like mollisols and calcareous and sandy soils are characterized by a low P-sorption

capacity and relatively high soil Ca-P content. Therefore, freshly released Pi by PSMs can remain longer in soil solution until its absorption by the roots (Duponnois et al. 2006).

12.7.2 Co- and Multiple Agent Inoculations

As both N and P are two major plant nutrients, combined inoculation of nitrogen fixers and P solubilizers may benefit plants better than either group of organisms alone. Inoculation of nitrogen-fixing organisms like *Rhizobium* and PSF was shown to have significant impact on wheat, chick pea, faba beans, beans, peas, green gram, etc., in increasing grain yield, growth, nutrient uptake (N and P), grain protein, etc., when compared to controls. However, in contrast, few studies imply that this combination did not show increase in dry matter or total P uptake (Kucey 1987). Further, a decrease in total N fixation is explained by high acidic conditions caused by PSF hindering rhizobial root colonization. Therefore, before going for field experiments, the compatibility between the two associate members must be checked in vitro.

12.7.3 Arbuscular Mycorrhizal Fungi (AMF) and PSMs

Mycorrhizal symbiosis is found in almost all ecosystems worldwide to improve plant fitness and soil quality through key ecological processes. Most of the major plant families form AM associations, the most common mycorrhizal type. Their origin and divergence has been dated back to more than 450 million years. Plant hormones as produced by soil microorganisms are known to affect AM establishment. The rhizosphere of a mycorrhizal plant can have features that differ from those of a non-mycorrhizal plant (Johansson et al. 2004).

The primary effect of AMF is the improvement of P uptake by plants due to the ability of external mycelium of AMF to act as bridge between roots and the surrounding soil microhabitats. This provides access to the phosphate ions from soil beyond the phosphate depletion zone surrounding the roots. The phosphate made available by PSMs may not reach the root surface due to limited diffusion. It was proposed that if the solubilized phosphates were taken up by AM mycelium, there will be increase in P supply to the plant. In particular AM inoculations improve the establishment of both inoculated and indigenous phosphate-solubilizing microorganisms (Medina et al. 2007).

The dual inoculation of PSMs and AMF may overcome the limitations imposed on the effectiveness of PSMs to enhance plant Pi uptake in soils with high Pi-fixation capacity. During this interaction, mycorrhizal plants release higher amounts of carbonaceous materials into the rhizosphere which is used as C source by PSMs. Recently, crop yield and N concentration in wheat plants were reported to have increased by >50 and 90 %, respectively, by the synergistic effect of *Glomus etunicatum* and *Burkholderia cepacia* BAM-6 (PSB) (Saxena et al. 2014).

12.7.4 PSMs as PGPR and Biocontrol Agents

Beneficial plant-microbe interactions in the rhizosphere are the determinants of plant health and soil fertility. Beneficial soil bacteria from the rhizosphere which have been shown to improve plant health or increase yield are usually referred to as plant growth-promoting rhizobacteria (PGPR) (Ahemad and Kibret 2014). The PGPR and the mechanisms by which it promotes the plant growth are ambiguous and are not fully understood but are thought to include the following characters (PGPR traits): (1) the ability to produce or change the concentration of plant hormones like indole acetic acid (IAA), gibberellic acid (GA), cytokinins and ethylene, (2) the asymbiotic N_2 fixation and symbiotic N_2 fixation, (3) the solubilization of mineral phosphates and other nutrients and (4) the antagonism against phytopathogenic microorganism by the production of siderophores, β-1,3-glucanases,

chitinases, antibiotics and cyanide (Figueiredo et al. 2011; Bhattacharyya and Jha 2012).

Apart from P solubilization, PSMs are also known to produce amino acids, vitamins and growth-promoting substances like IAA and GA (GA_3) which help in better growth of plants (Kishore 2007; Kishore et al. 2012). Production of plant growth regulators and biocontrol substances by PSMs in addition to P solubilization is an added advantage for being used as an efficient bioinoculants. Although there is a growing evidence that PSB and PSF augment plant growth due to biosynthesis of growth-promoting substances, future research in this direction is needed.

Naumova et al. (1962) reported that *A. chroococcum*, *B. megaterium* var. *phosphaticum* and *P. fluorescens* have accumulated in the medium some biologically active compounds such as auxin, gibberellins, vitamins, etc., which can stimulate plant growth and inhibit growth of fungi like *Fusarium* and *Alternaria*. Barea et al. (1976) reported that out of 50 isolates of PSB, 20 synthesized 3 types of plant hormones, and 43 produced cytokinin-like substances. Sattar and Gaur (1987) tested 8 PSB and fungal isolates such as *B. polymyxa*, *B. pulvifaciens*, *P. striata*, *A. awamori*, *A. niger*, and *P. digitatum* for synthesis of auxins and gibberellins.

At present, there is evidence supporting the role of this mechanism in plant growth enhancement. For example, several soil microorganisms, including bacteria, improve the supply of P to plants as a consequence of their capability for inorganic and organic P solubilization considering the fact that P availability is a limiting step in plant nutrition. This evidence suggests a fundamental contribution of P-solubilizing bacteria to plant nutrition and, therefore, to the improvement of plant growth performance. Several reports indicating plant growth promotion by PSMs using various PGPR traits are listed in Table 12.3. However, the intrinsic ability of PSMs for synthesizing the growth-promoting substances varies considerably under different ecological niches.

Biocontrol microorganisms adapt different mechanisms to inhibit plant pathogens. These mechanisms generally involve competition for nutrients, production of bacterial metabolites such as iron-chelating siderophores, etc. (Hussein and Joo 2014). The siderophore hypothesis postulates that PGPR exert their plant growth-promotion activity by depriving pathogen of iron. Siderophores from microorganisms can also induce systemic resistance in plants. In one study, P-solubilizing fungi *P. oxalicum* showed a strong antibiotic activity against a pathogenic fungus that severely attacks rape seed (*Brassica napus*) (Lipping et al. 2008). The combination of P-solubilization traits and biocontrol activity of PSMs was shown effective in promoting plant growth both in conventional and stressed soils of different agroecosystems (Jog et al. 2014).

Similarly, the ability of the most common rhizosphere microorganisms to produce cyanide is apparent. Cyanide production in one study was reported to be detrimental to plant growth. However, in a standardized gnotobiotic system, cyanide has been shown to be involved in the suppression of black root rot and several other pathogens like *Gaeumannomyces graminis* causing diseases in cereals. The contribution of this compound in the disease-controlling ability varies among different species and strains. The effect of enzymes like β-1,3-glucanases, chitinases, acyl homoserine lactones (AHL), ACC deaminase, proteases, cellulases and pectinases produced by PSMs and their role in inhibition of plant pathogens were proved in many studies (Table 12.4).

While the mechanisms of biocontrol activity have been well investigated, those responsible for the plant growth promotion by *T. harzianum* (a PSF) have not been extensively studied. In one study, Altomare et al. (1999) investigated the capability of *T. harzianum* T-22 in vitro (with plant growth-promotion and biocontrol activity) to solubilize insoluble minerals including RP. Apart from this, a PSF, *P. variabile* P16, was observed to increase glucose oxidase (GOD) production in the presence of polysaccharides, which were found to serve as activators of defensive system in this fungus (Petruccioli et al. 1999). In fact, GOD activity can play a significant role in antibiosis in soil environment by the production

Table 12.3 Few PSMs studied for PGPR traits and plant growth promotion

S. No	PSM	Plant	Plant response/additional traits	Reference
1	*Pseudomonas* sp.	*Brassica rapa*	Increased root elongation, biomass, no effect on P uptake/ IAA, ACC deaminase, siderophore	Poonguzhali et al. (2008)
2	*Serratia marcescens*	Wheat	Increased nutrient uptake, biomass/IAA, HCN, siderophore	Selvakumar et al. (2008)
3	*B. subtilis*	*Pinus roxburghii*	Increased shoot dry weight/IAA, siderophore, antagonist to *M. phaseolina*, *F. oxysporum*, *R. solani*	Singh et al. (2008)
4	*Acinetobacter* sp. (PSGB04), *Pseudomonas* (PRGBB06)	*Brassica napus*, Tomato	Increased root length, seedling vigour, dry mass/IAA, salicylic acid, N fixation	Indiragandhi et al. (2008)
5	*A. lipoferum* 3H	Rice	Increased P uptake, root length, fresh and dry shoot weight	Murty and Ladha (1987)
6	*Dyella ginsengisoli*, *Burkholderia kururiensis*, *Pandoraea* sp. ATSB30	Canola	Siderophore, IAA, salicylic acid, ACC deaminase	Anandham et al. (2008)
7	*Enterobacter* sp.	*B. juncea*	ACC deaminase, IAA, siderophore	Kumar et al. (2008)
8	*Burkholderia*	Paddy	ACC deaminase, IAA, siderophore, heavy metal solubilization	Jiang et al. (2008)
9	*Pseudomonas jessenii*	Mustard	ACC deaminase, IAA, siderophore, heavy metal solubilization	Rajkumar and Freitas (2008)
10	*Pseudomonas aeruginosa*	Black gram	ACC deaminase, IAA, siderophore	Ganesan (2008)
11	*Azotobacter* sp., *Mesorhizobium* sp., *Pseudomonas* sp., *Bacillus* sp.	–	IAA, siderophore, ammonia, HCN production, antifungal activity	Ahmad et al. (2008)
12	*P. aeruginosa*, *P. plecoglossicida*, *P. mosselii*	–	Siderophore, IAA, protease, cellulase, HCN	Jha et al. (2009)
13	*Mesorhizobium loti* MP6	*Brassica campestris*	HCN, IAA	Chandra et al. (2007)
14	*Pseudomonas* sp., *Bacillus* sp.	*Brassica campestris*	IAA, siderophore	Rajkumar et al. (2006)
15	*Bacillus cereus* MJ-1	Red pepper	Gibberellic acid	Joo et al. (2005)

H_2O_2 (enzymatically produced) which is cytotoxic to other microorganisms. Biocontrol abilities of some PSMs available are listed in Table 12.4.

12.8 PSM Biofertilizer-Production Technology

It is advised to isolate PSMs from selective soil samples depending on local needs. For example, acid-proficient PSMs are isolated from acidic soils. The visual detection of PSMs is done generally on Pikovskaya's agar medium amended with TCP or other insoluble phosphates (Pikovskaya 1948). NBRIP and bromophenol blue agar-assisted isolation was later proposed. The presence of clear halos around the colonies indicates P solubilization (qualitative assay). Colonies showing higher zone of solubilization are selected for isolation, but this does not indicate the intrinsic ability of the organism as the property is lost after frequent subcultures. Therefore, isolates are further evaluated in liquid

Table 12.4 Few PSMs studied for their ability as biocontrol agents

S. No	Microorganism	Host plant	Pathogen	Remarks	Reference
1	*P. oxalicum*	*Brassica napus*	*Sclerotinia sclerotiorum*	Antifungal compounds	Lipping et al. (2008)
2	*A. niger*, *A. awamori*	Tomato	*Fusarium oxysporum* f. sp. *lycopersici* (Fusarium wilt)	Organic acids	Khan and Khan (2001)
3	*P. aeruginosa* ID 4365	*Arachis hypogea*	*Colletotrichum falcatum*, *C. capsicum*, *Fusarium oxysporum*, *Sclerotium rolfsii*, *A. niger*	Siderophores, phenazines, HCN, IAA	Rane et al. (2008)
4	*P. aeruginosa*, *P. plecoglossicida*, *P. mosselii*	Tobacco, rice, groundnut, tea, sugarcane, chilli, mango, cotton, banana	*Rhizoctonia solani*, *Magnaporthe grisea*, *Macrophomina phaseolina*, *Sarocladium oryzae*, *Botrytis cinerea*, *Pestalotia theae*, *Colletotrichum falcatum*, *C. capsici*, *C. gloeosporioides*, *Fusarium oxysporum* f. sp. *cubense/vasinfectum*, *Cylindrocladium floridanum/ scoparium*	Protease, cellulase, IAA, HCN	Jha et al. (2009)
5	*P. putida*	Maize	*Alternaria alternata*, *Fusarium solani*	Chitinase, β-1,3-glucanase, salicylic acid, siderophore, HCN	Pandey et al. (2006)
6	*Mesorhizobium loti* MP6	*Brassica campestris*	*Sclerotinia sclerotiorum*	HCN, IAA	Chandra et al. (2007)
7	*T. harzianum* T22	Wheat, tomato, rice	*Gaeumannomyces graminis* var. *tritici*, wilt of tomato, blast of rice, *Streptomyces scabies*	Siderophores	Altomare et al. (1999)
8	*B. amyloliquefaciens*	Tomato	Control against mottle virus disease	Induced resistance	Murphy and Zehnder (2000)
9	*P. aeruginosa*, *B. subtilis*	Mung bean	Pathogens and nematodes causing root-knot disease, *Fusarium solani*, *Macrophomina phaseolina*, *Rhizoctonia solani*	–	Siddiqui et al. (2001)
10	*Serratia marcescens*, *P. fluorescens*, *B. pumilus*, *B. pasteurii*	Tobacco	Control against blue mould *Peronospora tabacina*	–	Zhang et al. (2004)

media containing insoluble phosphate by calorimetry and spectrophotometry (quantitative assay) (Morales et al. 2011). The detection of the presence of organic acids in cell-free extracts further confirms the selection of efficient organisms (Bashan et al. 2013). The efficient organisms selected in laboratory are identified by conventional (colony morphology, cell structure, biochemical tests) and molecular methods (PCR, RT-PCR, ARDRA, etc.) (Jaharamma et al. 2009). Greenhouse pot experiments, on selected plant species, using identified PSMs, are to be conducted for confirming increase in soil solution P and uptake by plant. Further thinning of isolates is done by checking the compatibility to indigenous microflora and other adverse environmental factors encountered during the course of fertilization (Bashan et al. 2013). Different inert carrier materials (lignite, talc, vermiculite, etc.) are employed for formulation of the selected PSM inoculants for easy dispersal. The quality evaluation of formulated PSM biofertilizers includes estimation of the number of cells per gram of the carrier material and permitted contamination

levels as per country norms. Also, the moisture-holding capacity and shelf life of the final product are estimated. After fulfilling all criteria as PSM inoculants, the fermenter-level mass production followed by carrier formulation and packaging is done for usage in the farmer's field.

12.9 Reasons for Failure of PSMs in the Field

In the general and more often the initial results obtained from plant experiments carried out in the greenhouse and laboratory contrast with those obtained in the farmer's field. This inconsistency is primarily due to the lack of fundamental microbiological knowledge. Commercial PSMs often fail, the reasons for which are attributed to several factors (Yarzabal 2010). In the carrier-based formulation, the nature of carrier material, number of cell population per gram and effectiveness of inoculants used play an important role.

The PSM inoculations are made by various methods like seedling root dip, seed coating and spraying. The survival and efficiency of inoculated bacteria depend on its root-colonizing ability. Hence, appropriate methods of application need to be employed. Inoculants used are often non-native and tend to be affected by local environmental factors. More or less, these inoculants show host and locality specificity (Gyaneshwar et al. 1998a). The nature of soil including temperature, moisture, pH and porosity can adversely affect the bacterial P-solubilization ability. Even if the bacteria are an efficient P solubilizer and if it fails to colonize the root, it can lead to solubilized P refixation. Therefore, before using a PSM as inoculants, thorough experimental analysis in field conditions apart from lab and greenhouse conditions is needed. Most of the PSMs are isolated using neutral and unbuffered media, although it is well known that both the acidity and buffering capacity of soils could limit microbially mediated P solubilization (Gyaneshwar et al. 1998a). PSM survival in a microcosm of the field environment should be examined during the initial stages of laboratory testing (Tang et al. 1995).

Antagonism and competition of the introduced PSMs in soil with other indigenous microflora is another factor affecting the P-solubilization efficiency. Introduced PSMs are vulnerable to reduced ability to survive, multiply and colonize in the rhizosphere. They are prone to competition for nutrients and predation with other microflora. These factors rapidly affect the introduced and native PSMs in soil. Hence, the selection of native and endemic efficient isolates is needed to harness maximum benefits.

The inoculum size of the added PSMs affects the efficiency and P-solubilizing ability (Lucy et al. 2004). Also in some instances, negative impacts were observed when inoculants were applied in large numbers (Harris et al. 2006). The initial inoculum's density and appropriate carrier formulation and dispersion determine delivery of the right number of viable cells and rhizosphere survivability.

12.10 Genetics of P Solubilization

Solubilization of mineral phosphates is predominantly by organic acids that is either by pH reduction or by phosphorus-associated cation chelation. Little is known about the genetic basis of the release of organic acids and mineral P-solubilization trait. A better understanding could pave the way for isolation, characterization and manipulation of genes involved in mineral and organic P solubilization. With an aim for the development of PSM strains (with enhanced P-solubilizing ability), few attempts were made to manipulate P-solubilizing genes through genetic engineering and molecular biotechnology followed by their expression in selected rhizobacterial strains (Table 12.5).

The rhizosphere contains a variety of carbon sources in varying amounts. The heterogenous microbial community in the vicinity produces different kinds of organic acids involved in nutrient mobility. Among the various organic acids, gluconic acid derived from direct glucose oxidation has been reported to be the major mechanism of P solubilization by Gram-negative bacteria and certain fungi (Scervino et al. 2011).

Table 12.5 Few PSMs and genes studied for genetic engineering and molecular studies

S. No	Organism	Gene studied	Expressed in	Function	Remarks	Reference
1	*Erwinia herbicola*	*mps*	*E. coli* HB101	Gluconic acid production, P solubilization	Gene involved in PQQ synthase for PQQ formation, a cofactor in GDH-PQQ catalyzing gluconic acid from glucose	Goldstein and Liu (1987)
2	*P. cepacia*	*gabY*	*E. coli* JM109	Gluconic acid production, P solubilization	Gene sequence similar to permease system mem protein. *gabY* plays alternate role in regulation of direct oxidation pathway	Babu Khan et al. (1995)
3	*Morganella morganii*	*nap A*	*Burkholderia cepacia* IS-16	Increased extracellular alkaline phosphatases	Induced under low Pi concentration	Fraga et al. (2001)
4	*E. coli*	*pho A*	–	Alkaline phosphatase	Induced under low Pi concentration (100–0.16 mM). Pho regulon involved as regulatory system along with sensor activator operon	Torriani-Gorini et al. (1993)
5	*Francisella tularensis*	*aepA*	–	Acid phosphatases	–	Reilly et al. (1996)
6	*Synechocystis*PCC6803	*mps*	*E. coli*		–	Gyaneshwar et al. (1998a)
7	*P. fluorescens* MF-0	*apo*	–	Acid phosphatases	Genes expressed maximally at 17 C in which the minimal and optimal temp are 0 and 28 °C, respectively	Burini et al. (1994)
8	*Shigella flexneri*	*apy*	–	ATP diphosphohydrolase or apyrase	–	Bhargava et al. (1995)
9	*E. coli*	*agp*	–	Periplasmic acid glucose-1-phosphate phosphatase	–	Touati and Danchin (1987)
10	*Serratia marcescens*	pKG3791	–	Produce gluconic acid	P solubilization	Krishnaraj and Goldstein (2001)
11	*Rahnella aquatilis*	pKIM10	*E. coli* DH5a	Produce gluconic acid	P solubilization	Kim et al. (1998)
12	*Enterobacter agglomerans*	pKKY	*E. coli* 109	P solubilization	Does not lower pH	Kim et al. (1997)

Biosynthesis of gluconic acid is mediated by oxidative metabolism of glucose by glucose dehydrogenase (GDH) and the reaction requires pyrroloquinoline quinine (PQQ) as a cofactor. The involvement of genetic/biochemical mechanisms for synthesis of GDH-PQQ holozyme varies on constitutive and inducible phenotypes among bacterial species (Goldstein 1994). Glucose, gluconate, mannitol and glycerol are the possible inducers of holozyme activity (Van Schie et al. 1987). Therefore, genes involved in biosynthesis/transport of PQQ can be cloned from various bacteria and transferred to other bacteria (Babu Khan et al. 1995). Many such genes responsible for organic acid synthesis might be involved in P solubilization. Even though a few genes are reported to be involved in P solubilization, the evaluation of actual genetic basis responsible is still remote.

The strategy of introduction of genes in natural rhizosphere-competent bacteria for overexpression of P-solubilization (both organic and inorganic) phenotype was proved successful in a few cases. With this approach, the need for mixing of P solubilizer and N fixer can be avoided by insertion of genes of required trait. For instance, the *apo*-GDH gene containing *Rhizobium* can be inserted with genes of PQQ biosynthesis making it an effective PSM in addition to its natural N_2-fixing ability. In an another approach, as adopted by Gyaneshwar et al. (1998b), *MPS* genes are screened directly in target bacteria by over-/under-expression of genes, followed by selection of transformants with MPS ability.

The genetical modification of PSMs has several advantages over transgenic plants owing to their ease of modification, adding several traits (PGPR) in a single organism, and their utilization for several crops instead of engineering crop by crop (Armarger 2002). All the available evidences indicate that the regulation of phosphatic enzymes is a complex process that requires considerable additional research. In any event, the existing knowledge about *Enterobacteriaceae* phosphatases constitutes a basis for better understanding and for further exploration of rules governing phosphatase expression in soil bacteria. The genes studied in different organisms for P solubilization are listed in Table 12.5.

12.11 Future Prospects and Conclusion

Further investigations are needed for evaluation of PSMs as biological inoculants for sustainable agriculture. As they play an important role in P nutrition of plants, several new stable combinations with other PGPR need to be studied. The biochemical/molecular basis of synergistic interactions within combinations is the subject of future research. The combination of PSMs and AMF proved to help withstand transplant shock in tissue culture plants and agroforestry seedlings are further needed to study in terms of molecular aspects. The response of different crops varies with PSM application basing on prevailing environmental factors that affect their survivability. PSMs resistant to different adverse environmental conditions can benefit plant growth in high temperature, drought, salinity and acidic conditions. The effect of such PSMs in improving antioxidant status of the plants mitigating adverse effects was recently studied (Ali et al. 2009; Xiao et al. 2013a).

Apart from high P-solubilization trait, PSMs with multiple PGP traits (siderophores, HCN, IAA, GA, β-glucanase, ACC deaminase, etc.) could confer better plant growth (Praveen Kumar et al. 2012). Reports on the application and dispersal of added PSMs on the plant and in the soil are very few. Efforts are under way to develop methodology of inoculants' application as liquid inoculants avoiding conventional carrier-based formulations (Leo Daniel Amalraj et al. 2010). Multiple inoculants with interspecific compatibility, survivability and root-colonizing ability have recently been evaluated in few agroforestry tree species (Kishore et al. 2012).

On the other hand, the genetic manipulation of PSMs to improve P-solubilizing capabilities and introduction of new PGP traits by rDNA technology offer a feasible approach for obtaining improved strains. Cloning of genes involved in the synthesis of organic acids and phosphatases

would be the first step in such genetic manipulation programmes. Sub-cloning of these genes into appropriate vectors followed by transfer and expression in target host strains along with some other important traits could result in new and efficient strains. Future research should concentrate on stability and performance of such genetically modified organisms once inoculated in soil. However, the putative risk involved in the release of GMO in soil is a matter of controversy with regard to possible horizontal transfer of inserted DNA to other soil microorganisms.

Several microorganisms are involved in P cycling in soils, but only 1 % can be cultivated under lab conditions, the remaining 99 % remain unstudied. Culture-independent methods developed in the recent past pave the way for the study of microbial ecology of P cycling microbes in soils. Development of PCR techniques making use of nucleic acid composition recently enabled consistent, precise culture media and growth-independent results (Peix et al. 2007) for detecting presence and quantifying expression of target genes in soil and rhizosphere. For example, specific primers are designed based on conserved regions directed at traits such as bacterial phytases (Jorquera et al. 2011).

References

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20

Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163:173–181

Ali SZ, Sandhya V, Grover M, Kishore N, Venkateswar RL, Venkateswarlu B (2009) Pseudomonas sp. strain AKM-P6 enhances tolerance of sorghum seedlings to elevated temperatures. Biol Fertil Soils 46:45–55

Altomare C, Noevell WA, Bjorkman T, Harman GE (1999) Solubilization of phosphates and micronutrients by the plant growth promoting and biocontrol fungus *Trichoderma harzianum rifai*. Appl Environ Microbiol 65(7):2926–2933

Anandham R, Indira GP, Madhaiyan M, Sa TM (2008) Potential plant growth promoting traits and bioacidulation of rock phosphate by thiosulfate oxidizing bacteria isolated from crop plants. J Basic Microbiol 48:439–447

Antoun H (2012) Beneficial microorganisms for sustainable use of phosphates in agriculture. Procedia Eng 46:62–67

Armarger N (2002) Genetically modified bacteria in agriculture. Biochimie 84:1061–1072

Azcon R, Medina A, Roldan A, Biro B, Vivas A (2009a) Significance of treated agro-waste residue and autochthonous inoculates (arbuscular mycorrhizal fungi and *Bacillus cereus*) on bacterial community structure and phytoextraction to remediate soils contaminated with heavy metals. Chemosphere 75:327–334

Azcon R, Peralvarez M, Biro B, Roldan A, Ruiz-Lonsano JM (2009b) Antioxidant activities and metal acquisition in mycorrhizal plants growing in a heavy metal multi-contaminated soil amended with treated lignocellulosic agrowaste. Appl Soil Ecol 41:168–177

Babu-Khan S, Yeo CT, Martin WL, Duron MR, Rogers RD, Goldstein A (1995) Cloning of a mineral phosphate-solubilizing gene from *Pseudomonas cepacia*. Appl Environ Microbiol 61:972–978

Barea JM, Navare E, Montoya E (1976) Production of plant growth regulators by rhizosphere phosphate-solubilizing bacteria. J Appl Bacteriol 40:129–134

Bashan Y, Kamnev AA, de Bashan LE (2013) A proposal for isolating and testing phosphate-solubilizing bacteria that enhance plant growth. Biol Fertil Soils 49:1–2

Bearden BN, Petersen L (2000) Influence of arbuscular mycorrhizal fungi on soil-structure and aggregate stability. Plant Soil 218:173–183

Bhargava T, Datta S, Ramakrishnan V, Roy RK, Sankaran K, Subrahmanyam YVBK (1995) Virulent Shigella codes for a soluble apyrase: identification, characterization and cloning of the gene. Curr Sci 68:293–300

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Burini JF, Gugi B, Merieau A, Guespin-Michel JF (1994) Lipase and acidic phosphatase from the psychrotrophic bacterium *Pseudomonas fluorescens*: two enzymes whose synthesis is regulated by growth temperature. FEMS Microbiol Lett 122:13–18

Butterly CR, Bunemann EK, McNeill AM, Baldock JA, Marschner P (2009) Carbon pulses but not phosphorus pulses are related to decrease in microbial biomass during repeated drying and rewetting of soils. Soil Biol Biochem 41:1406–1416

Cabello M, Irrazabal G, Bucsinszky AM, Saparrat M, Schalamuck S (2005) Effect of an arbuscular mycorrhizal fungus, *G. mosseae* and a rock-phosphate-solubilizing fungus, *P. thomii* in *Mentha piperita* growth in a soilless medium. J Basic Microbiol 45:182–189

Chandra S, Choure K, Dubey RC, Maheshwari DK (2007) Rhizosphere competent *Mesorhizobium loti* mp6 induce root hair curling, inhibit *Sclerotinia sclerotiorum* and enhances growth of Indian mustard (*Brassica campestris*). Braz J Microbiol 38:124–130

Chang CH, Yang SS (2009) Thermo-tolerant phosphate-solubilizing microbes for multi-functional biofertilizer preparation. Bioresour Technol 100:1648–1658

Chapuis-Lardy L, Le Bayon RC, Brossard M, Lopez-Hernandez D, Blanchart E (2011) Role of soil macrofauna in phosphorus cycling. In: Beunemann E, Oberson A, Frossard E (eds) Phosphorus in action: biological processes in soil phosphorus cycling, vol 26, Soil biology. Springer, Heidelberg

Collavino MM, Sansberro PA, Mroginski LA, Aguilar OM (2010) Comparison of in vitro solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth. Biol Fertil Soils 46:727–738

Dalal RC (1977) Soil organic phosphorus. Adv Agron 29:83–117

Duponnois R, Kisa M, Assigbetse K, Prin Y, Thioulouse J, Issartel M, Moulin P, Lepage M (2006) Fluorescent pseudomonads occurring in Macrotermes subhyalinus mound structures decrease Cd toxicity and improve its accumulation in sorghum plants. Sci Total Environ 370:391–400

Evans M (2012) Enhancing nutrient use efficiency. Arab Fertilizer 63:51–53

Figueiredo MVB, Seldin L, Araujo FF, Mariano RLR (2011) Plant growth promoting rhizobacteria: fundamentals and applications. In: Maheshwari DK (ed) Plant growth and health promoting bacteria. Springer, Berlin, pp 21–42

Food and Agriculture Organization of the United Nations (FAO) (2005) The state of food insecurity in the world: eradicating world hunger – key to achieving the Millenium development goals. FAO Corporate Document Repository. http://www.fao.org/docrep/008/a0200e/a0200e00.html. Accessed 20 Nov 2008

Fraga R, Rodriguez H, Gonzalez T (2001) Transfer of the gene encoding the Nap A acid phosphatase of *Morganella morganii* to a *Burkholderia cepacia* strain. Acta Biotechnol 21:359–369

Franco-Correa M, Quintana A, Duque C, Suarez C, Rodríguez MX, Barea JM (2010) Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. Appl Soil Ecol 45:209–217

Gadd GM, Sayer JA (2000) Fungal transformations of metals and metalloids. In: Lovley DR (ed) Environmental-metal interactions. American Society for Microbiology, Washington, DC, pp 237–256

Ganesan V (2008) Rhizoremediation of cadmium soil using a cadmium-resistant plant growth-promoting rhizopseudomonad. Curr Microbiol 56:403–407

Goldstein AH (1994) Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous phosphates by gram-negative bacteria. In: Torriani-Gorini A, Yagil E, Silver S (eds) Phosphate in microorganisms: cellular and molecular biology. ASM Press, Washington, DC, pp 197–203

Goldstein AH (1995) Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by gram negative bacteria. Biol Agric Hort 12:185–193

Goldstein AH, Krishnaraj PU (2007) Phosphate solubilizing microorganisms vs. phosphate mobilizing microorganisms: what separates a phenotype from a trait? In: Velazquez E, Rodríguez-Barrueco C (eds) First international meeting on Microbial Phosphate Solubilization. Springer Netherlands, pp 203–213

Goldstein AH, Liu ST (1987) Molecular cloning and regulation of a mineral phosphate solubilizing gene from *Erwinia herbicola*. Biotechnology 5:72–74

Gonzalez-Chavez MDA, Newsam R, Linderman R, Dodd J, Valdez-Carrasco JM (2008) Bacteria associated with the extraradical mycelium of an arbuscular mycorrhizal fungus in an As/Cu polluted soil. Agrociencia 42:1–10

Gunes A, Ataoglu N, Turan M, Esitken A, Ketterings QM (2009) Effects of phosphate-solubilizing microorganisms on strawberry yield and nutrient concentrations. J Plant Nutr Soil Sci 172:385–392

Gyaneshwar P, Kumar GN, Parekh LJ (1998a) Effect of buffering on the phosphate solubilization ability of microorganisms. World J Microbiol Biotechnol 14:669–673

Gyaneshwar P, Kumar GN, Parekh LJ (1998b) Cloning of mineral phosphate solubilizing genes from Synechocystis PCC 6803. Curr Sci 74:1097–1109

Halvorson HO, Keynan A, Kornberg HL (1990) Utilization of calcium phosphates for microbial growth at alkaline pH. Soil Biol Biochem 22:887–890

Hamdali H, Bouizgarne B, Hafidi M, Lebrihi A, Virolle MJ, Ouhdouch Y (2008) Screening for rock phosphate solubilizing Actinomycetes from Moroccan phosphate mines. Appl Soil Ecol 38:12–19

Hamdali H, Smirnov A, Esnault C, Ouhdouch Y, Virolle MJ (2010) Physiological studies and comparative analysis of rock phosphate solubilization abilities of Actinomycetales originating from Moroccan phosphate mines and of *Streptomyces lividans*. Appl Soil Ecol 44:24–31

Harris JN, New PB, Martin PM (2006) Laboratory tests can predict beneficial effects of phosphate-solubilizing bacteria on plants. Soil Biol Biochem 38:1521–1526

Hayat R, Ali S, Amara U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598

Hussein KA, Joo JH (2014) Potential of siderophore production by bacteria isolated from heavy metal: polluted and rhizosphere soils. Curr Microbiol 68(6):717–723

Indiragandhi P, Anandham R, Madhaiyan M, Sa TM (2008) Characterization of plant growth-promoting traits of bacteria isolated from larval guts of diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae). Curr Microbiol 56:327–333

Jaharamma M, Badri Narayanan K, Sakthivel N (2009) Genetic and functional diversity of phosphate solubilizing fluorescent pseudomonads and their simultaneous role in promotion of plant growth and soil health. In: Mahoney CL, Springer DA (eds) Genetic diversity. Nova Science Publishers, Inc., New York, pp 195–212

Jha BK, Pragash MB, Cletus J, Raman G, Sakthivel N (2009) Simultaneous phosphate solubilization potential and antifungal activity of new fluorescent pseudomonad strain, *P. aeruginosa*, *P. plecoglossicida* and *P. mosselii*. World J Microbiol Biotechnol 25:573–581

Jiang C, Sheng X, Qian M, Wang Q (2008) Isolation and characterization of a heavy metal-resistant *Burkholderia* sp. from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil. Chemosphere 72:157–164

Jog R, Pandya M, Nareshkumar G, Rajkumar S (2014) Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. Microbiology 160(4):778–788

Johansson J, Paul L, Finlay RD (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiol Ecol 48:1–12

Jones DL, Oburger E (2011) Solubilization of phosphorus by soil microorganisms: in phosphorus in action. Soil Biol 26:169–198

Joo GJ, Kim YM, Kim JT, Rhee IK, Kim JH, Lee IJ (2005) Gibberellins producing rhizobacteria increase endogenous gibberellins content and promote growth of red peppers. J Microbiol 43(6):510–515

Jorquera MA, Crowley DE, Marschner P, Greiner R, Ferna'ndez MT, Romero D, Menezes-Blackburn D, De La Luz Mora M (2011) Identification of β-propeller phytase-encoding genes in culturable *Paenibacillus* and *Bacillus* sp. From the rhizosphere of pasture plants on volcanic soils. FEMS Microbiol Ecol 75:163–172

JumpStart®. http://www.bioag.novozymes.com/en/products/canada/biofertility/JumpStart/Pages/default.aspx. Consulted 16 Apr 2012

Kaur G, Sudhakara RM (2014) Role of phosphate-solubilizing bacteria in improving the soil fertility and crop productivity in organic farming. Arch Agron Soil Sci 60(4):549–564

Kaushik BD (1995) Blue green algae for improvement in rice production. Seminar on natural resource management, HAU, Hisar, 11–13 Dec 1995

Khan MR, Khan SM (2001) Biomanagement of Fusarium wilt of Tomato by the soil application of certain phosphate solubilizing microorganisms. Int J Pest Manag 47:227–231

Kiikila O, Pennanen T, Perkiomaki J, Derome J (2002) Organic material as a copper immobilizing agent: a microcosm study on remediation. Basic Appl Ecol 3:245–253

Kim KY, McDonald GA, Jordan D (1997) Solubilization of hydroxyapatite by *Enterobacter agglomerans* and cloned *Escherichia coli* in culture medium. Biol Fertil Soils 24:347–352

Kim KY, Jordan D, McDonald GA (1998) Enterobacter agglomerans, phosphate solubilizing bacteria, and microbial activity in soil: effect of carbon sources. Soil Biol Biochem 30:995–1003

Kishore N (2007) Formulation evaluation and mass production of multiagent bioinoculants for agroforestry tree nurseries. PhD dissertation, Kakatiya University, Warangal

Kishore N, Ramesh M, Ram reddy S (2012) Evaluation of PGPR traits of some phosphate solubilizing microorganisms associated with four agroforestry tree species. Asian J Microbiol Biotechnol Environ Sci 14(2):193–204

Krishnaraj PU, Goldstein AH (2001) Cloning of a *Serratia marcescens* DNA fragment that induces quinoprotein glucose-dehydrogenase-mediated gluconic acid production in Escherichia coli in the presence of stationary phase *Serratia marcescens*. FEMS Microbiol Lett 205:215–220

Kucey RMN (1987) Increased phosphorus uptake by wheat and field beans inoculated with a phosphate solubilizing *Penicillium bilaii* strain and with vesicular-arbuscular mycorrhizal fungi. Appl Environ Microbiol 55:2699–2703

Kucey RMN (1988) Effect of *Penicillium bilaji* on the solubility and uptake of P and micronutrients from soil by wheat. Can J Soil Sci 68:261–270

Kumar V, Behl RK, Narula N (2001) Establishment of phosphate solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under greenhouse conditions. Microbiol Res 156:87–93

Kumar KV, Singh N, Behl HM, Srivastava S (2008) Influence of plant growth promoting bacteria and its mutant on heavy metal toxicity in *Brassica juncea* grown in fly ash amended soil. Chemosphere 72:678–683

Kumar V, Singh P, Jorquera MA (2013) Isolation of phytase producing bacteria from Himalayan soils and their effect on growth and phosphorus uptake of Indian mustard (Brassica juncea). World J Microbiol Biotechnol 29(8):1361–1369

Leo Daniel Amalraj E, Kishore N, Desai S, Venkateswarlu B (2010) Growth, shelf life and bioefficacy of liquid inoculants (PSB, *Azospirillum* spp and *Azotobacter* spp) formulated with polymeric additives. In: Sayyed RZ, Reddy MS, Sharma YR, Reddy KRK, Desai S, Rao VK, Podile AR, Reddy BC, Kloepper JW (eds) Plant growth promotion by rhizobacteria for sustainable agriculture. Scientific Publishers, New Delhi, p 609

Lipping Y, Jiatao X, Daohong J, Yanping F, Guoqing L, Fangcan L (2008) Antifungal substances produced by *Penicillium oxalicum* strain PY-1-potential antibiotics against plant pathogenic fungi. World J Microbiol Biotechnol 24:909–915

Lopez BR, Tinoco-Ojanguren C, Bacilio M, Mendoz A, Bashan Y (2012) Endophytic bacteria of the rock-dwelling cactus *Mammillaria fraileana* affect plant growth and mobilization of elements from rocks. Environ Exp Bot 81:26–36

Lucy M, Reed E, Glick BR (2004) Applications of free living plant growth-promoting rhizobacteria. Anton Leeuw Int J Gen 86(1):1–25

Maliha R, Samina K, Najma A, Sadia A, Farooq L (2004) Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms under in vitro conditions. Pak J Biol Sci 7:187–196

Mba CC (1997) Rock phosphate solubilizing Streptosporangium isolates from casts of tropical earthworms. Soil Biol Biochem 29:381–385

Medina A, Vassileva M, Barea JM, Azcon R (2006) The growth enhancement of clover by Aspergillus-treated sugar beet waste and *Glomus mosseae* inoculation in Zn contaminated soil. Appl Soil Ecol 33:87–98

Medina A, Jakobsen I, Vassilev N, Azcon R, Larsen J (2007) Fermentation of sugar beet waste by *Aspergillus niger* facilitates growth and P uptake of external mycelium of mixed populations of arbuscular mycorrhizal fungi. Soil Biol Biochem 39:485–492

Mendes GO, Zafra DL, Vassilev NB, da Silva IR, Ribeiro JI Jr, Costa MD (2014) Biochar enhances *Aspergillus niger* rock phosphate solubilization by increasing organic acid production and alleviating fluoride toxicity. Appl Environ Microbiol. doi:10.1128/AEM.00241-14, AEM.00241–14; published ahead of print 7 March 2014

Morales A, Alvear M, Valenzuela E, Castillo CE, Borie F (2011) Screening, evaluation and selection of phosphate-solubilising fungi as potential biofertiliser. J Soil Sci Plant Nutr 11(4):89–103

Murphy JF, Zehnder GW (2000) Plant growth-promoting rhizobacterial mediated protection in tomato against tomato mottle virus. Plant Dis 84(7):779–784

Murty MG, Ladha JK (1987) Differential colonization of *Azospirillum lipoferum* on roots of two varieties of rice (*Oryza sativa* L.). Biol Fertil Soils 4(1–2):3–7

Narsian V, Samaha SM, Patel HH (2010) Rock phosphate dissolution by specific yeast. Indian J Microbiol 50:57–62

Narula N, Saharan BS, Kumar V, Bhatia R, Bishnoi LK, Lather BPS, Lakshminarayana K (2005) Impact of the use of biofertilizers on cotton (*Gossypium hirsutum*) crop under irrigated agroecosystem. Arch Agron Soil Sci 51(1):69–77

Naumova AN, Mishustin EN, Marienko VM (1962) On the nature of action of bacterial fertilisers (Azotobacterin, Phosphobacterin), upon agricultural crops. Bull Acad Sci USSR 5:709–717

Ochoa-Loza FJ, Artiola JF, Maier RM (2001) Stability constants for the complexation of various metals with a rhamnolipid biosurfactant. J Environ Qual 30:479–485

Pandey A, Trivedi P, Palini LMS (2006) Characterization of phosphate solubilizing and antagonistic strain of *Pseudomonas putida* (BO) isolated from sub-alpine location in the Indian central Himalaya. Curr Microbiol 53:102–107

Park KH, Lee CY, Son HJ (2009) Mechanism of insoluble phosphate solubilization by *Pseudomonas fluorescens* RAF15 isolated from ginseng rhizosphere and its plant growth-promoting activities. Lett Appl Microbiol 49:222–228

Parks EJ, Olson GJ, Brickman FE, Baladi F (1990) Characterization of high performance liquid chromatography (HPLC) of the solubilization of phosphorus in iron ore by a fungus. Indian J Microbiol 5:18–19

Peix A, Velazquez E, Martynez-Molina E (2007) Molecular methods for biodiversity analysis of phosphate solubilizing microorganisms (PSM). In: Velazquez E, Rodrguez-Barrueco C (eds) First international meeting on microbial phosphate solubilization. Springer, Berlin, pp 97–100

Petruccioli M, Federici F, Bucke C, Keshavarz T (1999) Enhancement of glucose oxidase production by *Penicillium variabile* P16. Enzym Microb Technol 24:397–401

Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Microbiology 17:362–370

Poonguzhali S, Madhaiyan M, Sa T (2008) Isolation and identification of phosphate solubilizing bacteria from Chinese cabbage and their effect on growth and phosphorus utilization of plants. J Microbiol Biotechnol 18:773–777

Praveen KG, Kishore N, Leo Daniel Amalraj E, Mir Hassan Ahmed SK, Rasul A, Desai S (2012) Evaluation of fluorescent *Pseudomonas* spp. with single and multiple PGPR traits for plant growth promotion of sorghum in combination with AM fungi. Plant Growth Regul 67(2):133–140

Quiquampoix H, Mousain D (2005) Enzymatic hydrolysis of organic phosphorus. In: Turner BL, Frossardand E, Baldwin DS (eds) Organic phosphorus in the environment. CAB International, Wallingford, pp 89–112

Rajkumar M, Freitas H (2008) Effects of inoculation of plant growth promoting bacteria on Ni uptake by Indian mustard. Bioresour Technol 99:3491–3498

Rajkumar M, Nagendran R, Lee KJ, Lee WH, Kim SZ (2006) Influence of plant growth promoting bacteria and Cr 6+ on the growth of Indian mustard. Chemosphere 62:741–748

Rane MR, Sarode PD, Chaudhari BL, Chincholkar SB (2008) Exploring antagonistic metabolites of established biocontrol agent of marine origin. Appl Biochem Biotechnol 151:665–675

Redding MR, Shatte T, Bell K (2006) Soil-sorption-desorption of phosphorus from piggery effluent compared with inorganic sources. Eur J Soil Sci 57:134–146

Reid RK, Reid CPP, Szaniszlo PJ (1985) Effects of synthetic and microbially produced chelates on the diffusion of iron and phosphorus to a simulated root in soil. Biol Fertil Soils 1:45–52

Reilly TJ, Baron GS, Nano F, Kuhlenschmidt MS (1996) Characterization and sequencing of a respiratory burst inhibiting acid phosphatase from *Francisella tularensis*. J Biol Chem 271:10973–10983

Reyes I, Bernier L, Simard R, Antoun H (1999) Effect of nitrogen source on solubilization of different inorganic phosphates by an isolate of *Penicillium rugulosum* and two UV-induced mutants. FEMS Microbiol Ecol 28:281–290

Reyes I, Bernier L, Antoun H (2002) Rock phosphate solubilization and colonization of maize rhizosphere

by wild and genetically modified strains of *Penicillium rugulosum*. Microb Ecol 44:39–48
Richardson AE, Simpson RJ (2011) Soil microorganisms mediating phosphorus availability. Plant Physiol 156:989–996
Richardson AE, Hadobas PA, Hayes JE (2001) Extracellular secretion of Aspergillus phytase from Arabidopsis roots enables plants to obtain phosphorus from phytate. Plant J 25:641–649
Rodriguez H, Fraga R, Gonzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287:15–21
Rosenberg K, Bertaux J, Krome K, Hartmann A, Scheu S, Bonkowski M (2009) Soil amoebae rapidly change bacterial community composition in the rhizosphere of *Arabidopsis thaliana*. ISME J 3:675–684
Sattar MA, Gaur AC (1987) Production of auxins and Gibberellins by phosphate dissolving microorganisms. Zbl Mikrobiol 142:393–395
Saxena J, Minaxi, Jha A (2014) Impact of a phosphate solubilizing bacterium and an arbuscular mycorrhizal fungus (*Glomus etunicatum*) on growth, yield and P concentration in wheat plants. Clean Soil Air Water. doi:10.1002/clen.201300492
Scervino JM, Papinutti VL, Godoy MS, Rodriguez MA, Della Monica I, Recchi M, Pettinari MJ, Godeas AM (2011) Medium pH, carbon and nitrogen concentrations modulate the phosphate solubilization efficiency of *Penicillium purpurogenum* through organic acid production. J Appl Microbiol 110:1215–1223
Selvakumar G, Mohan M, Kundu S, Gupta AD, Joshi P, Nazim S, Gupta HS (2008) Cold tolerance and plant growth promotion potential of *Serratia marcescens* strain SRM (MTCC 8708) isolated from flowers of summer squash (*Cucurbita pepo*). Lett Appl Microbiol 46:171–175
Sessitsch A, Kuffner M, Kidd P, Vangronsveld J, Walter WW, Fallmann K, Puschenreiter M (2013) The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. Soil Biol Biochem 60:182–194
Sharan A, Shikha, Darmwal NS (2008) Efficient phosphorus solubilization by mutant strain of *Xanthomonas campestris* using different carbon, nitrogen and phosphorus sources. World J Microbiol Biotechnol 24:3087–3090
Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus 2:587
Siddiqui IA, Haque SE, Shaukat SS (2001) Use of rhizobacteria in the control of root rot-root knot disease complex of mungbean. J Phytopathol 149(6):337–346
Sims JT, Pierzynski GM (2005) Chemistry of phosphorus in soil. In: Tabatabai AM, Sparks DL (eds) Chemical processes in soil, SSSA book series 8. SSSA, Madison, pp 151–192
Singh H, Reddy MS (2011) Effect of inoculation with phosphate solubilizing fungus on growth and nutrient uptake of wheat and maize plants fertilized with rock phosphate in alkaline soils [J]. Eur J Soil Biol 47:30–34
Singh N, Pandey P, Dubey RC, Maheshwar DK (2008) Biological control of root rot fungus *Macrophomina phaseolina* and growth enhancement of *Pinus roxburghii* (Sarg.) by rhizosphere competent *Bacillus subtilis* BN1. World J Microbiol Biotechnol 24:1669–1679
Singh P, Kumar V, Agrawal S (2014) Evaluation of phytase producing bacteria for their plant growth promoting activities. Int J Microbiol 2014, Article ID 426483, 7 p. doi:10.1155/2014/426483
Song OR, Lee SJ, Lee YS, Lee SC, Kim KK, Choi YL (2008) Solubilization of insoluble inorganic phosphate by *Burkholderia cepacia* DA 23 isolated from cultivated soil. Braz J Microbiol 39:151–156
Sreenivas C, Narayanasamy G (2009) Role of earthworm (*Eisenia fetida*) and phosphate solubilising microorganism (*Aspergillus awamori*) in vermiphosphocomposting. Res Crops 10:293–300
Syed GD, Damare S (2013) Marine actinobacteria showing phosphate solubilizing efficiency in Chorao Island, Goa, India. Curr Microbiol 66(5):421–427
Tabatabai MA (1994) Soil enzymes In: Weaver RW, Angle JS, Bottomley PS (eds) Methods of soil analysis, part 2. Microbiological and biochemical properties. SSSA Book Series No. 5. Soil Science Society of America, Madison, pp 775–833
Tang WZ, Pasternak JJ, Glick BR (1995) Persistence in soil of the plant growth promoting rhizobacterium *Pseudomonas putida* GR12–2 and genetically manipulated derived strains. Can J Microbiol 4:445–451
Torriani-Gorini A, Yagil E, Silver S (1993) Phosphate in microorganisms: cellular and molecular biology. ASM Press, Washington, DC, p 427
Touati E, Danchin A (1987) The structure of the promoter and amino terminal region of the pH 2.5 acid phosphatase structural gene (appA) of *E. coli*: a negative control of transcription mediated by cyclic AMP. Biochimie 69(3):215–221
Van Schie BJ, Hellingwerf KJ, van Dijken JP, Elferink MGL, van Dijl JM, Kuenen JG, Konigns WN (1987) Energy transduction by electron transfer via a pyrroloquinoline quinone-dependent glucose dehydrogenase in *Escherichia coli*, *Pseudomonas putida*, and *Acinetobacter calcoaceticus* (var. *lwoffii*). J Bacteriol 163:493–499
Vassileva M, Serrano M, Bravo V, Jurado E, Nikolaeva I, Martos V, Vassilev N (2010) Multifunctional properties of phosphate solubilizing microorganisms grown on agro industrial wastes in fermentation and soil conditions. Appl Microbiol Biotechnol 85:1287–1299
Wan JHC, Wong MH (2004) Effects of earthworm activity and P-solubilizing bacteria on P availability in soil. J Plant Nutr Soil Sci 167:209–213
Xiao C, Zhang H, Fang Y, Chi R (2013a) Evaluation for rock phosphate solubilization in fermentation and soil–plant system using a stress-tolerant phosphate-

solubilizing *Aspergillus niger* WHAK1. Appl Biochem Biotechnol 169(1):123–133

Xiao CQ, Chi RA, Hu LH (2013b) Solubilization of aluminum phosphate by specific *Penicillium* spp. J Cent South Univ Technol 20(8):2109–2114

Yandigeri MS, Meena KK, Srinivasan R, Pabbi S (2011) Effect of mineral phosphate solubilization on biological nitrogen fixation by diazotrophic cyanobacteria. Indian J Microbiol 51(1):48–53

Yarzabal LA (2010) Agricultural development in tropical acidic soils: potential and limits of phosphate solubilizing bacteria, chapter 10. In: Dion P (ed) Soil biology and agriculture in tropics. Soil biology, vol 21. Springer, Berlin/Heidelberg, pp 209–233

Yi Y, Huang W, Ying G (2008) Exopolysaccharide: a novel important factor in the microbial dissolution of tricalcium phosphate. World J Microbiol Biotechnol 24:1059–1065

Yu X, Liu X, Zhu TH, Liu GH, Mao C (2012) Co-inoculation with phosphate-solubilizing and nitrogen-fixing bacteria on solubilization of rock phosphate and their effect on growth promotion and nutrient uptake by walnut. Eur J Soil Biol 50:112–117

Zhang S, Reddy MS, Kloepper JW (2004) Tobacco growth enhancement and blue mold disease protection by rhizobacteria: relation between plant growth promotion and systemic disease protection by PGPR strain 90–166. Plant Soil 262:277–288

Zhang H, Wu X, Li G, Qin P (2011) Interactions between arbuscular mycorrhizal fungi and phosphate-solubilizing fungus (*Mortierella* sp.) and their effects on *Kostelelzkya virginica* growth and enzyme activities of rhizosphere and bulk soils at different salinities. Biol Fertil Soils 47:543–554

Zhaoa K, Penttinen P, Zhang X, Xiaoling AO, Liu M, Yu X, Chen Q (2014) Maize rhizosphere in Sichuan, China, hosts plant growth promoting *Burkholderia cepacia* with phosphate solubilizing and antifungal abilities. Microbiol Res 169(1):76–82

Reproductive Strategies in Bryophytes

13

Virendra Nath and Pooja Bansal

Abstract

The chapter deals with the reproductive strategies in bryophytes: both asexual and sexual reproduction, fertilization and the reasons for the failure and their further development. The various factors involved in reproduction and their effects on reproductive biology with brief information on cladistic and molecular studies on phylogeny of bryophytes based on literature are provided.

Keywords

Biology • Bryophytes • Reproduction • Factors affecting reproduction • in vitro

13.1 Introduction

Bryophytes are the second largest group of plants after angiosperms and deserve a much more important place than they have today in biological research. They remained a neglected group of plants until the recent past in spite of their importance as model organisms in macroevolutionary population genetics and especially ecological research. Currently, *Physcomitrella patens* is one of the first bryophytes model organisms in genomic studies with faster life cycle than *Arabidopsis* and is particularly advantageous because of dominant gametophyte generation. This is increasingly used for studies on development and molecular evolutionary aspects of plants. To our knowledge, this is the only nonvascular primitive plant with its genome completely sequenced. Moreover, it is currently the only land plant with efficient gene targeting that enables gene knockout. The resulting knockout mosses are stored and distributed by the International Moss Stock Centre. Recent researches have shown the importance of mosses in transgenic pharmaceuticals production. Among its many assets, *P. patens* is able to produce human proteins (Hohe et al. 2002; Decker et al. 2003; Stefan et al. 2008) and is the only plant known used to produce the blood-clotting factor IX for pharmaceutical use.

V. Nath (✉) • P. Bansal
Plant Diversity, Systematics & Herbarium Division,
CSIR – National Botanical Research Institute,
Lucknow 226 001, Uttar Pradesh, India
e-mail: drvirendranath2001@rediffmail.com

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_13,

Bryophytes are primitive, nonvascular and seedless plants. These plants share a basically similar life cycle, bearing perennial and photosynthetically autonomous gametophyte as the dominant phase of their life cycle which gives rise to a permanently attached, unbranched, independent or partially dependent diploid sporophyte. The sporophyte remains physically attached to the gametophyte and is at least partially dependent physiologically on the maternal plant. Bryophyte biology provides a comprehensive, yet succinct, overview of the morphology, systematics, ecology as well as evolution of Bryophyta (mosses), Marchantiophyta (liverworts) and Anthocerotophyta (hornworts).

Although it is well established that bryophytes do not constitute a single, monophyletic lineage, these organisms share a fundamentally similar life cycle with a perennial and free-living photosynthetic gametophyte, alternating with a short-lived sporophyte that completes its entire development while attached to the maternal gametophyte. Thus, bryophytes are one of few successful groups of plants whose life cycle includes a functionally dominant, free-living haploid phase; thus, the evolutionary processes operating in these plants are of great general interest. Basic constraints in the life cycle are requirement of water for fertilization; terrestrial growth of the photosynthetic, perennial gametophyte; and the short-lived sporophyte which produces spores once in its lifetime in mosses. When the spores are released, they germinate, and protonema develops which is a photosynthetic filament of cells. As the protonema matures, it forms leafy buds that develop leafy gametophores which produce male (antheridia) and female (archegonia) gametangia.

The basic life history strategy in bryophytes has been thought to have combined perennial, dioecious gametophytes with the absence of asexual propagules and frequent production of sporophytes having long setae, dehiscent capsules and small spore. There are limited but valuable contributions present regarding the life cycle of bryophytes (Gayat 1897; Longton and Greene 1967, 1969; Longton 1969, 1980, 1988; Udar 1976; Zander 1979; Pujos 1992; Hadderson and Longton 1995). The basic pattern of life cycle within a group, i.e. Bryophyta, Marchantiophyta and Anthocerotophyta, is the same; however, in mosses and hornworts, the dependency of the sporophytic generation on the gametophyte is less than in liverworts as they are strongly chlorophyllous and manufacture a considerable amount of food owing to the presence of chloroplast and stomata in their capsule wall.

Extensive literature is available on the reproductive biology of flowering plants (Baker 1959; Doyle 1970; Husband and Schenoske 1996; Jhonston and Schoen 1996; Kaul et al. 2002; Goodwillie et al. 2005; Kudo 2006), but during the last few decades, the field of reproductive biology has emerged as one of the most important areas of research; therefore, attention has also been drawn to understand reproductive strategies adopted by nonflowering plants. Reproductive biology of bryophytes has been intensively studied by several workers (Saxton 1931; Gemmell 1950; Longton 1969, 1974, 1976, 1988, 1994; Longton and Greene 1969; Clarke and Greene 1970; Watson 1971; Crum 1972; Nakosteen and Hughes 1978; Smith 1978, 1979; During 1979; Anderson 1980; Crundwell 1981; Richardson 1981; Anderson and Snider 1982; Longton and Miles 1982; Wyatt 1982; Longton and Schuster 1983; Udar and Srivastava 1984; Wyatt and Anderson 1984; Chopra and Kumra 1988; Duckett and Renzaglia 1993; Nath and Asthana 2001, 2008; Roads and Longton 2003).

The subject of reproduction in bryophytes will be discussed under.

13.2 Asexual Reproduction

Reproductive biology figured prominently in discussions seeking to explain the slow evolution among bryophytes (Anderson 1963; Crum 1972). In particular, it was contended that asexual reproduction predominates over sexual

reproduction culminating in establishment from spores. It was pointed out that sporophytes of many species are rare or unknown, that establishment from spores is seldom observed in the field and that most species are able to reproduce by fragmentation of the gametophyte, with a minority also producing specialized asexual propagules. Thus, lack of effective genetic recombination through meiosis, resulting from a predominance of asexual reproduction accentuated by habitual self-fertilization in monoecious taxa, was viewed as crucial in robbing bryophytes species of evolutionary flexibility.

Bryophytes have developed several strategies of vegetative propagation, the leafy gametophore, and thus enabling an individual plant to form dense mats extending over considerable areas. Thus, asexual reproduction is important in population development and maintenance. Asexual methods of reproduction vary within the groups and to some extent from group to group. Besides, any part of the gametophyte, i.e. leaf, stem, rhizoid or any portion thereof and short specialized branches, and in fact any undamaged living cell of the plant or protonema may act as a vegetative reproductive structure.

In propagules an apical cell is present from which new gametophores will arise, and gemmae are the small structures derived from a secondary protonema (Imura and Iwatsuki 1990; Newton and Mishler 1994). However, Laaka-Lindberg et al. (2003) considered only those diaspores such as gemmae, whose germination will recapitulate the ontogeny of the whole plant as truly asexual.

Branching played an important role in the development of moss colonies and in the replacement of shoots within colonies, after breaking the contact between them (Meusel 1935).

In acrocarpous mosses, branches arise near the base or the apex of the shoot. However, pleurocarpous mosses produce relatively numerous short branches of determinate growth and less frequent, longer branches of indeterminate growth. In most of the mosses, these branching patterns are responsible for the development of colonies of their mature growth form, for their maintenance and for slow colony expansion. Regeneration is a method of asexual reproduction which occurs via detached leaves (Heald 1898; Meyer 1942; Gemmell 1953; Longton and Greene 1979) in *Physcomitrium turbinatum*, *Atrichum undulatum*, *Pleurozium schreberi*, etc. In these plants some gametophores were budded off directly from leaves and others from secondary protonema arising from leaves. Regeneration occurs from shoot fragment in *Bryum argenteum* (Longton and MacIver 1977). Several species of mosses, viz., *Bryum erythrocarpum*, *B. klinggraeffii*, *Leptobryum pyriforme*, *Pohlia lutescens* and *Pottia intermedia*, reproduce by means of rhizoidal tubers (Chopra and Rawat 1973).

Hitherto, asexual propagules appear to be most common in some species of *Bryum* and *Pohlia*. This method of asexual reproduction is common in mosses which grow as epiphytes or grows in arable fields (Whitehouse 1966; Watson 1971), and production and behaviour of these tubers are greatly influenced by light (Chopra and Rawat 1977).

In the case of hornworts, *Anthoceros* sp. produces tubers or tuberous swellings of thallus lobe apices which allow the taxon to survive in dry periods. However, in *Megaceros* sp. asexual reproduction takes place by fragmentation of frilly thallus borders. Asexual reproduction in most of the species of Marchantiales and Metzgeriales, viz., *Fossombronia* sp., *Riccia* sp. and *Sewardiella tuberifera*, occurs by means of tuber formation. However, several types of gemmae are also known in Hepatics, viz., discoid gemmae in *Marchantia polymorpha*, marginal discoid gemmae in *Radula nilgiriensis*, endogenous gemmae in *Riccardia* sp., stellate gemmae in *Blasia pusilla* and leaf gemmae in *Lophocolea minor* and *Cololejeunea formosana*. Caducous leaves are present in *Plagiochila* sp. and *Rectolejeunea* sp. which are derived from the leaf for asexual reproduction. Caducous broad branches are present in *Drepanolejeunea* sp. which is a modified stem. In *Lejeunea cardoti* and *Pellia neesiana*, fragmented stems are capable to give rise to a new plant.

13.3 Sexual Reproduction

13.3.1 Structure and Position of Sex Organs

As in all embryophytes, the sex organs are multicellular, offering some type of protection to the developing gametes, the sperms and egg. Gametangia are often accompanied by sterile unbranched filaments (i.e. paraphyses) and are surrounded by specialized leaves to form the perichaetium and perigonium. Lepp-Heino (2008) studied the reproduction and dispersal of Australian bryophytes.

1. *Antheridia*: In general, the antheridium has a stalk and antheridial body of different shapes usually spherical to elongate which on maturity dehisces and releases antherozoids. In Hepaticae, mainly leafy forms of antheridia are axillary in the male bract, and their number may vary from 1 to 2, while thalloid forms have embedded antheridia, usually singly, in the antheridial chamber inside the thallus tissue. However, in Anthocerotae, these are present in groups in the androecial chamber; and in mosses they occur in groups associated with paraphysis, terminal and usually protected by perigonial leaf.
2. *Archegonia*: Archegonium, in general, is a flask-shaped body with a swollen venter and projected neck. In Hepaticae, these are present in groups or may be single, protected by perianth or female bracts or involucres. In Anthocerotae, they are embedded in the thallus tissue while in mosses stalked and associated with paraphysis and perichaetial leaves.

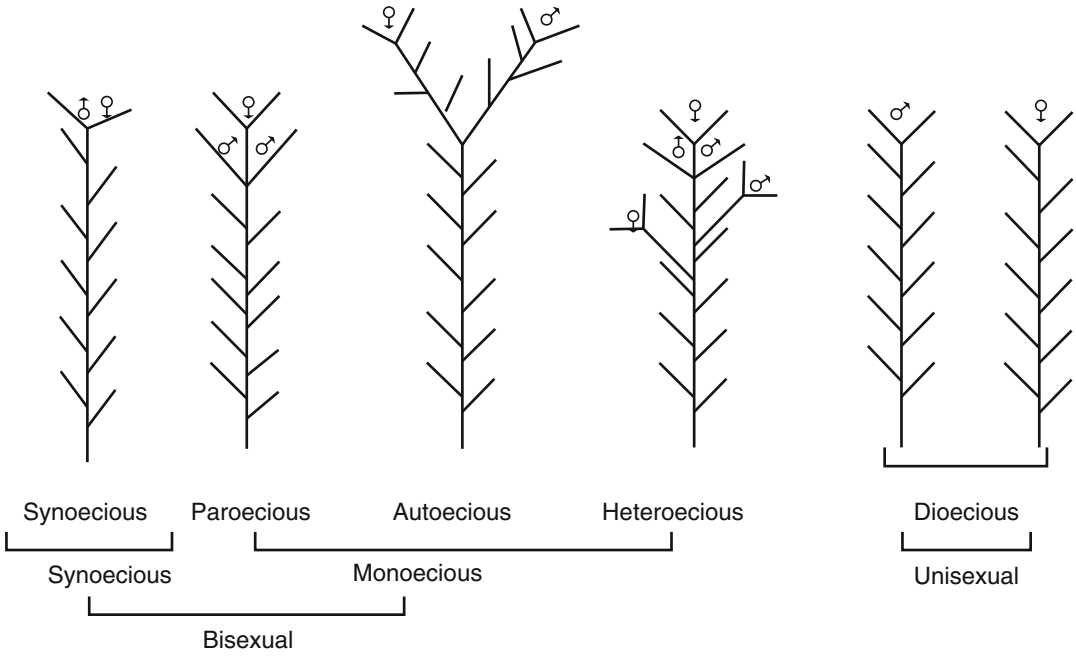
13.3.2 Sexuality

Bryophyte plants may be dioecious or monoecious. Female and male sex organs may be borne on one bisexual individual (monoicy) or two unisexual plants (dioicy). As regards the frequency of sexual reproduction, it confirmed that almost all monoecious mosses and the majority of dioecious species produce sporophytes freely, and fruiting is rare or absent in a substantial proportion of dioecious species, in parts of or throughout their range, through failure of males and females to grow side by side (Longton and Schuster 1983).

The patterns of sexuality in bryophytes discussed by Schuster (1966) are given below:

(a) *Dioecious sexuality*: In dioecious condition, the male and female reproductive organs are present on separate plant, and due to this, the chance of sexual reproduction is quite low. If the population of any particular area has only one sex, then sexual reproduction cannot take place. In such condition plants reproduce by asexual means only, and generally they are restricted or confined to a particular area.

(b) *Monoecious sexuality*: In monoecious condition, the male and female reproductive organs are present on the same plant, and on the basis of the position of sex organs, they are grouped in following categories:
   (i) *Autoecious*: The plants on one branch bear male receptacle and on the other branch bear female receptacle.
   (ii) *Paroecious*: The male reproductive part is present just below the female part.
   (iii) *Synoecious*: Both the sex organs are present on same bract.
   (iv) *Heteroecious*: In this, there is a combination of synoecious and autoecious conditions, and both antheridia and archegonia grow in close proximity; hence, there are fair chances of sexual reproduction.



13.4 Fertilization

For the bryophytes, water is an essential requirement for fertilization. It has been shown experimentally that mature antheridium dehisces explosively, and released spermatozoid spreads over the water surface and moves aimlessly. Their further movement is governed by chemotactic stimuli, i.e. sugar excreted by archegonia. Sometimes insects also help in the dispersal of spermatozoids. As per the information available, it is clearly evident that sperm dispersal distance is short in bryophytes. A maximum of not more than 50 cm travel of sperms in water availability has been observed in species with splash cups, while fertilization distances are not more than 10 cm in species without splash cups (Nath and Asthana 2008). In rare cases the distance travelled by antherozoids is also variable for various taxa. The sperms of liverworts are biflagellate, which enable them to swim short distances provided that at least a thin film of water is present. Their journey may be assisted by the splashing of raindrops. In 2008, Japanese bryologists discovered that some liverworts are able to fire sperm (*botanical ballistics* containing water up to 15 cm in the air), enabling them to fertilize female plants growing more than a metre from the nearest male (Pain 2010), viz., in *Polytrichum* sp. 60 cm, *Dawsonia* sp. 230 cm and *Mnium* sp. 50 cm. In case of epiphytic taxa, the spermatozoids are further transported by wind-carried water droplets.

13.4.1 Some Reasons for Failure in Fertilization

- The dioecious plants have less chance; however, monoecious plants have fair chances of sexual reproduction.
- In paroecious and synoecious condition, almost 100 % chances of sexual reproduction.
- Nonavailability of one sex.
- If time of maturation of sex organ is different, then there are less chances of sexual reproduction.
- Compatibility of male and female gametes.
- Viability of spermatozoids.
- One-time continuous production of spermatozoids in Hepaticae and Musci, while in Anthocerotae there is continuous production of antherozoids; hence, there are fair chances of fertilization.

13.5 Development and Structure of Sporophyte

After fertilization, the zygote is formed that develops into the sporophyte which is mainly differentiated into foot, seta and capsule.

Foot: It is embedded in the gametophyte and derives nourishment for developing sporophyte. It is variable in size. The foot is expanded in case of liverworts but not in mosses.

Seta: The stalk of the capsule remains small within the calyptra, but with maturity it rapidly elongates and pierces the calyptras to raise the capsule. In *Pellia epiphylla*, the seta elongates very rapidly. However, in Anthocerotae seta is absent or represented by a constriction only, whereas in Musci it is well developed, but in *Sphagnum* sp. and *Andreaea* sp., it is absent and replaced by pseudopodium. In case of liverworts, elongation of seta begins after the maturity of capsule and ends before the dispersal of spores. This elongation of seta is due to the elongation of cells not due to cell division. In mosses, the seta elongates very early for the conduction, and sporophyte has a well-developed elongated seta before the maturity of the capsule.

Capsule: In general the capsule has one to several cell-layer thick capsule walls enclosing the archespore which develop into spores and elaters in Hepaticae and Anthocerotae while into only spores in case of mosses.

13.6 Dependency of Sporophyte

The sporophyte of bryophytes in general is partially or fully dependent on the gametophyte. In liverworts, the sporophyte is totally dependent on the gametophyte as there is poor chlorophyll content, and the stomata are absent. Sporophyte is highly organized in Anthocerotae, though functional stomata are absent; hence, sporophyte remains functional for photosynthesis throughout the life. The sporophyte in mosses is rich in chlorophyll content, and functional stomata are also present; thus the sporophyte is partially dependent on the gametophyte.

13.7 Sporogenesis

During development, the zygote is differentiated into amphithecium and endothecium. In various groups, the fate of these layers is different. In liverworts and mosses, the capsule wall is developed from amphithecium. However, in hornworts, it is developed from the outer amphithecium. Endothecium develops spores and elaters in liverworts and columella in hornworts. Contrary to this, in all members of hornworts, inner amphithecium develops archespore, spores and elaters, whereas in case of Bryophyta, archespore and spores are developed from the outer endothecium and columella from the inner endothecium.

13.8 Dehiscence of Capsule

1. *Cleistocarpus*: In *Phascum* sp. and some members of Sphaerocarpales, Marchantiales, etc., the dehiscence of capsule takes place by the decaying of the capsule wall. In these groups spores are large and lesser in number; therefore, they are not dispersed to greater distance.
2. *Schistocarpus*: The capsule dehisces longitudinally through a single slit. The dehiscence of capsule in *Takakia* sp. and *Andreaea* sp. is four valves, and this type of dehiscence takes place in most of the members of Marchantiophyta. Further dispersal of spores is governed by the movement of the elongated seta or pseudopodium.
3. *Stegocarpous*: The capsule dehisces through the operculum or lid, and peristomial teeth help in the dispersal of spores as in most of mosses. In this case the spores dispersed to greater distances through wind, water or animals.

13.9 Spore Viability

Usually spores are viable for a short period, but in some cases they are viable for long periods, and during this period if they get suitable substratum, moisture, light, temperature and minerals,

then they germinate to form gametophyte, otherwise fail to germinate. In case of Lejeuneaceae, the spores are thin walled and viable for short period, and they germinate within the capsule, and multicellular spores with fully developed chloroplast are released which are ready for further development.

13.10 Spore Germination

Spores germinate under suitable condition of moisture, temperature and light. Initially, the spore enlarges due to the absorption of water and moisture. Resulting in the rupture of the spore coat or exospore, this phenomenon, i.e. dehiscence of the spore coat, is rather characteristic of certain genera (Fulford 1956; Nehira 1983).

In most of the members of Marchantiales and Anthocerotales, spores dehisce through triradiate mark, i.e. polar type of spores. However, in case of *Sphaerocarpos* sp. and *Riccia* sp., spores remain attached in tetrad form even at maturity thus dehisces at distal face. Subsequently, in *Monoselenium* sp., dehiscence of spore coat occurs at the point where both the faces unite. In case of *Cyathodium* sp., most of leafy liverworts and some mosses where spores are apolar, the inner content of the spores become enlarged and the spore coat ruptures irregularly.

13.11 Apogamy and Apospory: A Life Cycle Without Sex and Meiosis

The gametophytic generation in bryophytes is dominant which is defined as the plant that bears the sex organs which are essential for sexual reproduction, and the formation of sporophyte yields spores from which gametophytes can be regenerated. Bryophytes also exhibit alternative pathways to the normal life cycle under cultural conditions. These phenomena occur rarely in nature, and so their significance in their life cycle is meagre, but sometimes these can be induced in growing the plants under artificial culture medium.

Apogamy is the formation of a sporophyte directly from gametophytic tissue rather than following sexual reproduction. In mosses, apogamy has been reported to occur in haplophase as well as in diplophase. Springer (1935) was the first to report the formation of apogamous sporophyte from the leaf tips of naturally occurring diploid gametophytes of *Phascum cuspidatum*.

Apospory is the formation of gametophyte from the vegetative cell of sporophyte without the development of spores. Aposporous gametophytes differ from their haploid progenitors in the larger cells (Moutschen 1951) and larger gametangia (Marchal and Marchal 1911). Since apogamy and apospory can be induced, therefore these studies can be helpful in the understanding of the differential processes at cellular level and bases for alternation of generation. Tissue with haploid and diploid components can also be obtained and employed for comparative experimental studies.

13.12 Factors Involved in the Initiation and Maturation of Sex Organs and Affecting Reproductive Biology of Bryophytes

In bryophytes, growth of plant and the sexual reproduction is dependent on many external factors; these are light, temperature, humidity, carbohydrate, nitrogenous substance, growth regulators, pH, sex hormones, etc. Of all these, light and temperature are the most important factors for the initiation of the development of sex organs (Chopra and Sood 1973b; Awasthi et al. 2010a, b, c, 2011). These factors either affect the synthesis of growth hormones in the plant body or they remove the growth inhibitors.

Observations on seasonal reproductive cycles in a variety of mosses have yielded precise information on the periodicity of sex organ production, and sporophyte development, corresponding incisive investigation and information on hepatics are virtually existent. Periodicity of vegetative

growth and sexual reproduction in bryophyte is controlled by factors like light, temperature, humidity, hydration, carbohydrates, nitrogenous substances, growth regulators and pH, etc.

13.12.1 Duration of Light

On the basis length of daily light, illumination required for the initiation of gametangia is divided into two categories: long-day plants and short-day plants. *Marchantia polymorpha*, *Conocephalum conicum*, *Diplophyllum albicans*, *Lophocolea cuspidata*, *L. heterophylla*, *Lunularia cruciata*, *Pellia epiphylla*, *Preissia quadrata*, *Riccardia multifida* and *R. pinguis* are grown under long photoperiod thus considered as long-day plant (Anthony 1962; Benson-Evans 1961, 1964; Miller and Colaiace 1969; Voth and Hamner 1940; Wann 1925).

However, *Riccia glauca*, *Anthoceros laevis*, *A. husnoti*, *A. punctatus*, *Phaeoceros bulbiculosus*, *P. laevis* and *Notothylas orbicularis* are short-day plants as these require a short length of illumination (Hughes 1955; Benson-Evans 1961; Ridgway 1967). *Cryptothallus mirabilis*, *Riccia crystallina*, *R. gangetica* and *R. frostii* are day-neutral plants (Benson-Evans 1964; Chopra and Sood 1973a; Dua 1983; Vashistha 1985).

It is apparent that mosses are relatively lesser sensitive to photoperiod than are liverworts. *Polytrichum aloides*, *Funaria hygrometrica*, *Physcomitrella patens*, *Physcomitrium pyriforme*, *Leptobryum pyriforme*, *Bryum argenteum*, *B. coronatum*, *Barbula gregaria* and *Bartramidula bartramioides* are considered as day-neutral plant (Monroe 1965; Chopra and Rawat 1977; Nakosteen and Hughes 1978; Dietert 1980; Rahbar 1981; Kumra and Chopra 1983). On the other hand, *Sphagnum plumulosum* shows appearance of gametangia under short photoperiods (Benson-Evans 1964).

13.12.2 Quantum of Light

Liverworts are more shade tolerant/loving than mosses. An increase in light level increases the production of gametangia in liverworts such as *Conocephalum conicum*, *Pellia epiphylla*, *Riccardia pinguis*, *Riccia crystallina*, *R. gangetica* and *R. frostii* (Benson-Evans 1961; Dua et al. 1982) and mosses such as *Leptobryum pyriforme*, *Barbula gregaria*, *Bryum coronatum*, *Bartramidula bartramioides* and *Bryum argenteum* (Chopra and Rawat 1977; Rahbar 1981; Kumra and Chopra 1983).

13.12.3 Temperature

Temperature acts as a critical factor in the induction of gametangia in day-neutral plants. It also plays important role in the maturation of gametangia (Monroe 1965; Engel 1968; Nakosteen and Hughes 1978; Kumra and Chopra 1983). Contrary to this, some mosses do not require a specific temperature but exhibit response over a wide range of temperature. This is clearly seen in *Leptobryum pyriforme* which remains vegetative at 25 °C, and only a few plants turn fertile at 20 °C (Chopra and Kumra 1988). Similarly, when different temperatures are subjected to uninduced gametophytes of *Bryum argenteum*, *Barbula gregaria*, *Bryum coronatum* and *Bartramidula bartramioides*, then gametangia are produced in almost all the regimes (Kumra and Chopra 1983; Rahbar 1981). Nath et al. (2009) made an attempt to raise the plants of *Funaria hygrometrica* Hedw. in vitro in pure population and in bulk under controlled and aseptic conditions. Reproductive behaviour of this taxon, collected from Kempty Fall taxi stand, Mussoorie (Garhwal Hills), Guru Shikhar, Mount Abu (Rajasthan) and Pandav Caves and Pachmarhi (Madhya Pradesh), was also observed in culture conditions. It has been observed that low temperature (18–20 °C) plays a critical role for the onset of reproductive phase. This species is day-neutral in respect of photoperiod for gametangial induction.

13.12.4 Humidity

In case of *Marchantia polymorpha*, relatively high humidity hastens the sexual response,

whereas low humidity has the tendency to slow down the archegoniophore production (Klebs 1903; Wann 1925; Voth and Hamner 1940). Maturation of gametangia and sporophytes are greatly influenced by seasonal variation. Male gametangia mostly mature by the end of the dry season. Fertilization occurs during the wettest months, and sporophyte develops during the dry season, and dispersal of spores is confined mostly towards the end of the dry season. Female gametangia are receptive over the whole period with many mature gametangia before the start of the rainy season. However, male gametangia, in contrast to female, took longer time to develop and aborted in high numbers. Bryophytes in tropical rain forest are favoured by wet weather and mild temperatures (Maciel-Silva and Válio 2011).

13.12.5 Hydration

Plants of *Riccia crystallina*, when raised on media with different concentrations of agar (0.2–4 %), showed normal growth at 1 %, but higher concentrations inhibited vegetative growth and gametangium formation (Chopra and Sood 1973b). Similarly, female thallus of *Riccia frostii* remains sterile in liquid medium but, on agar culture, produces archegonia (Vashistha 1985).

13.12.6 Sugar and Carbohydrates

Suitable concentration of sucrose in culture medium has been found favourable for the initiation of buds in some case. The effect of sucrose is that it replaces light factor and bud formation takes place. An optimum concentration of sugar enhances the onset of bud and increases their number in suboptimal light intensity. Carbohydrate-nitrogen ratio is responsible for the formation of sexual branches in bryophytes (Wann 1925). Besides this, nutritional factor may also be involved in the production of male and female gametangia at different times. Awasthi et al. (2010c, 2012a) in their studies in vitro propagation of *Bryoerythrophyllum recurvirostrum* (Hedw.) Chen. and *Erythrodontium julaceum* (Schwaegr.) Par. by using spores as explants achieved well-developed gametophytes along with the development of gametangia (antheridia) by inoculating a range of concentration of inorganic media supplemented with and without sucrose under laboratory conditions.

Recently, Nath et al. (2012) carried out in vitro propagation of *Bryum coronatum* Schwaegr. by using spores as explants and achieved well-developed gametophytes along with the development of gametangia (antheridia) by inoculating a range of concentration of inorganic media supplemented with and without sucrose under laboratory conditions. However, Awasthi et al. (2012d) raised well-differentiated gametophytes of *Philonotis thwaitesii* Mitt. and *Brachythecium plumosum* (Hedw.) B.S.G. in vitro by inoculating their spores into a range of concentrations of inorganic media.

13.12.7 Nitrogenous Substances

Inorganic (i.e. nitrates) and organic (i.e. urea, amino acids, casein hydrolysate) nitrogenous sources greatly affect gametangium induction in bryophytes. In *Riccia crystallina*, as the concentration of urea increases, the production of archegonia too increases, but there is no effect on the antheridium formation (Chopra and Kumra 1988). Similarly, archegonial production and fresh weight of thalli increases with increase in the concentration of KNO_3.

13.12.8 Growth Regulatory Substances

Plant growth regulators are important supplements of growth medium, because they can induce specific developmental stages. Auxins and cytokinins are commonly used growth regulators, and gibberellic acid shows positive effect on bryophyte morphogenesis (Schumaker and Dietrich 1998; Cove et al. 2006; Sabovljevic et al. 2010).

It has been observed that IAA at 0.1 ppm indicates bud-forming activity in mutants in which

bud formation was completely arrested; however, higher concentration of IAA shows inhibitory effect. In case of *Anaectangium* sp. and *Entodon* sp., kinetin and IAA in combination increases number of buds. Coconut milk and watermelon juice promote protonemal growth but do not help in the formation of new buds.

In *Riccia crystallina*, *R. gangetica* and *R. frostii*, auxins promote archegonial production (Vashistha 1985), whereas in *Conocephalum conicum*, it induces receptacles. In female gametophytes of most of the bryophytes such as *Asterella angusta*, *Exormotheca tuberifera*, *Plagiochasma articulatum*, *Reboulia hemisphaerica* and *Pallavicinia canaras*, as far as endogenous level of IAA increases, the appearance of archegonia also increases; however, in male plants, a reverse change is observed (Rao and Das 1968).

Gibberellins also favour antheridium formation in *Riccia crystallina* and *R. gangetica*, *Barbula gregaria* and *Bryum coronatum* (Chopra and Kumra 1983). Thus, in most of the bryophytes, gibberellins are involved in antheridial induction. However, cytokinin enhances archegonial production and to some extent even antheridial formation in *Riccia crystallina* and *R. gangetica* (Chopra and Sood 1973b).

Recently, Awasthi et al. (2012b) also carried out in vitro the induction of protonemal buds and gametophore development in *Octoblepharum albidum* Hedw. by inoculating spores into a range of inorganic media supplemented with and without sucrose. The presence of sucrose in the medium was found obligatory for the bud induction and gametophore development. Synergistic effect of auxin and cytokinin stimulated the bud induction rapidly and also increases in number.

13.12.9 pH

pH value of the nutrient medium greatly affects growth and development of plants. In *Riccia crystallina*, maximal antheridial and archegonial production is exhibited at pH 4.5 and 6.5, respectively (Chopra and Sood 1973b); however, in *Riccia gangetica*, the values become 4.5 and 7.5, respectively (Dua 1983). However, in *Bryum argenteum* during the transition of vegetative phase to reproductive phase, a sharp reduction in pH of the medium is clearly observed (Bhatla 1981).

Reproduction is important for several aspects of the life and persistence of bryophytes. The term reproduction has been used in many ways in the literature (Mishler 1988a, b). It is readily apparent that different modes of reproduction are loosely associated with different habitats. On this basis, During (1979, 1992) proposed a series of life history strategies among bryophytes. The study of reproductive biology and its relationship to evolutionary processes in bryophytes thus remains an intriguing and challenging area of investigation and one that has considerable biological significance. Recent studies have clearly challenged early views, at least in demonstrating that a wide range of genetic variability exists within many moss species. Reproduction increases genetic diversity, and it is generally thought that sexual reproduction is most important due to frequent recombination at meiosis. Some bryophytes fail to produce sporophytes, and many others do so and often liberate spores in prodigious number (Longton and Schuster 1983). Current evidence suggests the occurrence of major differences among bryophytes in features such as frequency of sporophyte production, facility for establishment from spores and mating patterns, as well as in levels of ploidy and of intraspecific genetic variability. A major challenge for the future lies in attempting to discover pattern among various aspects of reproduction biology and genetics that may indicate the evolutionary processes operating in different groups of bryophytes.

Besides all this, a series of research papers published by Mishler and his group (1985, 1988a, b, 1994, 2000) explain the morphological development and phylogenetic basis of the species concept in bryophytes, reproductive ecology of bryophytes, cladistic analysis of molecular and morphological data and phylogenetic relationship of the green algae and bryophytes. Recently, bryophyte biology has also been discussed by

Shaw and Goffinet (2000). In morphological cladistic analysis, the classical group of bryophytes appear not to be monophyletic, in most cladograms with the liverworts being the sister group to all other land plants and the mosses more closely related to the tracheophytes than to the hornworts or liverworts (Mishler 2001). Forest et al. (2006) have unravelled the evolutionary history of the liverworts, multiple taxa, genomes and their cladistic analysis, needless to emphasize each achievement and advancement in knowledge on bryophyte biology and biotechnology, especially when it serves to protect genetic diversity.

Acknowledgements The authors are grateful to the director of National Botanical Research Institute, Lucknow, for providing us facilities, and the Council of Scientific and Industrial Research, New Delhi, for the financial assistance.

References

Anderson LE (1963) Modern species concept: mosses. Bryologist 66:107–111

Anderson LE (1980) Cytology and reproductive biology of mosses. In: Taylor RJ, Leviton AE (eds) The mosses of North America. Pac Div Am Assoc Adv Sci, San Francisco

Anderson LE, Snider JA (1982) Cytological and genetic barriers in mosses. J Hattori Bot Lab 52:241–254

Anthony RE (1962) Greenhouse culture of *Marchantia polymorpha* and induction of sexual reproductive structures. Turtox News 40:2–4

Awasthi V, Nath V, Asthana AK (2010a) *In vitro* propagation of the endemic and threatened Indian liverwort: *Cryptomitrium himalayense* Kash. Curr Sci 98(11):1440–1441

Awasthi V, Nath V, Asthana AK (2010b) Effect of some physical factors on reproductive behaviour of selected bryophytes. Int J Plant Reprod Biol 2(2):141–145

Awasthi V, Nath V, Asthana AK (2010c) *In vitro* study on micropropagation and reproductive behaviour of moss *Bryoerythrophyllum recurvirostrum* (Hedw) Chen. Int J Plant Reprod Biol 2(1):31–37

Awasthi V, Nath V, Pande N, Asthana AK (2011) *In vitro* study on growth and gametangial induction in the male clone of *Marchantia papillata* subsp. *grossibarba* (Steph.) Bischl. Int J Plant Reprod Biol 3(2):99–104

Awasthi V, Asthana AK, Nath V (2012a) *In vitro* propagation of an epiphytic pleurocarpous moss *Erythrodontium julaceum* (Schwaegr) Par. J Bryol 34(2):140–144

Awasthi V, Nath V, Asthana AK (2012b) *In vitro* induction of Buds in a moss *Octoblepharum albidum* Hedw. Phytomorphology 62(3&4):151–161

Awasthi V, Nath V, Pande N, Asthana AK (2012c) *In vitro* study on the growth and gametangial induction in a liverwort *Asterella wallichiana* (Lehm. et Lindenb.) Grolle. Int J Plant Reprod Biol 4(2):93–100

Awasthi V, Nath V, Pande N, Asthana AK (2012d) Morphogenetic studies and *in vitro* propagation of two mosses: *Philonotis thwaitesii* Mitt. and *Brachythecium plumosum* (Hedw.) B.S.G. Taiwania 57(1):27–36

Baker HG (1959) Reproduction methods as factors in speciation in flowering plants. Cold Spring Harb Symp Quant Biol 24:172–191

Benson-Evans K (1961) Environmental factors and bryophytes. Nature 191:255–260

Benson-Evans K (1964) Physiology of reproduction in bryophytes. Bryologist 67(4):431–445

Bhatla SC (1981) Involvement of pH in gametangial formation in the moss *Bryum argenteum* Hedw. Curr Sci 50:960–961

Chopra RN, Kumra PK (1983) Hormonal regulation of growth and antheridial production in three mosses grown *in vitro*. J Bryol 12:491–502

Chopra RN, Kumra PK (1988) Biology of bryophytes. Wiley Eastern India, New Delhi

Chopra RN, Rawat MS (1973) *In vitro* production of secondary gemmae on the protonema of *Bryum klinggraeffii* Schimp. Bryologist 76:183–185

Chopra RN, Rawat MS (1977) Studies on production and behaviour of protonemal gemmae in some Bryaceae. Bryologist 80:655–661

Chopra RN, Sood S (1973a) *In vitro* studies on the reproductive biology of *Riccia crystallina*. Bryologist 76(2):278–285

Chopra RN, Sood S (1973b) *In vitro* studies in Marchantiales I. Effects of some carbohydrates, agar, pH, light and growth regulators on growth and sexuality in *Riccia crystallina*. Phytomorphology 23:230–244

Clarke GCS, Greene SW (1970) Reproductive performance of two species of *Pohlia* at widely separated stations. Trans Br Bryol Soc 6:114–128

Cove D, Bezanilla M, Harries P, Quatrano R (2006) Mosses as model systems for the study of metabolism and development. Annu Rev Plant Biol 57:497–520

Crum H (1972) The geographic origins of mosses of North America's deciduous forest. J Hattori Bot Lab 35:269–298

Crundwell AC (1981) Reproduction in *Myurium hochstetteri*. J Bryol 11:715–717

Decker EL, Gorr G, Reski R (2003) Moss – an innovative tool for protein production. Bioforum Eur 2:96–97

Dietert MF (1980) The effects of temperature and photoperiod on the development of geographically isolated populations of *Funaria hygrometrica* and *Weissia controversa*. Am J Bot 67:369–380

Doyle WT (1970) The biology of higher cryptogams. Macmillan, London

Dua S (1983) Morphogenetic and physiological studies on some bryophytes. PhD thesis University of Delhi, New Delhi

Dua S, Singal N, Chopra RN (1982) Studies on growth and sexuality in *Riccia gangetica* Ahmad grown *in vitro*. Cryptog Bryol Lichenologist 3(3):189–199

Duckett JG, Renzaglia KS (1993) The reproductive biology of the liverwort *Blasia pusilla* L. J Bryol 17:541–552

During HJ (1979) Life cycle strategies in bryophytes – a preliminary review. Lindbergia 5:2–18

During HJ (1992) Ecological classifications of bryophytes and lichens. In: Bates JW, Farmer AM (eds) Bryophytes and lichens in a changing environment. Clarendon Press, Oxford, pp 1–31

Engel PP (1968) The induction of biochemical and morphological mutants in moss, *Physcomitrella patens*. Am J Bot 55:438–446

Forrest L, Christine E, Davis, Long DG, Crandel-Stroller BJ, Clark A, Hollinggsworth ML (2006) Unravelling the evolutionary history of the liverworts (Marchantiophyta): multiple taxa genomes and the analyses. Bryologist 109(3):303–334

Fulford M (1956) The young stages of the leafy Hepaticae. Phytomorphology 6:199–235

Gayat LA (1897) Recherches sur le développement de l'archégone chez les Muscinées. Ann Sci Nat Ser 83:161–258

Gemmell AR (1950) Studies in Bryophyta. I. The influence of sexual mechanism in varietal production and distribution of British musci. New Phytol 49:64–71

Gemmell AR (1953) Regeneration from the leaf of *Atrichum undulatum* (Hedw.) P. Beauv. Trans Br Bryol Soc 2:203–213

Goodwillie C, Kalisz S, Eckert CG (2005) The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanation and empirical evidence. Annu Rev Ecol Syst 36:47–49

Hadderson TA, Longton RE (1995) Patterns of life history variation in the Funariales, Polytrichales and Pottiales. J Bryol 18:639–675

Heald FDF (1898) A study of regeneration exhibited by mosses. Bot Gaz 26:169–210

Hohe A, Decker EL, Gorr G, Schween G, Reski R (2002) Tight control of growth and cell differentiation in photoautotrophically growing moss (*Physcomitrella patens*) bioreactor cultures. Plant Cell Rep 20:1135–1140

Hughes JG (1955) Physiology of sexual reproduction in the bryophytes. PhD thesis, University of Wales, Cardiff

Husband BC, Schenoske DW (1996) Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50:54–70

Imura S, Iwatsuki Z (1990) Classification of vegetative diasporas on Japanese mosses. Hikobia 10:435–443

Jhonston MO, Schoen DJ (1996) Correlated evolution of self fertilization and inbreeding depression: an experimental study of nine populations of Amsinckia (Boraginaceae). Evolution 50:1478–1491

Kaul V, Sharma N, Kaul AK (2002) Reproductive effort and sex allocation strategy in *Commelina benghalensis* L., a common monsoon weed. Bot J Linn Soc 140:403–413

Klebs G (1903) Willkürlicke Entwicklungsänderungen bei Pflanzen. Fischer, Jena

Kudo G (2006) Flowering phenologies of animal pollinated plants: reproductive strategies and agents of selection. In: Harder LD, Barett CH (eds) Ecology and evolution of flowers. Oxford University Press, Oxford, UK, pp 139–158

Kumra PK, Chopra RN (1983) Effect of some physical factors on growth and gametangial induction in male clones of three mosses grown *in vitro*. Bot Gaz 144:533

Lakka-Lindberg S, Korpelainen H, Pohjamo M (2003) Dispersal of asexual propagules in bryophytes. J Hattori Bot Lab 93:319–330

Lepp-Heino (2008) Reproduction and dispersal: Australian Bryophytes. Australian National Botanic Garden. Retrieved 22 Dec 2011

Longton RE (1969) Studies on growth and reproduction in mosses. Def Res Board Can Hazen Ser 38:6–9

Longton RE (1974) Geological differentiation in bryophytes. J Hattori Bot Lab 38:49–65

Longton RE (1976) Reproductive biology and evolutionary potential in bryophytes. J Hattori Bot Lab 41:205–223

Longton RE (1980) Physiological ecology of mosses. In: Taylor RJ, Leviton AE (eds) The mosses of North America. San Francisco, pp 77–113

Longton RE (1988) Life history strategies among bryophytes of arid regions. J Hattori Bot Lab 63:15–28

Longton RE (1994) Reproduction biology in bryophytes: the challenge and the opportunities. J Hattori Bot Lab 76:159–172

Longton RE, Greene SW (1967) The growth and reproduction of *Polytrichum alpestre* Hoppe on South Georgia. Phil Trans R Soc B252:295–322

Longton RE, Greene SW (1969) The growth and reproductive cycle of *Pleurozium schreberi* (Brid) Mitt. Ann Bot 33:83–105

Longton RE, Greene WE (1979) Experimental studies on growth and reproduction in the moss *Pleurozium schreberi* (Bird) Mitt. J Bryol 10:321–338

Longton RE, MacIver MA (1977) Climatic adaptation in Antarctic and Northern Hemisphere populations of a cosmopolitan moss, *Bryum argenteum* (Hedw). In: Llano GA (ed) Adaptations within Antarctic ecosystems. Smithsonian Institution, Washington, pp 899–919

Longton RE, Miles CJ (1982) Studies on the reproductive biology of mosses. J Hattori Bot Lab 52:219–239

Longton RE, Schuster RM (1983) Reproductive biology. In: Schuster RM (ed) Manual of bryology. The Hattori Botanical Laboratory, Japan

Maciel-Silva AS, Válio IFM (2011) Reproductive phenology of bryophytes in tropical rain forests: the sexes never sleep. Bryologist 114(4):708–719

Marchal E, Marchal E (1911) Aposporie et sexualité chez les mousses. Bull Acad Roy Sci Belg 9–10:750–778

Meusel H (1935) Wuchsformen und Wuchstypen der europäischen Laubmosse. Nava Acta Leopoldina 3(12):123–277

Meyer SL (1942) Physiological studies in mosses. IV. Regeneration in *Physcomitrium turbinatum*. Bot Gaz 104:128–132

Miller MW, Colaiace J (1969) The induction of sexual reproductive structures of *Marchantia polymorpha* grown under aseptic culture conditions. Bryologist 72:45–48
Mishler BD (1985) The morphological development, and phylogenetic basis of species concepts in bryophytes. Bryologist 88:207–214
Mishler BD (1988a) Relationships between ontogeny and phylogeny, with reference to bryophytes. In: Humphries CJ (ed) Ontogeny and systematics. Columbia University Press, New York, pp 117–136
Mishler BD (1988b) Reproductive ecology of bryophytes. In: Lovett Doust J, Lovett Doust L (eds) Plant reproductive ecology. Oxford University Press, London, pp 285–306
Mishler BD (1994) Cladistic analysis of molecular and morphological data. Am J Phys Anthropol 94:143–156
Mishler BD (2000) Deep phylogenetic relationships among "plants" and their implications for classification. Taxon 49:661–683
Mishler BD (2001) The biology of bryophytes. Am J Bot 88(11):2129–2131
Monroe JH (1965) Some factors evoking formation of sex organs in *Funaria*. Bryologist 68:337–339
Moutschen J (1951) Quelques cas nouveaux d'aposporie chez les mousses. Lejeunia 15:41–50
Nakosteen PC, Hughes KW (1978) Seasonal life cycles of three species of Fumariaceae in culture. Bryologist 81:307
Nath V, Asthana AK (2001) Reproductive biology of bryophytes. In: Johri BM, Srivastava PS (eds) Reproductive biology of plants. Narosa Publishing House, New Delhi, pp 148–174
Nath V, Asthana AK (2008) Reproductive diversity and evolution in bryophytes. Applied Bot Abstr 28(4):360–373
Nath V, Awasthi V, Asthana AK (2009) *In vitro* propagation and reproductive biology of moss *Funaria hygrometrica* Hedw. Int J Plant Reprod Biol 1(2):103–108
Nath V, Awasthi V, Asthana AK (2012) Culture studies and *in vitro* propagation of the moss *Bryum coronatum* Schwaegr. J Indian Bot Soc 91(4):391–397
Nehira K (1983) Spore germination, protonema and sporeling. In: Schuster RM (ed) New manual of bryology. The Hattori Botanical Laboratory, Nichinan, pp 343–385
Newton AE, Mishler BD (1994) The evolutionary significance of asexual reproduction in mosses. J Hattori Bot Lab 76:127–145
Pain S (2010) Botanical ballistics. New Sci 208(2792/3): 45–47
Pujos J (1992) Life history of *Sphagnum*. J Bryol 17:93–105
Rahbar K (1981) Morphogenetical and physiological studies on *Bartramidula bartramioides* Schimp and *Hyophila involuta* (Hook.) Jaeg. PhD thesis, University of Delhi, New Delhi
Rao MP, Das VSR (1968) Metabolic changes during reproductive development in liverworts. Z Pflanz Physiol 59:87–99
Richardson DHS (1981) The biology of mosses. Blackwell, Oxford
Ridgway JE (1967) Factors initiating antheridial formation in six Anthocerotales. Bryologist 70:203–205
Roads E, Longton RE (2003) Reproductive biology and population studies in two annual shuttle mosses. J Hattori Bot Lab 93:305–318
Sabovljevic A, Sabovljevic M, Grubisic D (2010) Gibberellin influence on the morphogenesis of the moss *Bryum argenteum* Hedw. *in vitro* condition. Arch Biol Sci 62(2):373–380
Saxton WT (1931) The life history of *Lunularia* with special reference to the archegoniophore and sporophyte. Trans RSS Afr 19:259–268
Schumaker KS, Dietrich MA (1998) Hormone-induced signalling during moss development. Annu Rev Plant Physiol Plant Mol Biol 49:501–523
Schuster RM (1966) The Hepaticae and Anthocerotae of North America. East of the hundredth meridian, vol I. Columbia University Press, New York
Shaw AJ, Goffinet B (2000) Bryophyte biology. Cambridge University Press, New York
Smith AJE (1978) Cytogenetics, biosystematics and evolution in the bryophyta. Adv Bot Res 6:195–276
Smith AJE (1979) Towards an experimental approach to bryophyte taxonomy. In: Clarke GCS, Duckett JG (eds) Bryophyte systematics. Academic, New York
Springer E (1935) Über apogame (vegetative enstandene) Sporogone an der bivalenten Rasse des Laubmooses *Phascum cuspidatum*. Z Indukt Abstamm Vererbungsl 69:249–262
Stefan et al (2008) The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. Science 319:65
Udar R (1976) An introduction to bryophyta. Shashi Dhar Malviya Prakashan, Lucknow
Udar R, Srivastava SC (1984) Reproductive biology of some Indian liverworts. Phytomorphology 33:37–46
Vashistha BD (1985) *In vitro* investigations on some Indian bryophytes. PhD thesis, University of Delhi, New Delhi
Voth P, Hamner K (1940) Responses of *Marchantia polymorpha* to nutrient supply and photoperiod. Bot Gaz 102:169–205
Wann FB (1925) Some of the factors involved in sexual reproduction of *Marchantia polymorpha*. Am J Bot 12:307
Watson EV (1971) The structure and life of bryophytes. Hutchinson University Library, London
Whitehouse HLK (1966) The occurrence of tubers in European mosses. Trans Br Bryol Soc 5:103–116
Wyatt R (1982) Population ecology of bryophytes. J Hattori Bot Lab 52:179–198
Wyatt R, Anderson LE (1984) Breeding systems in bryophytes. In: Dyer AF, Duckett JG (eds) The experimental biology of bryophytes. Academic, London, pp 39–59
Zander RH (1979) Patterns of sporophyte maturation dates in Pottiaceae. Bryologist 82:538–558

Cycads: An Overview

14

Anil K. Goel and J.S. Khuraijam

Abstract

Cycads are regarded as the "Living Fossils" and belong to a specialized group of plants having ancient lineage possessing great significance from the evolutionary point of view. During excavations, the cycad fossils located and accepted as related to the similar lineage as the present-day cycads have been known from the early Permian period, ca. 280–320 Myr ago. They had been popularly known as "Plant Dinosaur" and were generally known as the most dominant plant group in the Mesozoic period. The relict group of the seed plants was worldwide in their distribution and dominant in the plant world as the dinosaurs were then at the peak in the animal world. They were eaten by some herbivorous dinosaurs, such as *Stegosaurs*. During that period the plant group Bennettitales or cycadeoids was in abundance which had superficial resemblance with cycads but was more similar and closer to the angiosperms. The Jurassic period is generally called as the "Age of Cycads" because they were very common at that time. In fact the cycads represent the basal living lineage of the present-day seed-bearing angiosperms.

The taxonomic isolation and extreme antiquity of cycads have attributed them intrinsic interest and conservational importance which is disproportionate to the paucity of members because of their limited and disjunct distribution. The cycads are dioecious and very slow-growing plants; as a result they are getting extremely rare in the natural habitat. Due to their rarity, very attractive foliage and beautiful cones, cycads are considered as the prized collections in private and the public gardens leading to over exploitation from natural habitats and illegal trade in the international market. Therefore, international trade and the movement of

A.K. Goel (✉) • J.S. Khuraijam
Botanic Garden, CSIR – National Botanical Research Institute, Lucknow 226 001, Uttar Pradesh, India
e-mail: akgoelofcsirnbri@gmail.com

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_14,

cycads are regulated strictly under the rules governed by CITES. Botanic gardens have been recognized as prime custodians to play a vital role in maintaining the germplasm collection for the furtherance of taxonomic and *ex-situ* conservation studies on the cycads.

This paper stresses upon taxonomic and conservational significance of cycads and various reasons for the loss of cycad habitats world over including other important issues and parameters advocating for large-scale multiplication and *ex-situ* conservation programs in botanic gardens.

Keywords

Taxonomy • Conservation studies • Cycads • Living fossils • Angiosperms • Botanic gardens

14.1 Introduction

Cycads have endured the ages with very little changes. They are regarded as the emblem of changelessness and a heritage from the world too remote for human intelligence to grasp a group of plants which has seen many secrets of immeasurable past when the dinosaurs roamed the earth from 225 to 65 Myr ago. Cycads evolved during the Carboniferous or Permian period (ca 280–320 Myr), and cycad diversity probably reached its maximum during the Jurassic 150–200 Myr (Anderson et al. 2007; Pant 2002; Whitelock 2002). Present-day cycads have been an isolated group of flora possessing ancient lineage dating back to the Paleozoic era. They are thought to have evolved from the seed ferns millions of years ago. During the course of evolutionary process, the cycads moved from swampy habitats to the semiarid locations as most of their species are distributed today (Pant and Mehra 1962; Sharma and Goel 1991). The conifers and the cycads were among the first plants on earth to produce seeds.

At the end of Triassic and the beginning of the Jurassic period, the ancestors of modern cycads were numerous and diverse. They were comprised of an important part of the earth's flora and flourishing with the dinosaurs. Before the advent of man, it was the long-term climatic changes and short-term environmental catastrophes that altered the cycad population throughout the world. The climate became cool slowly as well as the continents drifted apart causing the fragmented distribution of the cycads as we see them today.

Descendants of cycads are thriving even today in constant evolutionary process. Cycads are very slow-growing plants, often producing one to two sets of fronds only once a year. So far 331 species of cycads have been reported from all over the world (Osborne et al. 2012). The lingering species of cycads are surviving in at least on five continents and on numerous islands in Indian, Pacific, and Atlantic Oceans (Chamberlain 1935). They are found in tropical and subtropical regions of both the Northern and Southern Hemispheres. They occur in South and Central America, Mexico, the Antilles, the United States of America, Australia, Melanesia, Micronesia, Japan, China, Southeast Asia, India, Sri Lanka, Madagascar, and southern and tropical Africa (Hurter and Glen 1996, 2001; Whitelock 2002). In terms of number of species, Australia is the richest country with a representation of 78 spp. in four genera followed by Mexico having 45 spp. in three genera and South Africa with 40 spp. under two genera (Donaldson 2003; Vovides et al. 2004). Their present distribution is mostly relictual, and several species are extremely rare or few might have gone extinct. The current scientific thinking is that the extant cycads are vigorously responsive to normal selection processes and that many of the still existing species are fairly recent in origin. Yet many of the cycad taxa have been recently discovered and described as new to science (Pant 2002).

Botanists are fascinated by these plants for various reasons since the time immemorial. They are intrigued by many unique and unusual characteristics expressed by cycads, viz., nitrogen-fixing upwardly growing coralloid roots with their root systems, neurotoxic and carcinogenic chemicals in the seeds, the adaptation to a point of dependence, and the reproductive biology. In addition the cycads exhibit left- and right-handedness in respect of the arrangement of scale leaves and their foliage in young condition twisted from left to right such that left- or right-handed cycads occur and called and shown to exhibit bioisomerism by Bahadur et al. (1977). They are strictly dioecious species and, specific pollinating insects add to the complexity and occasional reports of cycads undergoing sex change, are yet without any explanation. The phenomenon of thermogenesis (self-heating) in the male cones results to an increase in the temperature significantly during the period of pollen release, a process also observed in some aroids and resulting in the cone emitting volatile chemicals which attract specific pollinating beetles. In no other plant group, there is a puzzling of ancestral and derived characteristics as in cycads. Cycads are important plant groups for the study of evolution in plants.

14.2 Taxonomy of Cycads

In 1827, Robert Brown pointed out for the first time that what had till then been regarded as the female flower in cycads and conifers was actually a naked ovule. As a result of this remarkable discovery, the cycads and the conifers treated previously as dicotyledons came to be recognized as gymnosperms, as distinguished from the angiosperms where the seeds are enclosed in a carpel (Pant 2002).

However, the cycads have not been very well worked out taxonomically due to the severe loss to their habitats. In the past the cycads were not understood well and regarded as difficult groups for identification purposes. Recent studies on cycads in many countries have started to clarify the taxonomical identity with a substantial increase in the number of recognized taxa. As per the earlier reports, 158 species were recorded in the world list of cycads. The updated list now records nearly 331 species of cycads throughout the world (Osborne et al. 2012). Several complex species have been split, and many segregates are extremely rare and highly threatened species facing diverse range of threats.

Cycas media complex from northern Queensland can be cited as an example to amplify the same. Previously all the collection of this complex from Cape York Peninsula region had been identified and kept under *C. media*. The intensive field survey and studies undertaken during 1994 have revealed that six species of *Cycas* are found in this region and that *Cycas media* shows substantial geographical variation which allows three subspecies to be recognized. Three new taxa of *Cycas* were also isolated and described from this region and were having restricted distribution and regarded as extremely rare or threatened ones as per IUCN norms. Recently the discovery of a new species of *Cycas*, *C. annaikalensis* Singh and Radha in the year 2006 from Palghat district in Western Ghats also suggest the number of *Cycas* species may be more in India. Recently two more new species of *Cycas* were discovered from Southern India, *Cycas indica* Lindstrom and Hill and *Cycas swamyi* Singh and Radha (Lindstrom and Hill 2007; Singh and Radha 2008). The type of locality of *Cycas circinalis* Linn. (locally known as *Todda Panna* in Malabar region), the first ever known cycad taxon described by Carl Linnaeus in 1753, has been from the Malabar region in Kerala from India.

Studies have revealed that only ten genera of cycads have been described till date and treated under three families: Cycadaceae (one genus), Stangeriaceae (two genera), and Zamiaceae (seven genera). These genera have been mentioned below with the countries of their origin – *Bowenia* Hook. *f.* (Australia), *Ceratozamia* Brongn. (Mexico), *Cycas* Linn. (most widely spread genus among all cycads, distributed in Asia, Tropical Australia, Eastern Africa, Madagascar, and Western Pacific Islands), *Dioon* Lindl. (Mexico and Honduras), *Encephalartos*

Lehm. (South Africa), *Lepidozamia* Regel (Eastern Australia), *Macrozamia* Miq. (Australia), *Microcycas* (Miq.) A. DC. (Cuba), *Stangeria* T. Moore (Southwestern Africa), and *Zamia* L. (New World) (Norstog and Nicholls 1997; Jones 2002; Pant 2002; Whitelock 2002).

14.3 Distribution and Habitat

Cycads are basically the tropical and subtropical plants. If the distribution of present-day cycads is marked on a world map, it has been observed that they generally occur between the Tropics of Cancer and Capricorn or more precisely between 30° North latitude and 35° South latitude. It has been quite interesting to record that the majority of cycad species are found in the subtropics whereas only a few taxa in the equatorial habitats. Species found in the equatorial region generally occur at higher altitudes where the temperature and the humidity are usually lower. The cycads occurring in the rain forests of low elevation, where the humidity and the temperature are high, generally belong to the genus *Zamia*. But some species such as *Encephalartos ghellinckii* and *E. cycadifolius* have adapted to the habitats experiencing cold weather and moderate snowfall.

Cycads are distributed in nature in diverse habitats ranging from mesic to semiarid regions. Perhaps the most adapted taxa of cycads are thriving in the xerophytic conditions with extremely low seasonal rainfall and long hot summer periods. Such habitats are found in grasslands, sparse forests, woodlands, gorges, and rocky areas. In such habitats, a few taxa of *Encephalartos*, *Dioon*, *Cycas*, and *Macrozamia* thrive well. Some species of *Encephalartos* in South Africa occur at moderately higher elevations where snowfall and heavy frost takes place during winters (Donaldson 1995). Four genera of cycads are restricted to the single countries, with *Bowenia*, *Lepidozamia*, and *Macrozamia* occurring only in Australia and *Stangeria* only in South Africa. *Microcycas* is found only in Cuba. The cycads generally have a low tolerance for salt, but as an exception, *Zamia roezlii* has adjusted to grow in the coastal marshes that remain inundated with high-tide seawater. It grows in wet, heavy soils called muck. *Zamia pseudoparasitica* is the only species that has been found to be epiphytic in nature under the tropical and subtropical climate with pendent, leathery leaves up to 3.0 m long.

It is considered that the loss of cycad habitats is relatively faster in developing countries, but it is far greater in developed nations such as Australia and the United States. The threatened status is associated either with the destruction of habitat by fire and prolonged wars, etc. or removal of plants in wild for illegal trade particularly in China, India, Mexico, and South African nations. Cycads are subjected to over utilization for the commercial sporting, ornamental, educational, as well as scientific purposes.

14.4 Economic, Medicinal, and Traditional Significance of Cycads

The cycads, more particularly the *Cycas*, possess nutritive values which have also been known to the mankind since long. In the southern and eastern parts of India, Japan, SE Asia, Australia, and the other native regions, the people used it as a source of food. The stem starch is widely extracted and prepared into "Sago" in the various parts of South and Southeast Asia. The megasporophylls are also used as medicines for regulating the flow of vital energy and assuaging pain caused by functional disorders of various organs (Wang 1996). It is said to be beneficial to the kidneys. Seeds relieve hypertension. The roots of *Cycas* are used in rheumatic pains and colds. The stems of *Cycas circinalis* yield a gum. During a study of prehistoric uses of cycads, it was found that the simple techniques of leaching, rendering the cycad toxins harmless especially in *Macrozamias* in Australia, has been at least 4,000 years old (Beaton1990). There are evidences that the archeological remains of the collected cycad seeds in Australia may date back

to the late Pleistocene period. The seeds of *Cycas sphaerica* in Odisha region of India are used extensively by the locals as food and commonly sold in the local markets (Singh and Singh 2011).

Indigenous people living near the cycad localities have established immense knowledge on the traditional uses of cycads. Pinedo-Vasquez et al. (1990) documented four classes of uses in indigenous people of Mexico as edible, medicinal, religious, and ornamental. Osborne et al. (1994) recorded complex magical and medicinal uses of *Stangeria eriopus* in the South African region. Use of *Cycas* as food items has been reported from India, Japan, Myanmar, Vietnam, Sri Lanka, China, Malaysia, Philippines, and Madagascar (Pant 2002; Radha and Singh 2008; Wang 1996; Whiting 1963). Likewise other genera are used for different purposes in the countries where they grow naturally, viz., *Dioon* in Mexico and Honduras, *Encephalartos* in South Africa, and *Cycas*, *Macrozamia*, *Lepidozamia*, and *Bowenia* in Australia (Bonta et al. 2006; Douwes and Dalzell 2007; Hill and Osborne 2001; Perez-Farrera et al. 2006). In India, male cones of *Cycas beddomei* are used as a major ingredient in rejuvenating tonics. Pith ground in water and the paste thrown in ponds by locals for the faster multiplication and growth of the prawns. Male cones of *Cycas pectinata* in Assam are sold in the local market for treating asthma and piles (Das and Dutta 2007; Singh and Singh 2012) (Fig. 14.1).

14.5 Insects and Pests

The intimate association of cycads and insects was first noticed and reported by Thunberg in 1773 (Oberprieler 1989). Recently, the insect role in pollination has been discovered (Tang 1987; Terry 2001; Kono and Tobe 2007; Terry et al. 2012). Norstog and Stevenson (1986) proved evidences of insect pollination in some genera and species of cycads. Among the Indian cycad also, insect association are reported. *Trigona iridipennis* Smith, *Chilades pandava*, and *Derelomus* weevils are found to be associated with *Cycas sphaerica* in Andhra Pradesh (Raju 2009; Raju and Jonathan 2010).

In 1972, an insect *Aulacaspis yasumatsui* popularly known as the cycad aulacaspis scale insect was first reported in a *Cycas* sp. in Bangkok, Thailand (Takagi 1977). Cycad aulacaspis scale is a pest that infested on cycads which can cause severe damage leading to death of the host plant. The pest is easily spread by the movement of crawlers in the wind to other cycads.

An attack of *Aulacaspis yasumatsui* (Asian cycad scale) on *Microcycas calocoma* (Zamiaceae) was detected in the CSIR-NBRI Botanic Garden during August–Sept. 2007 which caused serious damage to the leaves. Quick removal of infected leaves and periodical spray

Fig. 14.1 Male cones of *Cycas pectinata* being sold in the local market in Meghalaya and Assam

Fig. 14.2 Leaf of *Microcycas calocoma* infected with Asian cycad scale

of systemic insecticides (at 1–2 weeks interval) helped in controlling this fast-spreading insect on this rare cycad species (Fig. 14.2).

14.6 Conservation

Most of the cycads are on the top of the list of endangered plants, and their future does not look very bright. But dedicated workers in several countries are putting their sincere efforts in many botanic gardens to ensure the survival of cycads. For taking up the studies on conservational aspects of cycads, scientists must also look into the availability of a particular species under *in-situ* and *ex-situ* conditions. They should also investigate the phenotypic and the genetic diversity and other significant aspects of cycad reproduction including the pollination, seed development, and standardization of micropropagation techniques. They are also regarded to play a significant role in nature acting as nitrogen-fixing agents due to the underground stems with apogeotropic coralloid root masses having blue-green algal symbionts such as *Anabaena* and *Nostoc* similar to the legumes. Intensive investigations should be conducted about the population distribution, the dynamics, and the other aspects such as influence of developmental activities and the encroachment by the alien plant species into the cycad habitats.

Nearly 64 % of cycads are threatened, which is the highest value of risk of extinction given to any group of organism (Barnosky et al. 2011; Nagalingum et al. 2011). Due to unharnessed developmental activities world over, the natural habitats of all the cycads are endangered and therefore strictly governed by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). *Ceratozamia*, *Cycas beddomei* (Nayar and Sastry 1987), *Encephalartos*, *Microcycas*, and *Stangeria* have been listed in Appendix-I of CITES. All other cycad taxa are included in Appendix-II, allowing their trade only under a license. Such practice has allowed the survival of *Encephalartos woodii* in South African botanic gardens. Research into *Stangeria* seed behavior at the University of Natal (Durban) made significant headway by successfully establishing a protocol for favorable *in vitro* germination seed storage methods, and the storage time were also standardized (Douwes 2004). In Mexico, cycads are considered a national conservation priority and are protected by national and international laws (INE-SEMARNAP 2000; CONABIO 2000; Diario Oficial 2000). Walters (2003) opined that a successful long-term conservation of cycads can only be achieved through combination of *in-situ* and *ex-situ* conservation. World over, many botanic gardens grow cycads; however, botanic gardens which house a very good representative of cycads are Jardin Botanico Fco J. Clavijero

(Mexico), Lowveld National Botanical Garden (South Africa), Montgomery Botanical Center (United States), Nong Nooch Tropical Botanical Garden (Thailand), Fairy Lake Botanical Garden (China), Royal Botanic Gardens, Kew (UK), and Royal Botanic Gardens, Sydney (Australia). Botanic Garden of National Botanical Research Institute, Lucknow, has the highest number of cycad collection in India. A good number can also be seen at Indian Botanic Garden, Kolkata, as well as in some private collections.

14.7 Ornamental Significance and Cultural Requirements

Cycads are tough and durable not only as survivors but also as ornamental plants of high intrinsic interest due to their long history and unique biology. They are among the finest accent plants, and whether punctuated as a collection or interspersed with the other group of plant species in a garden landscape, the cycads attract the crowds. They are relatively slow-growing objects and possess predictable dimensions, making them useful in many garden applications. Their palm-like beautiful and glistening leaves provide the tropical touch to the landscapes. Due to their unique ornamental significance, cycads are nowadays in great demand all over the world during the last two to three decades. As a result, their habitats in the nature have been adversely affected.

Cycads can be propagated with the help of suckers as well as the seeds. The seed germination takes place in 30–75 days depending upon the type of species and the size of seeds. Cycads thrive well in sandy porous soils with good aeration. The ideal soil mixture for cycads is a well-drained mixture of sterilized red soil (coarse sand) and leaf mold in equal quantities and a teaspoonful of bone meal per pot of 20 cm size. They should be kept in the semi-shady conditions in indirect sunlight. They need moderate irrigation. It will be better to feed the cycads with liquid fertilizer during the period of their growth.

Cycads are very hardy, and the healthy specimens are resistant to most of the pests and diseases. But the problems with diseases and the discoloration are generally the result of poor growing conditions or the other forms of stress. The occasional aphids, mealybugs, insects, and scales problems can be easily dealt by using conventional contact or systemic insecticides (malathion). In case they are susceptible to the fungal attacks, sprays of Bavistin are advised. Constant monitoring and rapid action are required to prevent the loss of cycad germplasm (Bailey 1963, Bailey and Bailey 1976; Brickell 1990; Cavendish 1969; Everett 1981; Graf 1981). Horticultural communities should come forward to look into the real problems associated with the cultural and conservational aspects of cycads which would be a positive step to ensure their survival in the future.

14.8 Cycads in India

Indian cycads are represented by a single genus *Cycas* with currently known nine species and one variety: *Cycas annaikalensis* Singh and Radha in Palghat area of Western Ghats, *Cycas beddomei* Dyer in Tirupati and Cuddapah hill regions of the Eastern Ghats, *Cycas circinalis* L. in Karnataka, Kerala, and Tamil Nadu states along the Western Ghats, *Cycas circinalis* var. *orixensis* Haines in Odisha, *Cycas indica* Lindstrom and Hill and *Cycas swamyi* Singh and Radha in Karnataka, *Cycas nathorstii* Schuster in Tamil Nadu, *Cycas pectinata* Ham. in Northeastern states, *Cycas sphaerica* Roxb. in the Eastern Ghats, and *Cycas zeylanica* (Schust.) Lindstrom and Hill in Andaman and Nicobar Islands (Hill 1995; Lindstrom and Hill 2007; Singh and Radha 2006, 2008). Except for *Cycas pectinata* Ham. and *Cycas nathorstii* Schuster, all the Indian cycads are endemic to India. Some of the exotic cycads such as *Cycas revoluta* Thunb., *C. rumphii* Miq, and several species of *Zamia* have been introduced in India under ornamental horticulture and frequently used for the landscaping purposes.

14.8.1 Germplasm Collection of Cycads in Botanic Garden at NBRI

NBRI Botanic Garden is maintaining a rich germplasm collection of over 5,000 taxa of various plant groups including RET species represented by 212 families from tropical and subtropical regions. In order to develop a Cycad House, the planting material of cycads is being consistently enriched through the exchange of germplasm from various botanic gardens all over the world. With the consistent germplasm exchange support from many botanic gardens, it could be possible to build up the germplasm collection of the following species of cycads for education, research and display: *Cycas annaikalensis* Singh and Radha, *C. angulata* R. Br., *C. beddomei* Dyer, *C. bifida* (Dyer) Hill, *C. circinalis* Linn., *C. cairnsiana* Muell., *C. conferta* Chirgwin, *C. debaoensis* Zhong and Chen, *C. diannanensis* Guan and Tao, *C. guizhouensis* Lan and Zou, *C. micholitzii* Dyer, *C. ophiolitica* Hill, *C. pectinata* Ham., *C. siamensis* Miq., *C. sphaerica* Roxb., *C. swamyi* Singh and Radha, *C. revoluta* Thunb., *C. rumphii* Miq., *C. taitungensis* Shen, Hill, and Tsou and Chen, *C. thouarsii* R. Br. ex Gaudich., *C. zeylanica* (Schust.) Lindstr. and Hill, *Stangeria eriopus* (Kunze) Baill, *Encephalartos altensteinii*, *E. aemulans* Vorster, *E. cycadifolius* (Jacq.) Lehm., *E. ferox* G.Bertol, *E. gratus* Prain, *E. ituriensis* Bamps and Lisowski, *E. lebomboensis* Verd., *E. natalensis* Dyer and Verd., *E. trispinosus* (Hook.) Dyer, *E. turneri* Lavranos and Goode, *Zamia erosa* Cook and Collins, *Z. angustifolia* Jacq., *Z. fischeri* Miq., *Z. furfuracea* L.f., *Z. integrifolia* L.f., *Z. loddigesii* Miq., *Z. muricata* Willd., *Z. portoricensis* Urb., *Z. pumila* L., *Z. roezlii* Linden, *Z. vazquezii*, *Dioon califanoi* De Luca and Sabato, *D. edule* Lindl., *D. holmgrenii* De Luca, Sabato and Torres, *D. mejiae* Standl. and Williams, *D. merolae* De Luca, Sabato and Torres, *D. purpusii* Rose, *D. spinulosum* Dyer ex Eichl., *Macrozamia communis* Johnson, *M. fawcettii* Moore, *M. moorei* Muell., *M. riedlei* (Gaudich.) Gardner, *Lepidozamia peroffskyana* Regel, and *Microcycas calocoma* (Miq.) A. DC. The germplasm resources of cycads have been the center of attraction for the students, teachers, researchers, and connoisseurs from all over the country. For the better display of these cycad species, a Cycad House had been installed in the botanic garden covering an area of 300 m^2 on October 2009. Male and female specimens of the available cycad taxa are planted together for further observations on the phenological and multiplication studies (Figs. 14.3 and 14.4).

14.8.2 Studies on Phylogenetic Relationship Between *Zamia* L. and *Microcycas* A. DC.

Ten species of *Zamia* and one species of *Microcycas* which were growing in the NBRI

Fig. 14.3 View of Cycad House at NBRI, Lucknow

Fig. 14.4 Some important cycad species in the botanic garden at NBRI: (**a**) *Cycas revoluta*, (**b**) *Encephalartos villosus* (female cone), (**c**) *Microcycas calocoma* (male cone), (**d**) *Stangeria eriopus*, (**e**) *Zamia loddigesii* (*male cone*), (**f**) *Zamia neurophyllidia*, (**g**) *Zamia pumila* (female cone)

Botanic Garden were used to study phylogenetic relationship among them. An RAPD Primer yielded a total of 17 amplicons out of which 16 were polymorphic among the 10 genotypes of *Zamia* and 1 of *Microcycas*. The amplicon patterns depict the genetic diversity among the cycad species and gave the phylogenetic relationship among these species. Difference in the amplicons pattern of the male and female of *Zamia pumila* suggests that molecular markers could also serve as an effective tool for sex determination in cycads (Fig. 14.5). Jing et al. (2007) used RAPD and SCAR molecular markers to link the sexuality in *Cycas tanqingii*. Chaw et al. (2005) described the phylogeny relationships of cycads (Cycadales) using chloroplast matK gene, trnK intron, and nuclear rDNA ITS region. In the study, the generic status of *Dyerocycas* and *Chigua* were unsupportable and were proven to be paraphyletic with *Cycas* and the *Zamia*, respectively. Later on, Lindstrom (2009) merged the genus *Chigua* into *Zamia*, and the two described species of *Chigua*, i.e., *C. bernalii* and *C. restrepoi*, are reported to be a single entity, i.e., *Zamia restrepoi*.

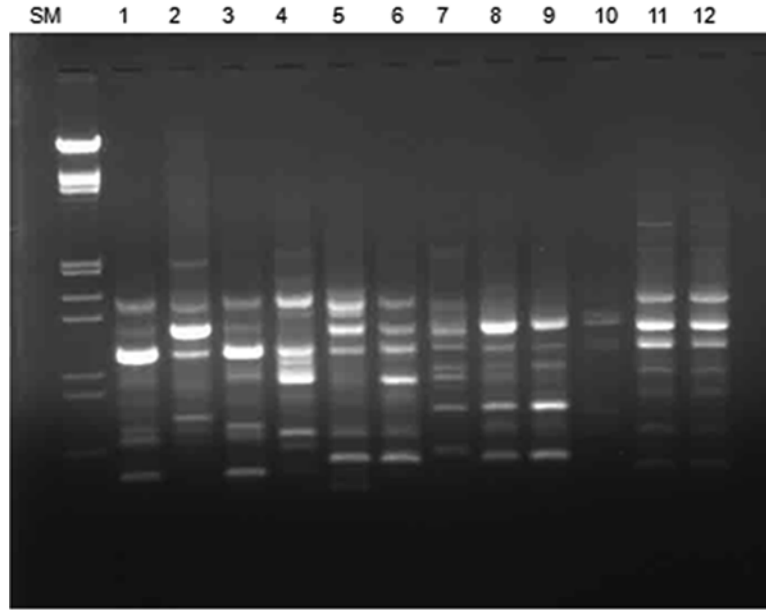


Fig. 14.5 RAPD profile of ten species of the genus *Zamia* and one species of *Microcycas*. (*1*) *Zamia floridana*, (*2*) *Z. loddigesii*, (*3*) *Z. angustifolia*, (*4*) *Microcycas calocoma*, (*5*) *Z. muricata*, (*6*) *Z. fischeri*, (*7*) *Z. vazquezii*, (*8*) *Z. neurophyllidia*, (*9*) *Z. roezlii*, (*10*) *Z. integrifolia*, (*11*) *Z. pumila* (male), and (*12*) *Z. pumila* (female)

14.9 Conclusion

Conservation of cycads should get top priorities with more attention to the studies about their ecological life responses. *Ex-situ* conservation in the botanic gardens is recognized as a defining characteristic, a very vital and significant component of overall strategy to conserve the vanishing cycads throughout the world (Goel 2003). Networking of the botanic gardens and institutions at international, national, and regional levels is extremely vital and essential. Botanic Gardens Conservation International (BGCI) at RBG, Kew (UK) is continuously working very hard in this direction. At present at least ten botanic gardens have been sincerely involved in the conservation and multiplication studies on cycads with their fair representatives in Australia, China, Italy, Mexico, South Africa, the UK, the USA, and Zimbabwe. Sharing of ideas, generated knowledge, expertise, results of multidisciplinary researches on cycads, and facilities for exchange of germplasm resources available with botanic gardens should be encouraged for making optimum utilization for their conservation, and the stringent rules of Convention of Biodiversity (CBD) may be liberalized for encouraging the R&D efforts in cycads. The cycad specialists from all over the world should be brought under one umbrella in the form of an international association. The botanic gardens should be committed to the long-term maintenance of the living collections of the cycad taxa. Organization of international seminars, workshops, conferences, and training and management courses and publication of newsletters and journals regularly at local and regional levels will certainly play a significant role in facilitating closer collaboration among cycad conservationists and will provide them a common platform with unique opportunity to discuss various issues about practical problems and issues related to the conservation of cycads. Then only man will be able to enjoy the beauty of cycads more closely and for longer duration. In order to create awareness among the masses about the conservation of cycads in South Africa, the government has released currency notes as well as the postal stamps depicting many rare cycad species.

Acknowledgments The authors are thankful to Dr. C. S. Nautiyal, Director, CSIR-National Botanical Research Institute, Lucknow, for extending the necessary facilities of R&D studies. Thanks are also due to the Ministry of Environment and Forests, Government of India, New Delhi, for the financial support under the Lead Garden Project for *ex-situ* conservation studies on RET species.

References

Anderson JM, Anderson HM, Cleal CJ (2007) Brief history of the gymnosperms: classification, biodiversity, phytogeography and ecology, vol 20, Strelitzia. South African National Biodiversity Institute, Pretoria

Bahadur B, Pratap RN, Vijay Kumar P (1977) Bioisomerism in cycads. Curr Sci 47:404–405

Bailey LH (1963) The standard cyclopedia of horticulture. The MacMillion Pub Co, New York

Bailey LH, Bailey EZ (1976) Hortus third – a concise dictionary of plants cultivated in the United States and Canada. Mac. Million Pub Co, New York

Barnosky AD, Matzke N, Tomiya S, Wogan GOU, Swartz B, Quental TB (2011) Has the Earth's sixth mass extinction already arrived? Nature 471:51–57

Beaton JM (1990) The improbable story of cycads as human food: archeological, anthropological and ethnographic evidence of more than 4000 years, Cycad'90 Abstracts: 1

Bonta M, Pinot OF, Graham D, Haynes J, Sandoval G (2006) Ethnobotany and conservation of Tiusinte (*Dioon mejiae* Standl. and L.O. Williams, Zamiaceae) in northeastern Honduras. J Ethnobiol 26(2):228–257

Brickell C (1990) Gardener's encyclopedia of plants and flowers. Darling Kindersley, London

Brown R (1827) Character and description of *Kingia* a new genus of plants found in south most coast of New Holland with observations on the structure of its unimpregnated ovulum and on the female flower of Cycadaceae and Coniferae

Cavendish M (1969) Encyclopedia of gardening. Paul Hamlyn, London

Chamberlain CJ (1935) Gymnosperms: structure and evolution. University of Chicago Press, Chicago

Chaw SM, Walters TW, Chang CC, Hu SH, Chen SH (2005) A phylogeny of cycads (Cycadales) inferred from chloroplast matK gene, trnK intron, and nuclear rDNA ITS region. Mol Phylogenet Evol 37(1):214–234

CONABIO (2000) Estrategia nacional sobre biodiversidad de Mexico. CONABIO, Mexico City

Das AK, Dutta BK (2007) *Cycas pectinata* – a wonderful native plant remedy for piles and asthma in Southern Assam, N.E. India. Ethnobotany 19:140–141

Diario Oficial (2000) Norma Oficial Mexicana NOM-059-Ecol-1994 Diario oficial de la Federacion, 16 de mayo de1994, CDLXXXVIII (10): 2–60. Mexico City

Donaldson JS (ed) (1995) Cycad conservation in South Africa. The Cycad Society of South Africa, Stellen bosch

Donaldson JS (2003) Cycads. Status, survey and conservation action plan, IUCN/SSC Cycad Specialist Group. IUCN, Gland

Douwes E (2004) *Stangeria eriopus* conservation project marks milestones. SABONET News 9(1):39

Douwes E, Dalzell CGM (2007) The ethnomedicinal use of cycads in South Africa. In: Vovides AP, Stevenson DW, Osborne R (eds) The 7th international conference on crystal biology: proceedings of Cycad 2005. Mem New York Bot Gdn 97:176–181

Everett TH (1981) The New York botanical garden illustrated encyclopedia of horticulture. Garland Pub., New York

Goel AK (2003) NBRI botanic garden- potential source of conservation and education. In: Janarthanam MK, Narasimhan D (eds) Plant diversity, human welfare and conservation. Goa University, Goa, pp 333–344

Graf AB (1981) Tropica – colour cyclopedia of exotic plants and trees. Roehrs Co. Inc, p 1136

Hill KD (1995) The genus *Cycas* (Cycadaceae) in the Indian region with notes on the application and typification of the name Cycas circinalis. Taxon 44:23–31

Hill KD, Osborne R (2001) Cycads of Australia. Kangaroo Press, Sydney

Hurter PJH, Glen HF (1996) *Encephalartos hirsutus* (Zamiaceae): a newly described species from South Africa. S Afr J Bot 61(3):226–229

Hurter PJH, Glen HF (2001) *Encephalartos relictus* (Zamiaceae): a new species from Southern Africa. Bothalia 31(2):197–212

INE-SEMARNAP (2000) Prep 6: protección, conservación y recuperación de la familia Zamiaceae (Cycadales) de México. INE-SEMARNAP, México DF

Jing JZ, Jin H, Li DL, Chen XK, Zhang Y (2007) RAPD and SCAR molecular markers linked to the sexuality of cycads (*Cycas tanqingii* D. Y. Wang). Sheng Wu Gong Cheng Xue Bao 23(6):1097–1101

Jones DL (2002) Cycads of the world. Smithsonian Institution Press, Washington, DC

Kono M, Tobe H (2007) Is *Cycas revoluta* (Cycadaceae) wind or insect pollinated? Am J Bot 94:847–855

Lindstrom AJ (2009) Typification of some species names in *Zamia* L. (Zamiaceae), with an assessment of the status of *Chigua* D. Stev Taxon 58(1):265–270

Lindstrom AJ, Hill KD (2007) The genus *Cycas* (Cycadaceae) in India. Telopea 11(4):463–488

Nagalingum NS, Marshall CR, Quental TB, Rai HS, Little DP, Mathews S (2011) Recent synchronous radiation of a living fossil. Science 334(6057):796–799

Nayar MP, Sastry ARK (eds) (1987) Red data book of Indian plants, vol 1. BSI, Howrah, p 367

Norstog KJ, Nicholls TJ (1997) The biology of the cycads. Cornell University Press, Ithaca

Norstog KJ, Stevenson DW (1986) The role of beetles in the pollination of *Zamia furfuracea* L. fil. (Zamiaceae). Biotropica 18:300–306

Oberprieler RG (1989) *Platymerus*, a forgotten weevil. Pellea 8:50–54

Osborne R, Grove A, Oh P, Mabry TJ, Ng JC, Seawright AA (1994) The magical and medicinal usage of Stangeria eriopus in South Africa. J Ethnopharmacol 43(2):67–72

Osborne R, Calonje MA, Hill K, Stanberg L, Stevenson D (2012) The World List of Cycads/La Lista Mundial de Cícadas. In: Stevenson D, Osborne R, Blake AST (eds) Proceedings of 8th international conference on Cycad Biology, Panama. Memoirs of The New York Botanical Garden. The New York Botanical Garden, New York 106 pp 480–508

Pant DD (2002) An introduction to gymnosperms, cycads and cycadales, vol 4, BSIP Monograph. Birbal Sahini Institute of Palaeobotany, Lucknow, p 380

Pant DD, Mehra B (1962) Studies in gymnospermous plants: *Cycas*. Central Book Depot, Allahabad

Perez Farrera MA, Vovides AP, Octavio-Aguilar P, Gonzalez-Astorga J, de la Cruz-Rodriguez J, Hernandez-Jonapa R, Villalobos-Mandez SM (2006) Demography of the cycad *Ceratozamia mirandae* (Zamiaceae) under disturbed and undisturbed conditions in a biosphere reserve of Mexico. Plant Ecol 187:97–108

Pinedo Vasquez M, Zarin D, Zipp P, Chota Inuma J (1990) Use values of tree species in a communal forest reserve in Northeast Peru. Conserv Biol 4:405–416

Radha P, Singh R (2008) Ethnobotany and conservation status of Indian *Cycas* species. Encephalartos 93:15–21

Raju AJS (2009) Nesting behaviour of the Baya Weaver bird, Ploceus philippinus (Ploceidae) and the life-cycle of the Plains Cupid butterfly, Chilades pandava (Lycaenidae) with the red-listed *Cycas sphaerica* and *C. beddomei* (Cycadaceae). J Threat Taxa 1(8):429–433

Raju AJS, Jonathan KH (2010) Reproductive ecology of *Cycas beddomei* Dyer (Cycadaceae), an endemic and critically endangered species of southern Eastern Ghats. Curr Sci 99(12):1833

Sharma SC, Goel AK (1991) *Cycas* – living fossil of horto-botanical interest. Indian J For 14(3):211–216

Singh R, Radha P (2006) A new species of *Cycas* from the Malabar Coast, Western Ghats, India. Brittonia 58(2):119–123

Singh R, Radha P (2008) A new species of *Cycas* (Cycadaceae) from Karnataka, India. Bot J Linn Soc 158:430–435

Singh R, Singh KJ (2011) The importance of Odisha *Cycas* in India. Biodiversity 12(1):21–27

Singh KJ, Singh R (2012) The ethnobotany of *Cycas* in the states of Assam and Meghalaya, India. In: Proceedings of 8th international conference on Cycad Biology, Panama. Memoirs of The New York Botanical Garden. The New York Botanical Garden, New York

Takagi S (1977) A new species of *Aulacaspis* associated with a cycad in Thailand (Homoptera: Cocoidea). Insecta Matsumurana New Ser 11:63–72

Tang W (1987) Insect pollination in cycad *Zamia pumila* (Zamiaceae). Am J Bot 74(1):90–99

Terry I (2001) Thrips and weevils as dual, specialist pollinators of the Australian cycad *Macrozamia communis* (Zamiaceae). Int J Plant Sci 162:1293–1305

Terry I, Tang W, Taylor A, Donaldson J, Singh R, Vovides A, Jaramillo AC (2012) An overview of cycad pollination studies. In: Proceedings of 8th international conference on Cycad Biology, Panama. Memoirs of The New York Botanical Garden. The New York Botanical Garden, New York

Vovides AP, Gonzalez-Hernandez MD, Perez-Farrera MA, Avendano Reyes S, Barcenas Pazos C (2004) A review of research on the cycad genus *Ceratozamia* Brongn (Zamiaceae) in Mexico. Taxon 53:291–297

Walters TW (2003) Off-site collections. In: Donaldson J (ed) Status survey and conservation action plan: cycads. IUCN (World Conservation Union), Gland/Cambridge, pp 48–53

Wang DY (1996) Random talking about cycads. In: Wang F, Liang H (eds) Cycads in China. Science & Technology Press, Guangdong, pp 1–8

Whitelock LM (2002) The cycads. Timber Press, Portland

Whiting MG (1963) Toxicity of cycads. Econ Bot 17:270–302

Angiosperms: An Overview

15

Bir Bahadur, T. Pullaiah, and K.V. Krishnamurthy

Abstract

This chapter provides an overview of the flowering plants or angiosperms. The unique features of angiosperms are described, and based on these features, the evolutionary and phylogenetic history of angiosperm is traced. The chapter also gives details on distribution, phytogeography, growth habit, life form classes, root and shoot systems, leaves, inflorescence, flower and reproductive biology. An account is also provided on the details of genetic diversity and species diversity found in angiosperms. Emphasis is laid on the recent APG system of classification. Plant genomes, model plants and a brief information on economic importance are also given.

Keywords

Angiosperms • Apomixis • Breeding systems • Classification • Economic importance • Evolution • Flower • Flowering plant genomes • Genetic diversity • Inflorescence • Life forms • Model angiosperms • Phytogeography

B. Bahadur
Sri Biotech Laboratories India Limited, Hyderabad, Telangana, India

T. Pullaiah (✉)
Department of Botany, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India
e-mail: pullaiah.thammineni@gmail.com

K.V. Krishnamurthy
Center for Pharmaceutics, Pharmacognosy and Pharmacology, School of Life Sciences, Institute of Trans-Disciplinary Health Science and Technology (IHST), Bangalore, Karnataka, India

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_15,

15.1 Introduction

The angiosperms (from the Greek words *angeion* = bottle, vessel, and *sperm* = seed), a term coined by Paul Hermann in 1690, represent the flowering plants classified under Angiospermae Lindl. (also known as Magnoliophyta). They represent a most diverse group of seed-bearing vascular land plants (Spermatophyta). They can be distinguished from the gymnosperms, the other seed-bearing group of plants by a number of characters: presence of a true flower; carpels that fully enclose ovules (future seeds) and that are distinguished into an ovary; style and stigma; simple embryo sac (=female gametophyte) instead of archegonia embedded in the female sporophytic tissue called nucellus and containing the female gamete or egg; very simple male gametophyte; double fertilization and the consequent presence of endosperm, a nutritive and regulatory tissue that controls the developing embryo; lack of free-nuclear proembryo; lack of true cleavage polyembryony; presence of fruits; true tunica-corpus organization of the shoot apical meristem; xylem vessel elements (derived phylogenetically from scalariform tracheary elements); sieve tube elements; companion cells; and tension wood. Any discussion on angiosperms should lay emphasis on these unique characters. In addition, all plants including angiosperms grow throughout their life and have no maximum size in the adult phase, as in most animals. In higher plants, localized meristematic tissues are present in root and stem tips and cambia and hence have continued growth.

15.2 Distribution and Phytogeography

Angiosperms are a very widely distributed group of plants. They occur virtually in all ecosystems of the world. In spite of their ubiquitous nature, their distribution is very uneven, with nearly 67 % of the world's flowering plants occurring in tropical zones. Table 15.1 provides a reasonable but provisional estimate of the number of angiosperm plant species in various parts of the world.

Table 15.1 Higher plant species diversity in terms of species number in different continents of the world

Latin America	85,000
Tropical and subtropical Africa	40,000–45,000
North Africa	10,000
Tropical Africa	21,000
Southern Africa	21,000
Tropical and subtropical Asia	50,000–55,000
India	15,000–17,500
Malaysia (including East India)	30,000
China	30,000
Australia	15,000
North America (including West Indies and Pacific Islands)	17,000–18,000
Europe	12,500

Based on Akeroyd and Synge (1992) and other sources

It is evident from this table that the richest region is South America, which accounts for one-third of the world's higher plants. When we consider the distribution of angiosperms in major ecosystems of the world, it is evident that tropical moist forest ecosystems (accounting for only 6–7 % of the earth's surface), found between the tropic of Cancer and the tropic of Capricorn, harbour the maximum number and diversity of angiosperms. It accounts for more than 50 % of the known angiosperms (Krishnamurthy 2003). Furthermore, these forests are also very rich in endemics, accounting for nearly 37,000 endemic plants, i.e. about 15 % of all plant species of the world, in an area of just 30,000 km^2 or 0.2 % of earth's land surface (Myers 1990).

The temperate forests that occur mainly in the northern hemisphere are dominated by deciduous hardwood (dicot) trees and to a lesser extent by evergreen broad-leaved hardwood trees. Approximately 1,200 species of trees (this includes some conifers also) are reported in these forests. Among these forests, those in Eastern Asia are the most diverse in terms of species diversity (Krishnamurthy 2003). The angiosperm species number in arid and semiarid ecosystems in the world is very poor and accounts for less than 2 % of the world's species. The same is true for the boreal forests (13×10^6 Km^2 as upland entities and 2.6×10^6 km^2 as peatlands). The arctic and alpine ecosystems (occupy 8 % of world's

Table 15.2 Species richness of various grasslands of South America

Type of grassland	Area (km^2)	No. of trees and shrubs	No. of subshrubs, half-shrubs, herbs and vines	No. of grasses	Total no. of species
Cerrado in north-western Sao Paulo	50	45	175	17	237
Cerrado in western Minas Gerais	15,000	C.200	C.300	73	C.600
Whole Cerrado region	2,000,000	429	181	108	718
Rio Branco savannas	40,000	40	87	9	136
Rupununi savannas	12,000	C.50	291	90	431
Northern Suriname Savannas	C.3000	15	213	44	272
Central Venezuelan Llanos	3	69	175	44	288
Venezuelan Llanos	250,000	43	312	200	555
Colombian Llanos	150,000	44	174	88	306

Data from Hornby (1992)

terrestrial surface, 5 % in arctic and 3 % in alpine) support only about 4 % of earth's flora with about 1,500 species in the arctic and 10,000 in the alpine regions. Grasslands occupy approximately 25 % of earth's land surface (according to some only 16–18 %), and these are dominated by grass and grass-like species, although in some areas herbaceous and shrubby as well as small trees may be present. The grasslands include steppes, prairies, llanos, cerrados, pampas, savannas and rangelands. Species diversity in grasslands is highly variable, the richest ones being those in South America and South Africa. The Pampas of Argentina and Uruguay have 400 species of grasses, and the Cerrados of Brazil have more than 300 species of plants (see details in Table 15.2). The African savannas contain around 2,500 plant species of which about 50 % are ecological endemics, while the rangelands of Australia are species poor and dominated by hummock grasses belonging to species of *Triodia* and *Plectrachne*.

The wetland ecosystems include freshwater wetlands (include bogs, fens, swamps, marshes, floodplains, lakes, etc.) which have only 0.014 % of earth's water and which occupy 5.3 million km^2 (see Krishnamurthy 2003) and the marine ecosystems. The total number of freshwater hydrophytic vascular plants in the world is 2,614 including angiosperms (Chambers et al. 2008) which constitute about 98 % of the total. They include submerged, floating and emergent hydrophytes that occur in water bodies as well as those that occur in swampy, boggy and marshy areas. There are about 20,000 marine plant species of which the marine angiosperms account for about 13 genera and 52 species only. The marine angiosperms, often called sea grasses, are rheophytes and belong only to monocot families. The mangrove ecosystem consists of intertidal forested wetlands characteristically located in littoral, sheltered and low-lying tropical and subtropical coast. Mangroves have a diverse collection of trees and shrubs that either form exclusive species or non-exclusive species. The former are found only in the mangroves, while the latter are not restricted to mangroves. According to Saenger et al. (1983), about 60 species belonging to 22 genera are exclusive, while 23 species belonging to 16 genera are non-exclusive. According to Tomlinson (1986), 54 species of mangroves are trees. Saenger et al. (1983) have further shown that there are 20 species of monocotyledons and respectively 110 and 28 species of dicotyledons in the mangroves of Asian and Atlantic coasts.

The patterns of distribution of angiosperms (and other plants) on the earth and the factors accounting for such observed patterns are covered under phytogeography. These patterns of distribution are often discussed only at the family and the lower hierarchical levels of genus and species. A *cosmopolitan* taxon of angiosperms is one that occurs across several continents. The other extreme type of distribution is called *endemic*, where taxa have a very restricted distribution.

Endemic angiosperms may be *paleoendemics* (once had a wide distribution but now have a very restricted distribution) or *neoendemics* (which are newly evolved taxa in a region and have not yet started to disperse themselves to a wider area). Narrow endemics occur in a very small area only. There are angiosperms which have a discontinuous or disjunct distribution. These taxa occur in different regions with no continuity between these different regions. These might have had once upon a time a continuous distribution but have become discontinuous due to sudden changes in the environmental condition (especially temperature) or development of barriers in the intermediate regions. A phenomenon related to discontinuous distribution is *vicariance/vicarism*. This refers to a group of species produced by allopatric speciation from one common ancestor. This often happens in a once continuous species that has become subsequently disjunct.

Angiosperms (and other plants) are also spoken of in terms of their original place of distribution in the world, and these, due to various factors, particularly anthropogenic, might have spread to other parts of the world. We speak of northern hemisphere taxa and southern hemisphere taxa. For example, with reference to Indian phytogeography, we speak of native Indian elements, Indo-Malayan elements, North African elements, tropical African elements, Mediterranean elements, European elements, tropical elements of the Old World, tropical American elements, subtropical elements, temperate elements, etc.

In this connection, mention should be made of *alien invasive* angiosperm species. These have rapidly colonized new areas of the world where they were not found earlier causing harm to local taxa by either eliminating them through competition or reducing their populations. Hundreds of alien invasive angiosperms have been reported in the recent past in different parts of the world including India. Some of the leading invasive taxa that have a cosmopolitan spread are *Ulex europaeus*, *Leucaena leucocephala*, *Lantana camara*, *Eupatorium adenophorum*, *Eichhornia crassipes*, *Parthenium hysterophorus*, etc.

15.3 Growth Habit and Life Form Classes

Angiosperms exhibit an enormous diversity of form and habit. Apart from the usual trees, shrubs and herbs, members belonging to Podostemaceae have vegetative plant bodies reduced to filamentous or thalloid dimensions. *Cuscuta* and *Cassytha*, which are *holoparasites*, have a plant body which is wiry and tendrillar. *Rafflesia*, another holoparasite, has a vegetative structure that is reduced to the dimensions of fungal hyphae. Some angiosperms are extremely big sized. For example, *Eucalyptus regnans* growing on Mount Baw Baw near Melbourne is officially recorded as 326 ft in height and around 25½ ft in girth. At the other extreme is *Wolffia*, the smallest angiosperm of just about 0.05 in. The *trees* have a stout main stem (axis) which may be branched or unbranched; they are capable of growing to great heights, and their trunks and main branches are very hard and woody. *Shrubs* do not have a tall and thick trunk (do not exceed a height of 20 ft), but instead a number of branches grow crowded together from near the ground giving the plant, often, a bushy appearance. *Herbs* are generally small in size with soft stems. The tallest of herbs may not extend beyond 10 ft. There are also *runners* (or *stolons*), *offsets*, *twiners*, *climbers* and *lianes*. A runner has a main, acaulescent stem that is fixed to the soil by a taproot. Its axillary branches have long and slender internodes, spreading horizontally on the surface of soil, and nodes that again get rooted and producing leaves and horizontally spreading branches. A runner that floats on water is an offset and an underground runner is called a sucker. Twiners have slender stems that coil or twine around supports and grow upward. The twining may be clockwise (CW) or anti-/counter-clockwise (CCW) and has been studied first by Charles Darwin (1865); it is a feature rarely described in taxonomic literature although Prain and Burkill (1936, 1939) utilized this character in the taxonomy of *Dioscorea*. According to Ornduff (2004), twining tracheophytes occur in about 58 unrelated angiosperm families, 46 of dicots comprising

137 genera and 289 species and 32 taxa in 15 genera belonging to 12 monocot families apart from *Gnetum* and some ferns. Narsaiah and Bahadur (1984) reported twining handedness in several Indian flowering plants and studied various characters including its effect on yield. It may be of interest to note that this condition might have arisen independently several times within the angiosperms. There are reports that the twining handedness also called asymmetry is related to yield (see Kihara 1972; Ornduff 2004). It may be of interest to point out that Bible (1976) noted differences in yield in terms of both fruit weight and number of fruits in right-handed than left-handed peppers and tomato plants, suggesting that the regulation of foliar spirality can be used to advantage for increasing productivity in crops. For more details, see Kihara (1972) and the work of Kihara's associates in Japan. Climbers are also weak stemmed like twiners but need the help of a climbing organ to get attached to the support; the climbing organ may be a tendril (which may be modified terminal bud, axillary bud, stipules, leaflets, leaf tip, petiole, etc.), a hook (a modified axillary bud or floral stalks), thorns/prickles or roots. Lianes are woody climbers.

Most angiosperms are autotrophic and are fixed in one place and obtain all their requirements from the abiotic environment. However, there are many angiosperms which depend on other living organisms, either partially or fully, for their requirements. To this category belong *epiphytes*, which live attached to other plant parts. They are hence called *space parasites*. Although they are capable of photosynthesis, they depend on the 'host' plant for space to occupy themselves and also partially for water and mineral requirements from the 'host' surface. Many orchids and bromeliads are epiphytes. The velamen roots of these taxa are capable of absorbing moisture from the external environment. Many epiphytes also have mycorrhizal association which provides some of their requirements. There are also parasitic angiosperms which may parasitize the host plant's roots (e.g. sandal plant *Santalum album*, *Orobanche*, *Rafflesia*, *Striga*, *Balanophora*, etc.) or stems (e.g. *Viscum*, *Loranthus*, *Cuscuta*, *Cassytha*, etc.). They have special *haustorial roots* through which they absorb food and other materials from the host. There are also *carnivorous* or *insectivorous* angiosperms that depend on insects for their nutrient requirements, particularly nitrogenous materials. They have special devises for capturing insects such as pitchers (*Nepenthes*), bladders (*Utricularia*), trigger-traps (*Dionaea*) or sticky leaves (*Drosera*). Another specialized group of angiosperms are *saprophytes*. These lack photosynthesis and require the help of fungi (often associated with roots) to satisfy their nutrient requirements. Examples of saprophytes are *Monotropa* and some Ericaceae.

Angiosperms are classified into four groups based on their life span:

1. *Annuals*: Annuals are plants that complete their entire life cycle within a season or year (e.g. paddy).
2. *Biennials*: Plants that live for two seasons, in the first season they live a vegetative life, make food materials and store them in underground organs and in the second season they reproduce using the stored materials (e.g. radish, carrot).
3. *Perennials*: Plants that live for a number of years but flower invariably every year (=*polycarpic*), they are mostly trees, shrubs and lianes. There are also perennial herbs like ginger, turmeric, banana, etc.
4. *Multiennials*: Plants are perennials but do not produce flowers every year. They flower only once in their lifetime and die soon after (e.g. talipot palm, species of *Agave*) (=*monocarpic*).

Angiosperms are also divided into many *life form classes* by Raunkiaer (1934). The Raunkiaer's system of classification of life forms is based upon the principle of position and degree of protection of perennating buds during the unfavourable or adverse seasons. The resultant product of analysis of life form classes found in any area is the biological spectrum. The life forms recognized by Raunkiaer (Fig. 15.1) are (1) *phanerophytes* which included the following subclasses, *mega-* and *mesophanerophytes*, *microphanerophytes*, *nanophanerophytes*, liana, epiphytes, parasites and stem succulents,

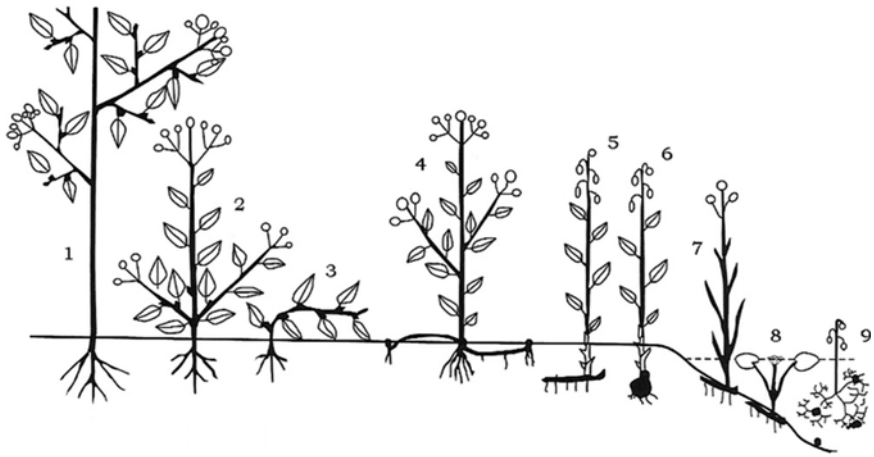


Fig. 15.1 Raunkiaer's life from classes: *1* phanerophytes, *2* and *3* chamaephytes, *4* hemicryptophytes, *5–8* cryptophytes, *9* therophytes (Raunkiaer 1934)

(2) *chamaephytes*, (3) *hemicryptophytes,* (4) *cryptophytes* and (5) *therophytes*. The normal world percentages of these five life form classes of angiosperms respectively are 46, 9, 26, 6 and 13. But these percentages differ at the regional level.

15.4 Vegetative Plant Body

The vegetative body is made of an aerial shoot system and an underground root system. The stem and the root form axial organs, while the leaves and flowers form the appendicular organs.

15.4.1 Root System

Plants typically depend on soil not only for support but also for uptake of water and nutrients such as nitrogen, phosphorus, etc. Root may be of the primary taproot system which extends deep into the soil and relies on lateral roots, more commonly characteristic of the dicotyledons, or of the adventitious/fibrous root system that replaces the taproot, the characteristic feature of all monocotyledons. These two main root systems provide the foundation for the plant growth and reproductive success. Adventitious roots can also arise in dicotyledons, but invariably in addition to the already existing taproot system. In some taxa, the roots show modifications such as tuberous and fleshy roots (as in carrot, radish or cassava); prop roots (as in *Ficus* species); stilt roots as in *Pandanus*, sugar cane or corn; contractile roots as in some *Oxalis* species; velamen aerial roots as in some epiphytic orchids; climbing roots; knee roots; pneumatophores as in some mangroves that grow in anoxic conditions; or root buttresses as in some huge tropical trees. Roots of many taxa are often closely associated with symbiotic fungi and bacteria. For instance, roots of legumes show association with root nodule bacteria belonging to *Rhizobium* and related genera, while many Amentiferae taxa have roots associated with the actinomycete, *Frankia*. In both of these cases, the association helps in nitrogen fixation. Roots of many angiosperms are associated with mycorrhizal fungi belonging to Glomales [ectomycorrhizae belonging to the Vesicular Arbuscular Mycorrhizae (VAM) or Arbuscular Mycorrhizae (AM) category; see more details on the subject in this volume] or to endomycorrhizal fungi as in orchids and Ericaceae. Roots may be affected by parasitic bacteria, fungi and nematodes of the rhizosphere.

Unlike the shoot, the root has not been studied in detail. The soil-root system interaction has been relatively ignored due largely to the underground/subterranean nature of the root. Also the

importance of root system architecture (RSA) needs to be understood (Christopher and Benfey 2012). This, according to the above authors, means spatial organization of the root system in its environment, which reflects its capability to extract resources (Lynch 1995, cited by Christopher and Benfey 2012). It has three important basic components: (1) the initiation of the root axes that causes branching, (2) the growth rate and path of each axis and (3) the expansion of root surface area. All these can produce highly complex topologies.

Shoot and root system crosstalk also ensures that carbon metabolism in the shoot is synchronized with nutrient cycles in the root and coordinates the nutrient homeostasis with growth (Jiao et al. 2009, cited by Christopher and Benfey 2012). The rootcap is a unique sensory organ and holds the key in plant-environment interactions for various biotic and abiotic soil conditions. The rootcap also performs the dual function of physical protection for root meristem and exudes various biochemicals into the rhizosphere, thereby attracting mutualistic microbes of the rhizosphere (Arnaud et al. 2010; cited by Christopher and Benfey 2012).

15.4.2 Shoot System

The shoot system is continuous with the root system and is made of stems (branched or unbranched) that bear leaves and flowers. The stem has nodes where leaves and lateral branches (if any) arise and internodes. Normally the nodes are highly telescoped regions of the stem, while internodes are elongated to various extents, contributing to the height of the plant. However, in some taxa, the internodes are also highly compressed and telescoped to result in what are called *acaulescent* plants. As indicated earlier, the shoot system may be unbranched or branched to various extents. The lateral branches develop from axillary buds that are located in the axil between the stem and the leaf. The branching may be *syll-eptic* or *proleptic* (Fig. 15.2) (Hallé and Oldeman 1970). The former is a characteristic of evergrowing or non-articulate growth seen in the continuously growing tropical plants. Here, there is a continuous development of a lateral meristem from terminal meristem to establish a branch, without an evident intervening period of rest of the lateral meristem. Proleptic branching is often a characteristic of rhythmically growing temperate plants showing articulate growth with a series of short and telescoped internodes. Here, there is a discontinuous development of a lateral meristem from a terminal meristem to establish a branch, with some intervening period of rest of the lateral meristem. Some plants show very strong apical dominance, with no or very few lateral branches arising from the main stem.

Shoot branching may be *sympodial* or *monopodial*. In sympodial system, the apex of the primary stem axis stops growing, for example, at the end of a growing season, and its function is taken over by the apices of one or more lateral branches. This type of branching is also sometimes called definite branching. In monopodial branching system, the apex of the primary stem axis continues to grow indefinitely. This type of branching is also called indefinite branching. Based on the branching patterns and thus the obtained crown geometric patterns, tropical tree architecture has been divided into 23 types (Fig. 15.3). Perhaps, these types are also seen in temperate trees and herbaceous and shrubby taxa. (Although it is easily noticed and striking in the shoot system, a similar, but in a less striking form, architectural pattern may occur in the root system.) Shoot architectural patterns maximize or at least optimize the amount of light harvested, CO_2 captured (both for photosynthesis) and O_2 taken in (for respiration); they also minimize or at least optimize transpirational water loss. Plant architecture is under strict genetic control, although is influenced also by certain environmental parameters. However, it should be mentioned that there is no correlation between plant architecture and broad systematic position. Some dicotyledon families are architecturally rich. For example, the small tropical Icacinaceae family (with about 300 species) has seven models of architecture, while a very large family like Leguminosae (*sensu lato*) (with about 12,000 species) preponderously has only one model of architecture (Halle and Oldeman 1970). Information on plant architecture has been

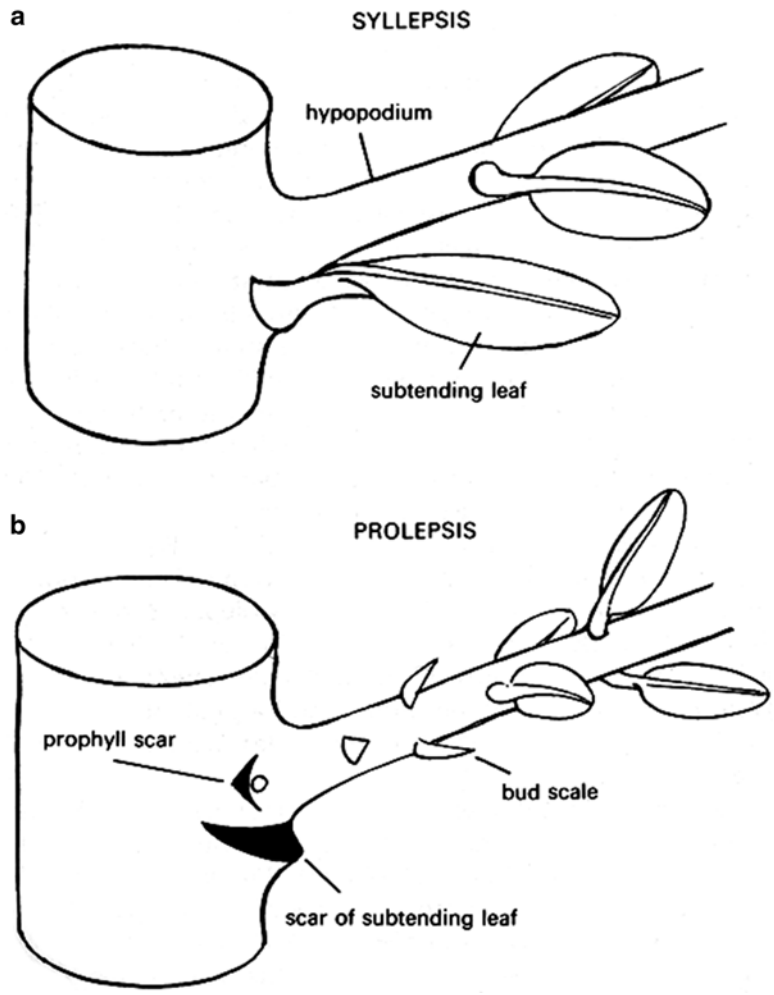


Fig. 15.2 Sylleptic and proleptic branching (Tomlinson 1983)

obtained from three different fields, using different approaches: descriptive, experimental and theoretical (see details in Tomlinson 1980, 1983; Raven 1986; Reinhardt and Kuhlemeir 2002; Krishnamurthy 2015). Plant architecture very strongly influences the suitability of a plant for cultivation, especially the spacing between any two plants. It also influences the yield and the efficiency with which it can be harvested.

Stems may also be found underground. These may be from the beginning underground or may arise as an aerial branch growing into the soil where their tips swell into tubers. The typical example for the latter is potato. Examples for the former include bulbs (as in onion, where the stem is surrounded by a number of leaves, fleshy or dry), corm (as in *Colocasia*, where the stem grows upright) and rhizome (as in ginger, where the stem grows plagiotropically). Under very arid and xerophytic conditions, the stem assumes the function of photosynthesis (as leaves are either absent or highly reduced to avoid/minimize transpirational loss of water) and becomes green; such stems are called *cladodes*. Since cladodes occur in unrelated angiosperm families like Cactaceae, Euphorbiaceae, Asparagaceae, Casuarinaceae, etc., it may be considered as a feature of convergent evolution.

15.5 Leaves

The leaves of angiosperms show the greatest diversity of types, form, size, shape and arrangement on the stem. A typical leaf has three parts: a

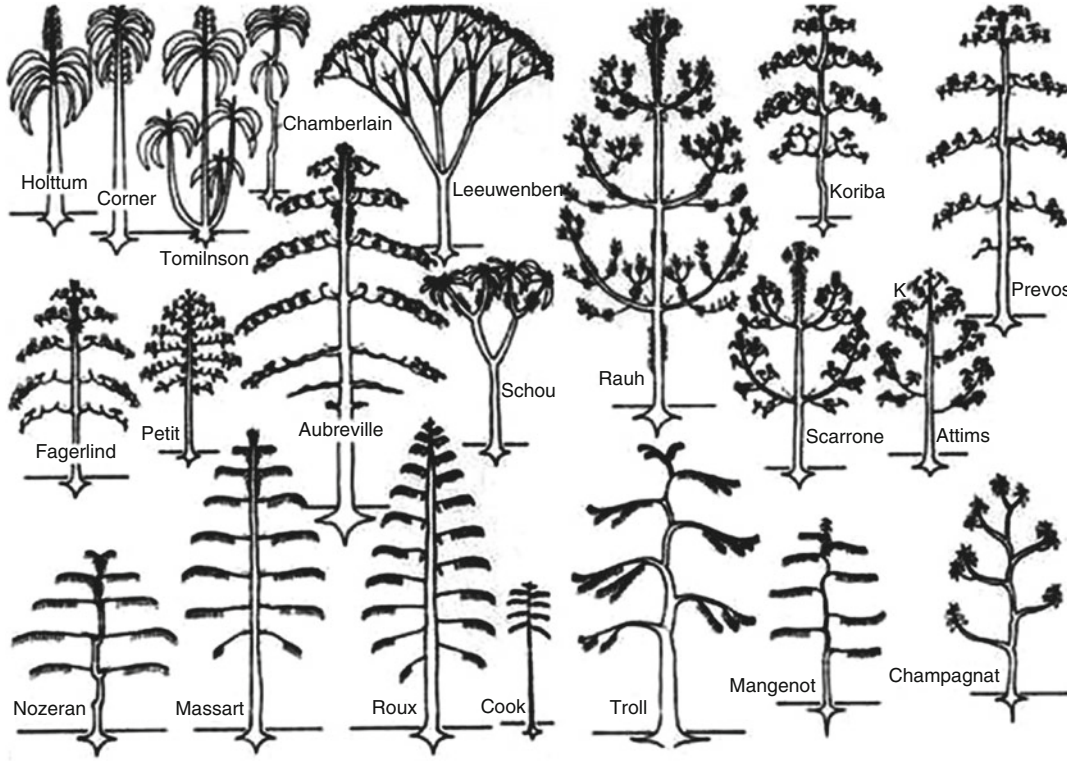


Fig. 15.3 The basic architectural models of tropical trees named after leading scientists. Root system is stylized, while the shoot system is shown in one plane (Hallé and Oldeman 1970; Oldeman 1974)

phyllopodium, the region of the leaf that joins with the stem, a petiole and a lamina or leaf blade. The phyllopodium in many monocots is in the form of a sheathing leaf base and a few dicots as well. In addition, the leaf consists of two stipules at the phyllopodial region. In some taxa, leaves are absent or modified into spines. The leaf blade, in xerophytic plants, may be absent and the petiole in such cases may become flat, green and phytosynthetic. Such leaf-like petiole is called a *phyllode* (e.g. species of *Acacia*). Leaves may be simple or compound. If only a single leaf blade is borne on the petiole, the leaf is called simple, but if there are more than three leaf blades each having a stalk of its own, the leaf is compound. The blade of the simple leaf may be entire or may be variously lobed. The shape, margin, tip, surface and texture of the lamina of a leaf vary considerably between taxa and are often characters of taxonomic importance. The shape may be linear, lanceolate, oblanceolate, elliptic, oblong, ovate, obovate, orbicular (or rotund), cordate, obcordate, reniform, cuneate, deltoid, sagittate, hastate, falcate or oblique. The laminal margin may be entire, undulate, dentate, crenulate, serrate, crenate, spiny, glandular, ciliate or dissected pinnately or palmately. The laminal apex may be acute, acuminate, obtuse, cuspidate, mucronate, retuse or emarginate. The surface of the lamina may be glabrous, pubescent, villous, hispid, scabrid, tomentose or glandular. The texture of the lamina may be fleshy or succulent, coriaceous, crustaceous or herbaceous. Compound leaves may be pinnately compound or palmately compound depending upon whether the leaflets are arranged like a bird's feather or like the fingers of a palm. Pinnately compound leaves are single pinnate, bipinnate or decompound. The pinnately compound leaves are either paripinnate (with a pair of leaflets ending at the tip of rachis) or imparipinnate (with a single leaflet at the end of the rachis).

Not uncommonly, the same plant bears different types of leaves. This phenomenon is called

heterophylly. Heterophylly may be caused by environmental variations. It is best illustrated by certain aquatic plants like *Cabomba* sp., *Limnophila heterophylla*, *Ceratophyllum* sp., etc. The submerged portions of stem bear variously dissected leaves, while those above water have entire leaves. In *Artocarpus integrifolia*, the leaves have different forms, the change in form resulting from a suppression of growth in some part of the blade. This is called *habitual heterophylly*. Species of *Eucalyptus* exhibit *developmental heterophylly* because the juvenile leaves are ovate or cordate and sessile occurring in pairs, while the adult plant leaves are narrow, lanceolate, petiolate and alternate. Leaf or a part thereof may also get modified into a pitcher as in the carnivorous *Nepenthes* for catching insects and in *Dischidia*, an epiphyte for collecting and accumulating water, or as a bladder, as in *Utricularia*, to trap insects.

Leaves, as already stated, vary very greatly in size. The largest leaf of angiosperms is shown by the Amazon lily (*Victoria regia* of Nymphaeaceae) and measures around 6 ft in diameter. Leaves of some banana varieties also measure around 6 ft, but only in length. Interestingly, the smallest leaf in *Nymphaea thermarum*, an endangered and endemic species of Rwanda, Africa, measures around 1 cm in diameter. Very large compound leaves are shown by some palms.

The regular order of arrangement of leaves on the stem is called *phyllotaxy* (or *phyllotaxis*) (Jean 1994). The most common phyllotaxy is spiral, with the angle of divergence between successive leaves as 137.5°. The other types of phyllotaxis are *distichous* (with leaves disposed at 90° from each other in pairs at each node), *opposite superposed* (leaves in pairs in each node, with each pair aligned one above the other along the same horizontal axis), *opposite decussate* (each successive pair of leaves at right angles to the next lower and the next higher) and *whorled* (with three or more leaves at each node). The majority of phyllotactic patterns noticed in flowering plants are based on spiral (or helical) arrangement of leaves. The single spiral that can be drawn through connecting the centres of all leaves in their order of succession is called the *ontogenetic* or *genetic spiral*. Phyllotaxy is often expressed by a fraction; the common fractions seen in angiosperms are $^{1}/_{2}$, $^{1}/_{3}$, $^{2}/_{5}$ and $^{3}/_{8}$, and the less common fractions are $^{5}/_{13}$, $^{8}/_{21}$, etc. This series of fractions belongs to the so-called Fibonacci summation series 0, 1, 1, 2, 3, 5, 8, 13, 21. In this series, the numerator and denominator of each succeeding fraction are the sum of numerators and denominators of the two preceding fractions. Each of these fractions represents the angle intervening between the centres of successive leaves, and this angle is 137.5° as indicated earlier. Important contributions on this subject have been made by Prof. T. A. Davis and Dr. Manoranjan Ghose of Indian Statistical Institute, Kolkata, on coconut and various other palms.

15.6 Internal Structure

The most important anatomical feature of angiosperms is the heteroxylous condition: the tracheary elements are of two categories, perforate vessel elements (=vessel elements) and imperforate tracheary elements (=tracheids). Although vessel elements are also present in *Selaginella* (Lycopsida), *Equisetum*, a few ferns (Pteropsida including *Psilotum*) and Gnetales (in all three genera, *Gnetum*, *Ephedra* and *Welwitschia*) (gymnosperms), they have evolved independently. Vessels of angiosperms (Austrobaileyales, magnoliids and eudicots and monocots) are evolutionarily derived from scalariformly pitted tracheary elements, while those of gymnosperms are derived from circular bordered-pitted tracheids. There are around 11 genera and 110 species of extant vessel-less angiosperms, which are considered as primitive flowering plants. The other anatomical features of angiosperms are the presence of sieve tube elements, companion cells that are ontogenetically related to sieve tube elements and the P protein (Fig. 15.4) containing sieve tube elements. The gymnosperms, in contrast, have only sieve cells, have no companion cells and have ER-containing sieve areas in sieve cells (no P proteins). The phloem of *Austrobaileya* alone, among angiosperms, is reported to lack

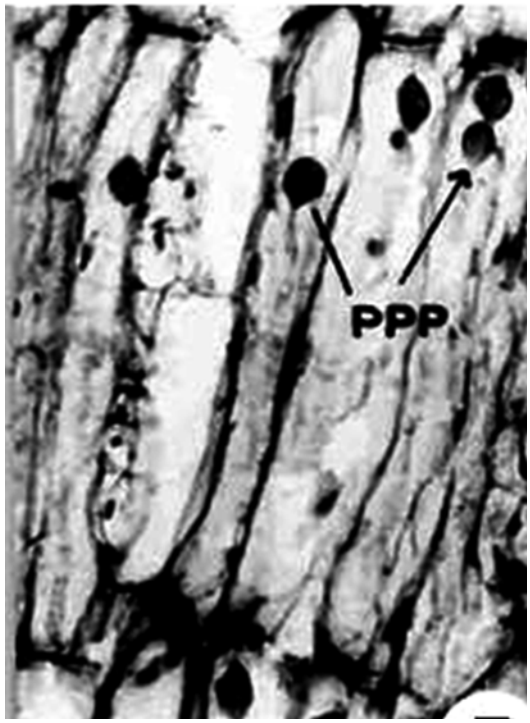


Fig. 15.4 Structure of secondary phloem in Tangential longitudinal section of *Dalbergia sissoo* showing P proteins (PPP) (Courtesy Dr. N. Venugopal)

sieve plates, but it was interpreted by others to have multiple sieve areas in the compound sieve plates; however, in this taxon, there are companion cells and P proteins (Fig. 15.4). Hence, the sieve tube elements of this taxon are considered as the most primitive.

Like gymnosperms, the dicots have a vascular cambium and secondary xylem and phloem derived from it. However, the monocots lack secondary growth contributed by a vascular cambium. Many arborescent monocots have a periderm similar to that in gymnosperms and dicots. A feature of distinction between the wood of gymnosperms and angiosperms is in the *reaction wood* (*RW*) formed in the leaning main trunk and branches. The RW of the former group is called *compression wood* (*CW*) and is formed on the lower side of the leaning stem or branch facing the ground, while the RW of dicots, called tension wood (TW), is formed on the upper side away from the earth. The TW is characterized by *gelatinous fibres*, while the CW has circular tracheids with pronounced intercellular spaces between them. There are also differences in the chemical composition of the cell walls of these two types of wood (Krishnamurthy 2007).

The shoot apical meristem (SAM) of angiosperms shows a distinct tunica-corpus organization. This concept is proved to be largely unsuitable for the characterization of the SAM of gymnosperms (barring *Gnetum*, *Ephedra* and a few conifers). The structure of the SAM in different vascular plant groups differs, suggesting its probable independent evolution in lycophytes, ferns, gymnosperms and angiosperms. Based on an analysis of the distribution and pattern of plasmodesmal (PD) network in the SAM of representative taxa of these vascular groups, Imaichi and Hiratsuka (2007) have concluded that SAMs of seed plants have low PD density per unit area, with no difference between SAMs showing tunica-corpus and cytohistological zonation organization. In contrast, SAMs of ferns have average PD densities, while Lycopsida have both fern and seed plant PD densities. Based on this analysis, the above authors have indicated the probable independent evolution of SAMs with single apical cells and SAMs with plural initial cells.

15.7 Inflorescence and Flower

The *flowers* of angiosperms are invariably borne on *inflorescences*. Only very rarely they are solitary (e.g. Malvaceae taxa). Even the solitary flowers are considered as derived characters, being derived from an inflorescence. The two fundamental models of vegetative growth habit of plants, i.e. monopodial and sympodial, are extended to the inflorescence also. In herbaceous taxa, the vegetative phase is replaced by a reproductive phase, while the other growth habits of many angiosperms, particularly of perennials, require the 'cohabitation of vegetative and floral buds along their shoots'. The monopodial model is seen in the racemose types of inflorescences, while the sympodial model is noticed in cymose types of inflorescences. Both these inflorescence models are regulated by genetic control (see

details in Reinhardt and Kuhlemeir 2002; Lifschitz and Eshed 2006). The specific variability in monopodial (racemose) and sympodial (cymose) inflorescence development and that between different sympodial meristems of the same plant (e.g. tomato) is likely to be caused by differential fine tuning of genes that control meristem identity and determinacy.

A typical flower consists of floral organs in specific patterns on a central axis, the *receptacle*, *calyx* (made of *sepals*), *corolla* (made of *petals*), *androecium* (made of *stamens*) and *gynoecium* (made of *carpels*). As already stated, the flower is unique to angiosperms, so also the carpel enclosing the ovules developing after double fertilization into fruits enclosing seeds. The emergence of flowers as reproductive units probably contributed substantially to the evolutionary success of angiosperms. The basic flower type is the one present in primitive angiosperms, the magnolioid flower, although the most primitive *Amborella* is dioecious. Endress (1994) highlighted three aspects of flower structure with different underlying rates of evolutionary change: (1) organization, (2) construction and (3) *mode*. The first one corresponds to the floral blueprint (=*bauplan*) that defines the number and position of floral organs (including the fusion of or between floral organs). The second one refers to the basic three-dimensional structure of the flower (=*gestalt*), and the third one indicates the later elaboration of specialized characteristics of the flower organs, such as the style and stigma and colour and smell. The blueprint is relatively stable in evolutionary terms. Growth patterns that decide the overall three-dimensional structure, particularly the overall size and shape of the flower, are relatively less stable, and the elaboration of specialized characters of the floral organs such as colour and smell is relatively fluid. All these three together reflect the diversity in flower-based reproductive strategies (see Soltis et al. 2002). The number of floral organs of each type is highly conserved. For example, most monocots have trimerous flowers, while dicots have tetramerous or pentamerous flowers. Another aspect of ground plan of evolutionary importance is the site of insertion of the outer organ relative to the ovary irrespective of the position of the ovary in the flower (hypogyny, perigyny or epigyny), and differential early growth of the flower can account for these different patterns. Despite much progress, we still do not know how spatial information is generated to set up the blueprint of the flower. Most flowers are hermaphroditic, and unisexual flowers of dioecious and monoecious taxa are derived. It seems that in many unisexual flowers, the male and female organ primordia arise, but are arrested at various stages. The evolution of flower and angiospermy is discussed briefly in a later section of this article.

15.8 Reproductive Development

The anther with four microsporangia is a *synapomorphy* (=derived character) of angiosperms, while in gymnosperms, the number varies from one in *Gnetum*, two in *Ginkgo* and conifers, two to six in *Ephedra* and several in cycads. The male gametophytes of angiosperms (pollen) are unique and highly reduced with just two cells (*vegetative* and *generative cells*) or three cells (vegetative cell and two male gametes). On the contrary, the male gametophyte of gymnosperms is more than three celled (barring *Gnetum* and *Welwitschia*). It has one (*Cycas*, *Welwitschia* and *Gnetum*), two (Pinaceae, *Ginkgo* and *Ephedra*), three or four (*Podocarpus*), five to seven (*Agathis*) or 18–20 (*Araucaria*) *prothallial cells*; they are altogether absent in Taxodiaceae, Cupressaceae, Cephalotaxaceae and Taxaceae. In addition, the male gametophyte contains a *tube cell*, a stalk cell and a *body cell* or instead two male gametes (occasionally many male gametes as in *Cupressus* and *Juniperus*). At the time of dispersal, the male gametophytes (pollen) of gymnosperms are one celled (some conifers), two celled (some conifers), three celled (most cycads, ginkgo, *Welwitschia* and *Gnetum*), four celled (*Pinus*), five celled (*Cedrus*, *Ephedra*) and multicelled (*Podocarpus* and *Araucaria*). Hence, basically gymnosperm microgametophyte is quite different from that of angiosperm. However, the argument that the three-celled pollen of *Gnetum* is equivalent to the three-celled pollen angiosperms is not acceptable (see Swamy 1974).

The gynoecium, or pistil, consists of one or more separate or fused carpels which are usually differentiated into stigma, style and ovary containing ovules. The stigma types, based on the morphology of the receptive surface and the secretions during the receptive period at the time of pollination, may be dry or wet and are of considerable taxonomic value. Ovules may be bitegmic as seen only in angiosperms and a synapomorphic character in angiosperms. Most gymnosperms have only one integument (unitegmic). Although *Gnetum* has three and *Ephedra* has two coverings around the nucellus, only the innermost of these coverings is the integument. The female gametophyte is unique to angiosperms; it is represented by an *embryo sac* embedded in the nucellus towards its micropylar locus. It has typically an egg apparatus (two synergids + one egg) located at the micropylar pole and a central cell with two polar nuclei (or a secondary nucleus) and three antipodals at the chalazal pole. In contrast, the gymnosperms have a nucellus in which a variable number of archegonia are embedded (except in *Welwitschia* and *Gnetum*); these archegonia have an egg cell (and a short neck). The structure of female gametophyte of even *Welwitschia* and *Gnetum* is quite unlike that of angiosperms.

Pollen, the carrier of male gametes, of angiosperms may be monads, diads, tetrads, octads and polyad (*Mimosa* and *Acacia*), filamentous (*Zostera maritima*) and specialized pollinia as in Asclepiadaceae and Orchidaceae. Size varies considerably; *Myosotis* (5 μm), large as in *Mirabilis* (177 μm) and 200 μm as in *Cucurbita pepo*. The outer wall is made of sporopollenin and displays a wide range of exine ornamentation that has adaptive value in dispersal, while the intine is cellulosic. Mature pollen may be two celled or three celled and are associated with gametophytic and sporophytic incompatibility systems, respectively.

In all angiosperms, the pollen, after pollination, is lodged on to the stigma at which it germinates and produces a pollen tube carrying the two male gametes (and often the vegetative cell also), which grow through the style before reaching the ovule. In contrast, the pollen grain of gymnosperms finally lands on the nucellus and germinates. There is single fertilization, in contrast to angiosperms where *double fertilization* (*syngamy* and *triple fusion*) leading to the formation of zygote and primary endosperm nucleus respectively takes place. Endosperm is absent in gymnosperms and its role is taken over by the nucellus itself. Although double fertilization has been reported in *Ephedra*, the second fertilization neither results in endosperm formation nor in embryo development. The so-called double fertilization in gymnosperms should not be confused with the one found in angiosperms and should not be attached much significance as some authors do (see, e.g. Friedman 1994).

Angiosperm embryogeny is ab initio cellular (the report of free-nuclear embryo in *Paeonia* is erroneous) in all taxa. But in the development of embryo in gymnosperms, there is always a free-nuclear phase, which may vary depending on the taxon. The report of ab initio cellular embryo in *Sequoia*, *Ephedra* and *Gnetum* is the result of faulty interpretation of the existing situation (Swamy 1973). While true cleavage polyembryony is the rule in gymnosperms, it is almost absent in angiosperms, being reported only in a few orchids. While the mature embryo has either two cotyledons (dicots) or one cotyledon (monocot) in angiosperms, their number varies from one, two, three, four or many in gymnosperms. Hence, embryogeny is totally different in angiosperms and gymnosperms. Because of the presence of carpels, angiosperms have a fruit enclosing seeds, while gymnosperms have only naked seeds.

15.9 Breeding Systems

These vary greatly as majority of angiosperms are bisexual that permits self-pollination of the same flower (autogamy) or by different flowers on the same plant (*geitonogamy*). Self-pollination provides reproductive assurance and guaranteed source of pollen as in *cleistogamy* and has automatic selection advantage, although there is the possibility of inbreeding depression which is harmful. There are several ways by

which outcrossing functions in angiosperms: heterostyly, dichogamy, inversostyly, enantiostyly, monoecy, dioecy and operation of incompatibility factors. *Self-incompatibility* is widespread among angiosperms and is revealed by the failure/inability of functional pollen from the same plant to germinate on the stigma or by the failure of the pollen tube to grow successfully in the style and achieve fertilization. Outbreeding systems of this sort, depending upon the presence of the style, were not possible prior to the evolution of the angiospermous flower and may have played a significant part in the rise of this class of plants to its present dominant position.

Self-incompatibility may be of gametophytic type (GSI) in which the pollen grains germinate but fail to grow down the style because of pollen rejection and is determined by self-incompatibility genes of both the maternal plant and the pollen grain. This mechanism is found in grasses and *Nicotiana*. In contrast, species showing *sporophytic self-incompatibility* (*SSI*) are polymorphic with two or three types of flowers in a species and are panmictic with stamens and stigmas of reciprocal heights as in *Eichhornia*, *Oxalis* sp., *Lythrum salicaria* (trimorphic) and *Oldenlandia umbellata*, *Primula vulgaris* (dimorphic) and several variations found in *Narcissus* and other species. Bir Bahadur and K.R. Shivanna's associates have studied various aspects of heteromorphic incompatibility in several Indian heterostylous plants.

We do not have much information on the operation of incompatibility in gymnosperms.

15.10 Apomixis

Flowering plants can choose between no less than three fundamentally different modes of reproduction: (1) outcrossing sex, (2) selfing sex and (3) asexuality. These influence the population structure and evolutionary potential in profoundly different ways. Perennial plants commonly use a combination of all three modes to fine-tune their reproductive strategy to changing ecological circumstances. Consequently, a propensity for asexual reproduction, *apomixis*, is a major feature of many flowering plants.

Approximately 450 species belonging to 40 families of angiosperms show apomixis, of which more than 250 species are from Poaceae (Pullaiah et al. 2008). Apomixis is common in perennial plants and in *ca*. 60 % of the British flora, but similar data is not available for other regions. Successful apomictic individuals may cover huge areas and live to great ages, favoured by 'symmetrical' selection. Apomixis was favoured by colonizing modes, for instance, post-glacially and continues to be so even today. Many alien invasive species of angiosperms are apomictic. For more information, see chapter on the subject in this volume.

Plants can also reproduce asexually through vegetative propagation, a process in which plants produce genetically identical offshoots (called clones) of themselves, which develop into independent plants. This asexual means of reproduction can occur naturally through specialized structures such as tubers, potato, rhizome as in ginger, runner as in mint and bulbs as in onion or artificially through grafting.

15.11 Genetic Diversity

Angiosperms exhibit enormous genetic diversity (also referred to as within-species diversity and intra- or infraspecific diversity). Genetic diversity can be measured in terms of number of genes (around 400,000 or more in many flowering plants; UNEP 1995), amount of DNA per cell (ranges from the relatively small 1C value of 0.0648 pg to 152.23 pg with a mean of 6.30 pg; Bennett and Leitch 2005), chromosome structure, size, shape and number (ranges in number from $2n=4$ in *Haplopappus gracilis*, *Brachyscome lineariloba* and *Colpodium versicolor*, the former two of Asteraceae and third of Poaceae, to $2n=530$ in *Poa littorosa*), size variation in plastid DNA (ranges from around 70 Kb in *Epifagus virginiana* to around 217 Kb in *Pelargonium hortorum*; Palmer 1991) or number of supernumerary or B chromosomes (see Krishnamurthy 2003).

The angiosperms may be diploids, polyploids (triploid, tetraploid, pentaploid, hexaploid, octoploid or still higher ploids) or aneuploids (show a decrease or increase in base number (n) by one chromosome). Polyploids are either *allopolyploids* or *autopolyploids*. Certain taxa possess a constant number (e.g. all *Quercus* sp. have $n=12$), while others show variations in number within the same species and different species of the same genus or family. For instance, in Malvaceae, the number varies from 10, 15, 20, 25 to 40 or from 12, 18, 24 to 30 or from 14, 28, 42, 56 to 84 and so on. Hybridization has played a major role in the evolution of chromosome number in angiosperms. In many angiosperms, there are conspicuous differences in *karyotypes*, often associated with differences in morphology of the chromosomes. There are also variations in chromosome size. Generally sex chromosomes are absent in angiosperms. As an exception, we can cite species of *Silene*.

Plant genomes of flowering plants display great diversity in view of frequent occurrence of polyploidy both in mono- and dicotyledons. The largest genome (150 Mbp) is found in *Paris japonica* (Melanthiaceae, Liliales). The smallest genome is found in *Genlisea margaretae* (63 Mbp) and *Utricularia gibba* (87 Mbp). This is an indication for the evolutionary forces (pressure) that may be at work for genome reduction (genome miniaturization) due to the loss of junk DNA. *Haplopappus gracilis* though has the lowest chromosome number and has a large genome size (239 Mbp). *Arabidopsis thaliana* has a genome size of 135 Mbp, while *Amborella trichopoda*, the most primitive living angiosperm, has a moderate genome size of 870 Mbp.

15.12 Species Diversity

The angiosperms constitute an extremely diverse group of vascular group exhibiting great species diversity. There are about 236,000–352,000 flowering plant species in the world (Krishnamurthy 2003). Another 275,000–300,000 species are estimated to be present on this earth, awaiting discovery. The known species are grouped in about 17,000 genera under about 200–600 families depending on the classification system. Orchidaceae with about 25,000–35,000 species and Leguminosae (sensu *lato*) with about 15,000–35,000 species are the largest families among angiosperms. In fact, approximately 30 families account for 62 % of the known angiosperms. Thirty-six families have only one species; Amborellaceae is one among these families. There are 57 genera with 500 or more species. The five largest genera are *Astragalus* (Fabaceae) with 3,270 species, *Bulbophyllum* (Orchidaceae) with 2,032 species, *Psychotria* (Rubiaceae) with 1,951 species, *Euphorbia* (Euphorbiaceae) with 1,836 species and *Carex* (Cyperaceae) with 1,795 species. The basal dicots have around 10,000 species, eudicots about 175,000 species and the monocots around 70,000 species. The dicots altogether are reported to account for 80 % of the total species.

Attention should also be focused on domesticated and cultivated species diversity of angiosperms. Domestication can be defined as the 'forceful' inclusion of wild populations of useful plant species, wholly or in part, into human society. Domestication involved (still involves) human intervention in the reproductive system of the plant that resulted in genetic and/or phenotypic changes. Domestication, hence, is the foundation stone of crop plant species diversity. Vavilov (1949–1950) indentified eight centres of origin of cultivated plants (or *centres of genetic diversity*) (China, Indian and Indo-Malayan centre, Inner Asiatic Centre, Asia Minor, Mediterranean centre, Abyssinian centre, Central American centre and South American centre), and these centres gave rise respectively to 166, 117, 55, 42, 83, 84, 38, 49 and 62 cultivated plant species. Vavilov's concept subsequently underwent modifications, and the number of centres was increased to 12. In the post-Vavilov period, several hundreds of plants have been domesticated.

The domesticated plants included cereals, millets, pulses, vegetables, oil-yielding plants, fruits, narcotics, sugar plants, spices and condiments, beverages, fibre-yielding plants, dye plants, wood-yielding plants, ornamentals and medicinal

and aromatic plants. Many of these different categories of domesticated/useful plant species have infraspecific taxa, varieties and land races.

A number of angiosperm species are threatened and are at the risk of extinction in the foreseeable future (UNEP 1995) due to deterministic and stochastic processes. The former are cause-and-effect processes such as glaciations, deforestation, habitat fragmentation, global warming, etc. The stochastic processes are chance events which may be distinguished into four types: demographic uncertainty, environmental uncertainty, natural catastrophes and genetic uncertainty. Based on the degree of threat, the IUCN has classified threatened angiosperms (also other plants) into a number of categories: extinct, endangered (including critically endangered), vulnerable, rare, indeterminate, insufficiently known, status unknown, candidate and safe. A census of threatened angiosperm species has also been made periodically by IUCN and other international and national bodies, and Red Lists have been prepared at international and national levels. The World Conservation Monitoring Centre (WCMC) is also assisting in this endeavour. It has been reported that 384 flowering plant species have disappeared/extinct from 1,600 CE to the present. Although Groombridge (1992) has listed the number of threatened taxa for different parts of the world, Asia 6,608, Europe 2,677, North and Central America 5,747, South America 2,061, Oceania 2,673 and Africa 3,308, no data is available for USSR. The total world threatened species are 23,274; the number has substantially increased in the intervening 22 years. Several conservation strategies and methods involving in situ and ex situ techniques are now being followed to protect and conserve threatened angiosperm species.

15.13 Classification

Angiosperms have been classified in the past in several ways and various classificatory systems and have been proposed and adopted. These include the artificial systems like that of Linnaeus, natural systems like that of Bentham and Hooker and phylogenetic systems like those of Eichler, Engler and Prantl, Hutchinson, Oswald and Tippo, Takhtajan, Arthur Cronquist, etc. Different countries of the world follow different systems of classification to document and manage their flowering plant resources. One of the latest phylogenetic systems of classification proposed is the APG system proposed by the Angiosperm Phylogeny Group. Recent molecular techniques and improved computer programs to analyse large sets of data with ease have provided more robust data on angiosperm plant phylogeny that are easily testable. These developments resulted in the formation of a group of people called the Angiosperm Phylogeny Group (APG). The APG system focused mainly on the level of families (with related families grouped into orders) because they are the groups around which most botanists organize their understanding of plant diversity. It need not be assumed, however, that different families or orders are equivalent in any evolutionary sense; rather, the APG organization signals a relative level in a hierarchy. Within any particular family, however, the system does presume, with some possible exceptions, that the genera included in it are all related and that the family itself is monophyletic (a lineage with all its members derived from a common ancestor); the same holds for the families included within a particular order. One of the main departures from the Cronquist system in the APG system is a less hierarchical arrangement of the higher-level groupings; Cronquist (1981) divided angiosperms into two classes: the monocotyledons (monocots), or Liliopsida, with five subclasses, and the dicotyledons (dicots), or Magnoliopsida, with six subclasses. The APG system does recognize some higher-level groupings but only at an informal level, such as eudicots, rosids and asterids. It continues to recognize the monocots as a monophyletic group; however, they are now seen as having evolved from within a more basal group of primitive dicotyledonous angiosperms. In contrast, Cronquist portrayed the monocots as being the sister group to all other dicotyledonous groups.

Following the original APG publication, more families were added to the molecular analyses, allowing these families to be placed in orders, and other new studies called for adjustments in the circumscription of particular families and orders. These changes were incorporated into an update in 2003 of the APG known as APG II, and the synopsis of angiosperm-plant classification presented here follows the APG II system. The number of recognized orders increased from 40 in the original APG system to 62 in APG II. It is important to recognize that modifications to the APG II system continue as new data become available. An update of APG II known as APG III appeared in 2009.

The basalmost group of APG system contain Amborellales, Nymphaeales and Austrobaileyales. Magnoliids contain four orders (Canellales, Laurales, Magnoliales and Piperales); the order Chloranthales is with one family Chloranthaceae and the Ceratophyllales with one family Ceratophyllaceae and genus *Ceratophyllum*; the monocots contain 11 orders (Acorales, Alismatales, Asparagales, Dioscoreales, Liliales, Pandanales, Petrosaviales, Arecales, Commelinales, Poales and Zingiberales), the eudicots has Sabiaceae (unplaced in an order), basal eudicots with four orders (Buxales, Proteales, Ranunculales and Trochodendrales) and core eudicots with Dilleniaceae (unplaced in an order) and orders Gunnerales and Saxifragales (position unresolved), rosids with 16 orders and asterids with 13 orders. Eudicots have mainly three-aperturate pollen and the lack of ethereal oil characteristic of most basalmost angiosperm groups. The core eudicots have stereotyped flowers than in basal eudicots, monocots or basal dicots; usually pentamerous, floral parts are arranged in whorls, members of one whorl alternating with the previous and next whorl of organs, flowers often bisexual and radially symmetrical (but zygomorphic flowers with bilateral symmetry are seen in some). The monocots are essentially herbaceous and lack cambial activity; have a single cotyledon, scattered vascular bundles and parallel venation; and are mostly with trimerous flowers.

15.14 Evolution of Angiosperms

Charles Darwin once called the origin and evolution of angiosperms an abominable mystery because of lack of not only clinching paleobotanical evidences but also the closest connecting extant taxa of seed plants. The earliest floral macrofossils are all at least ten million years younger than the first angiosperm microfossils (see Friedman et al. 2004). These have affinities to diverse flowering plant lineages including monocots, Platanaceae, Ceratophyllaceae, Nelumbonaceae, Nymphaeales, Laurales, Winteraceae, Chloranthaceae, Calycanthaceae, etc. Hence, as one botanist had put it, tracing the evolution of angiosperms has become an 'indoor pastime' and an 'armchair research'. Hence, in the pre-molecular biology era, many theories have been proposed to explain angiosperm origin, some purely theoretical and some based on weak 'evidences': Bennettitalean theory and its variations proposed/supported especially by Arber and Parkin (1907), Takhtajan (1969), Becker and Theissen (2003), Baum and Hileman (2006) and Theissen and Melzer (2007); Gnetalean theory proposed/supported by Wettstein (1901), Markgraf (1930), Fagerlind (1947) and Donoghue and Doyle (2000); Isoetalean theory proposed by Campbell (1928); Coniferahean theory proposed/supported by Eichler (1875), Engler (1892), Engler and Prantl (1924), Rendle (1904) and Doyle (1945) (see also Taylor et al. 2009); Pteridospermalean theory proposed/supported by Frohlich (2003), Melville (1969), Retallack and Dilcher (1981), Sun et al. (2001) and Zhang (1995); Pentoxylales theory proposed by Meeuse (1961); Caytonialean theory proposed by Thomas (1936) and Stebbins (1974); Durian theory proposed by Corner (1949); Chloranthoid theory proposed by Leroy (1983) and Stuessy (2004); Gonophyll theory proposed by Melville (1969) and supported by Stewart and Rothwell (1993); multiple origin hypothesis proposed by Anderson (1934), Wu et al. (2002) and Nair (1979); and Paleoherb theory proposed by Taylor and Hickey (1992, 1996) and supported by Burger (1981).

Fig. 15.5 Dendrogram of angiosperm phylogeny

In the last two to three decades, molecular phylogenetic studies (see full literature in Friedman et al. 2004; Soltis et al. 1999, 2000; Zanis et al. 2003) have indicated that extant gymnosperms may be sister to the flowering plants. Hence, for the time being, establishing angiosperm outgroups will continue to be critical to assessing character state polarities and homologies of the most important angiosperm features recorded at the beginning of this chapter. There is also conflict regarding which lineages constitute the earliest divergent angiosperms. Previous views focused on Magnoliaceae and its close relatives, but very recent analyses have indicated that the monotypic *Amborella* is sister to all other angiosperms (see full literature in Friedman et al. 2004) (Fig. 15.5) and that Nymphaeales is sister to all angiosperms exclusive of *Amborella*; further, Illiciaceae, Schizandraceae, Trimeniaceae and Austrobaileyaceae (Austrobaileyales) make up a clade that is sister to the remaining angiosperms.

The homologies of carpels, tetrasporangiate anthers, bitegmic ovules, three-celled male gametophytes, eight-nucleated female gametophyte, etc., which are believed to be synapomorphies of angiosperms, with those of nonflowering seed plants (i.e. gymnosperms), are totally unknown. It is generally assumed that the first flowers of angiosperms were hermaphroditic because the female flowers of *Amborella* produce staminodes, thus demonstrating a basically bisexual nature to its flower (it is also worth to indicate that unisexual flowers were common among the earliest angiosperm macrofossils). *Amborella* and many members of Austrobaileyales have helically arranged perianth lobes but not distinct sepals and petals which are characteristics of eudicots. Molecular genetics of floral organogenesis has provided some clues to the diversification of floral morphology; particularly the role of the homeotic transcription factors, the MADS-box genes (the MIKC type), is very significant in this connection. Also important is the role of floral organ identity genes (the ABC + DE genes; for details see Chap. 16 in this book), as their gene functions are mainly conserved across a breadth of taxonomic groups and hence can be used to identify widely conserved organ identity programmes (Friedman et al. 2004). It has been suggested that alterations in expression patterns of floral development genes gave rise to the angiosperm flower and all its variations. Investigations on the floral transcription factor *LEAFY* and the B-class MADS-box genes in gymnosperms, for example, have led to the proposal of the *mostly male theory* of floral origin (Frohlich 2003), which suggests that the male and female reproductive units were combined in the angiosperm flower by the emergence of ectopic ovules on microsporophylls in the originally male cones of a gymnosperm ancestor. Another theory of flower origin suggests a homeotic transformation of reproductive organ to either male or female from unisexual gymnosperm cones, resulting in the origin as an ancestral bisexual flower (Theissen et al. 2002; Theissen and Melzer 2007). In this hypothesis, changes in expression of the homologues to the B-class floral homeotic genes along the reproductive axis of the cone cause the required developmental transformation to form a flower.

Some botanists suggested that the angiosperms originated in the early Mesozoic or even the late Palaeozoic and that they underwent extensive diversification by the Aptian-Albian stages of the lower Cretaceous period. The best

available evidence from the fossil record indicates that the angiosperms originated during the early Cretaceous period around 130–135 million years ago. The ancestors of flowering plants diverged around 245–202 million years ago. These diversified enormously and got widely dispersed around 120 million years ago but replaced conifers as the dominant taxa only around 60–100 million years ago. Tricolpate pollen grains which are found in the more advanced dicotyledons are first reported from slightly younger Aptian rocks. Pollen sequences have clearly shown a pattern of increasing diversity in the Cretaceous. The progression of pollen from primitive types in older strata to more derived types in younger sediments indicates that angiosperms underwent much diversification during the Cretaceous (Raven and Axelrod 1974). Only a few records are available of dicotyledonous woods of the early Cretaceous age. They represent primitive types of xylem and lack of the features of advanced angiosperms. An investigation of fossil leaves reveals a pattern of increasing diversity during the Cretaceous period.

All available evidences lead to the conclusion that the angiosperms form one natural monophyletic branch of development (=*monophyletic*) and not *polyphyletic*. The presence of most characters listed in the introductory section of this chapter in all groups of angiosperms is a strong evidence for monophyletic origin. As Parkin (1923) had pointed out, independent origin of all these characters in different taxonomic groups of angiosperms is statistically very unlikely. Takhtajan (1969) is of the opinion that the orders Urticales, Casuarinales and Fagales so distinct at first sight from the Magnoliales are linked with the latter order by an intermediate group, the Hamamelidales. Similarly, Lemnaceae and Araceae distinct from one another are connected by the genus *Pistia*. One can cite numerous such examples. Defined broadly, the terms *monophyly* and *polyphyly* would differ in their meaning depending upon how far back in evolutionary history we go. If life arose once on earth, all organisms are ultimately monophyletic.

Simpson (1961) defined monophyly as the derivation of a taxon through one or more lineages from one immediately ancestral taxon of the same or lower rank. Such a definition would be true if say genus B evolved from genus A through one species of the latter, since in that case the genus would be monophyletic at the same rank (genus) as well as at the lower (species) rank. On the other hand, if genus B evolved from two species of genus A, it would be monophyletic at the genus level but polyphyletic at the lower rank. Most authors, however, adhere to a stricter interpretation of monophyly, namely, the group should have evolved from a single immediately ancestral species belonging to the group in question. There are thus two different levels of monophyly: a minimum monophyly (Melville 1983) wherein one supraspecific taxon is derived from another of equal rank (Simpson's definition) and a strict monophyly wherein one higher taxon is derived from single evolutionary species (Hennig 1966).

15.15 Model Angiosperms

With the advent of plant molecular biology and plant biotechnology during the last 20–25 years, a number of plants have been selected and developed as model organisms. Such plants that tend to have certain characteristics such as small body size, short generation time, small genome size, low levels of repetitive DNA, etc., have become popular choice as model plants for extensive work on genomics, comparative genomics, functional genomics and proteomics, phenomics and epigenomics. The model plants now commonly used across the globe are *Arabidopsis thaliana*, *Brassica napus*, *Oryza sativa*, *Zea mays*, *Nicotiana tabacum*, *Populus trichocarpa*, *Jatropha curcas*, *Ricinus communis*, *Hevea brazilensis*, *Manihot utilissima*, *Aquilegia coerulea*, *Phaseolus vulgaris*, *Cicer arietinum*, *Cajanus cajan*, *Medicago truncata*, *Capsella rubella*, *Lotus japonica*, *Glycine max*, *Amborella trichopoda*, *Vitis vinifera*, *Theobroma cacao*, *Malus domestica*, *Prunus amygdalus*, *Mimulus guttatus*, *Lemna gibba*, *Brachypodium distachyon*, *Linum usitatissimum*, etc.

15.16 Economic Importance

Most of the human diet is plant derived; in addition, a large fraction of raw materials for shelter, clothing and other life necessities of *Homo sapiens* is obtained from plant products. Humans use species of about 450 angiosperm families and in particular plants of 25 families: Anacardiaceae, Apiaceae, Arecaceae, Brassicaceae, Convolvulaceae, Cucurbitaceae, Dioscoreaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Lauraceae, Malvaceae, Meliaceae, Musaceae, Myrtaceae, Moraceae, Piperaceae, Poaceae, Rosaceae, Rubiaceae, Rutaceae, Solanaceae, Vitaceae and Zingiberaceae. Angiosperms provide man with food, fuel, fodder, shelter, clothing, drugs, beverages, paper, resins and gums and ornamental/fragrance plants. The most important are food plants, the cereal grasses (especially rice, wheat and corn), sugarcane, potatoes, tuberous vegetables and fruits. Vegetable fats, oils, waxes and masticatories are also of great value. Besides providing nourishment for man, fats and oils (fixed and essential) are used extensively in technology, medicine and the perfume industry. Several spices are supplied by angiospermous plants such as pepper, laurel, clove, cinnamon, vanilla and others. Plants containing stimulants are also important, especially tea, coffee and cacao. Likewise, plants containing narcotics are widely used in medicine (e.g. cocaine and morphine). Textile plants (e.g. cotton and flax) are very important for clothing. In addition, vegetable fibres serve a wide variety of purposes. Many trees yield useful wood, a number of which are of high commercial value. Chemicals of various kinds are also derived from various parts of plants, and more than 10,000 species of flowering plants are used in medicine. Ethnobotany deals with plants used by indigenous cultures all over the world.

The number of species used in industry is considerably less, but some of them, especially the tropical rubber trees, are exceptionally important. Angiosperm ornaments the land and provides the most natural aesthetic human environment. Gardening is the top leisure activity globally. Specific to gardening, there are so many botanic gardens all over the world. Plants have been used throughout human history not only as adornment for indoor and outdoor spaces of human habitation but also to alter microclimates for more comfortable habitation. For example, treelines and shrub borders have been used, particularly in the last millennium in Europe to provide windscreens for livestock and separation of pastures to secure livestock ownership. Landscaping has also been used for centuries as a method of microclimate amelioration for human habitation, wind protection, thermal buffering and atmospheric humidity modification. Plants have served as a source of interest and inspiration to humans particularly poets for millennia. Gardening for ornamental purposes and use of cut flowers for decoration have been noted as early as the Bronze Age by Egyptian, Cretan and Celtic cultures. Plants have been an important element of human art, with elements of plant architecture appearing as ornamentation for ceramics and other decorations in Neolithic and Bronze ages in many countries including China, Crete, Southern Africa, British Isles and Egypt and in the Mayan civilizations. For example, glyphs found in Middle Minoan pottery dating back to 1850 BC contain designs of olive, saffron, wheat, etc. In the history of art, plants played an important role as subjects in classical still-life paintings and may have reached a crescendo with obsessions during the eighteenth and nineteenth centuries (cited by Hogan and Taub 2011). Dendrochronology/tree ring dating deals with the scientific dating based on the analysis of patterns of tree rings in woods with applications in palaeoecology and archaeology (McGovern et al. 1995).

Due to space constraints, it is not possible to cover the unlimited information on the economic aspects of angiosperms.

References

Akeroyd J, Synge H (1992) Higher plant diversity. In: Groombridge B (ed) Global biodiversity – status of the earth's living resources. Chapman Hall, London, pp 64–87

Anderson E (1934) Origin of the angiosperms. Nature 133:462
APG II (Angiosperm Phylogeny Group II) (2003) An update of the Angiosperm Phylogeny group classification for the orders and families of flowering plants. APG II. Bot J Linn Soc 141:399–436
APG III (Angiosperm Phylogeny Group III) (2009) An update of the Angiosperm Phylogeny group classification for the orders and families of flowering plants. APG III. Bot J Linn Soc 161(2):399–436
Arber EAN, Parkin J (1907) On the origin of angiosperms. J Linn Soc Bot 38:29–80
Baum DA, Hileman LC (2006) A developmental genetic model for the origin of the flower. In: Ainsworth C (ed) Annual plant reviews, flowering and its manipulation, vol 20. Blackwell, Sheffield, pp 3–27, 304 pp
Becker A, Theissen G (2003) The major clades of MADS-box genes and their role in the development and evolution of flowering plants. Mol Phylogenet Evol 29:464–489
Bennett MD, Leitch I (2005) Genome size evolution in plants. In: Ryan Gregory T (ed) The evolution of the genome. Academic, Burlington, pp 90–112
Bible Bernad B (1976) Non-equivalence of left handed and right handed phyllotaxy in tomato and pepper. Hortic Sci 11:601–602
Burger WC (1981) Heresy revived: the monocot theory of angiosperm origin. Evol Theory 5:189–225
Campbell DH (1928) The phylogeny of angiosperms. Bull Torrey Bot Club 55:479–496
Chambers PA, Lacoul P, Murphy KJ, Thomaz SM (2008) Global diversity of aquatic macrophytes in freshwater. In: Balian EV, Lévêque C, Segers H, Martens K (eds) Freshwater animal diversity assessment. Springer, Dordrecht, Netherlands, pp 9–26
Christopher NT, Benfey PN (2012) Growth control of root architecture. In: Altman A, Hasegawa PM (eds) Plant biotechnology and agriculture:prospects for the 21st century. Academic, New York, pp 387–404
Corner EJH (1949) The Durian theory of the origin of the modern tree. Ann Bot 52:367–414
Cronquist A (1981) An integrated system of classification of flowering plants. Columbia University Press, New York
Darwin C (1865) The movements and habits of climbing plants. J Linn Soc (Bot) 9:1–118
Donoghue MJ, Doyle JA (2000) Seed plant phylogeny: demise of the anthophyte hypothesis? Curr Biol 10(3):R106–R109
Doyle J (1945) Developmental lines in pollination mechanism in the Coniferales. Sci Proc R Dublin Soc 24:43–62
Eichler AW (1875) Blüthendiagramme. W. Engelmann, Leipzig
Endress PK (1994) Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge
Engler A (1892) Syllabus der Pflanzenfamilien. Gebruder Borntraeger, Berlin
Engler A, Prantl K (1924) Die Naturlichen Pflanzenfamilien, 2nd ed. Duncker & Humblot, Leipzig
Fagerlind F (1947) Strobilus und Blute von *Gnetum* und die Moglichheit, aus ihrer struktur den Blutenbau der angiospermen zu deulen. Ark Bot 33A:1–57
Friedman WE (1994) The evolution of embryogeny in seed plants and the developmental origin and early history of endosperm. Am J Bot 81:1468–1486
Friedman WE, Moore RC, Purugganan MD (2004) The evolution of plant development. Am J Bot 91:1726–1741
Frohlich MW (2003) An evolutionary scenario for the origin of flowers. Nat Rev Genet 4:559–566
Groombridge B (ed) (1992) Global biodiversity- status of the earth's living resources. Chapman & Hall, London
Halle F, Oldeman RAA (1970) Essai sur L'architecture et la dynamique de croissance des arbres tropicaux. Masson, Paris
Hennig W (1966) Phylogenetic systematics. (Engl. Transl., D.D. Davis & R. Sangerl). University of Illinois press, Urbana
Hogan CM, Taub DR (2011) Introduction to plants. In: Cleveland CJ (ed) Encyclopedia of earth. Environmental Information Coalition, National Council for Science and the Environment, Washington, DC [First published in the Encyclopedia of Earth July 19, 2010; Last revised Date August 15, 2011; Retrieved September 27, 2012]
Hornby RJ (1992) Grasslands. In: Groombridge B (ed) Global biodiversity: status of the earth's living resources. Chapman & Hall, London, pp 280–292
Imaichi R, Hiratsuka R (2007) Evolution of shoot apical meristem structure in vascular plants with respect to plasmodesmatal network. Am J Bot 94:1911–1921
Jean RV (1994) Phyllotaxis: a systematic study of plant pattern morphogenesis. Cambridge University Press, Cambridge
Kihara H (1972) Right and left handedness in plants: a review. Seiken Ziho 23:1–37
Krishnamurthy KV (2003) Textbook of biodiversity. Science Publishers, Enfield, England
Krishnamurthy KV (2007) In pursuit of understanding the tension wood riddle. J Indian Bot Soc 86:14–26
Krishnamurthy KV (2015) Growth and development in plants. Scientific Publishers, Jodhpur
Leroy J-F (1983) The origin of angiosperms: an unrecognized ancestral dicotyledon, *Hedyosmum* (Chloranthales), with a strobiloid flower is living today. Taxonomy 32:169–175
Litschitz E, Eshed Y (2006) Universal florigenic signals triggered by *FT* homologues regulate growth and flowering cycles in perennial day-neutral tomato. J Exp Bot 57:3405–3414
Markgraf F (1930) Monographie der Gattung *Gnetum*. Bull Jard Bot Buitenzorg 10:253–310, Ser 3
McGovern PJ et al (1995) Dendrochronology. science in archaeology: a review. Am J Archeol 99(1):79–142
Meeuse ADJ (1961) The Pentoxylales and the origin of the monocotyledons. Koninkl Nederl Acad Van Wetenschappen Amsterdam C 64:543–559
Melville R (1969) Leaf venation patterns and the origin of angiosperms. Nature 224:121–125
Melville R (1983) Glossopteridae, Angiospermidae, and the evidence for angiosperm origin. Bull J Linn Soc 96:279–323

Myers N (1990) The biodiversity challenge: expanded hot spot analysis. Environment 10:243–256
Nair PKK (1979) The palynological basis for the triphyletic theory of angiosperms. Grana 18:141–144
Narsaiah G, Bahadur B (1984) Twining handedness in flowering plants. Indian J Bot 8:41–49
Oldeman RAA (1974) L'architecture de la foret guyanaise. Mem ORSTOM 73:1–204
Ornduff R (2004) Handedness in twining tracheophytes. In: Ramaswamy N, Reddy PN, Pullaiah T, Murthy GVS, Rao GP (eds) Gleanings in plant sciences. Regency Publications, New Delhi, pp 1–20
Palmer JD (1991) Plastid chromosomes: structure and evolution. In: Bogorad L, Vasil IK (eds) Cell culture and somatic cell genetics of plants, vol VIII A. Academic, San Diego, pp 5–53
Parkin J (1923) The strobilus theory of Angiosperm descent. Proc Linn Soc Lond 153:51–64
Prain D, Burkill IH (1936) An account of the genus *Dioscorea* in the East Part 1. The species which twine to the left. Ann Roy Bot Gdn Calcutta 14:1–210
Prain D, Burkill IH (1939) An account of the genus *Dioscorea* in the East Part 1. The species which twine to the right with addenda to part 1, summary. Ann Roy Bot Gdn Calcutta 14:211–528
Pullaiah T, Lakshminarayana K, Hanumantha Rao B (2008) Plant reproduction. Scientific Publishers, Jodhpur
Raunkiaer C (1934) The life-forms of plants and statistical plant geography. Clarendon Press, Oxford
Raven PH (1986) Evolution of plant life forms. In: Givnish TJ (ed) On the economy of plant form and function. Cambridge University Press, Cambridge, pp 421–492
Raven PH, Axelrod DI (1974) Angiosperm biogeography and past continental movements. Ann Mo Bot Gdn 61:539–673 (1981 republication)
Reinhardt D, Kuhlemeir C (2002) Plant architecture. EMBO Rep 3:846–851
Rendle AB (1904) The classification of flowering plants, 2nd ed. Cambridge University Press, Cambridge, England. (1930)
Retallack GJ, Dilcher DL (1981) Arguments for a glossopterid ancestry of angiosperms. Paleobiology 7:54–67
Saenger P, Hegerl EJ, Davis JDS (1983) Global status of mangrove ecosystems, Commission on ecology papers no. 3. IUCN, Gland
Simpson GG (1961) Principles of animal taxonomy. Columbia University Press, New York
Soltis PS, Soltis DE, Chase MW (1999) Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. Nature 402:402–404
Soltis DE, Soltis PS, Chase MW, Mort ME, Albach DC, Zanis M, Savolainen V, Hahn WJ, Hoot SB, Fay MF, Axtell M, Swensen SM, Prince LM, Kress WJ, Nixon KC, Farris JS (2000) Angiosperm phylogeny inferred from 18S rDNA, rbcL, and atpB sequences. Bot J Linn Soc 133:381–461
Soltis DE, Soltis PS, Albat VA, Oppenheimer DG, Depamphilis CW, Ma H, Frohlich MW, Theissen G (2002) Missing links: the genetic architecture of flowers and flower diversification. Trends Plant Sci 7:22–31
Stebbins GL (1974) Flowering plants: evolution above the species level. Harvard University Press, Cambridge, MA
Stewart WN, Rothwell GW (1993) Paleobotany and the evolution of plants, 2nd edn. Cambridge University Press, Cambridge, 521 pp
Stuessy TF (2004) A transitional-combinational theory for the origin of angiosperms. Taxonomy 53(1):3–16
Sun G, Zheng S, Dilcher DL, Wang Y, Mei S (2001) Early angiosperms and their associated plants from Western Liaoning, China. Scientific and Technological Education Publishing House, Shanghai, 227 pp
Swamy BGL (1973) Contributions to the monograph on *Gnetum* 1. Fertilization and proembryo. Phytomorphology 33:176–182
Swamy BGL (1974) Contributions to the monograph on *Gnetum* III. Some observations on microsporogenesis and germination of pollen. Phytomorphology 24:206–210
Takhtajan A (1969) Flowering plants origin and dispersal (trans: Jeffrey C). Oliver and Boyd, Edinburgh
Taylor DW, Hickey LJ (1992) Phylogenetic evidence for the herbaceous origin of angiosperms. Plant Syst Evol 180:137–156
Taylor DW, Hickey LJ (eds) (1996) Flowering plant origin, evolution, and phylogeny. Chapman and Hall, London, 403 pp
Taylor TN, Taylor EL, Krings M (2009) Palaeobotany the biology and evolution of fossil plants, 2nd edn. Academic, Amsterdam
Theissen G, Melzer R (2007) Molecular mechanisms underlying origin and diversification of the angiosperm flower. Ann Bot 100(3):1–17
Theissen G, Becker A, Winter KU, Muenster T, Kirchner C, Saedler H (2002) How the land plants learned their *floral* of BCs: the role of MADS-box genes in the evolutionary origin of flower. In: Cronick AC, Bateman RM, Hawkins JM (eds) Developmental genetics and plant evolution. Taylor & Francis, London, pp 173–206
Thomas HH (1936) Palaeobotany and the origin of Angiosperms. Bot Rev 2:397–418
Tomlinson PB (1980) Biology of trees native to tropical Florida. Published by the author, Petersham, Ma, USA
Tomlinson PB (1983) Tree architecture. Am Sci 71:141–149
Tomlinson PB (1986) The botany of mangroves. Cambridge University Press, New York
UNEP (1995) Global biodiversity assessment. Cambridge University Press, Cambridge
Vavilov N (1949–1950) The origin, variation, immunity and breeding of cultivated plants. Chronica Botanica. Waltham

von Wettstein R (1901) Handbuch der Systematischen Botanik. Gerbruder Borntraeger, Berlin

Wu Z-Y, Lu A-M, Tang Y-C, Chen Z-D, Li W-H (2002) Synopsis of a new "polyphyletic-polychronic-polytopic" system of the angiosperms. Acta Phytotaxonomica Sin 40:289–322

Zanis MJ, Soltis PS, Qiu Y-L, Zimmer E, Soltis DE (2003) Phylogenetic analyses and perianth evolution in basal angiosperms. Ann Mo Bot Gdn 90:129–150

Zhang HD (1995) Florology. Zhongshan University Press, Guangzhou, 234 p. (in Chinese)

Genetics of Flower Development

16

K.V. Krishnamurthy and Bir Bahadur

Abstract

The flower is a unique feature of flowering plants. Recent research on molecular biology has indicated that a flower is the result of expression and interplay of several genes operating in a sequence. At least four pathways trigger floral evocation: temperature pathway (vernalization and ambient pathways), light quality pathway, photoperiod pathway and gibberellin pathway. Production of different floral organs and the decision of boundary between them are controlled by several genes whose activity can be explained by ABC + DE model, as well as by the quartet model. This article also explains the genetic basis of all floral variations including floral symmetry. The genetic basis of termination of the floral meristem is also explained. Finally, the chapter discusses evolution of flower and floral organs from a genetic perspective.

Keywords

ABC + DE model • Floral evocation • Floral organ-identity genes • Flower • Flower evolution • Gibberellins • MADS-box genes • Photoperiod • Vernalization

16.1 Introduction

The flower is an exclusive feature of the angiosperms. Although the corresponding organs of Gnetales (of gymnosperms) are also called 'flowers', they are not true flowers. Detailed phylogenetic analyses based on morphological, anatomical and ontogenetic features have indicated that a flower is a modified vegetative shoot,

K.V. Krishnamurthy (✉)
Center for Pharmaceutics, Pharmacognosy and Pharmacology, School of Life Sciences, Institute of Trans-Disciplinary Health Science and Technology (IHST), Bangalore, Karnataka, India
e-mail: kvkbdu@yahoo.co.in

B. Bahadur
Sri Biotech Laboratories India Limited, Hyderabad, Telangana, India

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_16,

which is highly telescoped and with a determinate growth. This concept assumes that the floral organs are homologous to leaves and are appendicular on a cauline axis. The vascular system of the flower and its organs is grossly similar to that of a vegetative leafy axis, but specialized flowers have greatly modified vasculature due to various types of fusions (congenital or ontogenetic), cohesion and adnation between different floral parts and/or amplification or reduction of vasculature (Swamy and Krishnamurthy 1980). In some cases, phylogenetic abortion of floral parts is also the causative factor. However, there are others who have considered the angiosperm flower as axial/cauline in origin, based on a different set of phylogenetic analyses. As an example of this, we can cite the telome theory of Zimmermann (1952). Whatever may be the method phylogenetic origin of flower, the most characteristic feature of the flower (which is absent in the so-called flowers of Gnetales) is the carpel that fully encloses the ovule (the ovule is naked in gymnosperms).

Vegetative growth invariably culminates in production of the inflorescences or solitary flowers. During this process, there is a transformation of the vegetative shoot apical meristem (SAM) into a reproductive meristem, which may be an inflorescence or a floral meristem (FM) depending upon the plant species. Although what causes this transition is still not fully understood, sufficient advances have been made in our knowledge of floral initiation and flowering. In contemporary literature, the word 'flowering' denotes changes associated with formation of the floral or inflorescence primordium from a vegetative SAM, although it should be kept in mind that basic structural differences exist between the two. In the former, the SAM is transformed from an indeterminate to a determinate meristem, while in the latter, it continues to be indeterminate for some time and produces determinate floral meristems. Therefore, in this article, changes noticed in the floral meristem are treated separately wherever necessary from those noticed in the inflorescence meristem. However, the terms 'floral' and 'flowering' are used both in reference to individual flowers and of inflorescences.

Flowering is one of the most important 'decisions' made by plants during their development. Since they are sessile organisms, plants have to reproduce where they grow, and hence, to ensure their reproductive success, their flowering time has to be controlled under the most optimal and favourable environmental conditions. Besides this, the exact time of flowering is very critical for a number of other reasons. For instance, depending on their geographical location, plants must 'know' the season/time during which they can successfully complete seed set for their evolutionary survival. In outcrossing taxa, synchronized flowering of conspecifics is necessary to ensure pollen exchange for fertilization. In crop species, hastening or delaying flowering is vital for various reasons. Flowering time is also very important in apiculture and honey production. The control of flowering time has intrigued people for a very long time. Research aimed at understanding the multiple layers of control of flowering has been a very active area of study, at least in the last four decades (Amasino 2010; Salomé et al. 2011; Srikanth and Schmid 2011).

However, flowering is not an abrupt developmental decision of the plant, but it is the culmination of a cascade of closely related progressive developmental events such as the following: (1) acquisition of floral competence; (2) conversion of vegetative SAM into a floral apex; (3) initiation and differentiation of flower primordium; (4) differentiation and development of the different floral organs; (5) maturation of floral organs, especially of anthers and ovary/ovule and the male and female gametophytes contained in them; and (6) anthesis and opening of flowers and the receptivity for pollen receipt. All these events are controlled by genes, most of which have been identified through forward and reverse genetics, primarily in plants like *Arabidopsis thaliana* (Salomé et al. 2011).

16.2 Acquisition of Floral Competence

Acquisition of floral competence is often also referred to as *floral evocation*. The emergence of a floral primordium marks the expression of reproductive competence of the plant. Floral evocation not only involves physiological and molecular changes but also structural/anatomical changes in the concerned transitional vegetative SAM; some physiological and molecular changes also take place in the subjacent leaves, especially the flag leaf that is located below the flower/inflorescence. It is generally believed that a plant must attain certain amount of vegetative development, often known as *ripeness to flower*, before it can flower (Salisbury and Ross 2005). In fact, it has been believed by some investigators that there exists a packet of 'stem cells' in the embryonic SAM itself that is passively carried forward through the adult apex and then into the floral apex. Evident for this is shown by clonal analysis in maize plant, but most other investigators support the view that the vegetative SAM is determined florally only after producing a considerable number of leaves and nodes and internodes. A juvenile-to-adult transition is invariably a prerequisite to flowering especially in tree taxa. The probable unifying idea for the transition from vegetative to reproductive phase is that there should be a complete transformation in the activity of SAM from the one it had during vegetative phase of development.

16.2.1 Physiology of Floral Evocation

Many plants flower once the ripeness to flower is completed, without needing any further treatment. In such plants, the events associated with floral evocation often overlap with the final stages of vegetative maturation. However, in some species, floral evocation needs the presence of specific environmental cues, the most important of which are day length (i.e. *photoperiod*) and temperature (i.e. *thermoperiod*) (Kobayashi and Weigel 2007). In many perennial species, flowering is coordinated year after year by the more-or-less reliable seasonal fluctuations in photoperiod and thermoperiod cues.

16.2.1.1 Photoperiodic Control

Three major response groups of plants can often be recognized among photoperiodically sensitive plants: (1) *short-day plants* (*SDPs*) that flower only when exposed to light periods of less than a critical length in a 24-h light-dark cycle (since day length in excess of the threshold value will keep the SDPs in the vegetative state, these plants need to be appropriately called long-night plants (LNPs)); (2) *long-day plants* (*LDPs*) or short-night plants (SNPs) that flower only when the day length exceeds a certain threshold value; and (3) *day-neutral plants* (*DNPs*) that flower regardless of photoperiods, but only after a specific ripeness-to-flower phase. The last response group plants are far more in number than the other two groups (Salisbury and Ross 2005). Photoperiodic pathway of flowering is under genetic control. Single-gene mutants regulating flowering time have been isolated in a few plants such as *Arabidopsis*, maize, pea, sorghum, tobacco, etc. In pea plant, photoperiodic control of flowering is affected by genes such as *STERILE NODES* (*SN*), *DAY NEUTRAL* (*DNE*) and *PHOTOPERIODIC RESPONSE* (*PPD*), which are complementary to one another. Mutations in recessive alleles of any of these three genes result in an early-flowering day-neutral condition. In the facultative LDP *Arabidopsis*, more than 30 mutants that regulate photoperiodic effects and activate the flowering pathway integrators have been isolated. *FLOWERING LOCUS T* (*FT*), *LUMINIDEPENDENS* (*LD*) and MADS-box gene (MADS stands for first letters of *MCM1*, *AG*, *DEF* and *SRF* genes; *MCM1* in yeasts, *AG* and *DEF* in *Arabidopsis* and *SRF* in vertebrate animals) *SUPPRESSOR OF CONSTANS1* (*SOC1*) are the most studied genes (Putterill et al. 2004). The floral meristem-promoting effects of LDs are largely through the action of photoperiod-dependent regulator *CONSTANS* (*CO*), a transcription factor, whose action couples the circadian clock and the flowering pathway integrators *FT* and *SOC1*. The homologue of

FT in rice plant is *Hd3a*, while the homologue for the regulator of *FT*, i.e. *CO*, is *Hd1*. However, these act in distinctly different photoperiodic conditions. *HD1* activates flowering by activating *Hd3a* in SDs, and it delays the flowering by repressing *Hd3a* in LDs (Hayama et al. 2003). *SINGLE FLOWER TRUSS* (*SFT*) is a regulator of flowering time that encodes the tomato orthologue of *FT*. *SFT* interacts with the *SELF PRUNING* (*SP*) inhibitory function gene to regulate vegetative to reproductive transition in tomato, a day-neutral plant (Lifschitz and Eshed 2006). The *SP* gene is a homologue of *TFL1* and *CEN* and promotes the indeterminate state of the apical meristems. *CO* gene expression in *Arabidopsis* acts in a pathway that promotes the transcription of floral meristem-identity genes such as *LFY* and *TFL* (see a subsequent section of this article for more details on *LFY* and *TFL*) in response to an inductive LD treatment. *CO* and *LD* genes have already been cloned.

The main effect of a favourable light-dark cycle in the control of flowering is a change in the plant that will enable it to flower subsequently, even if there are unfavourable photoperiodic conditions at that time. This change in state is called *photoperiodic induction*. There is enough evidence to implicate the leaves in the sensing of day length, since masking of all leaves of a plant affects flowering. Exposure of even a single leaf to correct photoperiod is sufficient to induce flowering in many plants. It is generally believed that the photoperiodically induced leaves synthesize a floral stimulus that gets transmitted to the SAM and that this stimulating substance is synthesized at the influence of absolute length of dark period. This is evident from night-break experiments in which light is provided for short durations in the night. These experiments subsequently led to prove that *phytochromes* are involved in sensing photoperiods. Photoperiodic signalling pathways are mediated by both phytochromes A and B, as indicated by investigations made on phytochrome-deficient mutants of *Arabidopsis* such as *phytochrome B* (*phy B*), *phy A*, *phy C*, *pef 1* and *cryptochrome 2* (*cry2*). There are also several clock-associated genes such as *G1* and the downstream gene *CO*. Photoreceptors affecting the biological clock also affect flowering. Different photoreceptors are involved in different light conditions of both quality and irradiance. For instance, the blue light photoreceptors of the *ZTL/FKF1/LKP2* family have been shown to regulate the components of the clock and downstream genes acting in the photoperiod pathway (Boss et al. 2004).

Studies made on a late-flowering *indeterminate* (*id*) mutant of maize have suggested the presence of a gene involved in the production of this transmissible floral stimulus. This stimulus produced by the action of phytochromes in the leaves and transported to the SAM has been called *florigen* (Zeevaart 1976). All attempts so far made to chemically characterize/identify florigen have failed. Initially, florigen was considered as a hormone or a mixture of hormones, but it gradually got narrowed down to GA. A separate GA promotion pathway (probably related to photoperiod pathway) has been suggested, and this includes genes such as *GA1*, *GAI*, *RGA*, *FPF1* and *AtMYB33* involved either in GA biosynthesis or in signalling. Mutations that affect GA pathway are most inhibitory to flowering in plants grown under SDs. However, recent evidences suggest a link between GA and LD pathways. Mutations at the *EBS* locus cause early flowering in SDs and it represses *FT* expression. *SPY* gene which was a negative regulator of GA signalling is now found to interact with the LD pathway upstream of *CO* (Bernier and Périlleux 2005). The promotion of flowering due to application of GA, as well as the results of a study of GA-deficient mutant *ga1* (deficient in producing *ent*-kaurene, a precursor in GA biosynthesis) of *Arabidopsis*, provided strong evidence for the role of GA in flowering in several LDPs, but none of the SDPs under LD condition. The latter has been suggested as strong evidence against GA being the florigen, at least in SDPs. It has also been suggested by some investigators that inhibitory substances, instead of stimulatory florigen, produced in the leaves under noninductive conditions might be important in photoperiodic control of flowering and that this may even negate the promotive roles of favourable photoperiods. This '*inhibition hypothesis*' has been supported

by grafting experiments done, for example, in *Nicotiana*. Like the florigen, the chemical nature of the inhibitory substance is unknown. The inhibitory substance is called *antiflorigen*. Besides emphasizing that GA (especially GA_5) is unlikely to be universal florigen, Bernier and Périlleux (2005) have shown the role of sucrose, nitrate, glutamine and cytokinins in floral induction and flowering (e.g. in LD *Sinapis alba*).

16.2.1.2 Vernalization Control

It has been known for a long time that many annuals and biennials have a chilling requirement for flowering. This low-temperature treatment is commonly known as *vernalization*. It is believed that vernalization, like photoperiodism, exerts a specific effect on SAM during its transformation into floral meristem, although low temperature does not mimic photoperiodism. It is interesting to note that dividing cells are necessary for thermal induction and that the SAM is the main perceiving organ of the cue (Bernier and Périlleux 2005). It is also interesting to note that GA can substitute cold treatment since chilling increases the level of endogenous GA. Most of the work on the role of vernalization to flowering has been done on winter wheat, rye and *Hyoscyamus niger*. In the last species, subsequent to vernalization treatment, the plant must be maintained under LD conditions. Vernalization is believed to produce a stimulating substance called *vernalin*, and like florigen, the exact chemical nature of vernalin is yet to be deciphered. Vernalin is also considered to be a type of GA, but no solid conclusive evidence is available to date. Unlike in photoperiodism, there has not been much genetical studies on vernalization. The genes so far known act through repression of *FLC* gene, which is itself a repressor of flowering and which delays flowering in a rheostat-like manner. After the required amount of vernalization, *FLC* expression is abolished. *VIN3* and *MADS AFFECTING FLOWERING2* (*MAF2*) genes ensure that cold periods of insufficient duration will not cause flowering; the *VRN1* and *VRN2* genes are necessary for the maintenance of *FLS* repression after return to warm temperatures (Sung and Amasino 2004). *VRN1* gene is also apparently acting in an *FLC*-independent pathway to positively regulate the downstream gene *FT* (Boss et al. 2004).

16.2.1.3 Control by Other Environmental Cues

While photoperiodism and vernalization described above are classified as highly predictable or primary environmental factors participating in the control of flowering, there are other factors such as ambient temperature (perceived by all plant organs), irradiance (perceived by leaves/stem) and water availability (perceived by roots) which are considered as moderately predictable or secondary factors and mineral availability (perceived by roots), light quality (perceived by leaves/stem/SAM) and neighbouring vegetation (perceived by leaves/stem/*SAM*/roots) which are considered as unpredictable or tertiary factors (Bernier and Périlleux 2005). It should also be understood that the two primary factors mentioned above can substitute for each other and can also be replaced by secondary or tertiary factors. The promotion of flowering by a primary factor can also be reduced or even completely suppressed by another factor which may be another primary, secondary and tertiary factor (Bernier and Périlleux 2005). Some mutants of *Arabidopsis* (as well as a few other plants) that remained sensitive to both photoperiod and vernalization are classified as following 'autonomous' flowering mode. This autonomous mode includes several subsets of independent genes such as *FCA*/*FY*, *FVE*/*FPA*, *LD*/*FLD* and *FLK*. They promote flowering mainly through repression of *FLC*. While the vernalization and autonomous pathways work in unison to downregulate *FLC* expression, *FLC* is also positively regulated by *FRIGIDA* (*FRI*). Ambient temperature seems to control the expression of *PHY B* and *CRY2*. Light quality is obviously perceived by photoreceptors (*phy A* and *cry2*) involved in the *LD* pathway, which mediate the positive effects of far-red and blue wavelengths, respectively, on flowering; the *phy B* mutants exhibit a strong early flowering and are also temperature sensitive at 16 °C. Hence, *PHY B* is believed to act in light quality and 'ambient temperature' pathways by regulating

FT activity via an intermediate gene *PFT1*. *PHY B* is also believed to interact with the autonomous pathway since the early-flowering mutant of *phy B* requires *FCA* function.

16.2.2 Structural and Cytochemical Changes

It is now clear through studies employing diverse techniques like light and electron microscopy, cytochemistry and autoradiography that structural changes in the SAM are a major feature marking floral evocation (Nougarède 1967; Bernier 1988). These are supported by several biochemical studies.

During floral evocation, the SAM increases in diameter and becomes more conspicuous and often dome shaped. The tunica-corpus and the zonal organizations of SAM are changed into a mantle-core organization due to the redistribution of mitotic activity of the cells; the more-or-less layered mantle cells become more meristematic than the core cells, which look more parenchymatous (Bernier 1988). It is from the more rapidly cycling cells of the mantle region that floral organ primordia arise. The centrally located core has slowly cycling cells, and these are considered as a reservoir of cells that replenish the mantle, if need be. It is not clear whether the formation of the mantle-core organization is a cause for floral evocation or whether it is a part of the floral evocation event itself. Increased mitotic activity in the prospective mantle region of the meristem was observed to be synchronous with the arrival of the floral stimulus into it. In *Sinapis alba*, a plant that requires one LD cycle in about 18 h after floral evocation, for instance, there is a shortening of the *G2* phase of cycling cells; there is also a recruitment of nondividing *G2* cells into mitosis. Thus, a mosaic of rapidly cycling cells and relatively noncycling cells is resulted in the floral meristem. This is followed by a shortening of mitotic cycle duration of the rapidly cycling cells and an entry into *S* phase by some of the noncycling *G1* cells, thus causing a second wave of mitotic activity in about 62 h. This results in a synchronization of mitotic activity which is considered to be an important event in floral evocation. In *Silene coeli-rosa*, a taxon that requires at least seven LD cycles to flower, there is a shortening of *G1* and *G2* phases during photoinduction resulting in the accumulation of *G2* cells mainly in the mantle region of the meristem. This is followed by mitotic synchrony at 8–9 days under SD conditions. Here also, the net result is the synchronization of mitosis in mantle cells. However, in this species, there is a significant increase in the mitotic index in SAM as early as 0.5–1 h after induction began due to abbreviated *S* phases, which hastens the mantle cells through synthesis of DNA. An increase in DNA replication rate, thus, appears to be the earliest cytological event of photoinduction and floral induction in this species.

Cytochemical studies made on the transition apex indicate changes in RNA, protein and enzyme levels, and these have been implicated in floral evocation. Thus, transition to floral apex is associated with a general increase in macromolecular synthesis in the prospective floral apex. Some cytochemical studies combined with in situ hybridization have indicated a characteristic pattern of accumulation of gene transcripts for some ribosomal proteins like RPL2 and RPL 18 and a downregulation of arginine decarboxylase (ADC) at the distal end of the meristem, while cells at the base of the meristem showed an increased expression of transcripts.

There is also an increase in the amount of transcriptionally active dispersed chromatin in the nuclei of meristem cells, as revealed by ultrastructural studies made in *Sinapis alba* (Bernier et al. 1993). There is a restriction of RNA synthesis to rib meristem, and this indicates that the activation of cells in this region is one of the first events in floral evocation. This, in fact, either coincides with or just precedes the arrival of floral stimulus from the leaves to the meristem both in *S. alba* and *Pharbitis nil*. Perhaps, most of this RNA is mRNA. Many experiments involving the use of metabolic inhibitors have also shown the importance of RNA synthesis (perhaps mRNA) during floral evocation, emphasizing the importance of floral importance of mRNA, both in terms of its quantity and quality. Growing evidence is

there for the synthesis of and regulatory functions for microRNAs (miRNAs) during floral evocation and floral meristem specification. *ARGONAUTE 1* (*AGO1*), an essential factor in miRNA-mediated pathways, is required for *LFY* and *AP1* genes (Kidner and Martienssen 2005). Other studies indicate a role for a GA-regulated miRNA (mRNA159) in controlling floral initiation by regulating *LFY* transcript level and a role in floral organogenesis (Achard et al. 2004). Perhaps miRNA synthesis takes place after the arrival of the floral stimulus. *APETALA2* (*AP2*) and *Ap2*-like genes *TARGET OF EAT1* (*TOE1*) and *TOE2* (repressor genes) are potential targets for regulation by a group of miRNAs (especially miRNA172). The miRNA172 appears to regulate *AP2* (Chen 2004). The temporal upregulation of miRNA172 leads to temporal downregulation of *TOE1* and *TOE2* and thus relieves their repressive effects on floral meristem specification.

It is not yet clearly known whether the changing pattern of protein synthesis is important in photoperiodic floral induction, especially in the light of the fact that there is both the appearance of new proteins and disappearance of extant proteins. Although new RNA and proteins in vernalization-induced floral evocation have so far not been demonstrated, it is most likely that such an increase would be present. A comparison of mRNA populations from vegetative and floral tissues of tobacco plant showed the existence of a complex gene expression phenomenon during floral evocation in this plant also. Based on this study, it has been suggested that a few thousand genes might be required to specify floral evocation and development of floral organs.

Apical meristems of *S. alba* and tobacco harvested at different times after exposure to correct photoinductive cycles were used to make cDNA libraries. In *S. alba*, transcripts of three genes, *SaMADS-A* (necessary for floral meristem specification), *SaMADS-B* (essential for the differentiation of special cell types in the flower) and *SaMADS-D*, begin to accumulate rapidly and specifically in the apical meristem of photoinduced plants; *SaMADS-A* mRNA initially appears in the central part of the meristem, where the first signs of floral evocation are seen, as mentioned earlier. Thus, in floral evocation, expression of MADS-box genes could be the earliest molecular signal (see details in the next section).

16.2.3 Genetics of Floral Evocation

Molecular investigations have shown that floral evocation and control of floral meristem identity are regulated by floral meristem-identity genes, which are the earliest acting genes in reproductive development and which interact in complex regulatory networks with multiple feedback and feedforward loops (Kaufmann et al. 2010). The acquisition of floral identity by the meristem consists of a series of gene-controlled steps, and that mutation in one of the genes would cause arrest in the transition or deviation at the earliest point when the gene product is required. A detailed analysis of these genes has been made on plants such as *Arabidopsis thaliana*, *Antirrhinum majus* and a few others. This analysis has shown the operation of (1) chromatin regulators, (2) MADS-box genes that act as activators or repressors of floral meristem formation, (3) floral-identity genes and (4) integration and interaction of these genes in floral evocation and floral meristem specification.

The vegetative programme initiated in the embryo/seedling switches to an inflorescence or solitary flower programme. In *Arabidopsis* this programme transition can happen only if the wild *EMBRYONIC FLOWER2* (*EMF2*) gene, a chromatin regulator, is activated, while the mutant *emf 2* seedling bypasses the rosette vegetative phase to directly produce an inflorescence. It is likely that a similar mechanism might operate wherever flowers are produced directly from tissue culture explants/callus, although it is possible for the explant to have received sufficient photoperiodic induction before it is being used as an explant. In addition to *EMF2*, the other wild genes functioning as chromatin regulators such as *SPLAYED* (*SYD*), *TERMINAL FLOWER2* (*TFL2*), *atISWI*, *FIE* and *CURLY LEAF* (*CLF*) also inhibit floral initiation by acting as flowering repressors in the vegetative SAM (Wagner 2003). *EMF2* and *FIE* repress the expression, during

vegetative development, of genes that specify floral meristem like *LEAFY* (*LFY*) and *APETALA* 1 (*AP1*). Similarly, *SYD* acts as a repressor of *LFY-dependent* activity prior to floral transition. The MADS-box genes that activate the transition to flowering are *MAF5*, *XANTAL 1* (*XAL1*), *AGAMOUS LIKE* (*AGL*) *6*, *17*, *19*, *24*, *28*, *42*, *71*, *72* (with *AGL42*, *FYF*), etc., while those that repress are *FLC*, *AGL 15*, *18* (both together), *MADS AFFECTING FLOWERING 1–4* (*MAF1–4*), *SVP*, etc.

A detailed review of the genetical studies carried out on floral transition from vegetative status in grasses (Bommert et al. 2005) has revealed the operation of the following genes in this process: *BARREN INFLORESCENCE* 2 (*BIF2*), *BARREN STALK 1* (*BAI*) (in maize) and *LAX PANICLE* (*LAX*) (in rice). These control early developmental switches involved in the initiation of axillary meristems. *BIF2* is required for initiation and maintenance of all types of axillary meristems, while the other two to initiate inflorescence axillary meristems. After axillary meristems are induced by these genes, they acquire new identities. *FRIZZY PANICLE* (*FZP*) in rice and *BRANCHED SILKLESS 1* (*BD1*) in maize control meristem identity at the transition from SAM to FM (floral meristem). The other genes involved in meristem identity are the maize *REVERSED GERM ORIENTATION 1* (*RGO1*), *INDETERMINATE SPIKELET 1* (*IDS1*) and *INDETERMINATE FLORALAPEX 1* (*IFA1*).

Several genes are involved in conferring floral identity on the newly arising floral meristem in *Arabidopsis*; these include *LFY*, *AP2*, *AP1*, *CAULIFLOWER* (*CAL*) and *FRUITFUL* (*FUL*) (Lohmann and Weigel 2002). *LFY* is a key gene that is required not only for the formation of flowers on the inflorescence axis but also as an important integrator of flower-promoting pathways; *lfy* mutants are similar to wild plants throughout development but never produce complete flowers on the inflorescence and also have an increased number of secondary inflorescences. The *LFY* gene product is a factor that promotes the switching of SAM to a floral one. *LFY* encodes a sequence-specific DNA-binding transcription factor unique to the plant kingdom. The main role of *LFY* in the establishment of floral meristem is supported by the recent observation that auxin promotes *LFY* expression via the auxin-response factor *MONOPTEROS* (*MP*) (Yamaguchi et al. 2013). *AP1* and *LFY* also control the expression of genes involved in hormone pathways. *LFY* inhibits auxin biosynthesis and promotes auxin signalling in emerging floral meristems, while *AP1* regulates genes involved in GA metabolism and signal transduction. The *ap1* mutants produce flowers (with functional reproductive organs) with branched shoot-like features. The *cal* mutants are enhancers of *ap1*. The *ful* alleles, when combined with *ap1cal* double mutants, produced a nonflowering phenotype of continuously produced leafy shoots. While AP1, CAL and FUL proteins contain the DNA-binding MADS domain, AP2 protein contains AP2-DNA-binding domain.

The LFY mutant's homologue in *Antirrhinum* is *floricaula* (*flo*), and there is about 70 % identity between the predicted protein products of *LFY* and *FLO* genes. *ZEAFLO/LFY1* and *ZEAFLO/LFY2* (*ZFL1* and *ZFL2*) are the *LFY* homologues in maize, while *RFL* is its homologue, as well as of *FLO*, in rice plant. This idea of genes affecting the change from inflorescence meristem to floral meristem is also true in the case of *ap1*, *cal* and *usual floral organs* (*ufo*) mutants of *Arabidopsis*, the *squamosa* (*sqa*) and *fimbriata* (*fim*) mutants of *Antirrhinum majus* and the *falsiflora* (*fa*) and *anantha* (*an*) mutants of tomato. Certain MADS-domain transcription factors are also involved in maintaining the SAM by repressing floral initiation. An *Arabidopsis* MADS-box gene *SHORT VEGETATIVE PHASE* (*SVP*) functions as a repressor of the floral transitions; *svp* mutants flower earlier than the wild type, whereas *SVP*-ectopic over-expression dramatically delays floral transition (Hartmann et al. 2000; Vijayaraghavan et al. 2005) (Fig. 16.1). *SVP* is expressed through SAM of vegetative body and affects the activity of positive regulators of floral transition such as *AP1*, *CAL* and *SEP1* to *SEP4* (*SEPALLATA* series genes). The *Antirrhinum* homologue of *SVP* is *INCOMPOSITA* (*INCO*). Likewise, another *Arabidopsis* MADS-domain factor *AGL24*

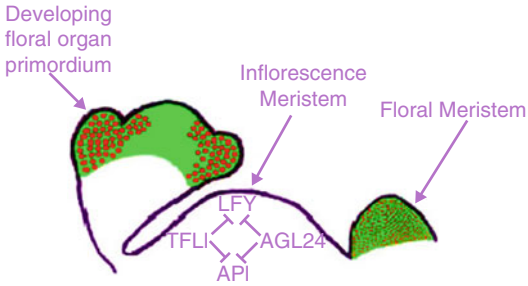


Fig. 16.1 Diagrammatic representation of young floral meristems on the flanks of the inflorescence meristem. *TFL1* and *AGL24* repress (denoted by T-bars) key floral meristem-identity genes *LFY* and *API* in the inflorescence meristem. Uniform accumulation of *LFY* and *API* transcripts in the young stage 2 floral meristem, to the right, is represented by uniform *green colour* with *red dots*. At stage 5 when floral organ primordia are being initiated, *API* expression (*red dots*) is restricted to the developing first whorl (sepal) and second whorl (petal) primordia, while *LFY* expression continues in all floral organ primordia (*green zone*) (Vijayaraghavan et al. 2005)

(closely related to *SVP*) represses floral meristem specification, since it promotes an inflorescence fate; it is expressed throughout the SAM and inflorescence meristem to a single layer. *LFY* and *AP1* repress *AGL24* (Yu et al. 2004). *FLOWERING LOCUS* C (*FLC*) is another floral repressor gene of the MADS-box category. It is repressed at the chromatin level and requires for it *HUA ENHANCER1-1* (*HEN1-1*). The closely related *SEP1*, *2*, *3* influence floral meristem identity, in addition to their main role as cofactors governing floral organ fate. This role is evident from occasional production of secondary flowers in the sepal axis of *sep1 sep2 sep3* triple mutants (Pelaz et al. 2000). Loss of floral meristem identity becomes more pronounced in quadruple mutants of various *sep* alleles combined with *ap1* mutants; for example, *sep1 sep2 sep4* triple mutant combined with *ap1* showed a cauliflower phenotype similar to *ap1cal* double mutant (Ditta et al. 2004). *SEP* genes are very diversified in the grass family; there are at least five *SEP* genes in rice and eight in maize (Bommert et al. 2005).

In the day-neutral tomato, several mutants are known. In these, inflorescence meristem forms sympodial vegetative shoots in which single flowers are separated or are replaced by leaves. These mutants include *falsiflora* (the tomato *LEAFY* gene), *jointless*, *blind*, *single flower truss*, *uniflora* or *macrocalyx* (Lifschitz and Eshed 2006).

We do not yet have a clearer idea about how the protein products of all the genes mentioned above control floral evocation and inflorescence/floral meristem identity at the cellular and molecular levels. Also complicated is our knowledge on how these genes are in turn controlled by genes that regulate phytochrome, florigen and vernalization. However, simple integration of all the involved factors, genes and pathways have been attempted (Fig. 16.2), although some favour separate schemes (Boss et al. 2004). Genes involved in photoperiodism (and phytochrome action), vernalization, floral hormones (especially GA) and inhibitors, if any, are called flowering pathway integrators (Vijayaraghavan et al. 2005). These integrators, especially *FT* and *SOC1*, function as positive regulators/promoters of floral meristem-identity genes, such as *LFY*, *AP1* and *CAL*, whose redundant activities in turn specify the floral meristem. *SOC1* also regulates *AGL24*, a promoter of inflorescence fate. *SOC1* also represses the precocious expression of floral homeotic *B*-, *C*- and *E*-class genes (see later) in inflorescence meristems and early floral meristems in a redundant manner with *AGL24* and *SVP*, respectively. LDs promote floral evocation through their effects on the photoperiod-dependent regulator *CONSTANS* (*CO*), a transcription factor, which stabilizes the CO protein (Valverde et al. 2004). Photoperiod perception and the *CO*-dependent transcriptional upregulation of *FT* occur in the leaves. Hence, a translocation of this information to the SAM is necessary to effect a change in the identity of the emerging lateral meristems. This is achieved, at least to some extent, by the movement of the FT RNA to the SAM perhaps along with other signals (Huang et al. 2005).

Critical studies made on tomato have revealed that the floral-promoting *SFT* signals are graft transmissible and complement all developmental defects of *sft* mutant plants. These also revealed that the *SFT* generates universal florigenic signals, i.e. the graft-transmissible signals generated in tomato can substitute for SD stimulus (e.g. in

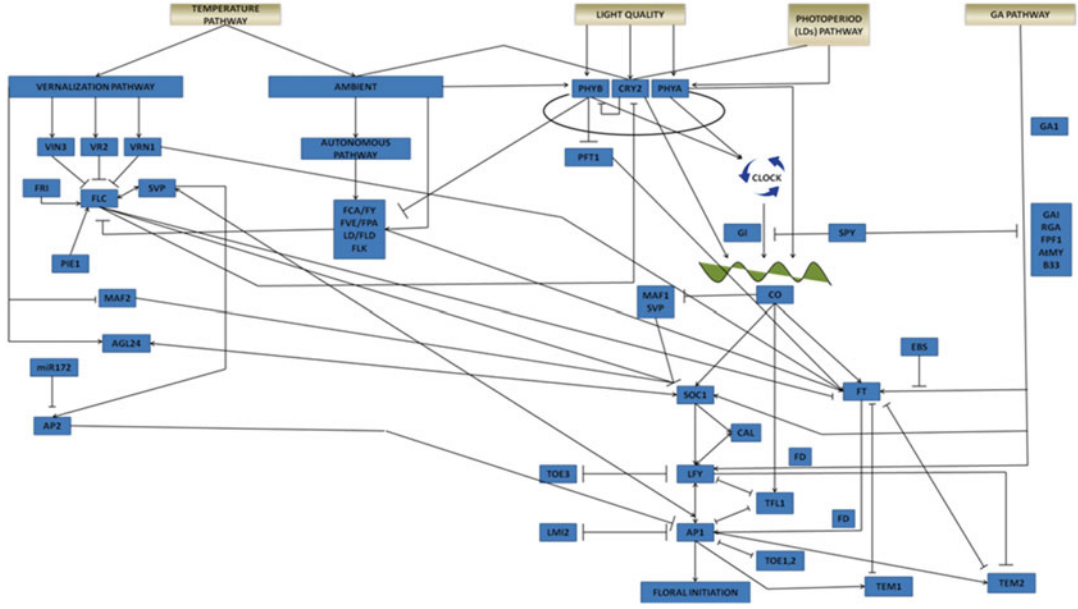


Fig. 16.2 An integrated model of the role of different genes and their interactions involved in floral initiation. The major pathways that involve these genes are also shown. → activators, ——I repressors (Based partly on Bernier and Périlleux 2005)

Maryland mammoth tobacco) and the systemic *SFT* signals can substitute for the LD stimulus in *Arabidopsis*. Lifschitz and Eshed (2006) have also dissected out the molecular components of the florigen pathway in tomato and *Arabidopsis*. Their yeast-two-hybrid screens uncovered four different SP-interacting proteins (SIPs): a NIMA-like protein kinase (SPAK) involved in cell division, 14-3-3 adaptor proteins, a bZIP G-box (SPGB) factor and an SP-specific interactor, SIP4. SPAK also interacts with the 14-3-3s which, in turn, also interact with SPGB and SIP4. *SP* and 14-3-3 share a SPAK-interacting site. With the exception of SIP4, other SIPs interact also with *TFL1*, *CEN* and *FT*. Abe et al. (2005) and Wigge et al. (2005) showed that one *Arabidopsis* homologue of SPGB is encoded by the late-flowering gene *FD* (in fact two FD-like genes have been identified) and that *FD* is partially required for the proper function of *FT*. Research data on *Arabidopsis* also suggested that to induce flowering, *FT* primary products must travel from leaves to SAMs (Abe et al. 2005) and this is most likely to be a leaf-induced FT-RNA, which may thus function as a florigenic signal (Corbesier and Coupland 2006). In tomato, the genes encoding the SPGB and 14-3-3 SP-interacting proteins are expressed in all leaves and all through development, thus marking it unnecessary for *SFT* RNA to travel. Accumulation of transcripts for flowering pathway integrators, and thus floral meristem-identity genes, a prerequisite for floral meristem specification and initiation, also requires the repression of *FLC* (a MADS-box gene). *FLC* interacts with another MADS-box gene (SVP) (Li et al. 2008), both of which jointly repress FT. The post-transcriptional regulation of FLC expression occurs through FCA, a nucleoprotein containing two RNA recognition motifs, an RNA-binding domain and a WW protein interaction domain, and FY, a WD-repeat protein. A link between the miRNA-driven post-transcriptional gene regulation and the flowering pathway integrators is suggested by the observation that at least one miRNA precursor gene MIR172-a2 is upregulated and the target *AP2*-like gene is downregulated after floral induction in a manner that is dependent on *CO* and *FT*.

Phytochrome-mediated and hormone signal transduction pathways, in addition to acting

through flowering pathway integrators, also control floral evocation and floral meristem establishment by regulating the activity of floral meristem-identity genes. These are evident through studies on *ap2*, *ap1*, *lfy* and *ag* mutants. Flowers of *ap2* or *ap1* mutant plants grown in SD showed inflorescence-like morphology, which is caused, at least partly, by *SPY* gene activity (Vijayaraghavan et al. 2005). This inflorescence-like morphology is suppressed by exogenous supply by GAs, indicating that floral meristem-determining factors are responsive to environmental and endogenous cues. Phytochromes and GAs affect maintenance of floral meristems once established, as evident from a study of *ag* and *lfy* mutant flowers in SDs (Vijayaraghavan 2001). A link between GA and phytochrome signal transduction and floral genes like *LFY*, *AP1*, *AP2* and *AG* has been shown by this study.

16.2.4 An Integrated Model for Floral Initiation

Bernier and Périlleux (2005) have analysed the work on the identification of the elusive 'florigen' involved in floral initiation in wild-type *Arabidopsis* in LD condition and concluded that it could be formed of both long-distance (sucrose and cytokinin) and short-distance signalling molecules (most components produced by genetic machinery except *CO* and *FT*). The former, as well as *FT*, move from leaves to SAM, while the latter are produced in the SAM itself and near it. Whether GAs act as long-distance signal or as short-distance signal or as both is yet to be resolved with certainly. These authors have proposed a model for floral initiation in *Arabidopsis*. They consider that sucrose is the most important factor and is considered to stimulate a number of cellular and molecular events in the SAM, after its hydrolysis through local invertases; cytokinins activate this hydrolytic activity, and together with products of sucrose hydrolysis, they increase the rate of cell division. Hexoses along with GAs participate in the upregulation of *LFY* expression, while *AP1* is activated by *FT*, which is itself positively regulated by *CO*. Activation of *SOC1* in the SAM might be due to other signals, possibly a cytokinin or GA. Although the model is not complete, the authors of this model consider it as a step closer to the identification of the elusive multifactorial 'florigen' at least in *Arabidopsis*. All recent data on the complexity of floral initiation are summarized and reviewed by Posé et al. (2012), Ó'Maoiléidigh et al. (2014) and Riechmann and Wellmer (2010, 2014).

16.3 Formation of Floral Organs

16.3.1 Categories of Floral Organs and Their Origin

A typical flower has four compressed whorls of floral organs: sepals, petals, stamens and carpels. In some flowers these organs are not present in whorls but in a spiral. Sepals and petals are sterile, while stamens and carpels are fertile organs of the flower. All these four categories of organs are borne on a receptacle, which is also known as thalamus or torus. The receptacle is considered by many as the axial region of the modified SAM and possesses nodes and internodes which are highly telescoped between the above four appendicular floral organs. The aggregation of sepals make up the calyx, that of the petals the corolla, of stamens the androecium and of carpels the gynoecium (often also called pistil). The stamens represent the male reproductive unit and produce the microgametophyte and male gametes, while the carpels represent the female reproductive unit and produce the macrogametophyte and egg. When the floral organs are arranged in whorls, members of successive whorls normally alternate in position with those of whorls directly above and below. If they are spirally arranged on the receptacle, the 'genetic spiral' is usually the same as that of the leaves.

All the floral whorls of a flower are generally produced in an acropetal succession by the floral meristem, with the carpel being the last to be produced; their production is precisely defined/determined according to the blueprint specific for each species. In some species, especially those that have an inferior ovary and a thalamus

overarching fully the ovary (e.g. in many Rosaceae and Rubiaceae), it is basipetal with carpels arising first and sepals the last. Some botanists use the word 'centripetal' instead of 'acropetal', thereby implying that initiation of floral whorls begins at the periphery of the flower and proceeds inwards and 'centrifugal for 'basipetal' wave (proceeding from the centre of the flower to the periphery). This differential direction of wave of floral organ differentiation has great significance in the sequence of expression of the genes that control the different organs (see later). In flowers possessing a large number of appendages of any one morphological category of floral organs, say, many stamens, these two types of waves of differentiation may be seen even within the whorls of the same category. However, these directional waves of differentiation are not always maintained in flowers of some taxa, posing problems to explain them on genetic grounds. For example, the development of stamens conforms to centripetal direction in taxa of families like Annonaceae, Magnoliaceae and Myrtaceae, while it is reverse in taxa of other families like Cactaceae, Hypericaceae and Malvaceae. It should, however, be emphasized here that the differential temporal factor in the differentiation of a floral whorl is only a transitory phase that is confined to the earlier stages of ontogeny and that the order of maturation of a floral whorl need not necessarily synchronize with that of its initiation (Swamy and Krishnamurthy 1980).

There is also great variation in the number of kinds of parts in a flower and the number of organs of a kind in a whorl. In male flowers carpels are absent and vice versa; in some both sepals and petals are absent or are represented only as perianth (made of tepals without distinction into sepals and petals). There is also great variation in the extent of fusion of organs of the same whorl or of different whorls. Much variation is also seen in the elaboration of organs, both in size and form. These variations have resulted in an almost endless variety of flower types, some simple, others complex; some considered as primitive, others as advanced; and some symmetric and others asymmetric and zygomorphic (Endress 1996). From an anatomical perspective, all floral organs are essentially derived from the activity of mantle cells. Invariably the first three mantle layers (as counted from periphery) L1, L2 and L3 are involved in organ differentiation. These three layers, respectively, give rise to the epidermis, outer layers and inner layers of the different floral organ primordia. The only part of the flower that has been reported to have an exclusive origin from a single meristem layer is the stylar part of the gynoecium, i.e. from the L2 layer. This is evident from a study of periclinal chimeras. However, the determinative decision that controls cells of these three layers to proceed to form specific floral organs is not rigidly fixed, at least in some taxa. Many recent investigators feel that the genesis of floral organs should not be based on cell lineage concept but on the position of individual cells or cell layers on the floral meristem. This is commensurate with our present understanding on the origin of leaves on SAM.

16.3.2 Interaction Between Floral Organs During Development

Although the four whorls of floral organs arise in specific sequence from the floral meristem both temporally and spatially, they do influence each other's differentiation on the floral meristem, suggesting a close interaction between them. In the cultured floral meristem of *Aquilegia formosa*, even under the most favourable culture media tried, the sepals suppressed the differentiation of the subsequently differentiating floral whorls. However, on surgical removal of sepal primordia from these cultured floral meristem, the meristem proceeded with normal growth and differentiation. Perhaps, some inhibitory substance(s) produced by the sepals inhibits the growth of the other floral organs. However, it is not clear as to how such inhibitory substances from sepal are overcome in the developing flower of an intact plant. Quite contrary results were obtained in the cultured floral meristem of tobacco plant. Here, the existing floral organ primordia promoted the differentiation of the next whorl of floral organs; hence, removal of the

existing sepal primordia arrested the differentiation of petals and suppression of petal primordia inhibited the differentiation of stamen primordia and so on. These observations on cultured tobacco floral meristem are considered to favour gene activation based on the operon model in such a way that inducing substances released by earlier-formed floral organs might possibly govern the developmental decisions involved in the formation of succeeding floral organs. However, data obtained from studies on *Arabidopsis* indicate that during floral organ ontogeny, inductive influences from adjacent whorls are not possibly required for appropriate floral organogenesis, but positional information bequeathed by the organ primordia are most likely to be involved in the development of floral organs. An experiment involving genetic knockout of petals and stamens in the flower of transgenic *Arabidopsis* and tobacco in which two floral organs were not surgically removed but were knocked out by ablation with the *DIPHTHERIA TOXINA* (*DTA*) gene fused to the promoter of a petal- and stamen-specific *APS* gene showed that sepal and carpel formation proceeded normally in the absence of these two whorls.

16.3.3 Genetic Control of Floral Organs

16.3.3.1 Control of Floral Organ Identity

The major question in the differentiation of floral organs is how the cells of the mantle region of the floral meristem faithfully establish their location in order to subsequently develop into the characteristic floral organs made up of the appropriate cell types; this is particularly very important for the stamens and gynoecium. Once the floral meristem has been specified, *AP1* and *LFY* activate floral organ-identity genes, which specify the four different types of floral organs. These floral organ-identity genes were discovered through the study of mutants of *Arabidopsis*.

Only in the last three decades or so, floral organ differentiation began to be studied by generating mutants that interfere with the ordered development of the concerned floral organ on the floral meristem. Since such mutations resulted in certain organs or a series of organs substituted by other organs not normally found in that position on the floral receptacle, they are called *homeotic* transformations, implying that what is newly formed does not confirm to what is being replaced. Thus, mutant flowers exhibit the subtle effects of floral organ-identity genes whose protein products are essential for the regular and patternized formation of organs on the floral meristem. The most important floral homeotic mutants of *Arabidopsis* and their phenotypes are shown in Table 16.1. On the basis of a critical

Table 16.1 Most important floral homeotic mutants of *Arabidopsis* (Krishnamurthy 2015)

Serial no.	Name of mutant	Phenotypic expression
1.	*Agamous* (*ag*)	An outer whorl of four sepals, two inner whorls of 10 petals (5 + 5) and a variable number of intermediate organs
2.	*Apetala1* (*ap1*)	Sepals transformed into bracts and flowers are formed in the axils of the bract-like organs
3.	*Apetala2* (*ap2*)	A gynoecium-like outer whorl, second and third whorls of stamens and a fourth whorl of carpels
4.	*Apetala3* (*ap3*)	The second whorl organs are transformed into sepals, the third into carpelloid stamens or into normal carpels
5.	*Floral* (*flo*)	Carpels are absent in the fourth whorl or are replaced by stamens or by stamen-carpel intermediate organs
6.	*Leunig* (*lug*)	Sepals are petaloid, staminoid or frequently carpelloid; petals with stamen characteristics; reduced number of stamens and carpels
7.	*Pistillata* (*pi*)	The second whorl of organs is transformed into sepals, the third is absent or carpel-like and the fourth is of carpels of abnormal size
8.	*Unusual floral organ* (*ufo*)	The outer whorl of sepals is followed by second and third whorls of sepals, petals, stamens or carpels; the third whorl carpels are fused with those of the fourth whorl

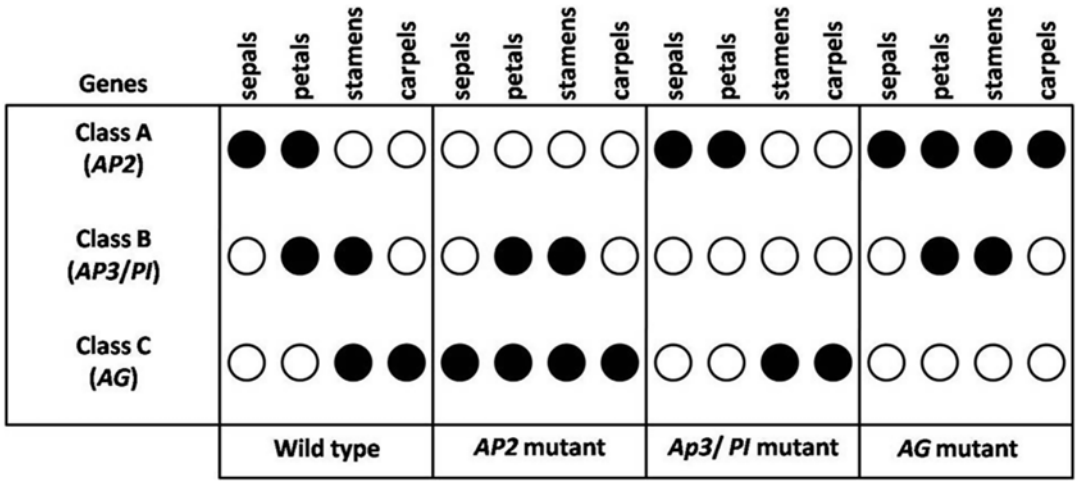


Fig. 16.3 Diagrammatic representation of the operations of the genes in wild type and three homeotic mutants of Arabidopsis. The genes active in each floral whorl are indicated by the *darkened circles. Hollow circles* indicate that the particular gene is absent in respective floral organs (Diagram based on Dr. Raghavan (2000))

analysis of the these phenotypes, a canonical working model called *ABC model* was developed (Coen and Meyerowitz 1991) in order to explain how the four whorls of floral organs are correctly specified (Fig. 16.3). This model is followed even today (Bowman et al. 2012); it invokes three major classes of homeotic genes, respectively, called *A*, *B* and *C*, each of which affects two different whorls on the floral meristem. The class-*A* genes (*AP1*, *AP2* and possibly *LUG*) affect the calyx (first) and corolla (second) whorls, class-*B* genes (*AP3* and *PISTILLATA* or *PI*) affect the corolla (second) and androecial (third) whorls and class-*C* genes (*AG*) affect the androecial (third) and carpel (fourth) whorls. Another important postulate of *ABC* model is that some genes control independently the floral organs in the first and fourth whorls, while a combination of gene products at the second and third floral whorls, respectively, determine the identity of the corolla and androecium. *A* and *C* genes, for example, act alone and, respectively, control sepal and carpel development in the first and fourth whorls, *B* controls petal development in combination with gene *A* in the second whorl and *C* controls the development of androecial whorl in combination with gene *B* in the third whorl. This model is very plastic in the sense that the products of *A*- and *C*-class genes cross-regulate each other in a mutually antagonistic way. This means that any region of the floral meristem where *AP2* gene expresses itself will not have the expression of *AGAMOUS* or *AG* gene, i.e. in the first and second whorls. Similarly, when *AG* gene expresses itself in the fourth whorl, *AP2* gene will not express in the regions of third and fourth whorls. But, when the activity of *A*-class gene is knocked off by a mutation in the *AP2* (i.e. in *ap2* mutants), the activity *C*-class genes will be abnormally high in the first and second whorls. Likewise, if the activity of *C*-class genes is removed by a mutated *AG* gene, then there is excessive activity of *A* genes in the third and fourth whorls. Thus, as per *ABC* model, it is easy to understand why an absence of *A*, *B* and *C* activities results in mutant phenotypes, respectively, represented by *ap2*, *ap3*/*pi* and *ag* (Fig. 16.3). The functioning of this model is confirmed in the double mutants *ap3*/*ap2* and *pi*/*ap2* in which *A* and *B* classes of genes are knocked out, and only activity of *C*-class gene is evident in all the four floral whorls, i.e. flowers consisting entirely of carpels. Similarly, when both *B* and *C* classes of genes are eliminated, for example, in *ag*/*pi* or *ag*/*ap3* double mutants, the flowers contain only sepals specified by *A*-class genes. Triple

mutants like *ap2*/*ag*/*pi* have 'flowers' with only leaf-like appendages since the activities of all the three classes of genes are absent (Raghavan 2000). Almost all genes required for the *ABC* functions (except *AP2*) are MADS-box genes that encode putative transcription factors.

Studies involving in situ hybridization techniques have also contributed to our understanding of floral organ-identity genes. Such studies have shown that *AG* mRNA is expressed almost exclusively in the stamen and carpel loci of the floral meristem. Similarly, the expression of *AP3* and *PT* mRNAs is restricted to petal and stamen domains. The fact that *AG* gene transcripts are present in all four floral organ primordia of an *ap2* mutant shows that *AP2* gene products function to negatively regulate *AG* gene activity (Dinh et al. 2012). In *Arabidopsis AP2* transcripts are regulated post-transcriptionally by miRNAs (Wollmann et al. 2010) to coordinate the specification of perianth versus reproductive organs; in *Petunia* and *Antirrhinum* a miRNA169/NF-YA module has a primary role in restricting the expression of C-class genes to the inner floral whorls.

Transgene technology has also added evidences to the *ABC* model. If the *AG* gene is overexpressed in *Arabidopsis* under the control of the CaMV255 promoter, the resultant effect is the swapping of the *AP2* gene products with high levels of *AG* gene products; the resultant floral phenotype on the transgenic plant is closely similar to that of the *ap2* mutant. This is direct evidence that *AG* gene functions to inhibit *Ap2* function. In the flower of transgenic *Arabidopsis*, the effective impact of the ectopically expressed *Ap3* gene is evident through the partial conversion of carpels to stamens with sepals being unaffected. On the contrary, when plants expressing both *Ap3* and *PI* are produced by crossing progenies harbouring chimeric constructs of the genes, flowers with two outer whorls of petals and two inner whorls of stamens are produced. Here, it is the expression of a combination of organ-identity genes that provides a strong evidence for the interaction of class-*B* genes with class-*A* and class-*C* genes in specifying petals and stamens, as predicted by the model.

Some refinements to the *ABC* model have been made by Ma (1997) (see Zahn et al 2005). The *ABC* model has been updated to include the *D* class ovule-specific genes and the *E*-class genes which express themselves in the three inner floral whorls and form quaternary protein complexes with the other floral homeotic genes needed for the correct organ identity (Fig. 16.4). *E*-function is stated to specify each of the four types of floral organs (Ditta et al. 2004). The *D* class genes are believed to have arisen through an angiosperm-specific duplication of an ancestral class-*C* gene. These studies have shown that although *ABC* genes are required, they are not sufficient to specify floral organ identity and hence required *D*- and *E*-functions also. In *Arabidopsis D*-function requires *SHATTERPROOF 1* or *2* (*SHP1*, *SHP2*, formerly *AGL1* and *AGL5*) and *SEEDSTICK* (*STK*, formerly *AGL11*), and *E*-function requires one of the three functionally redundant genes, *SEPALLATA1*, *2*, *3* (*SEP1*, *2*, *3* formerly *AGL2*, *AGL4*, *AGL9*) that are co-expressed with the *PI*, *AP3* and *AG* genes in the petals, stamens, carpel and ovules. Yet another *SEP* gene, *SEP4* (formerly *AGL3*), has been found (Ditta et al. 2004). At least one *SEP* class gene is required to superimpose sepal identity on vegetative leaf identity.

It is not very clear whether the *ABC* model, which was first described in eudicots, is applicable to lower dicots such as Ranunculaceae, Nymphaeaceae and Magnoliaceae where all floral organs, particularly perianth lobes and stamens, are produced in a spiral and not in whorls. Sharp gene expression boundaries exist during floral specification in eudicots (Fig. 16.5), but the same appears not to be true for at least some basal angiosperms (Theissen and Melzer 2007), where, for instance, the expression of the B-class genes is found outside of the petal and stamen domains. This spatially expanded B-class gene expression results in flowers with gradual transition between outer to inner tepals, inner tepals to stamens and stamens to carpels as well as in the production of intermediate organs (Soltis et al. 2005). These observations have led to the proposal of the *sliding* or *fading boundary hypothesis* which emphasizes that the underlying factor in floral diversity is

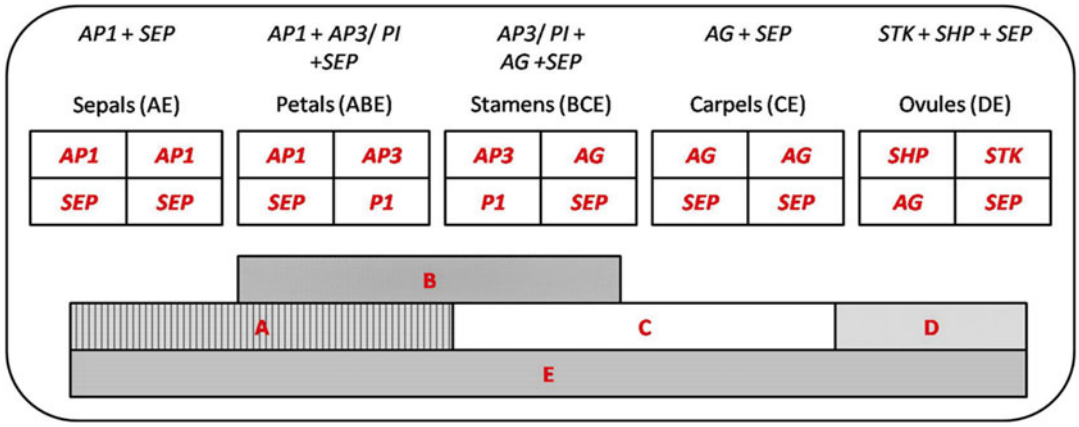


Fig. 16.4 ABC + DE model below and quartet model above. A-function genes (such as *AP1* of *Arabidopsis*) are necessary for the formation of sepals, B-function genes (*AP3* and *PI* in *Arabidopsis*) along with A-function genes are necessary for the formation of petals, B-function genes along with C-function genes (*AG* in *Arabidopsis*) are necessary for the formation of stamens and C- function genes alone are necessary for the formation of carpels. D-function (in *Arabidopsis STK* and *SHP1* and *SHP2*) and E-function (at least one of the four *SEP* genes) genes are necessary for the ovules and the whorls of the flower, respectively. In the quartet model (above, based on the ABC + DE model, used data from protein interaction). Here, the hypothesized quartets (*rectangles*), respectively, necessary for the different floral organs are shown (Based on Zahn et al. 2005)

change in the expression domains of floral homeotic genes which are expressed strongly at the centre and weakly at the edges of these expression domains (Bowman 1997). This hypothesis further proposes that the phenotypically identical sepals and petals (i.e. tepals making up the perianth whorl(s) of the monocot tulips) both express B-class orthologues, thus indicating that they might have evolved through a shift in the expression patterns of *B*-class genes to include the first whorl in addition to the second and third whorls. However, this is not true with the expression patterns of *B*-class genes in the tepals of many Liliaceae (including *Asparagus*), as well as in primitive eudicots such as Ranunculaceae and Magnoliaceae; this observation is against the sliding boundary hypothesis for all taxa which do not show a distinction between sepals and petals. In eudicots several genetic pathways promote the formation of sharp organ boundaries and of spatially well-defined expression domains of key floral regulators. Floral organ-identity factors seem to play a decisive role in this process. Another factor that contributes to the sharp boundary between organs in *Arabidopsis* might be the formation of feedback loops upon floral organ-identity gene activation (Theissen and Melzer 2007). This type of regulation would lead to amplification of small differences, resulting in a switch-like 'on-off' behaviour of gene expression. *B*- and *C*-function regulators in *Arabidopsis* have been shown to boost their own expression (Wuest et al. 2012). Moreover, they directly regulate several other genes that are required to maintain floral organ boundaries such as *RBE* and *SUP*, which themselves control floral organ-identity gene expression (Wuest et al. 2012). These mechanisms, either alone or together, could be important to the transition between the putative sliding boundaries found in flowers of some primitive angiosperms and the sharp boundaries seen in eudicots (Theissen and Melzer 2007).

Homeotic mutations in *Antirrhinum majus* and a few other angiosperms taxa have also been studied. In *Antirrhinum* these include *deficiens* (*def*), *globosa* (*glo*) and *sepaloidea* (*sep*) which have altered first three whorls of the flower to result in sepals, sepals and carpels instead of the normal sepals, petals and stamens, respectively. These mutants are phenotypically similar to *ap3* and *pi* mutants of *Arabidopsis*, and thus, their

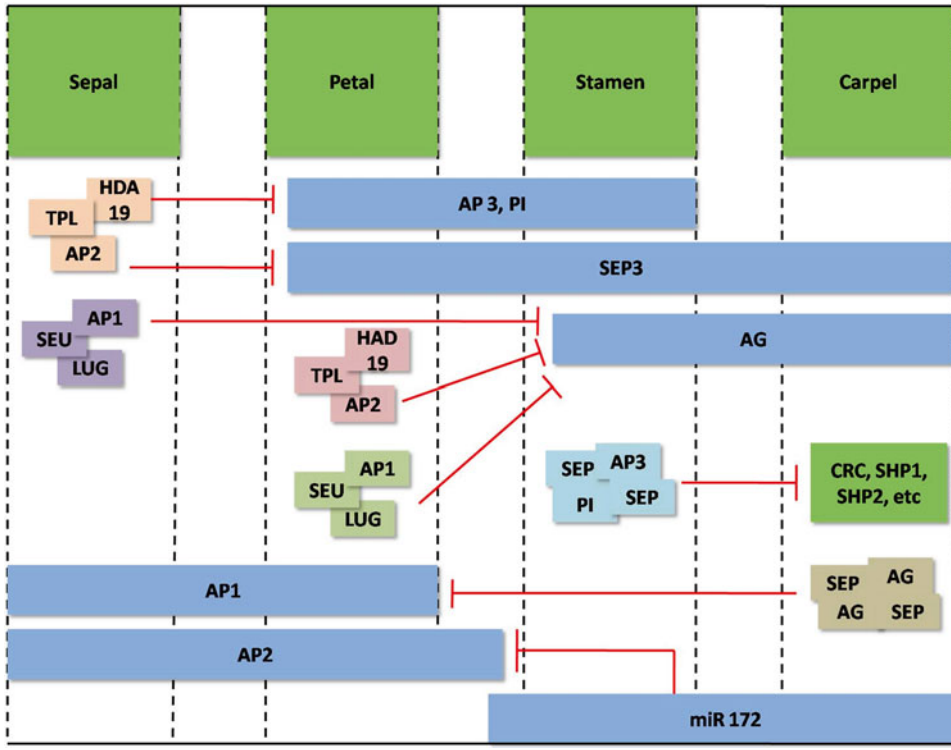


Fig. 16.5 Diagram showing floral organ boundary maintenance by the A-, B-, C- and E-function protein products. A-function proteins AP1 and AP2 promote first and secondary whorl organ fate. AP1 interacts with SEUSS (*SEU*) and LEUNIG (*LUG*), while AP2 forms complex with TOPLESS (*TPL*) and HISTONE DEACYLASE 19 (*HAD 19*). AP1 and AP2 repress C-function in the first two whorls. AP1 and AP2 also suppress the expression of B-class homeotic regulators AP3 and PI, as well as E-class SEP3 in sepals. AP3 and PI transcriptionally suppress the carpel-specific CRABS CLAW (CRC), SHP1 and SHP2 in the third whorl; AG suppresses (directly or indirectly) AP1 expression in the third and fourth whorls. miR172 suppress Ap2 mRNA accumulation in the liner two whorls. Organ boundaries (between sepals, petals, stamens and carpels) are shown by *vertical dotted lines*, ⊤ represents repression, while *squares* represent proteins (Diagram based on Ó'Maoileidigh et al. 2014)

genes belong to the *B* class that affects the second and third floral whorls (Zahn et al. 2005). The *plena* (*ple*) mutant of *Antirrhinum* is similar to the *ag* mutant of *Arabidopsis* and expresses itself in the third and fourth whorls of the flower; thus, its genes belong to class *C*. The mutation called *ovualata* (*ovu*) is semidominant and affects the first and second whorls of the flower, and thus the flowers are with carpels in place of sepals and stamens in place of petals; thus, it is similar to *ap2* mutant and belongs to class-*A* genes. The mutant *squamosa* (*sqa*) of *Antirrhinum* is orthologous to *ap1* mutant (A class) of *Arabidopsis*. Thus, ABC model is likely to be applicable to *Antirrhinum* also. This is substantiated by studies on the localization of clonal genes. In the wild-type flower, *DEF* and *GLO* gene transcripts are expressed in those very organs that are homeotic, i.e. in petals and stamens (however, in some flowering plants, the expression of these two transcripts are occasionally observed in whorls 1 and 4 or in non-floral organs). *PLE* transcripts get expressed in the third and fourth whorls of the floral meristem of wild-type flowers and thus are equivalent to *AG* gene of *Arabidopsis*. However, in *ovu* mutants, *PLE* transcripts are no longer restricted to inner two whorls but are also ectopically expressed in the outer two whorls. Hence, sex organs are formed in place of sterile floral organs in these *ovu* mutants.

An *AG*-like gene has been cloned from *Brassica napus*. This is expressed in transgenic

tobacco and converts the sepals into carpels and petals into stamens; hence, it produces *ap2* mutant-like phenotypes. Similarly, in transgenic tomato plants that express antisense RNA of tomato AG gene, the flowers have petaloid organs in place of stamens and pseudocarpels in place of carpels; this again supports the fact that the crucial function of *AG* gene is to control normal stamen and carpel development. On the contrary, when sense RNA is expressed in the transgenic plant, flowers with fruitlike tissues in the place of sepals and staminodes in place of petals are formed. In *Petunia hybrida*, a potential *B*-class gene called *FLORAL BINDING PROTEIN* (*FBP*) expresses itself only in the petals and stamens of wild-type flowers. If a chimeric *FBP* gene is introduced into transgenic *P. hybrida* in the sense orientation to cause a defect in class-*B* gene function, the flowers, as expected, showed sepals in place of petals and stamens in place of carpels. This plant also has spontaneously occurring green petal (*gp*) mutants in which petals are replaced by sepals, but, unlike in *ap2* mutant of *Arabidopsis*, a simultaneous replacement of stamens by carpels does not occur. In another of its spontaneously occurring mutant *blind* (*bl*), petals are partially converted into stamen tissue and sepals into carpelloid tissue. A gene sharing a sequence homologue to the *Antirrhinum DEF* gene has been found in *Petunia hybrida*, and this, under transgenic condition, restores to some extent the wild-type phenotype in the *gp* mutant. This shows that the inserted gene is vital for petal development in *Petunia*. That this transgene is involved in petal determination was verified by its ectopic expression in the wild-type *Petunia* that resulted in sepals in the second whorl, as in the mutant. Likewise, an ectopic expression of another *Petunia* gene, which is homologous to *AG* of *Arabidopsis* and *PLE* of *Antirrhinum*, leads to the recapitulation of the *bl* mutant genotype in transgenic *Petunia*. Thus, the *AG-DEF* homologue of *Petunia* is a class-*C* gene. There are also *AG*-like gene in tobacco and *DEF*-like genes in potato, which also get expressed in stamens and carpels, as expected of the *AG* gene, or in the petals and stamens, as expected of *DEF* gene.

The application of *ABC* model to grasses has been discussed in Bommert et al. (2005), Ciaffi et al. (2011) and Yoshida and Nagato (2011). In this discussion, the authors have referred to the regions where lodicules, stamens and a pistil develop as whorls 2, 3 and 4, respectively. Because the homology of petal and lemma is controversial, the authors have avoided defining whorl 1. ABC class MADS-box genes have been isolated from several grass species such as rice, maize and barley by homology cloning. The *B*-class genes in grasses are SI1 (*SILKY1*) (maize) and *SPW1* (*SUPERWOMAN 1*) (rice) (acts on whorls 2 and 3) which encode AP3-like proteins of *Arabidopsis. WPI1* of wheat is orthologous to *PI*, while in rice *OsMAD2* (which expresses in three inner whorls) and *OsMADS4* (which expresses in whorls 2 and 3) are orthologous to PI. *OsMADS3* expresses in whorl 3 strongly to produce stamens, while its expression in whorl 4 to produce carpel needs to be elucidated. In contrast to class-*B* genes, the function of class-*C* genes seems to have diversified in grasses. Class-*C* genes of grasses should be orthologous to *AG* genes of *Arabidopsis* and should negatively regulate the expression of class-A genes. Maize has two class-C genes, *ZAG1* and *ZMM2*, which are closely related to each other and act on whorls 3 and 4. *DROOPING LEAF* (*DL*) gene of rice specifies the carpel, and in the mutant *dl*, the carpels are replaced by stamens homeotically and completely. *DL* encodes for a *YABBY* protein, and this is the first finding that a *YABBY* gene controls organ specification in the flower, similar to the MADS-box genes. DL is most closely related to the *CRABS CLAW* (*CRC*) gene from the *Arabidopsis YABBY* gene family. *DL* orthologenes are also present in maize and barley. *DL* and *SPW1* antagonistically regulate each other's expression, so also *DL* and *OsMADS4*. *FT* interacts with bZIP transcription factor *FD* in yeast. Photoperiodic induction occurs in the leaves and activates *CO* that stimulates *FT* expression; this expression is not detected in SAM, but only in the vascular tissue suggesting that the *FT* mRNA or protein or both move to the SAM where *FT* interacts with *FD* to upregulate *SOC1* within hours of induction. Later *FD*/*FT* act redundantly

with *LFY* to activate *AP1* (or according to some directly *AP1*). FT is a small protein of 23 KDa and thus can move through plasmodesmata from its place of production to place of action.

Another molecular model that explains the floral organ identity is the *floral quartet model* (Figs. 16.4 and 16.5). This advances the genetic *ABC* model by integrating floral MADS-box genes and the molecular data demonstrating interaction between floral MADS-domain proteins (Zahn et al. 2005). According to this model, MADS-domain proteins form specific heteromeric complexes of different proteins for each floral organ (Theissen and Saedler 2001). This model is supported by observation that ectopic expression of *AP1*, *AP3*, *PI* and *SEP* and *AG*, *AP3*, *PI* and *SEP* proteins results in homeotic conversion of leaves into petals or stamens, respectively. It is believed that these quaternary complexes of MADS-box genes may be involved in activating or repressing target genes by binding to their promoters (Theissen and Saedler 2001; Zahn et al. 2005).

It is not clear how a limited number of transcription factors interact to control floral organ development. It is believed that a sequence-specific binding of the transcription factors to DNA might lead to transcription of genes in each floral whorl. Because of the close overlapping in the activities of the floral organ-identity genes, the molecular basis of floral organ development appears to be more complicated than evident from the above-mentioned simpler statements (see Raghavan 2000). We also do not have much information as to how the transcription factors select the genes for binding, or whether these genes make the specific protein products for each of the specific floral organs or they make only some intermediate products of great value in organ determination. We still have a long way to go to completely understand the genetic basis of floral organ identity and formation.

16.3.3.2 Genetics of Floral Organ Number

There is very little data regarding genes whose expression controls the correct number of units of each organ to be initiated on the floral meristem. Mutation in *CLV*, *PERIANTHIA* (*PAN*), *WIG* and *FLORAL ORGAN NUMBER* (*FON*) genes of *Arabidopsis* (also the rice *FON1* gene) causes an increase in the number of units present in some floral whorls, while mutation in *REVOLUTA* (*REV*), *SUPERMAN* (*SUP*) and *TOUSLED* (*TSL*) genes leads to reduced organ number in whorls either due to the formation of incomplete/aborted structures or due to the occasional loss of floral organs. In *fasciata* (*fas*) mutants, there are fewer petals and stamens, but more sepals in their respective whorl than the wild-type flowers (carpel number in not changed). A *CLV*-like signal pathway (present in *Arabidopsis*) is likely to operate in maize also where supernumerary lateral floral organs have been reported (Bommert et al. 2005), since the maize *FASCIATED EAR2* (*FEA2*) gene encodes a *CLV2* homologue. *FEA2*, like *CLV2*, acts to restrict stem cell population, and *fea2* mutants produce additional floral organs. The same authors have also recorded that *THICK TASSEL DWARF1* (*TD1*) gene causes additional floral organs in maize. Is a change in floral organ number correlated with the size of the floral meristem? While it is true for *clv* and *wig* mutants of *Arabidopsis* and of *fea2* mutants of maize, *pan* mutants go through ontogenetic changes during development, independent of changes in size, cell number or cell pattern of the floral meristem. From the above evidence, it may be concluded that the *PAN* gene can be considered to act directly in the process by which cells assess their position within the floral meristem and probably it is the gene that controls floral organ number.

16.3.3.3 Genetics of Floral Symmetry

As already indicated, flowers of many taxa are radially symmetric and actinomorphic (e.g. *Arabidopsis thaliana*). There are also taxa whose flowers are not radially symmetric, but are zygomorphic. The latter categories of taxa are well suited to study the genetic control of floral symmetry. For example, *Antirrhinum majus* has been used for this purpose. Mutants that affect floral symmetry in this taxon have been screened to see variations from the dorsiventral symmetry (but not radial) that its wild-type flowers have. In this

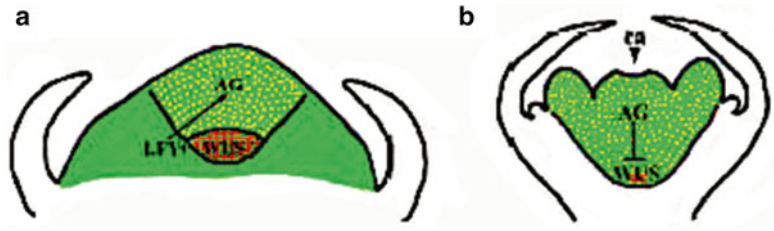


Fig. 16.6 The *WUS-AG* feedback loop controls floral meristem determinacy. (**a**) In stage 3 floral meristem WUS (denoted by the *red* hatched area) enhances LFY-mediated expression of *AG* (denoted by *yellow dots*). (**b**) Enhanced *AG* expression in stage 6 flowers at the time of carpel (*ca*) initiation terminates stem cell activity by repressing *WUS* expression (Vijayaraghavan et al. 2005)

flower, the asymmetry is due to the unequally sized floral organs, especially of the dorsal and lateral petals, as well as due to the number of fertile stamens. The mutants such as *cyc* have radially symmetric flowers and also lack the genetic functions associated with domains of the lateral and dorsal petals and stamens. This is evident by the RNA expression pattern of *CYC* gene in wild-type flowers, whose transcripts are restricted to the above-mentioned domains. Thus, *CYC* gene promotes the larger size of dorsal petals and retards dorsal stamen growth, and this confuses the mechanism of action of *CYC* in determining dorsiventrality of the flower. It is believed that *CYC* acts in unison with *DICHOTOMA* (*DICH*) gene to result in dorsiventrally symmetrical flowers.

16.4 Termination of Floral Meristem

It was mentioned earlier that both the vegetative SAM and floral meristem have a population of 'stem cells' that serve as 'founder cells' for the various floral primordia. It was also mentioned that unlike the inflorescence meristem, the floral meristem terminates once all floral organs have been initiated. The termination of the floral meristem is brought about by *WUS-AG* feedback loop (Lenhard et al. 2001; Lohman et al. 2001). There are interactions between *LFY*, *WUS* and *AG* genes in the core region of the floral axis, and these provide a mechanism to explain the differential effects of stem cell regulation in SAM versus floral meristem. The induction of *AG* and *WUS* is dependent on *LFY*, thereby meaning that *LFY* actually regulates 'stem cells' in the floral meristem (Fig. 16.6a). The *AG* protein thus formed represses *WUS* transcription and terminates the floral meristem (Fig. 16.6b). The *WUS-AG* feedback loop seen in floral axis is different from the *WUS-CLV3* feedback loop seen in SAM as the former controls the determinate native, while the latter, by acting on the central mother cell zone, controls the indeterminate nature of SAM (Laux 2003; Vijayaraghavan et al. 2005).

16.5 Evolution of Flower and Floral Organs: A Genetic Perspective

Angiosperms represent the most recently evolved land plant group, but their origin and early evolutionary history are comparatively poorly known for various reasons than the other vascular plant groups. The most important reason is the total uncertainty about the identity of the closest seed plant relative to the flowering plants. Recent phylogenetic studies carried out through the use of molecular data have revealed that extant gymnosperms may be a sister group to angiosperms and that establishing angiosperm outgroups based on characteristic polarities and homologies of angiosperms features like closed carpel, tetrasporangiate anthers and the second ovular integument (in many angiosperms) is still very critical. These studies have indicated Ranales as the earliest angiosperm group with monotypic *Amborella*

trichopoda probably sister to all angiosperms and Nymphaeales sister to all remaining flowering plants. Hence, reconstruction of the morphology of the earlier (ancestral) flower has often been done with comparisons among these most extant primitive angiosperm lineages. It is often assumed that a hypothetical ancestral flower should be hermaphrodite without any sepal-petal dichotomy (i.e. possessing only perianth) in the spirally arranged floral leafy whorls. There is also a general view that sepals, petals and perianth might have had separate evolutionary origins.

Attempts have been made recently to usher in molecular genetic information into the phylogenetic derivation of the flower, as this information have been believed to provide some vital clues to the diversification of floral morphology. Such studies invoke the role of homeotic transcription factors, the MADS-BOX genes, but it is the floral-identity genes (*ABC* model) that have received greater attention in evolutionary studies (as they receive in developmental studies), since their functions are mainly conserved across vast taxonomic groups and thus can be used to identify widely conserved organ identity programmes (Friedman et al. 2004). The occurrence of *B*-class mutants in monocots, particularly in orchids where the complex perianth is patterned by the differential expression of multiple B-class gene paralogues (Mondragon-Palomino and Theissen 2011) and in the 'inside-out' flowers of *Lacandonia schismatica*, where centrally located stamens are surrounded by carpels (Alvarez Buylla et al. 2010) may be cited as examples of conserved presence of the floral-identity genes in angiosperms. Genetic studies are consistent with not only the dependent origins of petals but also with the independent origins of sepals, petals and tepals. It has been suggested that the expression patterns of MADS-BOX and other floral genes got altered in order to result in the angiosperm flower and its further evolution. For example, there are varied expression profiles for *LFY* homologues in diverse species. Also, recent studies of the protein from many plant species elucidate how there are changes in the conserved DNA-binding domain, over evolutionary time. Both these could contribute to its likely diverse functions (Maizel et al. 2005). The functional characterization of members of the *CLAVATA* signalling pathway and *LEAFY* homologues implies that fundamental mechanisms such as regulation of floral meristem size, flowering and inflorescence development are conserved between dicot and monocot species (Bommert et al. 2005). Similarly, expression studies of *LEAFY HULL STERILE1* (*LHS1*), a rice MADS-box gene, have indicated that this gene is conserved in many grasses. Both *B*- and *C*-class orthologues of floral-identity genes are found in gymnosperms, with C-class genes expressed in both male and female reproductive structures and *B*-class genes expressed in male reproductive tissues (see Bowman et al. 2012 for full literature). Thus, the specification of stamens and carpels seems to have its origin in the common ancestor of seed plants. None of the *ABC* genes appears to exist outside seed plants, suggesting that their origin lies in extensive gene duplication of an ancestral MADS-box gene in the lineage leading to seed plants (Floyd and Bowman 2007). Investigations on LFY and B-class MADS-BOX genes in gymnosperms have led to the proposal of the mostly male theory of floral origin (Frohlich 2003), which states that the male and female reproductive units were combined in the angiosperm flower by the emergence of ectopic ovules on a set of microsporophylls in original unisexual cones of a gymnosperm ancestor. Another hypothesis for the origin of flowers has suggested a homeotic transformation of reproductive organs to either male or female unisexual gymnosperm cones, leading to the evolution of an ancestral hermaphrodite flower (Theissen et al. 2002). Further studies are needed on floral genes of angiosperms and their gymnosperm homologues to test either of the above two hypotheses.

References

Abe M, Kobayashi Y, Yamamoto S, Yamaguchi A, Daimon Y, Ichinoki H, Ikeda Y, Notaguchi M, Goto K, Araki T (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science 309:1052–1056

Achard P, Herr A, Baulcombe DC, Harberd NP (2004) Modulation of floral development by gibberellins-regulated micro-RNA. Development 131:3357–3365

Alvarez-Buylla ER, Ambrose BA, Flores-Sandoval E, Vergara-Silva F, Englund M, Garay-Arroyo A, Garcia-Ponce B, de la Torre-Barcena E, Espinosa-Matias S, Martinez E et al (2010) B-function expression in the flower center underlies the homeotic phenotype of *Lacandonia schismatica* (Triuridaceae). Plant Cell 22:3543–3559

Amasino R (2010) Seasonal and developmental timing of flowering. Plant J 61:1001–1013

Bernier G (1988) The control of floral evocation and morphogenesis. Annu Rev Plant Physiol Plant Mol Biol 39:175–219

Bernier G, Périlleux C (2005) A physiological overview of the genetics of flowering time control. Plant Biotechnol J 3:3–16

Bernier G, Havelange A, Houssa C, Petitijean A, Lejeune P (1993) Physiological signals that induce flowering. Plant Cell 5:1147–1155

Bommert P, Satoh-Nagasawa N, Jackson D, Hirano H-Y (2005) Genetics and evolution of inflorescence and flower development in grasses. Plant Cell Physiol 46:69–78

Boss PK, Bastow RM, Mylne JS, Dean C (2004) Multiple pathways in the decision to flower: enabling, promoting, and resetting. Plant Cell 16:518–531

Bowman JL (1997) Evolutionary conservation of angiosperm flower development at the molecular and genetic levels. J Biosci 22:515–527

Bowman JL, Smyth DR, Meyerowitz EM (2012) The ABC model of flower development: then and now. Development 139:4095–4098

Chen X (2004) A microRNA as a translational repressor of *APETALA2* in *Arabidopsis* flower development. Science 303:2022–2025

Ciaffi M, Paolacci AR, Tanzarella OA, Porceddu E (2011) Molecular aspects of flower development in grasses. Sex Plant Reprod 24:247–282

Coen ES, Meyerowitz EM (1991) The war of the whorls: genetic interactions controlling flower development. Nature 353:31–37

Corbesier L, Coupland G (2006) The quest for florigen: a review of recent progress. J Exp Bot 57:3395–3403

Dinh TT, Girke T, Liu X, Yant L, Schmid M, Chen X (2012) The floral homeotic protein *APETALA* 2 recognizes and acts through an AT-rich sequence element. Development 139:1978–1986

Ditta G, Pinyopich A, Robles P, Pelaz S, Yanofsky MF (2004) The *SEP4* gene of *Arabidopsis thaliana* functions in floral organ and meristem identity. Curr Biol 14:1935–1940

Endress PK (1996) Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge

Floyd SK, Bowman JL (2007) The ancestral developmental tool-kit of land plants. Int J Plant Sci 168:1–35

Friedman WE, Moore RC, Purgganan MD (2004) The evolution of plant development. Am J Bot 91:1726–1741

Frohlich MW (2003) An evolutionary scenario for the origin of flowers. Nat Rev Genet 4:559–566

Hartmann U, Hohmann S, Nettesheim K, Wisman E, Saedler H, Huijser P (2000) Molecular cloning of *SUP*, a negative regulator of the floral meristem in *Arabidopsis*. Plant J 21:351–360

Hayama R, Yokoi S, Tamaki S, Yano M, Shimamoto K (2003) Adaptation of photoperiodic control pathways produces short-day flowering in rice. Nature 422:719–722

Huang T, Bohlenius H, Eriksson S, Parcy F, Nilsson O (2005) The mRNA of *Arabidopsis* gene FT moves from leaf to shoot apex and induces flowering. Science 309:1694–1696

Kaufmann K, Pajoro A, Angenet GC (2010) Regulation of transcription in plants: mechanism controlling developmental switches. Nat Rev Genet 11:830–842

Kidner CA, Martienssen RA (2005) The role of *ARGONAUTE1* (*AGO1*) in meristem formation and identity. Dev Biol 280:504–517

Kobayashi Y, Weigel D (2007) Move on up, it's time for change: mobile signals controlling photoperiod-dependent flowering. Genes Dev 21:2371–2384

Krishnamurthy KV (2015) Growth and development in plants. Scientific Publishers, Jodhpur

Laux T (2003) The stem cell concept in plants: a matter of debate. Cell 113:281–283

Lenhard M, Bohnert A, Jurgens G, Laux T (2001) Termination of stem cell maintenance in *Arabidopsis* floral meristems by interactions between *WUSCHEL* and *AGAMOUS*. Cell 105:805–814

Li D, Liu C, Shen L, Wu Y, Chen H, Robertson M, Helliwell CA, Ito T, Meyerowitz E, Yu H (2008) A repressor complex governs the integration of flowering signals in *Arabidopsis*. Dev Cell 15:110–120

Lifschitz E, Eshed Y (2006) Universal florigenic signals triggered by FT homologues regulate growth and flowering cycles in perennial day-neutral tomato. J Exp Bot 57:3405–3414

Lohman JU, Hong RL, Hobe H, Busch MA, Parcy F, Simon R, Weigel DA (2001) Molecular link between stem cell regulation and floral patterning in *Arabidopsis*. Cell 105:793–803

Lohmann JU, Weigel D (2002) Building beauty: the genetic control of floral patterning. Dev Cell 2:135–142

Ma H (1997) To be, not to be, a flower–control of floral meristem identity. Trends Genet 14:26–32

Maizel A, Busch MA, Tanahashi T, Perkovic J, Kato M, Hasebe M, Weigel D (2005) The floral regulator *LEAFY* evolves by substitutions in the DNA binding domain. Science 308:260–263

Mondragon-Palomino M, Theissen G (2011) Conserved differential expression of paralogous *Deficiens*- and *Globosa*-like MADS-box genes in the flowers of Orchidaceae refining the 'orchid code'. Plant J 66:1008–1018

Nougarède A (1967) Experimental cytology of the shoot apical cells during vegetative growth and flowering. Int Rev Cytol 21:203–351

Ó'Maoiléidigh DS, Graciet E, Wellmer F (2014) Gene networks controlling *Arabidopsis thaliana* flower development. New Phytol 201:16–30

Pelaz S, Ditta GS, Baumann E, Wisman E, Yanofsky MF (2000) B and C floral organ identity functions require *SEPALLATA* MADS-box genes. Nature 465:200–203

Posé D, Yant L, Schmid M (2012) The end of innocence: flowering networks explode in complexity. Curr Opin Plant Biol 15:45–50

Putterill J, Laurie R, Macknight R (2004) It's time to flower: the genetic control of flowering time. Bioessays 26:363–373

Raghavan V (2000) Developmental biology of flowering plants. Springer, New York

Riechmann JL, Wellmer F (2014) Flower development methods and protocols. Humana Press/Springer, New York

Salisbury F, Ross C (2005) Plant physiology. Easton Press, Norwalk

Salomé PA, Bomblies K, Laitinen RAE, Yant L, Mott R, Weigel D (2011) Genetic architecture of flowering-time variation in *Arabidopsis thaliana*. Genetics 188:421–433

Soltis PS, Endress PK, Chase MW (2005) Phylogeny and evolution of angiosperms. Sinauer, Sunderland

Srikanth A, Schmid M (2011) Regulation of flowering time: all roads lead to Rome. Cell Mol Biol Life Sci 68:2013–2037

Sung S, Amasino RM (2004) Vernalization and epigenetics: how plants remember winter. Curr Opin Plant Biol 7:4–10

Swamy BGL, Krishnamurthy KV (1980) From flower to fruit-embryology of angiosperms. Tata McGraw-Hill, New Delhi

Theissen G, Melzer R (2007) Molecular mechanisms underlying origin and diversification of the angiosperm flower. Ann Bot 100:603–619

Theissen G, Saedler H (2001) Plant biology floral quartets. Nature 409:469–471

Theissen G, Becker A, Winter KU, Munster T, Kirchner C, Saedler H (2002) How the land plants learned their floral ABCs: the role of MADS box genes in the evolutionary origin of flowers. In: Cronk QC, Bateman RM, Hawkins JM (eds) Developmental genetics and plant evolution. Taylor & Francis, London

Valverde P, Mouradov A, Soppe W, Ravenscroft D, Samach A, Coupland G (2004) Photoreceptor regulator of *CONSTANS* protein in photoperiodic flowering. Science 303:1003–1006

Vijayaraghavan U (2001) How plants pattern flowers: lessons from molecular genetic studies of flowering in *Arabidopsis thaliana*, a model plant. Curr Sci 80:233–243

Vijayaraghavan U, Prasad K, Meyerowitz E (2005) Specification and maintenance of the floral meristem: interactions between positively-acting promoters of flowering and negative regulators. Curr Sci 89:1835–1844

Wagner D (2003) Chromatin regulation of plant development. Curr Opin Plant Biol 6:20–28

Wellmer F, Riechmann JL (2010) Gene networks controlling the initiation of flower development. Trends Genet 26:519–527

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmidt M, Lohmann JU, Weigel D (2005) Integration of spatial and temporal information during floral induction in *Arabidopsis*. Science 309:1056–1059

Wollmann H, Mica E, Todesco M, Long JA, Weigel D (2010) On reconciling the interactions between *APETALA2*, miR172 and *AGAMOUS* with the ABC model of flower development. Development 137:3633–3642

Wuest SE, Ó'Maoiléidigh DS, Rae I, Kwasniewska K, Raganelli A, Hanczaryk K, Lohan AJ, Loftus B, Graciet E, Wellmer F (2012) Molecular basic for the specification of floral organs by *APETALA3* and *PISTILLATA*. Proc Natl Acad Sci U S A 109:13452–13457

Yamaguchi N, Wu MF, Winter CM, Berns MC, Nole-Wilson S, Yamaguchi A, Coupland G, Krizek BA, Wagner D (2013) A molecular framework for auxin-mediated initiation of flower primordia. Dev Cell 24:271–282

Yoshida H, Nagato Y (2011) Flower development in rice. J Exp Bot 62:4719–4730

Yu H, Ito T, Wellmer F, Meyerowitz EM (2004) Repression of *AGAMOUS-LIKE24* is a crucial step in promoting flower development. Nature Genet 36:1557–1561

Zahn LM, Leebens-Mack J, de Pamphlis CW, Ma H, Theissen G (2005) To B or not to B a flower: the role of *DEFICIENS* and *GLOBOSA* orthologs in the evolution of the angiosperms. J Hered 96:225–240

Zeevaart JAD (1976) Physiology of flower formation. Annu Rev Plant Physiol 27:321–348

Zimmermann W (1952) Main results of the telome theory. Paleobotanist 1 (Birbal Sahni Memorial Volume): 456–470

Pre-fertilization: Reproductive Growth and Development

17

K.V. Krishnamurthy

Abstract

This chapter deals with details on anther and male gametophytic development, ovule and female gametophytic development, events leading to double fertilization, pollen germination and pollen tube and syngamy and triple fusion. Since basic embryological developmental details are already detailed in earlier literature, attention is focused only on recent data, particularly molecular data pertaining to these aspects. Special attention has been given to genetic control of anther tapetum, endothecium and anther dehiscence, microsporogenesis, microgametogenesis, chalazal behaviour and function and female gametophytic development. The importance of cell cycle events in syngamy and triple fusion is highlighted.

Keywords

Anther dehiscence • Chalaza • Embryo sac mutants • Endothecium • Female gametophyte • Male gametophyte • Ovule • Pollen tube • Syngamy • Tapetum • Triple fusion

17.1 Introduction

The angiosperm *flower* typically has four whorls of lateral organs: *sepals*, *petals*, *stamens* and *carpels*. The outer whorls of sepals and petals are sterile and often do accessory functions in reproduction, while the inner whorls of stamens and carpels, respectively, are the male and female reproductive organs producing the male and female gametophytes and gametes. There is great variation in the number of stamens from zero in female flowers to one to many depending on the plant species. The stamens are free, fused to one another variously to form one to many bundles or attached to the petals or to the carpels. Each stamen typically has a stalk (*filament*) and an *anther*, the two being attached to each other by a *connective*. Staminal nectaries may be present on the filaments or on the anthers of several species of

K.V. Krishnamurthy (✉)
Center for Pharmaceutics, Pharmacognosy and Pharmacology, School of Life Sciences, Institute of Trans-Disciplinary Health Science and Technology (IHST), Bangalore, Karnataka, India
e-mail: kvkbdu@yahoo.co.in

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_17,

unrelated families (Chaturvedi and Bahadur 1985). The number of carpels ranges from one to many, free from one another (*apocarpous*) or fused (*syncarpous*) to form the gynoecium (or *pistil*). A typical gynoecium has a basal *ovary* bearing *ovules* on special placental tissue (of various types), an apically situated *style* and a *stigma* at the tip of the style. There is great variation in the size, shape and number of style and stigma depending on the taxon.

17.2 Anther and Male Gametophyte

The anther is the actual male sexual region of the stamen. The term *microsporangium* is often used as a synonym of anther, but the former term has a much wider connotation and also represents the homologue of the microspore-producing structures of other vascular groups, particularly the pteridophytes (Swamy and Krishnamurthy 1980; Krishnamurthy 2015). Though there are a number of similar developmental features between the anther and the microsporangium of other vascular plants, the male gametophytic organization and behaviour are significantly different. The gametophytic cycle in angiosperms shows extreme abbreviation in time and space, and the male gametophyte or pollen is often composed of just two cells, a *vegetative cell* and a *generative cell*. Anther and pollen development is a critical phase in the life cycle of the angiosperms, and it involves precisely controlled cellular processes including cell division, cell differentiation and cell death due to diverse range of genes and their interaction (Sanders et al. 1999; McCormick 2004; Scott et al. 2004; Ma 2005).

A typical anther is tetrasporangiate although uni-, bi- and octa-sporangiate conditions are also known; these sporangia coalesce to form two sacs or *thecae* in tetrasporangiate taxa and one in uni- and bi-sporangiate taxa, containing the pollen grains. The microsporangia are surrounded by an epidermal layer followed on the inside by the wall layers; the latter are made up of an *endothecium*, *middle layers* and a *tapetum* covering the sporangial locule (Fig. 17.1).

The anther primordium in transectional view is almost squarish to rectangular and is made of homogeneous parenchymatous tissue, covered by an epidermal layer. The *archesporial tissue* differentiates as a single or a group of two to a few adjacently located cells in the hypodermal position at the four corners of the anther primordium. This tissue, in fact, extends vertically from base to the apex of the sporangium. The cells of this tissue are distinct from the rest of the anther tissue by their larger size and greater avidity for nuclear and cytoplasmic stains. The archesporial cells divide periclinally to form outer *primary parietal cells* and inner *primary sporogenous cells*. Both these may undergo further periclinal (and a few anticlinal) divisions to respectively form the wall layers and the *sporogenous cells* (Fig. 17.1); rarely the latter directly function as sporogenous cells. Based on variations in anther wall development and the number of wall layers present, four types are recognized by Davis (1966): *basic*, *dicot*, *monocot* and *reduced* types. One of the earliest genes required for cell division and differentiation in the anther is the *SPOROCYTELESS* (*SPL*)/*NOZZLE* (*NZZ*) gene (Schiefthaler et al. 1999; Yang et al. 1999). In the *spl/nzz* mutant, archesporial initiation occurs normally, but male sporocyte differentiation is halted and anther development fails to continue. The mutant genes of *EXTRA SPOROGENOUS CELLS* (*EXS*)/*EXCESS MICROSPOROCYTES1* (*EMS1*) alter the number of archesporial cells. Two other genes *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1* (*SERK1*) and *SERK2* also have redundant functions during the earlier stages of anther development and, when mutated, result in more sporogenous cells (Albrecht et al. 2005; Colcombet et al. 2005).

17.2.1 Endothecium

The endothecium forms a single layer of hypodermal wall tissue; occasionally, more than one layer may be present in some taxa or may be totally absent as in *cleistogamous* flowers, aquatic plants and extreme saprophytes. The cells of

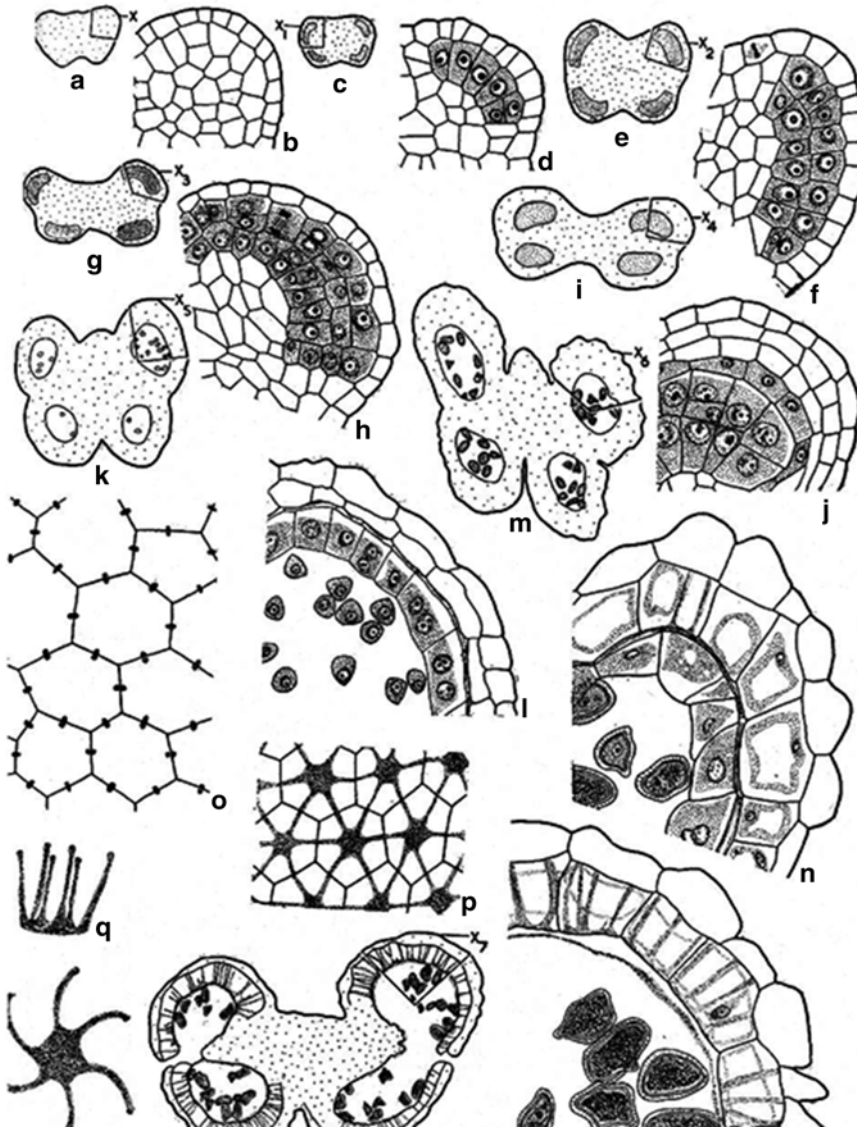


Fig. 17.1 (**a**–**t**), (**a**–**n**) *Trachyspermum ammi*, (**o**–**t**) *Cuminum cyminum*. Microsporangium (**a**, **c**, **e**, **f**, **j**, **k**, **m**). Outline diagrams for (**b**, **d**, **f**, **h**, **j**, **l**) and (**n**), respectively, showing development of anther. (**b**, **d**, **f**, **h**, **j**, **l**, **n**) Enlargements of portions marked *X*, *X1*, *X2*, *X3*, *X4*, *X5* and *X6* in (**a**, **c**, **e**, **g**, **i**, **k**) and (**m**), respectively. (**o**, **p**) Endothecial cells showing thickenings (from whole mounts) (**q**, **r**) lateral and surface views of endothecial thickenings. (**s**) Outline diagram of mature anther (**t**, **s**). (**t**) Same, enlargement of portion marked (Sehgal 1965)

endothecium are often radially elongated and develop special banded thickening in the inner tangential walls and rarely on radial walls also when the sporangium fully matures (Fig. 17.1). The thickening material is not callose but an α-cellulose; in some it may be slightly lignified. Transcriptional activity is required for the differentiation of endothecium as is evident from the localization of poly(A)-RNA in rice microsporangia by *in situ* hybridization using [^{3}H] poly(U) as a probe (Raghavan 2000). Just before meiosis poly(A)-RNA concentration decreases sharply in the epidermis and middle layers, a large amount of this is retained in the endothecium. Even after

the completion of meiosis in the microspore mother cell, some amount of poly(A)-RNA is retained in the endothecium. In rice and wheat anthers, the histone *H3* gene also activates the endothelial differentiation, particularly in the wild-type and transgenic rice; however, the mechanism of this differentiation is not yet clear. The importance of endothecium in anther dehiscence and the way in which the latter occurs are detailed on a subsequent page of this article.

17.2.2 Tapetum

As already stated, the innermost wall layer of the microsporangium is the tapetum. To start with, it borders on the sporogenous cells, and because of its strategic position between the other wall layers and the sporogenous cells, it assumes great significance and importance. Although it is found as a single layer all around the sporogenous tissue, it has been shown to have a *dual origin* (Fig. 17.2). The tapetal cells towards the outer sector of the microsporangium are derived from the primary parietal tissue, while those towards the centre of the anther are derived from the connective tissue. Although evidences of dual origin of tapetum are lost eventually and become a homogeneous layer in many taxa, there are differences in cell size, shape, number of cell layers, nuclear size, shape and ploidy or time of differentiation, etc. between proximal and distal tapeta (Periasamy and Swamy 1966).

Two distinct types of tapeta are known in angiosperms: (1) *glandular*, *secretory* or *parietal tapetum* in which the cells retain their walls and persist *in situ* without much change in shape and position until they perish by *programmed cell death* (*PCD*) (Fig. 17.1). The tapetal PCD, as the PCD seen in many other plant cells, is a highly orchestrated event that occurs synchronously with pollen mitotic division and formation of pollen exine (Sanders et al. 1999). It is relatively rapid and shows chromatin condensation, DNA fragmentation and mitochondrial and cytoskeletal disintegration (Papini et al. 1999; Love et al. 2008); (2) *periplasmodial tapetum*, in which the cells lose their inner tangential and radial walls due to enzymatic action of the tapetal cells themselves followed by the coalescence of the protoplasts of all tapetal cells to form a viscous fluid that flows into and fills the sporangial cavity all around the developing microspore mother cells. The former type is more common

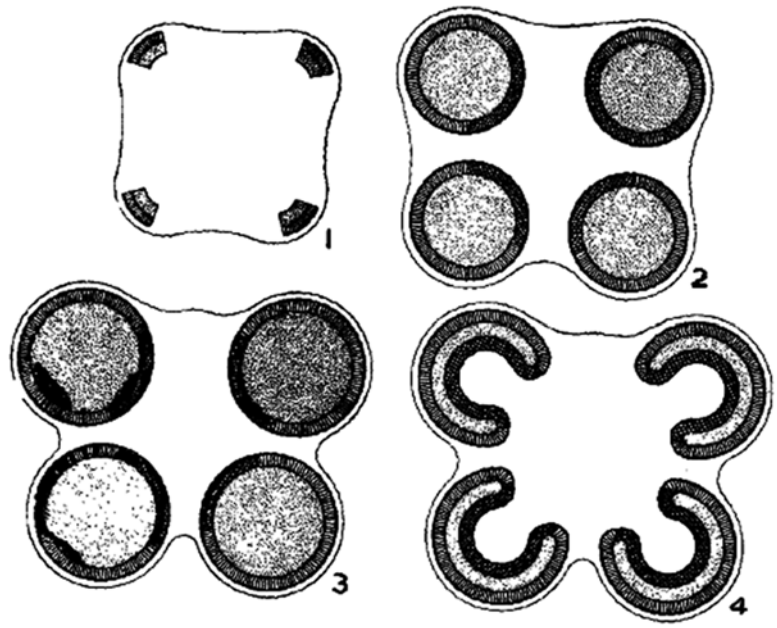


Fig. 17.2 Development of anther (*1–4*) to show dual origin of anther tapetum. Single-hatched portion of the anther tapetum is of parietal origin, while double-hatched portion is derived from the connective tissue (Periasamy and Swamy 1966)

in dicots, while the latter in the monocots. The glandular tapetal cells are richly protoplasmic, and their nuclei are prominent and metabolically active; in some taxa, nuclei increase in number (two to eight), become polyploidal (due to nuclear fusion or endomitosis) or become polytenic (up to 16 times increase in DNA content). Crystals, starch, lipids, mitochondria, Golgi bodies, ER, membrane-bound ribosomes, plastids, etc. are reported in the tapetal cells. The cell walls are cellulosic. The walls of periplasmodial tapetal cells, before the formation of periplasmodium, have more pectin than cellulose. The periplasmodium is an organized structure. It gets dehydrated before its complete degradation. A third type of tapetum is often recognized and is named *amoeboid tapetum* (some botanists mistakenly call the periplasmodial tapetum as amoeboid tapetum; see Swamy and Krishnamurthy (1980) for discussion on this). In this type, the cells radially elongate conspicuously and protrude into the sporangial cavity, without, however, losing their cell walls. This type is associated with some types of male sterility.

The tapetum has been considered as a nurse as well as a regulatory tissue for the developing male gametophyte. Many indirect evidences are there to implicate the tapetal cells as sources of deoxyribosides which would then be used for DNA synthesis by the microspores, although actual transfer of these from tapetal cells could not be directly demonstrated. There are circumstantial evidences to indicate that carbohydrates and pollen reserves may result, at least partially, from the transfer of soluble sugars and peptides or amino acids from the tapetal cells. In many plants, there is a close correspondence between tapetal disintegration and the appearance of pollen reserves.

The most important function of the tapetum is to supply pollen wall and pollen coat polymers (Piffanelli et al. 1998). The glandular tapetal cells contain in their cytoplasm numerous bodies, often attached to the lipid membrane-bound, electron-dense organelles known as *pro-ubisch*, *pro-sphaeroid* or *proorbicule bodies*. The shape of these bodies varies considerably: granular, rod-shaped, star-shaped, circular, perforated disc-like or compound multiperforate platelike. They accumulate as *ubisch bodies* near the plasma membrane before disappearing from inside the cell. They are then immediately seen on the exine of the microspores, where they get integrated as *sporopollenin* (Fig. 17.3). Hence, ubisch bodies are often considered as transport forms of sporopollenin. The periplasmodial tapetum, after excessive dehydration, gets deposited on the surface of microspores/pollen grains to form *tryphine*, a complex mixture of lipoidal substances. There is also a deposition of *pollenkitt*. Tapetum controls male fertility/sterility through its timely/untimely production of the enzyme *callase* (=β-1,3-glucanase). In fertile anthers, it is produced by the tapetum when the callose wall around the microspore tetrad needs to be dissolved to release the individual microspores, while in sterile anthers, the enzyme is often produced precociously to dissolve the callose wall around the microspore mother cell before it undergoes meiosis. Some tapetum sequences from anther cDNA libraries of *Brassica napus* and *Arabidopsis* specify β-1,3-glucanase. Genes that encode proteinase inhibitors of β-1,3-glucanase action have been isolated from anthers.

In situ hybridization with [^{3}H] poly(U) has revealed that mRNA accumulation is one of the metabolic activities that prepares tapetal cells for their function. Commensurate with this high metabolic activity, the tapetal cells show the activities of a number of genes. At least five tapetum-specific mRNAs and two mRNAs that are also seen in other anther tissues (TA series mRNAs) were demonstrated by in situ hybridization and by the use of chimeric gene constructs in transgenic plants even as early as 1990 (Koltunow et al. 1990). These mRNAs get accumulated and lost in the same temporal sequence during tapetum ontogeny and have been identified from a cDNA library of tobacco. One of these is *TA29* whose product is a glycine-rich cell wall protein that is likely to be involved in exine formation. Subsequent studies have revealed the expression products of several other genes.

An *Arabidopsis* gene, *MALE STERILITY2* (*MS2*) (Wilson et al. 2001; Ito and Shinozaki 2002), is expressed in the tapetum, and the

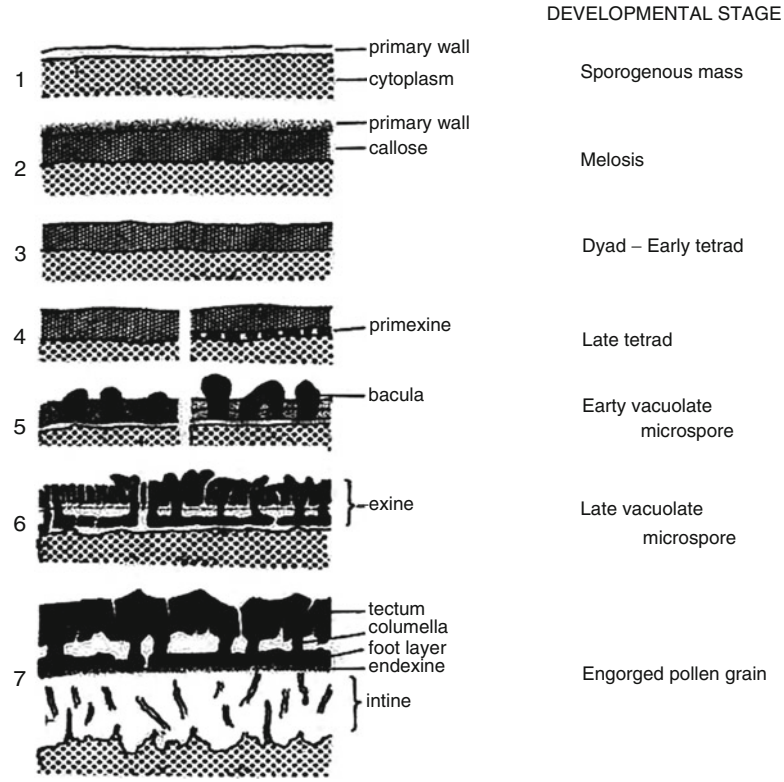


Fig. 17.3 Summary of pollen wall developmental stages (*1–7*) (sporoderm) ontogeny of *Sorghum bicolor*. Corresponding developmental stages in the anther locule are also mentioned opposite to each figure (Adapted from Christensen et al. 1972; Swamy and Krishnamurthy 1980)

sequence similarity of this gene's product to a protein that converts fatty acids to fatty alcohols has implicated this gene to pollen exine formation (Aarts et al. 1997). Its rice orthologue is *DEFECTIVE POLLEN WALL* (*DPW*) (Shi et al. 2011). Loss of function of the *FACELESS POLLEN1*/*WAX2*/*YRE*/*CER3* gene causes defects in exine; this gene is likely to encode a putative enzyme of unknown function presumably involved in pollen wall formation (Ariizumi et al. 2003). The other rice genes important in tapetal function are *WAX-DEFICIENT ANTHER1* (*WDA1*), *OsC6* and *PERSISTENT TAPETAL CELL1* (*PTC1*). Fairly recently, *Arabidopsis* genes encoding the cytochrome P450 enzymes of *CYPTO3A2* and *CYP704B1* have been shown to be involved in the biosynthesis of sporopollenin (mutants have severe to moderate defects in exine deposition) (Morant et al. 2007; Dobritsa et al. 2009). De Azevedo Souza et al. (2009) have shown that *ACYL CoA SYNTHETASE5* (*ACoS5*) encodes a fatty acyl synthetase that plays a vital role in exine formation and sporopollenin biosynthesis in *Arabidopsis*; the *acos5* mutant is totally male sterile with pollen lacking recognizable exine. Genes that co-regulate along with ACoS5 in pollen exine formation in *Arabidopsis* such as *DIHYDROFLAVONOL4-REDUCTASE LIKE1* (*DRL1*)/*TETRAKETIDE* α-*PYRONE REDUCTASE1* (*TKPR1*) (Grienenberger et al. 2010) are also very important, as they affect male sterility (Tang et al. 2009). *DRL1*/*TKPR1* is involved in flavonoid metabolism and plays a pivotal role in sporopollenin precursor biosynthesis. It was also reported recently that the enzymes closely related to chalcone synthase (CHS) encoded by *At1gO2050* [*LESS ADHESIVE POLLENS* (*LAP6*)/*POLYKETIDE SYNTHASEA* (*PKSA*)] and *At4g34850* (*LAP5*/*PKSB*) catalyses the sequential condensation of a starter acyl-CoA substrate with malonyl-CoA molecules to produce alkylpyrone *in vitro* (Dobritsa et al. 2010). *PKSA* and *PKSB* are specifically and transiently expressed in tapetal cells during microspore development in *Arabidopsis* anthers, mutants of *PKS* genes displayed exine defects and a double

pksa pksb mutant was completely male sterile with no apparent exine; these results show that hydroxylated α-pyrone polyketide compounds generated by the sequential action of *ACoS5* and *PKSA/B* are potential and previously unknown sporopollenin precursors (Kim et al. 2010).

The other genes which are involved in tapetum development and function are *ABORTED MICROSPORES* (*AMS*) (Sorensen et al. 2003), the rice orthologue *TATETUM DEGENERATION RETARDATION* (*TDR*) (Li et al. 2006), *TAPETAL DETERMINANT1* (*TPD1*) (Yang et al. 2003), *DYSFUNCTIONAL TAPETUM* (*DYT1*) (Zhang et al. 2006), the rice orthologue *UNDEVELOPED TAPETUM* (Jung et al. 2005), *DEFECTIVE IN TAPETAL DEVELOPMENT AND FUNCTION1* (*TDF1*) (Zhu et al. 2008), *MYB80* (formerly-*MYB103*) (Higginson et al. 2003; Li et al. 2007; Zhang et al. 2007), *ECERIFERUM1* (*CER1*) (Shi et al. 2011) and *MS1* (Wilson et al. 2001). *TDF1* encodes *MYB*; *tdf1* mutant also shows enlarged tapetum with increased vacuolation (Phan et al. 2011) and causes arrest of microspore development. Early tapetal initiation is affected by the downstream genes *EXTRA SPOROGENOUS CELLS* (*EXS*)/*EXCESS MICROSPOROCYTES1* (*EMS1*) (Cannales et al. 2002; Zhao et al. 2002) and *TPD1*. Mutants in these genes have an absence of tapetal and middle layers. Mutations in *SERK1* and *SERK2* genes result in the lack of a tapetal layer. *MYB33* and *MYB65* also act redundantly to facilitate tapetal development around meiosis stage; it has been shown that the expression of *MYB33* is regulated by miRNAs (Millar and Gübler 2005). These genes are not affected in the dyt1 mutant indicating that they are upstream of *DYT1* (Zhang et al. 2006). In the *dyt1* mutant, tapetum occurs (also meiosis), but tapetum development is abnormal with enlarged vacuoles in its cells. *DYT1* (by encoding basic-helix-loop-helix proteins) has been proposed to be involved in the regulation of many tapetal genes, either directly or indirectly, including *AMS* and *MS1* (Zhang et al. 2006). The *ams* (its wild gene *AMS* also encodes basic-helix-loop-helix proteins) mutant has premature tapetal degeneration because of its abnormally enlarged and vacuolated cells.

Detailed studies have been done on the role of *MS1* gene in tapetal development and pollen wall biosynthesis (Yang et al. 2007). Early events in anther development in *ms1* mutant are normal and that the *MS1* acts, through encoding PHD transcription factors, late in pollen development after tapetal initiation and is downstream of *DYT1* (Zhang et al. 2006). *MS1* coordinates the expression of late genes associated with pollen wall formation and which are involved in the biosynthesis of components of the phenyl-propanoid pathway, long-chain fatty acids and phenolics, which are required for sporopollenin biosynthesis. In the *ms1* mutant, tapetal PCD does not occur, but tapetal degeneration occurs by necrosis (Vizcay-Barrena and Wilson 2006); there is also down-regulation in the expression of a member of cys proteases in *ms1* mutants. These proteases are likely to be critical to the progression of PCD, and in their absence, possibly in association with a lack of tapetal secretion, PCD does not occur. *MS1* also controls the synthesis of pollen coat (oleoresin gene family, lipid transfer proteins or LTPs, ACP lipids and phenyl-propanoid pathway); it does not directly regulate genes associated with pollen wall biosynthesis (due to its timing of expression) but acts *via* one or a number of additional transcriptional factors (TFs) including *MYB99* and two *NAM* genes that contain a conserved NAC domain (Yang et al. 2007). Based on an analysis of transcript levels within *tdf1* and *ams* mutants, Zhu et al. (2008) suggested that *TDF1* functions upstream of *AMS* and that *AMS* is upstream of *MYB80*. Xu et al. (2010) identified 13 genes as direct targets of *AMS*, but *MYB80* was not among them. Transcript levels of *MS1*, *MS2* and *A6* are downregulated in the *MYB80* mutant, suggesting that they act downstream of *myb80*. It is not known if the three genes are directly or indirectly regulated by *MYB80. MYB80* is recently shown (Phan et al. 2011) to directly target a glyoxal oxidase (GLOX1), a pectin methyl esterase (VANGUARD1) and an A1 aspartic protease (UNDEAD), all of which are expressed in the tapetum and microspores. The timing of PCD in tapetum is likely to be regulated by *MYB80*/*UNDEAD* system. The overall genetic

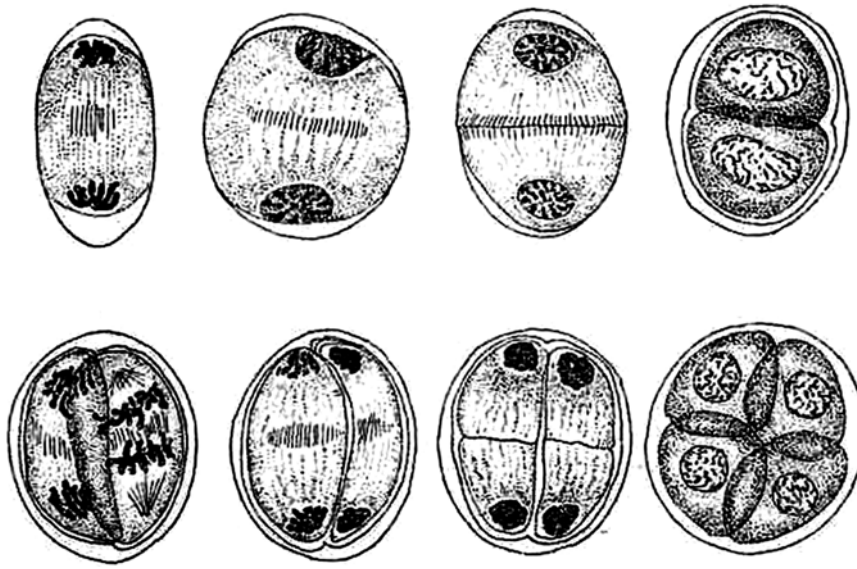


Fig. 17.4 Successive divisions of microspore mother cell of *Lilium regale* (Gerassimova-Navashina 1951)

regulation of sporopollenin synthesis and pollen exine development is reviewed by Ariizumi and Toriyama (2011).

17.2.3 Microsporogenesis and Microgametogenesis

The sporogenous cells either directly or after a few divisions give rise to microspore mother cells (MMCs). The MMCs possess thin cellulosic cell walls with plasmodesmal connections, not only between themselves but also with the tapetal cells. Dictyosomes and plastids (without starch grains) are characteristically present in the cells. Most DNA synthesis in MMCs is done during premeiotic interphase, but a meager amount is also synthesized during zygotene-pachytene. Similarly, active RNA and protein synthesis takes place during premeiotic stage with a fall during meiotic prophase. There is a decline in ribosomal population after the initiation of meiosis, but the population is restored after homotypic division. There is also a reorganization of mitochondria and plastids in the microspore, as they are partly degraded during meiosis. Just at the onset of meiosis in MMCs, a callose wall is deposited inner to the original cellulosic wall. Any irregularity in callose deposition/metabolism results in male sterility. Callose deposition starts on the walls of MMCs close to tapetum and gradually extends to the more centrally located cells of the anther. Initially, the callose wall is incomplete leaving many gaps in the wall through which massive cytoplasmic channels between adjacent MMCs (but not with tapetum cells) are established. These channels reach their maximum development during zygotene-pachytene and help establishing near synchronicity in meiosis in all MMCs of a sporangium. Callose deposition is considered as a necessary prerequisite for meiotic induction and continuance (Krishnamurthy 1977, 2015). Callose is highly impervious to most molecules and thus is a highly isolating and insulating material. The plasmodesmal connections are sealed off towards the end of metaphase I in taxa with successive division and at anaphase II in plants with simultaneous division.

Two types of meiotic division are known in MMCs, either of which results in the formation of a tetrad of four microspores. In *successive division*, a centrifugally extending cell plate and then a wall are promptly laid down between the daughter nuclei at the end of each of the two divisions (Fig. 17.4). In the *simultaneous division*, the separation of all four microspore nuclei is

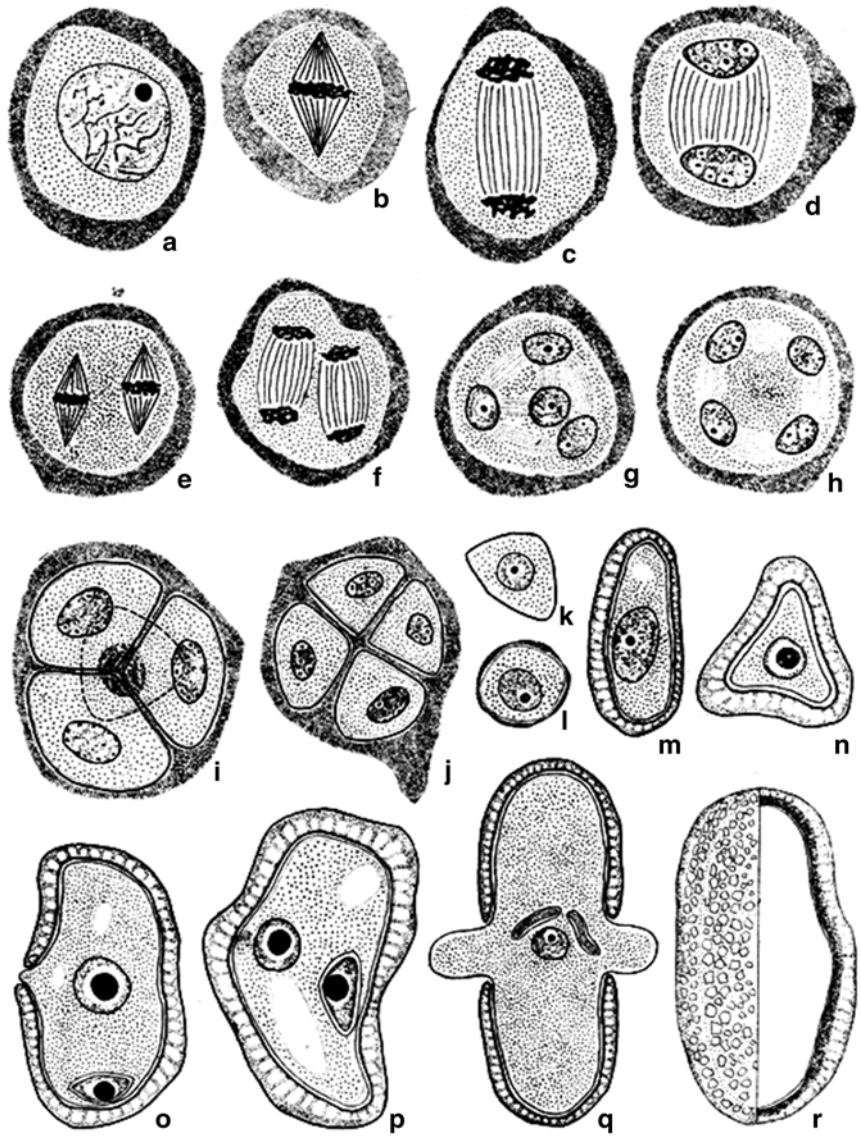


Fig. 17.5 *Trachyspermum ammi*. Microsporogenesis and male gametophyte; (**a**–**j**). Simultaneous meiotic division in microspore mother cell leading to tetrad formation; (**k**–**n**) Uninucleate microspore. (**o**–**p**) Two-celled pollen. (**q**) Three-celled pollen; (**r**) Palynogram (Sehgal 1965)

effected through centripetally extending furrows at the end of the second division (Fig. 17.5a–j). The callose wall around the tetrad is heterogeneous and layered. The outermost layer is the most well developed. Three more concentric layers follow this on the inside distinguished from each other by their variable density. The fifth layer is the innermost and the least dense of all. It surrounds and isolates the four microspores and cell plates. Each microspore is individually surrounded by the *primexine*. Soon after meiosis, callose wall around the microspore tetrad is degraded by β-1,3-glucanase into D-glucose and oligomers of D-glucose of different lengths, which may be used by the microspores for various purposes (such as nutrition and pollen wall formation). As a result of callose degradation, the individual microspores are separated out of the

tetrads. β-1,3-glucanase is present in low quantities in the tapetum even during meiosis in MMCs, but increases suddenly during late tetrad stage to cause the separation of microspores. In some angiosperms, failure of microspores to separate out of the tetrads results in the formation of *permanent tetrads* or *compound pollen grains*. In some Mimosaceae and Orchidaceae, polyads of 8–32 grains called *massulae* are formed. An extreme case of adherence of all pollen grains of an entire microsporangium is seen in many Asclepiadaceae and the resultant structure is called a *pollinium*.

The studies made so far show that both the diploid sporophytic tapetal cells and the haploid gametophytic microspore contribute to pollen wall synthesis (Ariizumi and Toriyama 2011). Exine formation is stated to commence from the late tetrad stage with the laying down of the primexine between the callose wall and the plasma membrane of the microspore (Paxson-Sowders et al. 1997) (except at the germinal pore region where it is absent). The microspore just released from the tetrad does not have an *exine* (the outer wall of the pollen). The primexine is distinguished from the callose by its electron opacity. It has a matrix, presumably made up of cellulose, and radially directed rods, the *probaculae* and *profoot layer*. The deposition of *sporopollenin* begins immediately after release of microspores from the tetrad, and its source is from the tapetum, as already detailed. The characteristic pattern of the sporoderm is determined by features already imprinted in the primexine during the period of enclosure in the tetrad (Blackmore et al. 2007). However, a few investigators believe that the initial exine pattern laid down in the microspore is controlled by the plasma membrane and that callose causes this imprinting by acting as a template (and not the primexine). After the first division of the microspore, exine formation is almost complete. At later stages of pollen ontogeny, pectocellulosic *intine* and *tryphine* are deposited (Piffanelli et al. 1998). Intine formation first begins in the vicinity of the germinal aperture(s) and from there spreads all around the microspore; this growth is said to be associated with dictyosome activity in coordination with the plasma membrane. Thus, intine is programmed entirely by the haploid, male gametophytic genome and is made of pectocellulose, while the exine is organized both through tapetal inputs and microspore activity.

Under typical conditions, the microspore nucleus occupies a central position, while the cytoplasm has many small vacuoles spread almost evenly (Fig. 17.5k–n). Just before division, the nucleus moves towards a side that is generally opposite to the furrows. Mitochondria and plastids are displaced to the cytoplasm opposite to the nucleus. During interphase, active ribosomal RNA synthesis takes place. A conspicuously large vacuole appears in the cytoplasm opposite to the nucleus. The nucleus then divides followed by a curved callose wall to result in a small lens-shaped daughter cell (appearing spindle shaped in cross-sectional view) called the *generative cell* (*GC*) and a conspicuously larger cell called *vegetative cell* (*VC*) (Fig. 17.5o, p). Thus, the division is asymmetric. The callose wall separating the GC from VC is highly transitional and is retained only for about 10–20 h. GC soon gets pinched off from the microspore wall and becomes embedded in the cytoplasm of the VC, by which time its callose wall is also lost. This may or may not be accompanied by a change of shape of the GC. This separation is effected by the growth of callose wall in between the plasma membrane of the GC and the intine of pollen grain. The new location of GC obviously provides a new environment for interaction between GC and VC. At this stage, the pollen is said to be mature in most taxa. The GC is surrounded by a double membrane, by a distinct cellulosic wall or by the retention of the original callose wall depending on the species. The GC is less dense due to very poor or even no RNA and proteins. Minute vacuoles filled with water or lipid materials are also present. The DNA content of its nucleus is very high (rises to 2C level), but the nucleolus is not very conspicuous. Axial microtubules have been recorded and these are important in controlling the shape of the GC. However, there is some disagreement regarding the cytoplasmic organelles of the GC, probably because of species-dependent variations. Mitochondria,

dictyosomes, lipid bodies and ER have been reported. Plastids have not been detected in many species, although reported in a few taxa. In general, GC is poor in organelle content and variety. In contrast, the VC shows dense cytoplasm due to greater amount of RNA and proteins. The nucleus is invariably lobed and poorer in DNA content (mostly at the 1C level) and has a relatively large nucleolus. Thus, the nucleus of GC switches on DNA synthesis, but there is no appreciable RNA or protein synthesis as transcription is slowed down, while the nucleus of VC switches off DNA synthesis but without interfering with transcription (Raghavan 2000). VC may have starch or oil as a major storage product.

The division of the GC into two male gametes or sperms takes place either in the pollen itself (in about 25 % of the angiosperms) (Fig. 17.5q) or in the pollen tube. Hence, the pollen grains are liberated from the anther at the two- or three-celled condition. Division of GC in the pollen grain is due to normal mitosis followed by cytokinesis through cell plate formation or through furrowing. The mechanism of division of GC in the pollen tube is not very clear because of difficulties in studying due to spatial restraints; it appears to be normal mitosis. The organelles reported in GC are also recorded in the two sperms. DAPI staining and fluorescence microscopy have indicated the absence of plastid DNA in the sperm cells (in 82 % of species surveyed).

17.2.4 Genetics of Microsporogenesis and Microgametogenesis

Cytochemical, autoradiographic, biochemical and molecular studies on RNA and protein synthesis have indicated that pollen development is controlled by a temporal and spatial programme of differential gene expression. The period leading up to the first division of the microspore is marked by major contribution of rRNA in the total RNA synthesized. This is consistent with the opinion that among the multiple copies of 5s RNA genes that control pollen development, some are switched off after the peak synthesis, while a few persist for an additional period. Quantitative variation in mRNA populations has also been noted during pollen development. The mRNA that gets accumulated in the mature pollen may serve as templates for the first proteins in germination. Both qualitative and quantitative differences are detected in the proteins synthesized during different stages of pollen development. Such proteins include lysine- and arginine-rich histones which accumulate in the GC and sperm nucleus, and they are linked to transcription of the haploid microspore/pollen genome. Stress proteins such as extensins and arabinogalactan-rich proteins, which are important in incompatibility reactions, are also synthesized by the developing pollen.

All the above imply active gene expression. The genes involved in pollen development have been isolated and characterized, especially in *Arabidopsis*, *Brassica napus*, *B. oleracea*, cotton, *Lilium*, *Oenothera*, *Petunia*, tomato, *Tradescantia* and *Zea mays*. The isolated genes were found to be members of small gene families present in one or two copies in the genome, and none appeared to belong to large multigene families (Raghavan 2000). As already indicated, both sporophytic and gametophytic genes are involved. Transcripts of two distinct sets of gametophytic genes are shown to be activated in specific temporal and spatial patterns. Transcripts of the first set, commonly called *early genes,* become active at the tetrad stage or at the latest when microspores are released from the tetrads, but these have only short periods of activity. Some of the early genes are importantly needed for coding cytoskeleton elements. One of the very early products is the *DEFECTIVE IN EXINE PATTERN FORMATION 1* (*DEX1*) gene protein; it is a putative membrane-associated protein with predictable protein-binding domains. The *dex1* mutant in *Arabidopsis* delays primexine formation, and hence, the sporopollenin synthesized by the tapetum is abnormally deposited on the mutant microspore surface (Paxson-Sowders et al. 1997). Recently, Kim et al. (2011) have shown that ER- and Golgi-localized phosphatases gene *A2* (*PLA2*) plays critical roles in *Arabidopsis* pollen development

and germination. These authors have characterized three to four *Arabidopsis PLA2* paralogues and found that they are expressed during pollen development, germination and pollen tube growth. Suppression of *PLA2* using RNA interference approach resulted in pollen lethality and inhibition of tube growth.

It was already shown that there are phenotypic differences between the GC and VC. The genetic basis of these phenotypic differences has been analyzed by using transgenic molecular markers and in situ hybridization techniques with cloned genes. An associated asymmetry in gene expression is seen along with the asymmetric cell division that results in GC and VC. Mitotic division is not a prerequisite for expression of VC-specific gene(s) but a symmetric division silences gene expression in GC. Twell (1995) has shown that ablation of VC of transgenic tobacco pollen by the cytotoxin *DTA* gene linked to the tomato pollen-specific gene inactivates the GC and prevents its function (Raghavan 2000). How the VC controls the activity of GC is not clear. The *late genes* become active after the microspores divide and their activity continues till pollen tube growth (Stinson et al. 1987). The proteins encoded by late genes include pectin lyases, pectin esterase, polygalacturonase, protein kinases, ascorbate oxidase, thioredoxins, actin-depolymerizing factors, zinc finger class proteins, RNA helicases, pollen allergins, ATPase, osmotin, stress proteins, PR-proteins, malate synthase, superoxide dismutase, etc.

Attention should also be focused on the MIKC*-type type II MADS-box genes that affect development of male gametophyte. Combinations of double and triple mutants of *agl65*, *agl66*, *agl104* MADS-box genes give rise to several pollen phenotypes with disturbed viability, delayed germination and aberrant pollen tube growth (Adamczyk and Fernandez 2009; Smaczniak et al. 2012). The gene products form a protein interaction and regulatory network controlling pollen maturation. These also regulate transcriptome dynamics during pollen development.

A detailed account on tapetal genes (i.e. sporophytic genes) was already provided. Most, if not all, of them affect the pollen development in diverse ways, either directly or indirectly. For example, mutation in *AMS* and *DYT1* genes causes degeneration of microspores. In *Arabidopsis*, a candidate gene called *QUARTET* (*QRT*) is required for the separation of microspores from the tetrads. It is probably a tapetal gene. A mutation of this gene causes a patchy formation of callose between the microspores in the tetrad (Preuss et al. 1994); there is also a fusion of the microspores through their developing exine due to a failure of pectin degradation. Another well-studied gene is the *DUO POLLEN1* (*DUO1*) gene (see Zheng et al. 2011). It encodes a male germ cell-specific R2R3Myb protein (Rotman et al. 2005) that is required for the expression of the *Arabidopsis thaliana* G2/M regulator cyclin B1;1 (*CYCB1*;*1*) in the male germline (Brownfield et al. 2009a, b), suggesting an integrative role for *DUO1* in cell specification and cell cycle progression that is necessary for twin sperm cell production. *DUO1* mRNA is directly targeted by miRNA159, which leads to its degradation. Whether *APC*/*C* is required for *DUO-1*-dependent *CYCB1*;*1* regulation is unknown (Zheng et al. 2011). Mutants in both *APC8* and *APC13* had pleiotropic phenotypes resembling those of mutants affecting miRNA biogenesis. Zheng et al. (2011) have shown that these *apc*/*c* mutants have reduced miR159 levels and increased *DUO1* and *CYCB1*;*1* transcript levels and that APC/C is required to recruit RNA polymerase II to *MIR159* promoters. Thus, in addition to its role in degrading *CYCB1*;*1*, APC/C stimulates production of miR159, which downregulates *DUO1* expression, leading to reduced CYCB1;1 transcription. Both *MIR159* and *APC8* protein accumulated in unicellular microspores and bicellular pollen, suggesting that spatial and temporal regulation of miR159 by *APC*/C ensures mitotic progression. Consistent with this, the percentage of mature pollen with no or single sperm-like cells increased in *apc*/*c* mutants and plants overexpressing *APC8* partially mimicked the *DUO1* phenotype. Thus, *APC*/*C* is an integrator that regulates both miRNA-mediated transcriptional regulation of *CYCB1*;*1* and degradation of *CYCB1*;*1* (Zheng et al. 2011) (Fig. 17.6).

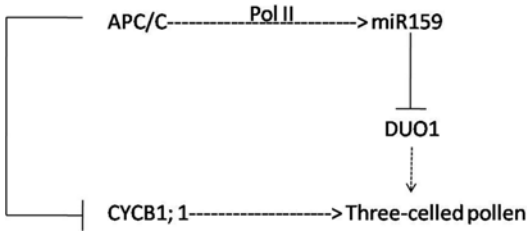


Fig. 17.6 A model for the dual roles of APC/C in regulating cyclin B1;1 during male gametophyte development (Based on Zheng et al. 2011)

17.2.5 Anther Dehiscence

Almost simultaneously with the maturation of microsporangia, the wall layers aligned in the groove between the adaxial and abaxial pairs of sporangia fail to undergo histological modifications. This linear strip of tissue is the *stomium* which predetermines the place of future anther dehiscence. Due to the continued bulging out of the distal anther wall, the stomium appears to be seated in a furrow. The cells of the stomium form the weak zone in the anther wall. The few parenchyma cell layers that separate the adaxial and abaxial sporangia that form a septum are resorbed towards anther maturity causing the merger of the sporangia on either side of the anther. At about this time, the stomial cells slightly elongate radially obviously due to the pressure exerted by the bulging anther wall on either side. Meanwhile, the mechanical action of the endothecium causes an evagination of the anther wall along the stomial direction. Finally, the anther dehisces to release the pollen. In some taxa, pollen is released through apical pores or valves in the sporangia, while in cleistogamous and aquatic taxa, the pollen is released through the disintegration of the anther wall.

A critical analysis of anther-specific cDNA clones in tobacco supports the contention that the whole programme of anther wall differentiation, degeneration of middle layers and anther dehiscence consists of a cascade of temporal and spatial gene expression events in the anther wall (Raghavan 2000; Krishnamurthy 2015). The cDNA clones implicated in this programme are *TA56*, encoding a thiol endopeptidase, and *TA20*, encoding an unknown protein. By following the expression of these two clones by in situ hybridization, it was shown that *TA56* transcripts accumulated in the anther wall in the prospective stomial region at a very early stage of anther development. As the anther matures, there is an appreciable decrease in the intensity of hybridization signals in the cells in and around the stomium. At the same time, the cells around the connective tissue acquire the hybridization signal. *TA20* transcripts are seen in all layers of anther wall to start with, but with anther maturation, they are concentrated in the connective cells around the vascular bundles. Selective expression of TA56 gene transcripts in the stomial region suggests a role for endopeptidase in anther dehiscence (Koltunow et al. 1990). When the stomial region alone is ablated with a cytotoxic gene fused to the *TA56* gene promoter, anther development was normal, but fails to dehisce (Beals and Goldberg 1997), again supporting the above contention. Transcripts of anther-specific cDNA clones isolated from tomato anthers are also expressed in the wall layers, particularly in epidermis and endothecium. The protein encoded by these genes show homology to Kunitz trypsin inhibitor (KTI) and pectinase enzyme.

The events in anther dehiscence are also mediated by structural features of the filament since the former is dependent on the latter for the transport of water and nutrients. Dehiscence is largely a desiccatory process, and any histological feature promoting rapid water loss from the anther or disruption of water to the anther might facilitate dehiscence. Open stomata, a weakly developed cuticle, prominent intercellular space system and xylem lacunae of the filament are some of these histological features. There are hygroscopic and cohesive mechanisms involved in desiccatory anther dehiscence. Hygroscopic mechanisms depend entirely upon volumetric changes in the cell walls, whereas cohesion mechanisms involve volumetric changes in the cell lumen, the cell walls merely undergoing passive deformation. Cohesive mechanisms largely involve cohesive forces between water molecules in the cell lumen. Dehiscence occurs when cohesive forces are exceeded. In hygroscopic

mechanisms, adhesive forces are important. Although most people accept cohesive mechanism, both appear to be important.

At the time of dispersal, pollen grains are partly dehydrated with a water content of 10–30 %. Further water loss occurs during pollen transport resulting in a condition similar to that in dry seeds. The pollen becomes metabolically poor in activity due to disorganization of the membrane systems of 'vegetative cell' organelles and plasmalemma. The effect of dehydration is also evident on the cell walls. Apparently, this dehydration helps the dispersal capability of pollen. Proline accumulation in the pollen cytoplasm and the presence of some stress proteins in the cell walls characterize such dehydrated pollen.

17.3 Ovule and Female Gametophyte

The female gametophyte develops in a structural unit called *megasporangium*, the female counterpart of the microsporangium. This term is generally employed for the megaspore (sometimes also called the *macrospore*) bearing units of vascular plants, but the same unit of seed-bearing plants (spermatophyta) is called the *ovule*, which contains the *nucellus*, enclosed by one or two *integument*s. It is in the nucellus that the female gametophyte gets differentiated. Unlike the non-ovule-bearing vascular plants (pteridophytes), fertilization of the *egg* (the female gamete developed inside the female gametophyte) and the consequent development of the embryo is initiated while the ovule (future seed) is still attached to the parent sporophyte.

17.3.1 Configuration of Ovule

The ovule primordium is initiated as a tiny protuberance on the placental tissue of the ovary. While the primordium is growing in size, a small annular tissue thickening appears just above the point of attachment of the primordium to the placenta. This point corresponds to the location of the *chalaza* or base of the ovule. This annular belt grows at a relatively faster rate than the protuberance and soon encloses the latter leaving a pore at the apex called the *micropyle*. The central protuberance becomes the *nucellus*, while the annular belt becomes the *integument*; in some taxa, an additional integument is formed in the same way as the first. If the primordium continues to grow straight throughout its course without showing any change of direction, the configuration of such an ovule is said to be *orthotropous* or *atropous*. In such an ovule, at maturity, the *funicle*, or stalk of the ovule, the *chalaza* [that part of the ovular tissue adjacent to the base of the integument(s)] and the micropyle lie along the same vertical axis. Changes in the direction of growth of the ovule primordium result in other ovular configurations such as *anatropous*, *campylotropous*, *hemitropous* and *amphitropous* where the imaginary lines connecting the positions of funicle, chalaza and micropyle form different types of triangles. Details on structural variations in the ovules of angiosperms are summarized in Kapil and Vasil (1963), Swamy and Krishnamurthy (1980) and Bowman (1984).

17.3.2 Nucellus

As already stated, the ovular tissue enclosed by the integument(s) forms the nucellus. Depending on the extent of this sporophytic tissue, two major types of ovules are recognized: (1) *tenuinucellate*, where the nucellus is represented only by a few cells, and (2) *crassinucellate,* where the nucellus is massive. In the former type, the hypodermal female archesporial cell directly functions as the *megaspore mother cell*, while in the latter it cuts off a *parietal cell* which undergoes repeated divisions to not only form a massive nucellus but also to push the megaspore mother cell deep inside the nucellus. Crassinucellate condition may also be contributed by active cell division of apically located chalazal cells. In some cases, the nucellar epidermis at the micropylar pole divides repeatedly periclinally to add to the mass of the nucellus. This condition is

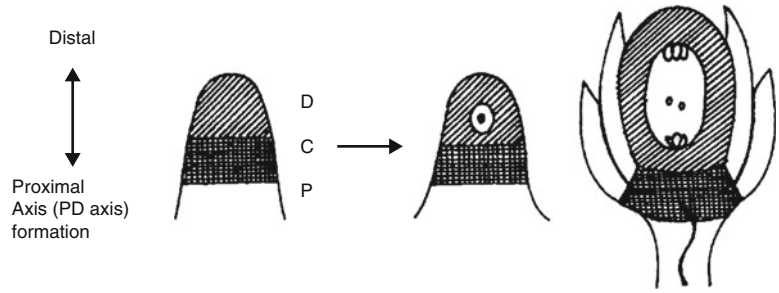


Fig. 17.7 Diagrammatic representation of the proximo-distal polarity in a developing ovule showing the three pattern elements. The proximal (*P*) domain forms the funicle, the central or chalazal (*C*) domain forms the chalaza-integument complex and the distal (*D*) domain forms the nucellus and embryo sac. C domain may possibly have two subdomains (Modified from Grossniklaus and Schneitz 1998)

called *pseudocrassinucellate* (Davis 1966); here, also the megaspore mother cell is pushed deep in the nucellus. Nucellus is totally lost after fertilization in all tenuinucellate ovules and in many crassinucellate and pseudocrassinucellate taxa, but in a few as in *Piper nigrum*, it may persist in the seed as *perisperm*.

17.3.3 Chalaza

That part of the ovule that is subjacent to the base of the integument may be designated as the *chalaza*. It is the region of the ovule from where the integuments originate and where there is no distinction into nucellus and integument(s). The chalaza indicates a pole of the ovule that serves as a seat of very vital metabolic activities from the very beginning of ovule organization to even during post-fertilization stages. It is very difficult to delimit the boundaries of chalaza either morphologically or physiologically. The importance of chalaza in the establishment of different ovular configurations was already drawn attention to. Its importance as the point of origin of the integument(s) has also been indicated. It is also the location from where additional 'integumentary' structures like *arils* arise in arillate taxa. Attention may also be drawn to the already indicated fact that the basal increase in nucellar volume in some crassinucellate ovules is contributed by the apically located cells of the chalaza. The vascular trace to the ovule also terminates at the base of the chalaza; further branching and ramification of the integumentary vasculature, if present, is also seen in the chalaza both before and after fertilization (Krishnamurthy 2015). Most pronounced growth of the embryo sac invariably takes place only along the chalazal direction. Ovules of some species exhibit the differentiation of a histologically distinctive pad of cells in the chalazal region called *hypostase* (also called *postament*, *podium* or *pseudochalaza*) whose cells are often thick walled; it is believed to play a role in the supply of nutrients to the growing embryo sac, as well as in stabilizing the moisture status of the ovule.

Thus, the chalaza serves as an important topocentre of the ovule all throughout the development of the ovule and the seed. Data on molecular biology of ovule/seed development have indicated that the ovules develop and mature into seeds by maintaining a distinct proximo-distal axis (Grossniklaus and Schneitz 1998) and that this axis is characterized by a three-tiered arrangement of pattern elements: *distal* (or *nucellar*), *chalazal* (or *central*) and *proximal* (*funicular*) domains (Fig. 17.7). Chalazal domain can be further subdivided into two subdomains (Baker et al. 1997), based on its role in the production of either the inner or outer integument. About a dozen genes are already known to affect the integuments in *Arabidopsis* by operating at the chalazal domain (Table 17.1).

Table 17.1 Chalaza as a topocentre of operation of genes involved in ovule/seed development

S. no.	Wild-type genes whose mutant forms are known to be involved	Effect seen in mutants
1.	Inner no outer integument (*INO*)	Only inner integument formed; no outer integument
2.	*Bell 1* (*BEL 1*)	Both the integuments not organized; inner totally absent, while the outer represented by an amorphous entity or collar-like structure
3.	*Superman* (*SUP*) (also known as *FLO10* gene)	Development of outer integument affected; grows asymmetrically around the ovule
4.	*Short integument1* (*SIN1*) (maternal gene)	Integument development affected; no clear distinction between inner and outer integuments; they are extremely short. The gene also causes defects in embryo-like funnel-shaped cotyledon or masses of unorganized tissue
5.	*Aintegumenta* (*ANT*)	Lacks integuments. Also, there is no embryo sac formation
6.	*Huellenlos* (*HLL*)	Lacks integuments. Also, there is no embryo sac formation
7.	*Aberrant testa* shape (*ATS*)	Produces a single-fused integument and hence no distinction between the two integuments
8.	*Unicorn* (*UCN*)	Produces supernumerary integuments
9.	*Blasig* (*BAG*)	Affect cell division and cell shape in integuments
10.	*Strubbelig*	Affect cell division and cell shape in integuments
11.	*FBP7, FBP11* (MADS-box genes) (in Barley and Petunia)	Affect ovule determination and development in carpel; no endosperm development

All the genes in mutant form affect development (data compiled from different sources)

17.3.4 Archesporium, Megasporogenesis and Female Gametophyte

Although comprehensive reviews on the female gametophyte have been published previously (Maheshwari 1950; Swamy and Krishnamurthy 1980; Willemse and van Went 1984; Haig 1990; Huang and Russell 1992; Russell 2001; Yadegari and Drews 2004), in this article a consolidated account is provided laying emphasis on molecular biological aspects. The female *archesporium* is always differentiated at the nucellar apex in the hypodermal position. Invariably only one archesporial cell gets differentiated, although there are taxa where more than one cell may be formed in an ovule. It is a very conspicuous cell, larger than other nucellular cells, with a deeply staining cytoplasm, a large nucleus and high nucleolar RNA and with plasmodesmal connections with adjacent nucellar cells. It cuts off through a periclinal wall an outer parietal cell and an inner sporogenous cell as already detailed; it remains near the surface or fairly deep inside depending, respectively, on tenuinucellate or crassinucellate ovules. The sporogenous cell increases in size and becomes the *megaspore mother cell* (*MMC*) or *megasporocyte* (Fig. 17.8).

The MMC elongates parallel to the long axis of the nucellus. Just prior to the initiation of meiosis, the plasmodesmal connections that the MMC had with the nucellar cells are cut off, and a callose wall is deposited around it. A failure of callose deposition results either in female sterility or in unreduced apomictic development (Krishnamurthy 1977, 2015). The deposition of a callose wall is intrinsically related to the position of the functional megaspore and the type of *embryo sac* (female gametophyte) development. There is an unequal deposition of callose on the MMC, which, in fact, is related to the polarization of its organelles and the cell as a whole. Callose is laid down first and thickest on the portion of the wall of MMC where the functional

megaspore is destined to be formed, i.e. in the chalazal pole in monosporic *Polygonum*, chalazal half in bisporic *Allium*, micropylar pole in monosporic *Oenothera*, chalazal half in bisporic *Endymion* and all around MMC in tetrasporic types of embryo sacs. Very significant ultrastructural changes and regroupings of organelles of MMC form the hallmark of the changeover from the diploid to the subsequent haploid state. The mitochondria and plastids dedifferentiate at the early meiotic prophase only to redifferentiate at the megaspore tetrad stage; they also became highly dispersed and far removed from one another in early prophase and again get regrouped at the tetrad stage. The cytoplasmic and nucleolar RNA concentrations decrease with the onset of meiosis due to a prophase diminution or total loss of ribosome content. A new array of ribosomes is formed with the initiation of meiosis along with a steady increase in the number of nucleoli of the nucleus of MMC through budding. Initiation of the production of polysomes and appearance of paracrystalline inclusions are also seen towards the end of meiosis.

Three basic types of embryo sac development are conventionally recognized in angiosperms. In the monosporic type, the MMC undergoes the heterotypic division of meiosis to form two cells separated by a thick callose wall, each of which again divides (homotypic division) to form a tetrad of four haploid cells or megaspores. In the monosporic *Polygonum* type, the chalazal megaspore alone is functional, while in *Oenothera* type, the micropylar megaspore alone is functional. In the bisporic type, the MMC undergoes the heterotypic meiotic division to form a dyad where homotypic division proceeds either in the chalazal (*Allium* type) or micropylar dyad (*Endymion* type) only to form a binucleate (two megaspores) functional cell. In both the mono- and bisporic types, the non-functional megaspores/cells undergo PCD. The only functional megaspore (nucleus) in monosporic and the two functional megaspores (nuclei) in bisporic types further divide to form the mature embryo sac (female gametophyte). The major unanswered question in the above four subtypes of female gametophytic ontogeny is the following: what determines the selection of the functional megaspore (cell)? Although, as stated earlier, there are differences in the pattern of callose deposition, it is not known whether it is the cause or the effect of this determination. It is also to be mentioned that the non-functional megaspore-containing cells are always smaller than the functional cells and that there is a definite asymmetry involved in their production; asymmetric division is definite to decide the different fates of the two resultant cells. In the tetrasporic types, both the heterotypic and homotypic divisions of meiosis are not accompanied by cell walls, and hence, a cell with four megaspore (nuclei) called *coenomegaspore* is formed. All the four megaspores here contribute to the formation of the mature embryo sac. Thus, in these three basic types of female gametophytic ontogeny, the formation/identification of the functional megaspore(s) is varied with reference to the heterotypic or homotypic meiotic divisions; hence, these two divisions form an important criterion in embryo sac ontogenies. In view of this, a modified classification of embryo sac types was proposed by Swamy and Krishnamurthy (1975, 1980). Among the tetrasporic types, further classification is based on the total number of nuclei, their relative position in the mature embryo sac, nuclear fusion and the consequent ploidy of the involved components of the embryo sac, etc.

Once the required number of nuclei is formed during *megagametogenesis*, depending on the type of female gametophyte, their organization sets in (Fig. 17.8). Invariably, three of the nuclei at the micropylar pole organize into the cells of the *egg apparatus*, with an *egg cell* in the centre with two *synergids*, one on either side of the egg. Two nuclei move to the middle part of the embryo sac and get organized as part of the *central cell*; these two nuclei are the *polar nuclei*. At the

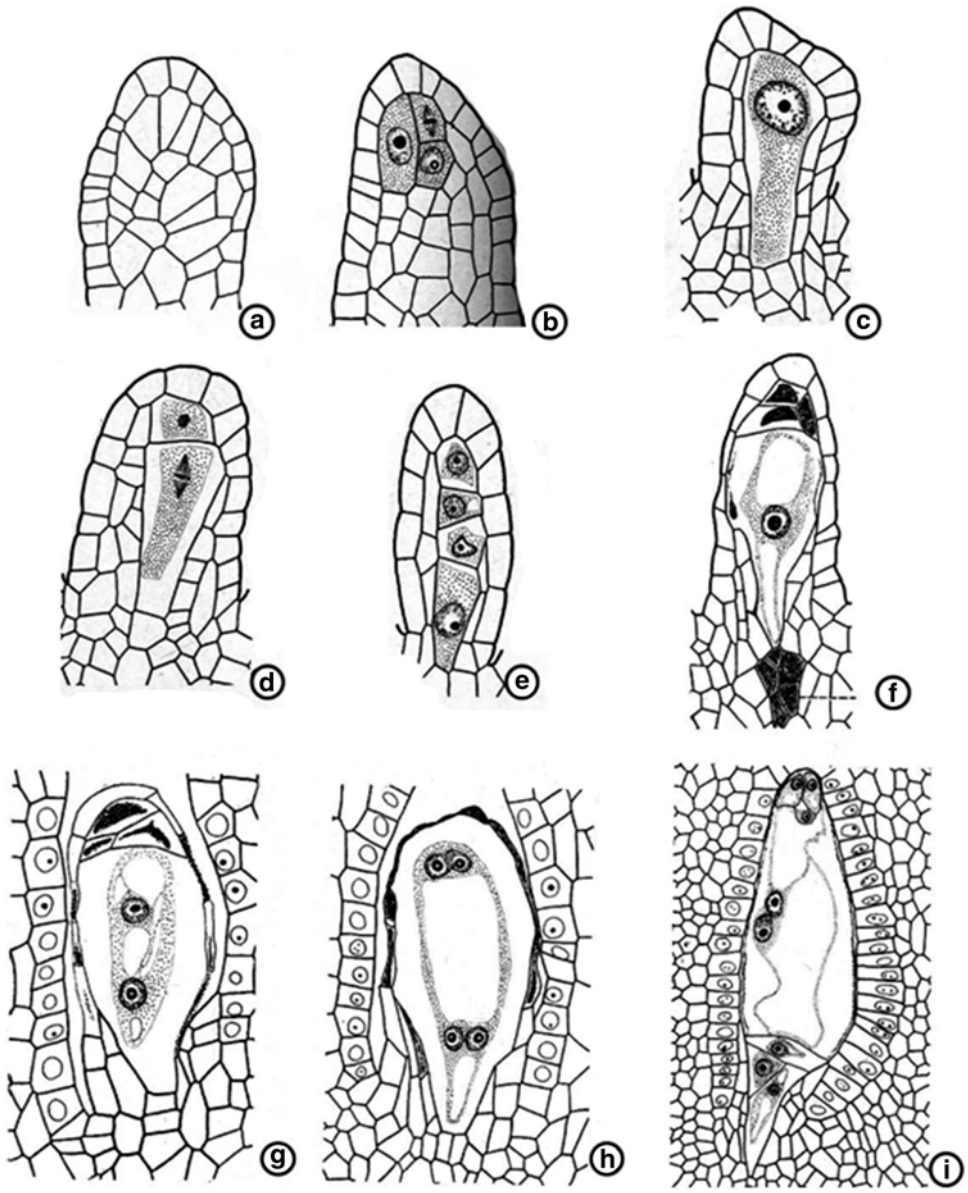


Fig. 17.8 Monosporic *Polygonum* type of embryo sac development as found in *Trachyspermum ammi* and *Cuminum cyminum*. (**a**) L.S. of ovule primordium. (**b**) L.S. of ovule primordium with three archesporial cells. (**c**) L.S. of ovule with enlarged archesporial cell. (**d**) Division of archesporial cell. (**e**) Megaspore tetrads. (**f**) Functional megaspore along with three degenerating megaspores. (**g**) Two nucleate embryo sac. (**h**) Four nucleate embryo sac. (**i**) Mature embryo sac showing egg apparatus at the micropylar end three antipodals at the chalazal end and a central cell with two polar nuclei (Sehgal 1965)

chalazal end of the embryo sac, three nuclei organize themselves into the *antipodal cells*. The egg nucleus is towards its chalazal end, while a large vacuole occupies its opposite end. The egg cell normally has a wall only at its micropylar facet with the chalazal region devoid of it. Whether a thin wall is formed at the chalazal region of the egg initially and then disappeared or whether from the beginning there is no wall in this region is a matter of controversy, since both conditions

have been known in literature. The absence of a wall is necessary for the easy transfer of male gamete from the synergid. The ER of the egg cell is oriented parallel to its plasmalemma over a large part of it, but in more numbers around the nucleus; ribosomes are also concentrated near the nucleus. A very prominent nucleus is seen in the egg cell. Large amount of cytoplasmic DNA is present in the egg cell along with the nuclear protein, histone.

The two synergids are saccate and pyriform, and their nucleus is situated at the micropylar end of the cell with a large vacuole occupying at its chalazal pole. The micropylar region has a characteristic structure called *filiform apparatus* (*FA*). The FA is a special type of wall ingrowth that is a characteristic of transfer cells. The wall ingrowths are finger- or platelike and are made of fibrous polysaccharides. They have a central core with tightly packed cellulose microfibrils surrounded by a sheath of non-microfibrillar material. The presence of FA functionally implicates the synergids as transfer cells, but the direction of translocation, whether towards or out of these cells, is not clear; it is more probable that it is out of these cells that substances, especially chemotropic substances, are released towards the micropyle probably to attract and direct the pollen tubes. Cell ablation studies in synergids of *Torenia fournieri* show their control on both pollen tube guidance and reception (Higashiyama et al. 2001). The material synthesized and released by the synergid consists of a homogeneous osmiophilic substance indicating its non-cellulosic composition, but it is likely to be a carbohydrate that shows positive reaction to periodic acid-Schiff's reagent. In *Nicotiana*, one of the two synergids becomes receptive to pollination and starts to accumulate more loosely bound calcium (Tian and Russell 1997). Synergids have a maximum concentration of ER towards their micropylar end, and its density gradually gets reduced towards the chalazal end; dictyosomes are more concentrated in the middle part of the cell, while spherosomes are evenly spread over the entire synergid cytoplasm (Swamy and Krishnamurthy 1980). Only one synergid is present in *Peperomia* type, while they are absent in the *Plumbago* and *Plumbagella* types of embryo sacs. In the latter two types, the egg cell itself has a filiform apparatus. Synergid haustoria are seen in some species.

The central cell is the largest cell of the female gametophyte. It normally has two polar nuclei lying juxtaposed to each other or exhibiting various degrees to fusion or total fusion to form a diploid *secondary nucleus*. Only one polar nucleus is present in the *Oenothera* type, while more than two nuclei occur in *Penaea*, *Plumbago* and *Peperomia* types. The central cell is highly vacuolated, and its cytoplasm has a high amount of active dictyosomes and ER, especially around the nuclei. There is abundant cytoplasmic RNA, mostly ribosomal. Free ribosomes and many mitochondria are present, especially near its micropylar end. The vacuole(s) of central cell is a major reservoir of sugars, amino acids and inorganic salts. Starch is abundantly present in the cytoplasm of the central cell. The wall of central cell in maize is multilayered and pectocellulosic, while in cotton it is rich in pectic substances. In many taxa, the lateral walls show transfer cell morphology. Normally, there are three antipodal cells or nuclei located at the chalazal end of the embryo sac. Antipodal cells with more than one nucleus, syncytial antipodals, endoreduplicated antipodal nuclei (reaching up to 1024C level) showing polytenic chromosomes and/or nucleolar DNA amplification, increased number of antipodals (up to about 300 reported in the grass, *Sasa paniculata*) or ephemeral antipodals characterize some taxa. The antipodal cells often have been suggested to play some role in the control of endosperm development, at least in some grasses, although often considered as inert structures without any obvious function. Some fairly recent studies indicate their involvement in secretion, absorption and transport of nutrients to the central cell before and after fertilization. This is evident from the presence of active nucleoli, ribosome-polysome pattern, active ER, transfer cell morphology, plasmodesmata (between antipodals and central cell), etc. Antipodal haustoria develop in some taxa.

17.3.5 Gene Expression in Female Gametophyte Development and Function

The developing and the fully developed female gametophytes are physiologically very active and probably express hundreds of genes. Gene expression during megagametogenesis has a dual function: (1) orchestrating the female gametophytic programme during the division of the functional megaspores and (2) assigning characteristic fates to the female gametophytic cells formed (see Raghavan 2000). Although until the mid-1990s the *gametophytic factor1* (*gf1*) mutant alone was described in *Arabidopsis*, in the subsequent years, a large number of gametophytic mutants have been isolated; such mutants are very difficult to identify since half of the genome of embryo sac carries a wild-type allele such that the plants appear fertile (Brukhin et al. 2005). These mutants are deficient in one or more of the developmental processes/functions ascribed to the embryo sac. With the introduction of protocols for marked insertional mutagenesis and studying chromosomes carrying multiple markers, the process of identification of gametophytic mutants has become easier (see Brukhin et al. 2005 for more details on the protocols). There is a differential gene expression in the different cells of the embryo sac, although derived from a single source, as indicated by differences in mRNA profiles and ribosomal populations of these cells. There is also a differential gene expression at different stages of gametophytic development such as megaspore specification, initiation of megagametogenesis, mitotic progression, establishment of polarity, migration of polar nuclei to the centre of the embryo sac, fusion of polar nuclei, cellularization of the components nuclei of the embryo sac, antipodal cell death and degeneration of synergids (Brukhin et al. 2005). Steffen et al. (2007) have identified 71 *Arabidopsis* genes through a differential expression screen based on reduced expression in *determinant infertile1* (*dif1*) ovules which lack female gametophytes. Of these, 11 were exclusively expressed in the antipodals, 11 exclusively or predominantly in central cells, 17 exclusively or predominantly in the synergid cells, one exclusively in egg and three in multiple cells. Most of the gametophytic mutants have been isolated from *Arabidopsis*, but a few have also been known from maize (Table 17.2).

Table 17.2 Embryo sac mutants known

S. no	*Arabidopsis thaliana* mutant class	Name of mutants
1.	Mitotic	*ada*, *agp18*, *ana*, *ant*, *apc2*, *astlik* (*alk*), *bel1*, *cki1*, *eda1-eda23*, *emd fem2*, *fem3*, *fem5*, *fem9-fem16*, *fem18-26*, *fem 29-fem31*, *fem33-fem38*, *gfa4*, *gfa5*, *gfl*, *hma*, *kupalo* (*kuo*), *msd*, *nomega*, *prolifera* (*prl*), *rbr1*, *sin*, *swa1*, *tya*
2.	Karyogamy	*amon* (*amn*), *apis* (*aps*), *eda24-eda41*, *gfa2*, *gfa3*, *gfa7*, *nan*, *pri*.
3.	Cellularization	*dam*, *fem4*, *fem6-fem8*, *fem11*, *fem13*, *fem15*, *gfa2*, *gfa3*, *jum*, *nja*, *wlg*.
4.	Degeneration	*gfa2*, *fem1*, *fem14*, *nan*, *yarilo* (*yar*)
5.	Fertilization	*fer*, *srn*, *une1-une18*
6.	Maternal effect	*ash*, *aya*, *bga*, *cap1*, *cap2*, *ctr1*, *didilia* (*did*), *dme*, *fie*, *fis1* (*mea*), *fis2*, *fis3* (*fie*), *kem*, *lpat2*, *mea*, *mea1-mea70*, *prl*, *zal*
	Zea mays mutant class	Name of the mutants
7.	Mitotic	*ig*, *hdd*
8.	Fertilization	*zmeal*
9.	Maternal effect	*mel1*

Most mutants fall under the mitotic class. These mutants affect the initiation or control and regulation of any of the three mitotic divisions involved in embryo sac ontogeny from the functional megaspore in *Arabidopsis* (Polygonum type). The phenotypic effects of these mutants are the unusual number of nuclei or an aberrant distribution of nuclei in the developing embryo sac (Christensen et al. 1997, 1998, 2002; Pagnussat et al. 2005). The most important and interesting mutant of this class is *nomega*, where the embryo sac is arrested at the two-nucleate stage. This mutant illustrates how variable expressivity of a mutation influences the degree

of segregation ratio distortion and ovule sterility. Although *nomega* is a gametophytic mutant and 50 % of the ovules should contain mutant embryo sacs, only 30 % of ovules were aborted (Kwee and Sundaresan 2003). The *NOMEGA* gene product shows a high degree of homology to the *APC6*/*CDC16* subunit of the anaphase-promoting complex and is involved in chromosome separation (and cytokinesis). It is to be mentioned here that *APC*/*C* functions as an E3 ubiquitin ligase in the ubiquitin-mediated proteolysis pathway, which controls several key steps in the cell cycle. Another mutant of this class is *hadad* (*hdd*) (Moore et al. 1997). Cellularization and mitotic progression are not coupled to each other in this mutant indicating that the developmental programmes controlling nuclear division and cellularization are independent. The second class of embryo sac mutants is the *karyogamy class* mutants. In these mutants, the fusion of polar nuclei is arrested, and there is often a delay in the degeneration of antipodals and synergids (for a feature of another class of mutants, see below) (Christensen et al. 2002). The most important mutant of this class is the *gfa2* mutant. The *GFA2* gene encodes a J-domain-containing protein which is associated with mitochondrial function involved in nuclear fusion. The *cellularization class* of mutants forms the third class of mutants and they show defects in cell formation around embryo sac nuclei once they are formed; they also affect cell polarity and cell shape (especially of the egg and synergids) depending on the mutant (Moore 2002; Christensen et al. 1998). The *fem4* mutant is a good example of this class; in this mutant, the egg cell is not pear shaped and the synergids have altered polarity and shape. The *degeneration class* mutants show defects in the degeneration of three non-functional megaspores, the synergids and/or the antipodals and are probably involved in the PCD process. The *gfa2* mutation that affects karyogamy can also belong to this class. It affects synergid degeneration and the failure of polar nuclei fusion (Moore 2002). In the *fertilization class* of mutants, the pollen tube does not stop after entering into one of the synergids but continues to grow and fails to release its contents; or many tubes enter into the embryo sac, but none is involved in fertilization (Huck et al. 2003). The mutants *feronia* (*fer*) and *sirene* (*srn*) are examples of this class (Rotman et al. 2003); in these, the embryo sac development is normal. The *maternal-effect class* of mutants shows their effects after double fertilization and details on these are provided in Chap. 18 of this volume.

Genetic studies have revealed functions for several type I MADS-box genes in embryo sac development (Masiero et al. 2011) (type I MADS-box genes are a heterogeneous group and have only the ~180 bp DNA sequence encoding the MADS domain in common – see Smaczniak et al. 2012). A large-scale expression analysis revealed that 38 out of 61 type I MADS-box genes are active in female gametophyte (and seed) development (Bemer et al. 2010; Wuest et al. 2010). Some of them exhibit highly specific expression patterns in particular cells. However, for many of them, no direct function has been given so far, probably due to genetic redundancy. The AGL80 protein and DIANA (DIA; AGL61) protein form a functional protein dimer and control the differentiation of the central cell (Steffen et al. 2008).

17.4 Double Fertilization

Fusion of male and female gametes is *fertilization*. In the gymnosperms, of the two male gametes contained in the pollen tube, one fuses with the egg and the other degenerates, and hence, there is only *single fertilization*. In contract, in angiosperms, both the sperms in the pollen tube are involved in fertilization, the first with the egg (*syngamy*) to result in *zygote* and the second with the polar nuclei/secondary nucleus (*triple fusion*) to result in *primary endosperm nucleus* (*PEN*). This process is *double fertilization,* and it is an exclusive and defining feature of the angiosperms (Raghavan 2003). Double fertilization provides not only the required stimulus for embryo and endosperm development but also for the development of the ovary into fruit and of the ovule into seed. Events that happen prior to double fertilization in the stigma, style, pollen on the stigma,

pollen tube in the style and ovule and in the different components of the embryo sac are all very important for viable double fertilization and post-fertilization changes. These changes are due to long-distance signalling between pollen (on the stigma) and the female tissues. These events are often covered under *progamic phase* (Raghavan 2000). Auxin and ACC, the precursor of ethylene, can partly mimic the progamic event effect, but other yet unidentified pollination factors are needed to induce the full postpollination syndrome (Zhang and O'Neill 1993; O'Neill 1997). A large body of knowledge concerning double fertilization have already been reviewed (Lord and Russell 2002; Willemse and van Lammeren 2002; Higashiyama et al. 2003; Raghavan 2003; Weterings and Russell 2004), and only the most notable information are provided here.

17.4.1 Pollen on the Stigma

Pollination brings the pollen to the stigma. Pollen adhesion to the stigmatic surface is determined by the degree of wetness and/or the surface features of the stigma and pollen exine. Stigmas are classified into *wet stigma*, characterized by stigmatic exudates produced either by the stigma itself or by the stylar canal cell from where it gets transported to the stigma surface, and *dry stigma,* where the stigmatic papillae are invested on their surface by a proteinaceous *pellicle* (a physiological equivalent of stigmatic exudate). Wet stigma is generally important for two-celled pollen, while the dry stigma is important for three-celled pollen. The pellicle contains, in addition to proteins, amino acids, lipids, phenolics, sugars, minerals, water, CA^{2+}, etc. and shows high esterase, acid phosphatase and a few other enzyme activities, which are also reported in stigmatic exudates.

The time interval between pollination and pollen germination varies in different species, relatively immediately in herbaceous taxa but generally after a long time in arborescent taxa. Correspondingly, the rate of pollen tube growth is faster in herbs than in trees, and the time between pollination and fertilization is shorter in the former and prolonged (over days or even months) in the latter. It was already mentioned that the pollen grains are dehydrated during release from the anther, but once they land on the stigma, they get hydrated from stigmatic exudates/pellicle and undergo volumetric increase or *harmomegathy*. The rapidity of pollen hydration depends on the nature of stigma, gradual and slow in dry type and rapid in wet type. For example, in rye, within 3 min after arriving on the stigma, the pollen may take up 6×10^{-8} cm^3 of water indicating a flow of 3.5×10^{-10} cm^3/s^{-1} at the pollen-stigma interface. Soon after hydration, rapid changes take place in the vegetative cell of the pollen. The pollen wall proteins are released onto the stigma and the range of proteins released is very wide. Around 26 different proteins have been known to be released from rye and around 40 proteins in *Brassica oleracea*, when compared to control pollen not kept in contact with stigma. Since some of these proteins released by pollen are highly phosphorylated, it is possible that protein phosphorylation could account for signal transduction in compatible pollen transfer. To start with, these proteins do not get bound to the stigma but soon do so to initiate a close interaction. Since the stigma receives various kinds of pollen grains, some compatible and most others incompatible, there must exist some kind of a physiological mechanism to ensure that only compatible pollen grains are allowed by the stigma to germinate and produce an effective pollen tube. This mechanism is often referred to by the term *recognition*. Compatibility or otherwise has been shown to be mainly the result of interaction of the proteins released by the pollen with the proteins of the stigma (pellicle or exudate), and this reaction is similar to the antibody-antigen reaction. Ca^{2+} is also involved in pollen recognition-rejection reaction by serving in cell signalling. Hence, compatible pollen grains produce transient CA^{2+} peaks in the stigmatic papillae (for instance, in *Brassica napus*) adjacent to the applied pollen grains. In some cases of incompatibility, pollen tube may pass the stigma but are arrested in the style. Studies in *Arabidopsis* mutants have shown that pollen-stigma interactions are regulated by specific components of the pollen wall tryphine. In the male-sterile *pop1* (for

'defective in pollen-pistil interaction') mutant, the pollen grains fail to get hydrated on the stigma and germinate, although under *in vitro* conditions they are able to germinate and hence non-germinability is not due to loss of viability/fertility. The mutant grains lack long-chain lipids as well as tryphine. *CER1* locus mutants of *Arabidopsis* also have pollen that do not hydrate on the stigma and lack tryphine with the normal component of lipids. The cytological changes in the pollen immediately after hydration are also striking. The plasma membranes and other membrane systems become more resolvable, the mitochondria regain normal appearance, profiles of ER appear, vacuolation begins with normal tonoplasts around the vacuoles, protoplasmic streaming revives, etc. The vegetative nucleus moves towards the germinal pore – just before the formation of the pollen tube.

17.4.2 Pollen Germination and Pollen Tube

The metabolic changes associated with pollen germination include efflux of metabolites and increased respiration and rates of RNA and protein synthesis. In many species, the mature pollen also contains stored mRNA that codes for the first proteins needed for germination and pollen tube growth, although they are not enough to code for all the essential proteins required. In some taxa, rRNA and tRNA are produced during germination. A battery of genes that are needed to produce cell wall degrading enzymes like polygalacturonase, pectin lyase and pectin esterase as well as other enzymes like ascorbic acid oxidase, receptor kinases, etc. is also activated. Pollen germination also involved the formation of a pollen tube and, hence, the nature and mode of action of cytoskeletal elements (actin filaments or MFs and microtubules or MTs) that provide the motive force for germination are of great importance (Tiwari and Polito 1990a, b). Both cytoskeletal elements are organized as short fibres inside the pollen grain, and these represent their precursors either as reservoirs of protein subunits or as units for the assembly of longer filaments (Cai et al. 2005a, b). The loose network of MFs occurring throughout the vegetative cell is soon replaced by an entangled web of fibrils converging towards the germinal aperture. Both these cytoskeletal elements organize themselves as longer bundles and enter into the emerging tube. In the tube, they are mainly structured in bundles that approximately have the same direction as the tube axis. MTs are more abundant in the terminal part of the tube close to the growth region. Although the synthesis of new actin and tubulin may take place during tube growth (Mascarenhas 1990; Sorri et al. 1996), the level of actin and tubulin during tube elongation is steady. The polarization of both cytoskeletal filaments is initiated with the emergence of the pollen tube. We, however, do not have any information on the organization sites of these.

A part of the intine confronting the germinal area protrudes and grows out as the pollen tube. Local secretion of hydrolytic enzymes is involved in wall dissolution confronting this area. Thus, pollen tube is not a real cell but a transporter of the sperms to the female gametophyte. Most generally, a single tube is formed from a pollen grain, but more than one tube (up to 14) as well as branching of the single tube (but only one branch carries the sperms) are reported in some taxa. A fairly recent model recognizes four distinct overlapping cytological zones (but not rigidly fixed zones) in the pollen tube (although not in all plants): an apical growth zone, a nuclear zone, a zone of vacuoles and a callose plug zone. The unipolar growth of the tube is restricted to the apical growth zone of about 4–7 μm where local secretion of wall materials takes place. The tube wall is made up of cellulose embedded in a noncrystalline polysaccharide matrix, probably pectin. Callose is absent in the wall at tube tip, but forms a layer inside the tube wall a little behind the tip. Thus, the wall at the proximal part of the tube has pectin in the outer, cellulose in the middle and callose in its inner layers. Immunofluorescence cytochemical studies using pectin monoclonal antibodies have shown two patterns of pectin deposition, one as periodic annular deposits found coating the pollen tube walls of species with solid styles and the other as a more uniform sheath as in tubes of species with hollow styles. Pollen tube

tip is richer in esterified pectins than the proximal parts. The plasma membrane at the growing tube tip is connected to the tubular and smooth ER. Mitochondria, amyloplasts and secretory Golgi bodies and vesicles produced by them are present in abundance. Golgi-derived vesicles are of two types: one is 0.1–0.3 μm in diameter, bound by unit membranes and rich in polysaccharides and the other is 0.01–0.05 μm in diameter and rich in RNA. The former play an important role in building the wall at the growing tip (3,000–5,000 vesicles are produced per minute), while the function of the latter is unknown. The wall materials are polar transported to the tube tip through cytoplasmic streaming, as evidenced by studies using cytochalasin B which inhibits wall deposition, tube growth and streaming but not vesicle production; also the apical zone is without streaming as otherwise the vesicles would be retransferred to the basal part of the tube and would not be available for wall growth at the tube tip. Proton microprobe analysis, fluorescence using chlortetracycline that selectively fluoresces Ca^{2+} ions, and ^{45} Ca autoradiography reveal that the pollen tube growth is also associated with polar electric currents and polar distribution of CA $^{2+}$ ions. The tube tip cytoplasm also has enzymes like phosphatases, amylases, dehydrogenases, invertase, oxidases, transferases, pectinase, synthetases, lyases, ligases and lipases.

Attention was drawn already to the cytoskeletal elements that accumulate towards prospective tube-emerging germ pore region of the pollen and the way in which they are present. Thus, the pollen tube, from the beginning, contains filamentous cytoskeletal components that form the structural basis of its internal organization (Cai et al. 2005b). They regulate and promote most of their biological functions, the most important of which is transporting the sperms towards their correct destination. They control tube growth and help the tube's cytoplasm to dynamically reorganize itself during tube growth. The presence of microtubules in the growth region is debated. Immunocytochemical studies have demonstrated their presence as short and twisted structures. EM studies have not demonstrated them there; probably they are not produced there (Del Casino et al. 1993; Lancelle et al. 1987; Cai et al. 2005b). Actin bundles that are present in the tube cytoplasm (Tang et al. 1989) are likely to be generated by the action of villin-like proteins (Vidali et al. 1999) but are not present in the tube apex where only a mesh of short actin filaments (G-actin) are present (Miller et al. 1996). The main aspect of pollen tube growth regulation process is the transformation of the G-actin of the tube axis to the actin bundles of pollen tube body. Ca^{2+} is a central factor in this transition as it controls many distinct activities such as helping in fusion of secretory vesicles in the tube tip, polymerization of actin in cooperation with other protein factors, conversion of actin filaments into bundles, inhibition of myosin activity, etc. The model of Cai et al. (2005b) based on the above details is given in Fig. 3.8, but the role of microtubules is not included in it for want of sufficient data. Microfilaments, however, are implicated by Cai et al. (2005a, b) in apical secretion and thus in tube elongation, cytoplasmic streaming and organelle transport, directional movement of cell wall materials and transport of sperms.

17.4.3 Pollen Tube Growth Through Gynoecial Tissue

The pollen tube grows penetrating the cuticle of stigma surface cells, perhaps through the productions of cutinase, and enters into the stigma. Some studies indicate that the tube does not penetrate the cuticle of stigma papillary cells from which the surface exudates or pellicle is removed enzymatically indicating that the pollen grain produces a precursor of the cutinase enzyme, which then gets activated by a 'factor' present in the stigmatic exudate/pellicle. If the stigma cells are ablated by the introduction, a stigma-specific gene fused to the cytotoxic *BARNASE* gene, a few pollen grains germinate on the stigmatic surface, but the pollen tubes do not penetrate into the style. This block to penetrate into style becomes totally restored by the application of an exudate of the wild-type stigma. The vital factor(s) in the exudates necessary for pollen tube penetration appears to be lipids, probably cis-unsaturated tri-

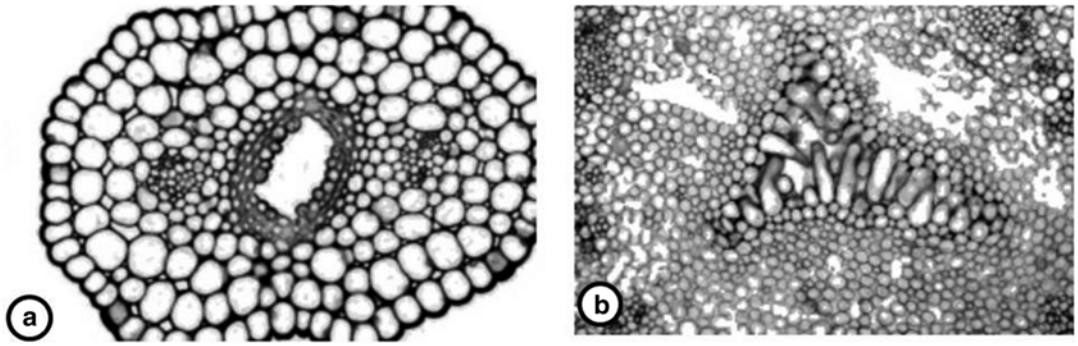


Fig. 17.9 (**a**) T.S. of hollow- or open-type style of showing canal cells or transmitting tissue living the canal, over the surface of which pollen tubes grow towards the ovule. (**b**) T.S. of solid- or closed-type style of showing loosely packed transmitting tissue cells in between which the pollen tubes grow towards the ovule (Leins and Erbar 2010)

glycerides. In the presence of these lipids, pollen tubes are even able to penetrate leaves, and hence, lipids appear to decide the recognition/rejection reaction. Studies carried out also indicate that the components of this pollen recognition system are present even in other floral organ, but are segregated to the stigmatic surface by the action of genes such as *FIDDLEHEAD* (*FDH*) of *Arabidopsis*.

Once the pollen tube crosses the stigma to enter into the apical part of the style, its further growth depends on the structure of the style. In styles with a centrally located canal (*hollow* or *open style*), the glandular cells lining the canal, called *canal cells*, act as the transmitting tissue to guide the pollen tube's ectotrophic growth along their surface (Fig. 17.9a) (e.g. Liliaceae members). The canal cells have an 8–14 um thick secretory zone on the side facing the canal. The canal cells are often multinucleate/polyploidal commensurate with their secretory activity. Their thin transverse walls have plasmodesmata, while the longitudinal walls are thick. The material secreted by the canal cells are released into the canal. In solid or closed styles (e.g. Malvaceae, Solanaceae), the pollen tube passes in the intercellular substance present in between cells located in the central region of the style, and all these cells are considered as making up the transmitting tissue (Fig. 17.9b). A half-closed type of style is reported in some members of Cactaceae and in *Artabotrys* (Annonaceae), where there is only a rudimentary type of transmitting tissue. Irrespective of the style type, the style supplies regulatory substances, boron and nutrients in the form of sugars, proteins, lipids and minerals necessary for pollen tube growth. The intercellular substance of solid style and the secretion of hollow style are also very important in controlling incompatibility reactions. Especially important in this connection are the arabinogalactan-rich and hydroxyproline-rich proteins (extensins), which are found through immunocytochemistry in the extracellular matrix of transmitting cells and which are very important players in deciding the compatibility or otherwise of the tubes.

Some experiments have shown that the growth of the pollen tube through the style is mediated not only by the stylar matrix and its chemicals but also by electrical or mechanical signals that interact with the stylar matrix (Raghavan 2000). As per a model for tube direction proposed, pollen tube growth through style is reckoned as an authentic directional cell substrate adhesion molecule present in the stylar matrix. This adhesion molecule is shown to be homologous to human vitronectin.

Soon after crossing the style, the pollen tube generally grows along the placenta inside the ovary and reaches the funicular region from where it grows into the micropyle. Such a growth into the micropyle is often guided by a special glandular structure called *obturator*, which may be funicular, ovary wall or placental in origin

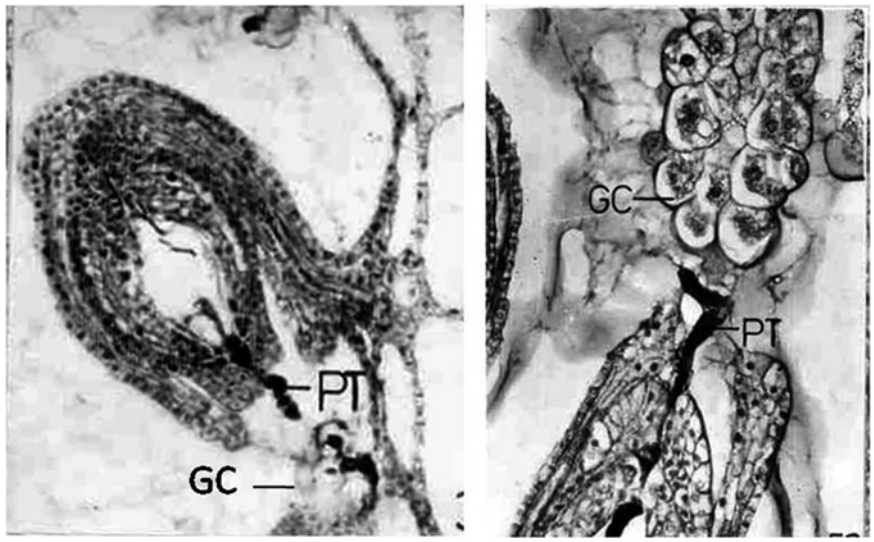


Fig. 17.10 L.S. of portion of an ovary of *Ottelia alismoides* showing the guidance of pollen tube (*PT*) by the ovary wall obturator (*GC*) (Photography courtesy of Dr. R. Indra)

(Fig. 17.10). In the vast majority of taxa, the pollen tube enters into the ovule through the micropyle (*porogamy*), while in a few others (e.g. Casuarinaceae), it passes through the raphe along the vascular trace, reaches the chalaza and grows (often after branching) towards the embryo sac (*chalazogamy*). Some believe that the entry of the pollen tube into the embryo sac is done through the enzymatic secretions of the tube that dissolve the embryo sac wall at the point of contact, but others, on the basis of EM studies, have shown that the synergid secretions cause the pore on the embryo sac through which the pollen tube enters. The synergid that receives the pollen tube is believed to show signs of degeneration long before the entry of pollen tube into it, probably to provide least resistance to its entry (Higashiyama et al. 2000), while the other is still intact. It is also believed that the FA of this synergid, as already stated, controls chemotactically this entry. On entering into this synergid, the tube abruptly stops (what causes this sudden cessation of growth is not known), a subterminal or terminal pore is formed in the tube or tube tip gets ruptured (Weterings and Russell 2004) and its contents are emptied into the cytoplasm within 2–3 min (Rotman et al. 2003) (Fig. 17.11). The release of contents is believed to be due to low oxygen tension in the embryo sac. The other synergid usually undergoes a slight enlargement before fully degenerating (Krishnamurthy 2015).

Some information must be provided at this point on pollen tube attraction and its guidance from the stigma to the embryo sac. Initially, only a single chemical cue emanating from the ovule was suggested in the above function (Mascarenhas 1993; Johnson and Preuss 2002). But since there is a long distance between stigma and embryo sac, several consecutive cues might be required (Lush 1999). In the transmitting tissue and the ovary wall, the pollen tube growth might be governed by extracellular matrix components and arabinogalactan-rich proteins, respectively (Lennon et al. 1998; Sanchez et al. 2004). From the ovary wall/septum/placenta, the pollen tube is attracted towards the ovule and enters into the micropyle, and this involves attraction from both sporophytic and gametophytic tissues (Hülskamp et al. 1995; Shimizu and Okada 2000). Septum-to-integument GABA gradient produced by sporophytic tissues of *Arabidopsis* (Palanivelu et al. 2003) has been implicated. Also the mature and fully formed female gametophyte may involve guidance factors produced separately by funcicle and micropyle (as evident from studies on floral gametophytic *megamata* mutants) (Shimizu and

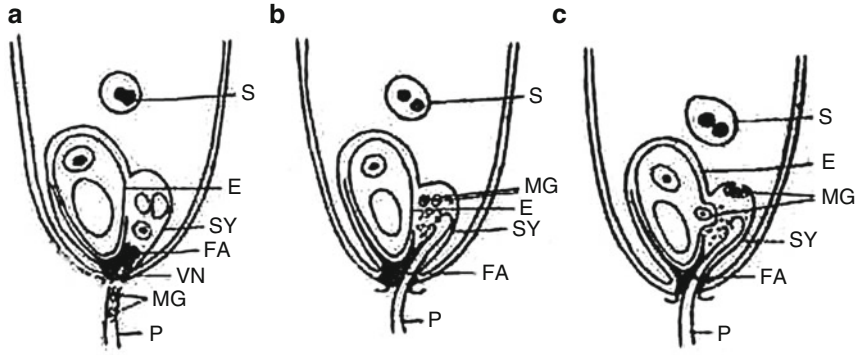


Fig. 17.11 Diagrammatic representations of the entry of the pollen tube into the embryo sac (**a**), discharge of the male gametes into one of the two synergids (**b**) and their subsequent migration to their respective destinations (**c**). *E* Egg, *FA* filiform apparatus, *MG* male gametes, *P* pollen tube, *S* secondary embryo sac nucleus, *Sy* synergid, *VN* vegetative nucleus (Jensen 1973)

Okada 2000). Culture of isolated embryo sacs of *Torenia fournieri* is shown to attract pollen tubes from a distance of ~100–200 μm (Higashiyama et al. 1998, 2001, 2003). Laser ablation of both synergids alone failed to attract pollen tubes showing that synergids are the sources of pollen tube attraction signal and that the competence of pollen tube to respond to this directional signal requires its growth in the gynoecial tissue. The identity of the synergid-derived attractant is not yet clear, but it may be calcium as indicated earlier; calcium alone is not likely to be involved, but sugars or peptides could also be involved (see detailed literature in Weterings and Russell 2004). Studies made on *feronia* (*fer*) mutant of *Arabidopsis* indicate that the signal from synergid might be lost rapidly so as to allow only one tube into each ovule to avoid polyspermy and/or heterofertilization (syngamy and triple fusion through sperms from two different pollen tubes) (Huck et al. 2003). Hence, according to these authors, repulsion of additional pollen tubes is not the likely operating factor here as initially thought of by Shimizu and Okada (2000). Since the other synergid is intact, both the rapid-loss-of-signal theory and repulsion theory must be verified with further work before accepting/rejecting either of them or both of them (Weterings and Russell 2004).

But what about the receptors of the pollen tube that perceive the signal(s)? It is likely that a family of Rho small GTPases (Rop) may be involved in changing growth direction of pollen tubes (Yang 2002) and in maintaining polar growth. Rop regulates a tip-focused cytosolic Ca^{2+} gradient, promoting the formation and dynamics of tip-localized F-actin (Li et al. 1999; Fu et al. 2001). It is likely that in response to directional cues from the ovule and synergid, Rop localization and activation are reoriented in the direction of this signal, i.e. towards the micropyle (Zheng and Yang 2000). Further studies are needed to verify this model.

17.4.4 Syngamy and Triple Fusion

In some taxa, the vegetative nucleus remains in the pollen grain itself and does not move into the pollen tube. In two-celled pollen, the GC and, in the three-celled pollen, the two male gametes enter into the tube. The cytoskeletal elements are responsible for directing their movement inside the tube. Wherever the vegetative nucleus enters into the tube, it is also carried by the action of cytoskeletal elements to varying distances in the tube and it lies in the neighbourhood of GC/sperms. It does not undergo DNA synthesis and degenerates sooner or later through PCD; rarely, it greatly enlarges in size and becomes lobed and polyploidal. The exact function of VC is not known, although some believe that it coordinates the delivery of the two sperms to the female gametophyte (Weterings and Russell 2004). The

two sperms derived from GC always lie close to one another and invariably are connected through plasmodesmata. Such a close unit is often designated as 'male germ unit', and this unit condition might help in easy sperm delivery. Of the two sperms formed, one is larger, rich in mitochondria and poor in plastids, while the other has the contrasting features. The former is likely to be involved in triple fusion, while the latter in syngamy (Weterings and Russell 2004). This is called *preferential fertilization*.

Immediately after the pollen tube contents are released into a synergid, pores are formed in the plasma membrane between this synergid and the egg as well as between it and the central cell. The sperms are definite cells; they soon get separated from one another. Two actin 'coronas' are shown to be formed from the middle of this synergid, one terminating near the egg nucleus and the other near the central cell (Weterings and Russell 2004). These 'coronas', together with myosin that is acquired on the surface of sperm cells, appear to mediate sperm movement. Probably as a result of this, the sperms show repeated changes in shape, which, according to some, indicates that they reach their destinations through autonomous movement, probably directed through their microfilaments. Soon after double fertilization, the actin 'coronas' disappear (Fu et al. 2000). The mechanism of syngamy and triple fusion has been discussed according to *mitotic hypothesis* and in connection with cell cycle events (Batygina and Vasilyeva 1998; Weterings and Russell 2004). For successful fertilization, the cell cycles of the male and female gametes must be synchronized; the two nuclei fuse in either *G1* or *G2* depending on the species. Hence, the sperms at the time of dispersal of three-celled pollen may be in *G1*, *G2* or *S* phase (Friedman 1999). In bicelled pollen cell cycle, synchrony between the two sperms and the female target cells is achieved in the pollen tube. In maize and many members of Poaceae, gametes tend to fuse in *G1*, while in others in *G2*. In tobacco, the pollen is disseminated in the two-celled stage with the GC possessing 2C DNA complement (*G2*). In the pollen tube (after 8–12 h subsequent to pollination), the GC divided to result in two sperms, which are in 1C condition (*G1*), as it approaches the ovary part. In the degenerated synergid, the sperms complete the *S* phase and appear to fuse only when they enter *G2*. The sperm destined to fuse with the egg nucleus reaches earlier, probably because of the shorter distance it has to travel, but *syngamy* is completed much later than *triple fusion*. The product of syngamy is the diploid zygote. Increased cytoplasmic calcium in the egg cell is necessary and sufficient to induce egg activation; whether this increase induced during fertilization is caused by the sperm fusion event or by a factor present in the sperm cytoplasm is not clear. The sperm that is involved in triple fusion travels to the central cell where it fuses with the polar nuclei/secondary nucleus to result in primary endosperms nucleus (PEN).

References

Aarts MG, Hodge R, Kalantidis K, Florack D, Wilson ZA, Mulligan BJ, Stiekema WJ, Scott R, Pereira A (1997) The Arabidopsis MALE STERILITY2 protein shares similarity with reductases in elongation/condensation complexes. Plant J 12:615–623

Adamczyk BJ, Fernandez DE (2009) MIKC* MADS domain heterodimers are required for pollen maturation and tube growth in Arabidopsis. Plant Physiol 149:1713–1723

Albrecht C, Russinova E, Hecht V, Baaijens E, de Vries S (2005) The Arabidopsis thaliana somatic embryogenesis receptor-like kinases1 and 2 control male sporogenesis. Plant Cell 17:3337–3349

Ariizumi T, Toriyama L (2011) Genetic regulation of sporopollenin synthesis and pollen exine development. Annu Rev Plant Biol 62:437–480

Ariizumi T, Hatakeyama K, Hinata K, Sato S, Kato T, Tabata S, Toriyama K (2003) A novel male-sterile mutant of Arabidopsis thaliana, faceless pollen-1, produces pollen with a smooth surface and an acetolysis-sensitive exine. Plant Mol Biol 53:107–116

Baker SC, Robinson-Beers K, Villanueva JM, Gaiser JC, Gasser CS (1997) Interactions among genes regulating ovule development in Arabidopsis thaliana. Genetics 145:1109–1124

Batygina TB, Vasilyeva VE (1998) Sexual reproduction in flowering plants: periodization of egg cell and zygote development and possible types of karyogamy. In: Bhatia B, Shukla AK, Sharma HL (eds) Plant form and function. Angkor Publishers (P) Ltd, New Delhi, pp 170–198

Beals TP, Goldberg RB (1997) A novel cell ablation strategy blocks tobacco anther dehiscence. Plant Cell 9:1527–1545

Bemer M, Heijmans K, Airolde C, Davies B, Angenent GC (2010) An atlas of type I MADS box gene expression during female gametophyte and seed development in Arabidopsis. Plant Physiol 154:287–300

Blackmore S, Worthey AH, Skvarla JJ, Rowley JR (2007) Pollen wall development in flowering plants. New Phytol 174:483–498

Bowman F (1984) The ovule. In: Johri BM (ed) Embryology of the Angiosperms. Springer, New York, pp 123–157

Brownfield L, Hafidh H, Borg M, Sidorova A, Mori T, Twell D (2009a) A plant germline-specific integrator of sperm specification and cell cycle progression. PLoS Genet 5:e1000430

Brownfield L, Hafidh H, Durbarry A, Khatab H, Sidorova A, Doerner P, Twell D (2009b) Arabidopsis DUO POLLEN3 is a key regulator of male germline development and embryogenesis. Plant Cell 21:1940–1956

Brukhin V, Curtism MD, Grossnicklaus U (2005) The angiosperm female gametophyte: no longer the forgotten generation. Curr Sci 89:1844–1852

Cai G, Casino CD, Romagnoli S, Cresti M (2005a) Pollen cytoskeleton during germination and tube growth. Curr Sci 89:1853–1860

Cai G, Ovidi E, Romagnoli S, Vantard M, Cresti M, Tiezzi A (2005b) Identification and characterization of plasma membrane proteins that bind to microtubules in pollen tubes and generative cells of tobacco. Plant Cell Physiol 46:563–578

Cannales C, Bhatt AM, Scott R, Dickinson H (2002) EXS, a putative LBR receptor kinase, regulates male germline cell number and tapetal identity and promotes seed development in Arabidopsis. Curr Biol 12:1718–1727

Chaturvedi A, Bahadur B (1985) Gland crested anthers in some angiosperms. J Swamy Bot Cl 2:79–86

Christensen JE, Horner HT Jr, Lersten NR (1972) Pollen wall and tapetal orbicular wall development in Sorghum bicolor (Gramineae). Am J Bot 59:43–58

Christensen CA, King EJ, Jordan JR, Drews GN (1997) Megagametogenesis in Arabidopsis wild type and the Gf mutant. Sex Plant Reprod 10:49–64

Christensen CA, Subramanian S, Drews GN (1998) Identification of gametophytic mutations affecting female gametophyte development in Arabidopsis. Dev Biol 202:136–151

Christensen CA, Gorsich SW, Brown RH, Jones LG, Brown J, Shaw JM, Drews GN (2002) Mitochondrial GFA2 is required for synergid cell death in Arabidopsis. Plant Cell 14:2215–2232

Colcombet J, Boisson-Dermier A, Ros-Palau R, Vera CE, Schroeder JI (2005) Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR KINASES 1 and 2 are essential for tapetum development and microspore maturation. Plant Cell 17:3350–3361

Davis GL (1966) Systematic embryology of angiosperms. Wiley, New York

de Azevedo Souza C, Kim SS, Koch S, Kienow L, Schneider K, McKim SM, Haughn GW, Kombrink E, Douglas CJ (2009) A novel fatty Acyl-CoA synthetase is required for pollen development and sporopollenin biosynthesis in Arabidopsis. Plant Cell 21:507–525

Del Casino C, Li Y, Moscetelli A, Scali M, Tiezzi A, Cresti M (1993) Distribution of microtubules during the growth of tobacco pollen tubes. Biol Cell 79:125–132

Dobritsa AA, Shrestha J, Morant M, Pinot F, Matsuno M, Swanson R, Moller BL, Preuss D (2009) CYP70481 is a long-chain fatty acid omega-hydroxylase essential for sporopollenin synthesis in pollen of Arabidopsis. Plant Physiol 151:574–589

Dobritsa AA, Lei Z, Nishikawa S, Urbanczyk-Wochniak E, Huhman DV, Preuss D, Sumner LW (2010) LAP5 and LAP6 encode anther-specific proteins with similarity to chalcone synthase essential for pollen exine development in Arabidopsis. Plant Physiol 153:937–955

Friedman WE (1999) Expression of the cell cycle in sperms of Arabidopsis: Implications for understanding patterns of gametogenesis and fertilization in plants and other eukaryotes. Development 126:1065–1075

Fu Y, Yuan M, Huang BQ, Yang HY, Zee SY, O'Brien TP (2000) Changes in actin organization in the living egg apparatus of Torenia fournieri during fertilization. Sex Plant Reprod 12:315–322

Fu Y, Wu G, Yang Z (2001) Rop GTPase-dependent dynamics of tip-localized F-actin controls tip growth in pollen tubes. J Cell Biol 152:1019–1032

Gerassimova-Navashina HN (1951) Pollen grains, gametes and sexual process in angiosperm. Transact Bot Inst Acad Sci USSR 7:294–355 (in Russian)

Grienenberger E, Kim SS, Lallemand B, Geoffroy P, Heintz D, de A Souza C, Heitz T, Douglas CJ, Legrand M (2010) Analysis of TETRAKETIDE α-PYRONE REDUCTASE function in Arabidopsis thaliana reveals a previously unknown, but conserved, biochemical pathway in sporopollenin monomer biosynthesis. Plant Cell 22:4067–4083

Grossniklaus U, Schneitz K (1998) The molecular and genetic basis of ovule and megagametophyte development. Semin Cell Dev Biol 9:227–238

Haig D (1990) New perspectives on the angiosperm female gametophytes. Bot Rev 56:236–274

Higashiyama T, Kuroiwa H, Kawano S, Kuroiwa T (1998) Guidance in vitro of the pollen tube to the naked embryo sac of Toreniafournieri. Plant Cell 10:2019–2031

Higashiyama T, Kuroiwa H, Kawano S, Kuroiwa T (2000) Explosive discharge of pollen tube contents in Torenia fournieri. Plant Physiol 122:11–13

Higashiyama T, Yabe S, Sasaki N, Nishimura Y, Miyagishima S, Kuroiwa H, Kuroiwa T (2001) Pollen tube attraction by the synergid cell. Science 293:1480–1483

Higashiyama T, Kuroiwa H, Kuroiwa T (2003) Pollen-tube guidance: beacons from the female gametophyte. Curr Opin Plant Biol 8:36–41

Higginson T, Li SF, Parish RW (2003) AtMYB103 regulates tapetum and trichome development in Arabidopsis thaliana. Plant J 35:177–192

Huang BQ, Russell SD (1992) Female germ unit: organization isolation and function. Int Rev Cytol 140:233–296

Huck N, Moore JM, Federer M, Grossniklaus U (2003) The Arabidopsis mutant feronia disrupts the female gametophytic control of pollen tube reception. Development 130:2149–2159

Hülskamp M, Schneitz K, Pruitt RE (1995) Genetic evidence for a long-range activity that directs pollen tube guidance in Arabidopsis. Plant Cell 7:57–64

Ito T, Shinozaki K (2002) The MALE STERILITY1 gene of Arabidopsis, encoding a nuclear protein with a PHD-finger motif, is expressed in tapetal cells and is required for pollen maturation. Plant Cell Physiol 43:1285–1292

Jensen WA (1973) Fertilization in flowering plants. Bioscience 23:21–27

Johnson MA, Preuss D (2002) Plotting a course: multiple signals guide pollen tubes to their targets. Dev Cell 2:273–281

Jung KH, Han MJ, Lee YS, Kim YW, Hwang I, Kim YK, Nahm BH, An G (2005) Rice undeveloped Tapetum 1 is a major regulator of early tapetum development. Plant Cell 17:2705–2722

Kapil RN, Vasil IK (1963) Ovule. In: Maheshwari P (ed) Recent advance in the embryology of angiosperms. International Society of Plant Morphologists, Delhi

Kim SS, Grienenberger E, Lallemand B, Colpitts CC, Kim SY, de Azevedo Souza C, Geoffroy P, Heintz D, Krahn D, Kaiser M, Kombrink E, Heitz T, Suh D-Y, Legrand M, Douglas CJ (2010) LAP6/POLYKETIDESYNTHASE A and LAP5/POLYKETIDE SYNTHASE B encode hydroxyalkyl α-pyrone synthases required for pollen development and sporopollenin biosynthesis in Arabidopsis thaliana. Plant Cell 22:4045–4066

Kim YJ, Zheng B, Yu Y, Won SY, Ma B, Chen X (2011) The role of mediator in small and long noncoding RNA production in Arabidopsis thaliana. EMBO J 30:814–822

Koltunow AM, Truettner J, Cox KH, Wallroth M, Goldberg RB (1990) Different temporal and spatial expression patterns occur during anther development. Plant Cell 2:1201–1224

Krishnamurthy KV (1977) Meiotic induction is plants: hypothesis. Biology 1:15–17

Krishnamurthy KV (2015) Growth and development in plants. Scientific Publishers, Jodhpur

Kwee HS, Sundaresan V (2003) The NOMEGA gene required for female gametophyte development encodes the putative APC6/CDC16 component of the anaphase promoting complex in Arabidopsis. Plant J 36:853–866

Lancelle SA, Cresti M, Hepler PK (1987) Ultrastructure of cytoskeleton in freeze- substituted pollen tubes of Nicotiana tabacum. Protoplasma 140:141–150

Leins P, Erbar C (2010) Flower and fruit: morphology, ontogeny, phylogeny, function and ecology. Science Publishers, Schweizerbart

Lennon KA, Roy S, Hepler PK, Lord EM (1998) The structure of the transmitting tissue of Arabidopsis thaliana (L.) and the path of pollen tube growth. Sex Plant Reprod 11:49–59

Li H, Lin Y, Heath RM, Zhu MX, Yang Z (1999) Control of pollen tube tip growth by a Rop GTPase-dependent pathway that leads to tip-located calcium influx. Plant Cell 11:1731–1742

Li N, Zhang DS, Liu HS, Yin CS, Li XX, Liang XQ, Yuan Z, Xu B, Chu HW, Wang J, Wen TQ, Huang H, Luo D, Ma H, Zhang DB (2006) The rice tapetum degeneration retardation gene is required for tapetum degradation and anther development. Plant Cell 18:2999–3014

Li SF, Iacuone S, Parish RW (2007) Suppression and restoration of male fertility using a transcription factor. Plant Biotechnol J5:297–312

Lord EM, Russell SD (2002) The mechanisms of pollination and fertilization in plants. Annu Rev Cell Dev Biol 18:81–105

Love AJ, Milner JJ, Sadanandom A (2008) Timing is everything: regulatory overlap in plant cell death. Trends Plant Sci 13:589–595

Lush WM (1999) Whither chemotropism and pollen tube guidance? Trends Plant Sci 4:413–418

Ma H (2005) Molecular genetic analyses of microsporogenesis and microgametogenesis in flowering plants. Annu Rev Plant Biol 56:393–434

Maheshwari P (1950) Embryology of angiosperms. McGraw-Hill, New York

Mascaenhas JP (1993) Molecular mechanisms of pollen tube growth and differentiation. Plant Cell 5:1303–1314

Mascarenhas JP (1990) Gene activity during pollen development. Annu Rev Plant Physiol Plant Mol Biol 41:317–338

Masiero S, Colombo L, Grini PE, Schnittinger A, Kater MM (2011) The emerging importance of type I MADS box transcription factors for plant reproduction. Plant Cell 23:865–872

McCormick S (2004) Control of male gametophyte development. Plant Cell 16:142–153

Millar AA, Gübler F (2005) The Arabidopsis GAMYB-like genes, MYB33 and MYB65, are microRNA-regulated genes that redundantly facilitate anther development. Plant Cell 17:705–721

Miller DD, Lancelle SA, Helper PK (1996) Actin filaments do not form a dense mesh work in Lilium longiflorum pollen tube tips. Protoplasma 195:123–132

Moore JM (2002) Isolation and characterization of gametophytic mutants in Arabidopsis thaliana. Ph.D thesis, State University of New York at Stony Brook

Moore JM, Viele-Calzada JP, Gagliano W, Grossniklaus U (1997) Genetic characterization of hadad, a mutant disrupting megagametogenesis in Arabidopsis thaliana. Cold Spring Harbor Symp Quant Biol 62:35–47

Morant M, Jergensem K, Schaller H, Pinot F, Mollerm BL, Werck Reichhart D, Bak S (2007) CYP703 is an ancient cytochrome P450 in land plants catalyzing

in-chain hydroxylation of lauric acid to provide building blocks for sporopollenin synthesis in pollen. Plant Cell 19:1473–1487

O'Neill SD (1997) Pollination regulation of flower development. Annu Rev Plant Physiol Plant Mol Biol 48:547–574

Pagnussat GC, Yu HJ, Ngo QA, Rajani S, Mayalagu S, Johnson CS, Capron A, Xie LF, Ye D, Sundaresan V (2005) Genetic and molecular identification of genes required for female gametophyte development and function in Arabidopsis. Development 132:603–614

Palanivelu R, Brass L, Edlund AF, Preuss D (2003) Pollen tube growth and guidance is regulated by Pop2, an Arabidopsis gene that controls GABA levels. Cell 114:47–59

Papini A, Mosti S, Brighigna L (1999) Programmed-cell-death events during tapetum development of angiosperms. Protoplasma 207:213–221

Paxson Sowders DM, Owen HA, Makaroff CA (1997) A comparative ultrastructural analysis of exine pattern development in wild-type Arabidopsis and a mutant defective in pattern formation. Protoplasma 198:53–65

Periasamy K, Swamy BGL (1966) Morphology of the anther tapetum of angiosperms. Curr Sci 35:427–430

Phan HA, Iacuone S, Li SF, Parish RW (2011) The MYB80 transcription factor is required for pollen development and the regulation of tapetal programmed cell death in Arabidopsis thaliana. Plant Cell 23:2209–2224

Piffanelli P, Ross JHE, Murphy DJ (1998) Biogenesis and function of the lipidic structures of pollen grains. Sex Plant Reprod 11:675–680

Preuss D, Rhee SY, Davis RW (1994) Tetrad analysis possible in Arabidopsis with mutation of the QUARTET (QRT) genes. Science 264:1458–1460

Raghavan V (2000) Developmental biology of flowering plants. Springer, New York

Raghavan V (2003) Some reflections on double fertilization, from its discovery to the present. New Phytol 159:565–583

Rotman N, Rozier F, Boavida L, Dumas C, Berger F, Faure JE (2003) Female control of male gamete delivery during fertilization in Arabidopsis thaliana. Curr Biol 13:432–436

Rotman N, Durbarry A, Wardle A, Yang WC, Chanboud A, Faure JE, Berger F, Twell D (2005) A novel class of MYB factors controls sperm-cell formation in plants. Curr Biol 15:244–248

Russell SD (2001) Female gametogenesis: ontogenesis of the embryo sac and female gametes. In: Bhojwani SS, Soh WY (eds) Current trends in the embryology of angiosperms. Kluwer Academic Publication, Dordrecht, pp 89–100

Sanchez AM, Bosch M, Bots M, Nieuwland J, Feron R, Mariani C (2004) Pistil factors controlling pollination. Plant Cell 16:S98–S106

Sanders PM, Bul AQ, Weterings K, McIntire KN, Hsu YC, Lee PY, Truong MT, Beals TP, Goldberg RB (1999) Anther developmental defects in Arabidopsis thaliana male-sterile mutants. Sex Plant Reprod 11:297–322

Schiefthaler U, Balasubramanian S, Sieber P, Chevalier D, Wisman E, Schneitz K (1999) Molecular analysis of NOZZLE, a gene involved in pattern formation and early sporogenesis during sex organ development in Arabidopsis thaliana. Proc Natl Acad Sci U S A 96:11664–11669

Scott RJ, Spielman M, Dickinson HG (2004) Stamen structure and function. Plant Cell 16:46–60

Sehgal CB (1965) The embryology of Cuminum cyminum L. and Trachyspermum ammi (L.) Sprague (=Carum copticum Clarke). Proc Natl Inst Sci India 31:175–201

Shi J, Tan H, Yu XH, Liu Y, Liang W, Ranathunge K, Franke RB, Schreiber L, Wang Y, Kai G, Shanklin J, Ma H, Zhang D (2011) Defective pollen wall is required for anther and microspore development in rice and encodes a fatty acyl carrier protein reductase. Plant Cell 23:2225–2246

Shimizu KK, Okada K (2000) Attractive and repulsive interactions between female and male gametophytes in Arabidopsis pollen tube guidance. Development 127:4511–4518

Smaczniak C, Immink RGH, Angenent GC, Kaufmann K (2012) Developmental and evolutionary diversity of plant MADS-domain factors: insights from recent studies. Development 139:3081–3098

Sorensen AM, Krober S, Unte US, Huijser P, Dekker K, Saedler H (2003) The Arabidopsis ABORTEDMICROSPORES (AMS) gene encodes a MYC class transcription factor. Plant J 33:413–423

Sorri O, Äström H, Raudaskoski M (1996) Actin and tubulin expression and isotype pattern during tobacco pollen tube growth. Sex Plant Reprod 9:255–263

Steffen JG, Kang IH, Macfariane J, Drews GN (2007) Identification of genes expressed in the Arabidopsis female gametophyte. Plant J 51:281–292

Steffen JG, Kang IH, Portereiko MF, Lloyd A, Drews GN (2008) AGL61 interacts with AGL80 and is required for central cell development in Arabidopsis. Plant Physiol 148:259–268

Stinson JR, Eisenberg AJ, Willing RP, Pe ME, Hanson DD, Mascarenhas JP (1987) Genes expressed in the male gametophyte of flowering plants and their isolation. Plant Physiol 83:442–447

Swamy BGL, Krishnamurthy KV (1975) Embryo sac ontogenies in angiosperms—an elucidation. Phytomorphology 25:12–18

Swamy BGL, Krishnamurthy KV (1980) From flower to fruit- embryology of angiosperms. Tata McGraw Hill, New Delhi

Tang XJ, Lancelle SA, Hepler PK (1989) Fluorescence microscopic localization of actin in pollen tubes: comparison of actin antibody and phalloidin staining. Cell Motil Cytoskeleton 12:216–224

Tang LK, Chu H, Yip WK, Yeung EC, Lo C (2009) An anther-specific dihydroflavonol 4-reductase-like gene

(DRL1) is essential for male-fertility in Arabidopsis. New Phytol 181:576–587
Tian HQ, Russell SD (1997) Calcium distribution in fertilizes and unfertilized ovules and embryos sacs of Nicotiana tabacum L. Planta 202:93–105
Tiwari SC, Polito VS (1990a) The initiation and organization of microtubules in germinating pear (Pyrus communis L.) pollen. Eur J Cell Biol 53:384–389
Tiwari SC, Polito VS (1990b) An analysis of the role of actin during pollen activation leading to germination in pear (Pyrus communis L.): treatment with cytochalasin D. Sex Plant Rep 3:121–129
Twell D (1995) Diphtheria toxin-mediated cell ablation in developing pollen: vegetative cell ablation blocks generative cell migration. Protoplasma 187:144–154
Vidali L, Yakota E, Cheung AY, Shimmen T, Helper PK (1999) The 135KDa a actin-binding protein from Lilium longiflorum pollen is the plant homologue of Villin. Protoplasma 209:283–291
Vizcay Barrena G, Wilson ZA (2006) Altered tapetal PCD and pollen wall development in the Arabidopsis ms1 mutant. J Exp Bot 57:2709–2717
Weterings K, Russell SD (2004) Experimental analysis of the fertilization process. Plant Cell 16:107–118
Willemse M, van Lammeren A (2002) Fertilization, from attraction to embryo. Biologia (Bratisl) 57:13–22
Willemse MTM, van Went JI (1984) The female gametophyte. In: Johri BM (ed) Embryology of angiosperms. Springer, Berlin, pp 159–196
Wilson ZA, Morroll SM, Dawson J, Swarup R, Tighe PJ (2001) The Arabidopsis MALESTERILITY (MS1) gene is a transcriptional regulator of male gametogenesis, with homology to the PHD-finger family of transcription factor. Plant J28:27–39
Wuest SE, Vijverberg K, Schmidt A, Weiss M, Gheyselinck J, Lohr M, Wellmer P, Rahnenführer J, von Mering C, Grossnicklaus U (2010) Arabidopsis female gametophyte gene expression maps reveals similarities between plant and animal gametes. Curr Biol 20:506–512
Xu J, Yang CY, Yuan Z, Zhang DS, Gondwe MY, Ding ZW, Liang WQ, Zhang DB, Wilson ZA (2010) The ABORTED MICROSPORES regulatory network is required for postmeiotic male reproductive development in Arabidopsis thaliana. Plant Cell 22:91–107
Yadegari R, Drews GN (2004) Female gametophyte development. Plant Cell 16:S133–S141
Yang Z (2002) Versatile signaling switches in plants. Plant Cell 14:S375–S388
Yang WC, Ye D, Xu J, Sundaresan V (1999) The SPOROCTELESS gene of Arabidopsis is required for initiation of sporogenesis and encodes a novel nuclear protein. Genes Dev 13:2108–2117
Yang SL, Xie LF, Mao HZ, Puah CS, Yang WC, Jiang L, Sundaresan V, Ye D (2003) TAPETUM DETERMINANT1 is required for cell specialization. Plant Cell 15:2792–2804
Yang C, Vizcay Barrena G, Conner K, Wilson ZA (2007) MALESTERILITY1 is required for tapetal development and pollen wall biosynthesis. Plant Cell 19:3530–3548
Zhang XS, O'Neill SD (1993) Ovary and Gametophyte development are coordinately regulated by auxin and ethylene following pollination. Plant Cell 5:403–418
Zhang W, Sun YL, Timofejeva L, Chen C, Grossniklaus U, Ma H (2006) Regulation of Arabidopsis tapetum development and function by DYSFUNCTIONAL TAPETUM (DYT1) encoding putative bHLH transcription factor. Development 133:3085–3095
Zhang ZB, Zhu J, Gao JF, Wang C, Li H, Li H, Zhang HQ, Zhang S, Wang DM, Wang QX, Huang H, Xia HJ, Yang ZN (2007) Transcription factor AtMYB103 is required for anther development by regulating tapetum development, callose dissolution and exine formation in Arabidopsis. Plant J 52:528–538
Zhao DZ, Wang GF, Speal B, Ma H (2002) The excess microsporocytes1 gene encodes a putative leucine-rich repeat receptor protein kinase that controls somatic and reproductive cell fates in the Arabidopsis anther. Genes Dev 18:2021–2031
Zheng ZL, Yang ZB (2000) The Rop GTPase switch turns on polar growth in pollen. Trends Plant Sci 5:298–303
Zheng B, Chen X, McCormick S (2011) The anaphase-promoting complex is a dual integrator that regulates both microRNA-mediated transcriptional regulation of cyclinB1 and degradation of cyclin B1 during Arabidopsis male gametophyte development. Plant Cell 23:1033–1046
Zhu J, Chen H, Li H, Gao JF, Jiang H, Wang C, Guan YF, Yang ZN (2008) Defective in Tapetal development and function 1 is essential for anther development and tapetal function for microspore maturation in Arabidopsis. Plant J 55:266–277

Post-fertilization Growth and Development

18

K.V. Krishnamurthy

Abstract

This chapter deals with post-double-fertilization growth and development in the angiosperms, particularly emphasizing the molecular genetic aspects. The patternized development of mature embryo starts with the polarized zygote. The importance of maternal gene control on early embryogeny and on endosperm development is highlighted. The non-maternal genetic control of embryogenesis, laying emphasis on pattern genes, and endosperm development is also discussed in detail. Particular attention is also focused on histological differentiation of the embryo, an aspect that was paid least attention in the past. The physical and chemical factors involved in fruit development and ripening are discussed; also discussed are the genetic control of fruit development and ripening. The importance of chalaza in seed development, not focused much in the past, is also detailed in this chapter.

Keywords

Chalaza • Climacteric respiration • Embryogenesis • Endosperm • Fruit ripening • Maternal genes • Pattern genes • Zygote

18.1 Introduction

Double fertilization induces not only sudden and rapid changes but also gradual and delayed changes in the ovary and ovule contained in it. These together constitute the post-fertilization changes. The ovary develops into a *fruit* and the ovule into a *seed*. The stigma and stylar portions of the gynoecium normally absciss off, as also the other floral parts such as stamens, petals and sepals. But in some taxa, not only one or more of these floral parts persist but also grow to a variable extent along with the fruit/seed in the post-fertilization phase. Often these parts influence the development of the fruits/seeds in a substantial way. The *embryo* arises from the fertilized egg and the *endosperm* from the central

K.V. Krishnamurthy (✉)
Center for Pharmaceutics, Pharmacognosy and Pharmacology, School of Life Sciences, Institute of Trans-Disciplinary Health Science and Technology (IHST), Bangalore, Karnataka, India
e-mail: kvkbdu@yahoo.co.in

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_18,

cell that has the (fertilized) *primary endosperm nucleus (PEN)*. The transition from the maternal to the zygotic state and the subsequent establishment of an embryo-specific developmental pathway underline dramatic gene expression programmes (Okamoto and Kranz 2005). Not only are the timing of these changes, but also the paternal and maternal contributions to this transition are of fundamental importance and interest. In animals, the timing of zygote gene activation varies considerably (at the two-celled embryo stage in mice, four-celled stage in *C. elegans* and at the mid-blastula stage in *Xenopus* and zebra fish), and early embryonic development largely depends on maternal mRNA and proteins. Among angiosperms only fragmentary data are available, that too mainly from *Arabidopsis* and maize. In the former taxon, the very early post-fertilization development is largely under maternal control (Vielle Caldaza et al. 2000). Expression analysis of 16 genes during early post-fertilization development in the latter taxon revealed that only maternally inherited alleles were detected during 3 days after fertilization (Grimanelli et al. 2005). However, the general opinion is that there might be no apparent maternal control during early embryogenesis (Tzafrir et al. 2004). Some parental alleles are expressed early during development in *Arabidopsis* (Köhler et al. 2005). The gpf-mRNA——from a paternally inherited transgene——was reported to appear as early as 4 h after in vitro fertilization to coincide with male chromatin condensation followed by translational activity 6 h after fertilization in the maize zygote (Scholten et al. 2002). Depending on individual genes, considerable variation can occur regarding the contributions of maternal and paternal alleles to early embryo and endosperm development and timing of their development. The importance of maternal control is dealt with in detail subsequently in this article.

18.2 Embryogenesis

18.2.1 Introduction

The embryo is a miniature sporophyte. It is the end product of zygotic ontogeny when the fertilized ovule becomes the seed. The embryo is the most important and integral component of the seed. A 'seed' without an embryo serves no purpose (Krishnamurthy 1994, 2015). The subject of embryogenesis has now attained a fascinating uplift because of inputs from several disciplines. Particular attention is to be drawn here to the results obtained through the techniques of isolation of viable gametes and development of an in vitro fertilization (IVF) system, whereby a zygote is produced by electrical fusion of an isolated egg cell with an isolated sperm cell. Studies on the behaviour of these zygotes which develop into an asymmetrical two-celled proembryo, a few-celled proembryos and transition-stage embryos have provided vital information on early embryogenesis (Kranz et al. 1991, 1998; Kranz and Lörz 1993; Scholten et al. 2002; Hoshino et al. 2004; Okamoto et al. 2004). A procedure for isolating the basal and apical cells from two-celled maize proembryo was established, and then these isolated cells were used as starting points for detecting genes that are up- or down-regulated in the apical or basal cell (Okamoto et al. 2005).

The seemingly simple process of embryogenesis has now turned out to be very intriguing and full of exciting problems (Evenari 1984). Embryogenesis involves a cascade of several developmental episodes that start in the zygote and occur in an ordered sequence that result in the mature embryo, which becomes imprinted with the structural and functional organization of the adult sporophytic body. Embryogenesis entails interplay of an ordered integration and ontogenetic coordination of several factors; cell multiplication becomes progressively associated with the origin of new centres of localized growth, which, in turn, cause the development of specific parts and organs of the plant. Although several morphologically distinguishable contours (filamentous, globular, cordate, torpedo and mature embryos) form at different stages of embryogenesis, the entire gamut of events need to be looked upon as a continuous process in which any given stage of embryogenesis is intimately related to the previous stage as well as to the stage that follows it (Swamy and Krishnamurthy 1980; Krishnamurthy 1994). This relationship is not to be conceived only in terms

of cell lineages and tier systems but in terms of positional information that operates on any cell in the embryo. During successive stages of development, different chemical, structural and functional components are fabricated under the direction of a set of genes to establish distinct patterns at each of the different stages of embryogenesis. The interaction of developmental, structural, biochemical, physiological, functional and genetic (and also epigenetic) factors/controls during embryogenesis is highly complicated and involves mechanisms not yet fully delineated (Krishnamurthy 2015).

18.2.2 The Zygote

As already stated, the zygote is the fusion product (syngamy) of one of the two male gametes carried by the pollen tube with the egg cell present in the female gametophyte. The egg cell which was comparatively poor in cytoplasmic content and fairly quiescent metabolically prior to syngamy undergoes sudden and rapid changes soon after syngamy, resulting in changing the many properties of the egg cell during the first few hours. Pollination and fertilization alone cannot directly account for all changes noticed in the egg cell that becomes the zygote, but changes more than these are involved. The changes noticed include among others the following: number and position of organelles, regrouping and increase in the ER, increase in starch grains in plastids, elaboration and addition in the number of mitochondrial cristae, generation of new ribosomal and polysomal populations, increase in dictyosome activity, clumping of all organelles around the nucleus at the chalazal pole, formation of a wall all around the cell (within 36–50 h depending on the species) and momentary reduction in cell size (within 8–10 h after fertilization) due to shrinkage (up to half its original size in some taxa) followed by subsequent zygotic enlargement (Krishnamurthy 1994). In most angiosperms, the zygote enters into division immediately after syngamy, but in some it undergoes a period of rest (up to 7 days in tobacco and up to several months in *Pistacia*). The delay may be due to delayed fusion of male and female nuclei or due to delayed formation of a complete callose wall around the zygote. This post-fertilization enclosure of the zygote by a callose wall provides the necessary insulation and isolation in the female genetophytic milieu because of its different genetic makeup.

One of the most characteristic features of the zygote and the embryo derived from it is their polarity. This polarity is inherited from the egg cell, but it is accentuated by syngamy (see also Okamoto and Kranz 2005). The polarity of the egg and zygote in turn is largely due to the polar electric field/gradient that already existed in the embryo sac (called *topophysic effect*, sensu Evenari 1984) (Krishnamurthy 1994). Sexually derived zygote and embryo alone bequeaths this polarity but not adventitiously developed embryos or embryoids derived from cultured explants (Swamy and Krishnamurthy 1981; Krishnamurthy 1999). The evidences for zygotic polarity are its cytological organization and its asymmetric division to result in two unequal cells which have entirely different fates (Mansfield and Briarty 1990; Pritchard 1964; Schulz and Jensen 1968; Tykarska 1976; Schel et al. 1984; Lindsay and Topping 1993). A possible mechanism involved in the establishment of zygotic polarity is the crucial subcellular localization of mRNAs in zygotes (Okamoto and Kranz 2005).

18.2.3 Embryogenesis

Wardlaw (1965) considered the zygote and the embryo derived from it as a complete, specific *diffusion reaction system* that operates in conformity with the laws of physical chemistry and mathematics. It is also a gene-determined reaction system operating under the sustaining environmental conditions prevailing at the micropylar milieu of the embryo sac. The division of the zygote, as already mentioned, is asymmetric and results in two unequal cells, a smaller *apical cell* and a larger *basal cell*; the smaller apical cell is endowed with most of the zygotic cytoplasm, while the larger basal cell inherits the large vacuole and only scanty cytoplasm of the egg cell. The embryo is formed by subsequent cell divi-

sions of these two cells, which show considerable variations in their extent of contributions to the final embryo. Based on these, five major types and many subtypes of embryogeny are recognized in angiosperms (Johansen 1950; Maheshwari 1950; Créte 1963). A number of classical embryologists believe that the most characteristic aspect of embryogenesis is the orderly and almost predictable sequence of cell divisions and their predetermined fate in organizing the embryo during embryogeny in any given species. This enabled Souèges (1937) to establish his 'laws of embryonomy', which are followed faithfully in embryogeny. The four main laws are (1) *law of origins* (2) *law of numbers,* (3) *law of dispositions* and (4) *law of destinations* (see Johansen 1950; Krishnamurthy 2015 for detailed statements of these laws). This approach of Souèges is often called the *cell lineage concept* or *mosaic theory* (Street 1976). This concept/theory gained great support for many years and contributed greatly to the recognition of the major types of embryogenesis and the variations under them. As a result, many embryologists tended to assess the type of embryogeny from the first few divisions of proembryo and to allocate the ontogeny into one of the recognized types without looking beyond the globular stage (Krishnamurthy 2015).

The concept of cell lineage and its fixity has been questioned in the last three to four decades since even within the same taxon, distinct variations were observed in early embryogenesis and cell lineages (Periasamy 1977, 1994). This led to the proposal of the *regulative theory of embryo organization* by those who worked on *somatic embryoidogenesis*; according to this proposal, the cell segmentation patterns during early embryogeny are controlled solely by physical factors and that the constituent cells of developing embryos do not inherit distinct and specific cytoplasmic potentialities but remain undetermined and uncommitted. An embryogenetic 'field' may exist in combination with a *position effect*, i.e. a given cell acts in the embryo in relation to the surrounding cells. In other words, it is not the cell or cell group itself that determines the future histogenic region of the embryo it gives rise to but only the position that the cell or cell group occupies in the developing embryo; this is called *positional information* or *Wolpert model* (Wolpert 1970, 1971, 1981). Therefore, according to this theory, allocation of parts of the mature embryo to initials at the 8–16-celled stage of the young embryo appears to be based on topographical correspondence of the initials with the parts of the mature embryo rather than on the actual proof of derivation of parts from the initials. Thus, according to this theory, the end product (i.e. mature embryo) is more important than the means by which the end product is produced. In the embryo of *Arabidopsis*, Scheres et al. (1994) used a transgenic marker consisting of the maize transposable element *ac* incorporating a CaMV35S promoter-GUS gene fusion and traced the hypocotyls, root and root meristem back to compartments in the four tiers of cells in the cordate embryo and that there were no restricted cell lineages for the root and hypocotyl.

The dicot embryo, as already mentioned, shows four distinct morphological contours during its development: filamentous, globular, cordate and torpedo shapes. The first contour results from the variable number of transverse divisions in the zygote. The second contour is initiated by two successive divisions at right angles to each other in one, two or rarely more of the more basal cells of the filamentous embryo (Periasamy 1977, 1994). The globular embryo initiates the *epiphysis* at the prospective shoot pole and the *hypophysis* at the prospective root pole (Fig. 18.1). The differentiation of these two polar entities is a very important morphogenetic step because the further orderly development of the embryo depends only if these are formed at the two opposite poles of the globular embryo (Swamy and Krishnamurthy 1975, 1977, 1978). In the absence of these, there is no transition of the globular embryo into the cordate embryo, and if epiphysis does not differentiate, the cotyledons are not differentiated (see Krishnamurthy 1988 for detailed evidences and arguments in this regard). All the morphological contours of embryo also involve conspicuous symmetry changes in the embryo.

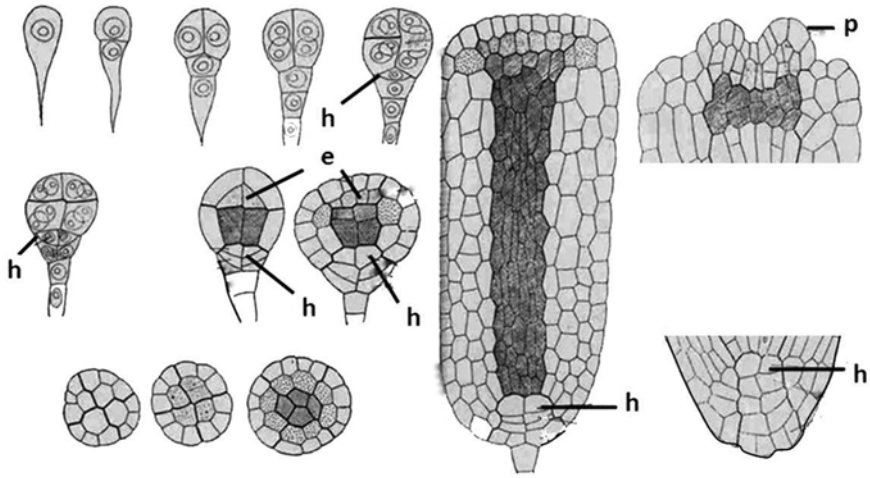


Fig. 18.1 Various stages in the development of the dicot embryo, as represented by *Sphenoclea zeylanica*. Stippled cells represent epicotyls (including epiphysis); single-hatched and cross-hatched cells represent the plerome. *E* epiphysis, *h* hypophysis, *p* leaf primordium (Swamy and Padmanabhan 1961)

From filamentous to globular stage, the embryo is typically radially symmetrical; from globular to cordate stage, the embryo changes from radial to bilateral symmetry (because of two cotyledons) in dicots, while in monocots, because of the presence of only one cotyledon, the change from globular embryo involves only unilateral symmetry. This symmetry change has been shown to be controlled by appropriate changes in the surrounding endosperm (Krishnamurthy 1988).

During embryogeny, with progressive cell divisions, there is a synthesis of DNA, virtually, by all cells of the embryo to be followed by division of their nuclei. Hence, all cells of the young embryo will have the 2C level of DNA. In the embryos of many legumes and a few other taxa, prospective cotyledonary cells continue to synthesize DNA by endoreduplication after division, resulting in a progressive increase in the DNA content of cells of full-grown cotyledons to a level as high as 64 °C in species of *Pisum* and 256 °C in *Phaseolus vulgaris*. It is, however, not clear whether the increase in DNA content represents endoreduplication of the entire genome or selective amplification of certain sequences such as the storage protein genes.

18.2.4 Histological Differentiation

Most classical embryologists believed that histogenesis in the embryo starts only during late embryogenesis and that it becomes morphologically obvious only after the late globular or early cordate stage. Consequently, most of them began their histogenetic studies only from late globular or early cordate stage and considered that histogenetic study up to these stages is of no consequence (Krishnamurthy 1994, 2015). This was also partly due to their belief that 'histological differentiation' means only the origin of the different meristems such as protoderm, ground meristem, procambium and root and shoot apical meristems. It should, however, be emphasized here that the visual recognition of histogenesis is, in fact, preceded by distinct biochemical and histochemical changes, which cannot be normally seen but can only be demonstrated by special staining protocols, EM studies, autoradiography, etc. Therefore, any attempt to deal with differentiation in the developing embryo should encompass not only the temporal and morphological aspects but also the physical, physiological and bio- and histo-chemical and genetic basis of the histogenetic differentiation phenomenon.

The *protoderm* is perhaps the first histogenetic region to differentiate in the developing embryo. Although the time of its origin and differentiation slightly varies with taxa, invariably it gets differentiated in the octant stage. The differentiation of protoderm is related to the cutting off of an internal cell in the embryo. Probably in view of this relationship, Periasamy (1977, 1994) attached great significance to the internal cell formation in the proembryo and attempted to classify angiosperm embryogeny on this basis. According to him, the increasing exposure of newly formed cells from the zygote to the internal environment of the future embryo, rather than to the external environment of the endosperm, makes the segmentation of the first internal cell and the consequent differentiation of the protoderm an important morphogenetic event. The differentiation of the protoderm further increases exposure of the embryonal cells to its internal environment. The time of differentiation of protoderm appears to be dependent on the regulatory effect of the extra-embryonal environment, especially the one prevailing at the micropylar milieu of the embryo sac, but studies on somatic embryoidogenesis indicated that the basic control over its differentiation probably lies in the embryo itself (Swamy and Krishnamurthy 1981; Krishnamurthy 1999).

Almost simultaneously with the differentiation of protoderm, both hypophysis and epiphysis become initiated at the opposite poles of the globular embryo. The hypophysis refers to a group of cells that becomes differentiated at the prospective root pole, while the epiphysis is a similar group of cells at the prospective shoot pole. The hypophysis later forms an integral part of the root apical meristem and constitutes the base for the organization of the quiescent centre (see detailed account on quiescent centre in Chap. 4 of this book) (Swamy and Krishnamurthy 1975). Similarly, the epiphysis later forms an integral part of the shoot apical meristem as the base for the relatively quiescent central mother cell zone (Swamy and Krishnamurthy 1977). The cells of hypophysis and epiphysis have identical histology, ultrastructure and cytochemistry: they are larger than their adjacent cells, less densely cytoplasmic, more vacuolated and poorer in RNA and protein content. They are large nucleated and relatively quiescent mitotically; those cells, which do divide, have a very greatly prolonged mitotic cycle. These two regions remain more or less unaffected through further embryogeny, although the number of their constituent cells slightly increases and continues into the seedling and into the mature plant as well. Thus, claims of disappearance of these two zones in the mature embryo and their reappearance in the seedling apices are likely to be based on faulty observations made on non-median longitudinal sections. During the organization of the two meristems in the mature embryo and seedlings, there is a change from the random distribution of cell divisions to a concentration in the respective poles around hypophysis and epiphysis to form radicular meristem and shoot apical meristem. Hence, hypophysis and epiphysis may be considered as the second formative tissues, next to promeristem, in the embryo. Once the cotyledons are initiated, the shoot apex, in most taxa, lapses to a state of quiescence in the mature embryo; however, in some taxa like the legumes, the embryonic shoot apex produces a number of leaf primordia even before germination.

The above description and discussion holds good for the dicotyledons. In the monocots, there is only one cotyledon which apparently looks 'terminal', while the shoot apical meristem appears to have been pushed to a 'lateral' position. However, detailed studies made in several monocots both by Prof. B.G.L. Swamy and his school at Chennai (see Swamy and Krishnamurthy 1980) and by Prof. B. Haccius and her students at Mainz University, Germany, have shown that both shoot apex and the cotyledon are terminal in origin and that due to the more pronounced growth of the cotyledonary sector, the near quiescent shoot apical sector built around the epiphysis apparently 'becomes lateral'.

The *ground meristem* and the *provascular meristem* are blocked out almost simultaneously during embryogeny. Both are derived from the central core of the late globular or early cordate embryo stage by way of differential cell enlargement, stainability and vacuolation. Depending on

the species, the ground meristem may produce only the cortex or both cortex and pith (Krishnamurthy 1994, 2015). In those species where cortex alone is derived from ground meristem, the peripheral layer of cells of the central meristematic core of late globular embryo produced the cortex through periclinal division. Where the ground meristem produces both the cortex and pith, the central core has a progenitor layer on its peripheral part, while the cells in its central region undergo enlargement and vacuolate to produce the pith.

Our knowledge on *procambialization* and *vascular differentiation* in the embryo is very meagre largely due to lack of identifying unique features of procambium and also to the lack of very early biochemical markers for the differentiating procambial cells. Many workers in the past have designated the entire central core of cells of the embryonic axis as procambium (Esau 1965), and such a designation implies that the procambium gives rise to not only vascular tissues but also to the nonvascular pith tissue (if present), conjunctive parenchyma and pericyle. It is true that in embryos there is a blocking out of the so-called provascular tissue in their central axial region at the late globular/heart-shaped stage, but it should not be confused with procambial differentiation. The blocking out takes place as a continuous structure from the embryo axis into the central core of the cotyledons (Fig. 18.1) leading to statements such as the following: 'the procambium of the cotyledons, hypocotyls and radicle is one continuous tissue systems'. Detailed and critical studies made in Prof. B.G.L. Swamy's laboratory in Chennai (see Swamy and Krishnamurthy 1980; Krishnamurthy 1994) have shown that procambialization of the embryo is noticed the earliest in the cotyledon(s) which forms its median vascular trace, while the blueprint for the procambium in the radicle is laid down much later when the radicular apical meristem organizes itself. In the latter, the metaxylem locus becomes histologically very distinctive. At this stage, the hypocotyl part of the embryo exhibits a conspicuous developmental lag in spite of having a central core of cells. This condition emphasizes that (1) the root and hypocotyl are independently derived organs of the embryo and (2) the procambium of the cotyledon(s) has no connection with that of the radicle or the hypocotyl as they get differentiated belatedly, particularly that of the radicle. Whereas the origin of the procambium in the cotyledon(s) and in the radicle is traceable to their respective meristems, that of the hypocotyl is not related to any apical meristematic unit but from its own constituent cells at appropriate loci. Vascularization of the procambium in the hypocotyl is also correspondingly delayed.

18.2.5 Genetic Control of Embryogenesis

18.2.5.1 Maternal Genetic Control

Attention was already drawn briefly in the introductory section to the maternal effect on early post-fertilization development. The maternal-effect gene mutants affect post-fertilization development. A mutant phenotype of this kind that depends on the genotype of the female gametophyte, but independent of the paternal contribution, is referred to as gametophytic maternal effect (Grossniklaus and Schneitz 1998). The basis of maternal effects may be due to (1) mutation in genes that are expressed during embryo sac development, but whose products are required after fertilization for embryo and endosperm development; (2) abnormal mitochondria or plastids that are usually inherited from the mother plant; (3) alterations in gene dosage; for example, it may be caused by haploinsufficiency in the endosperm, which inherits two mutant alleles from the mother but only one paternal wild type from pollen in outcross; (4) the mutation probably affects a stored factor present in the cytoplasm of the egg and/or central cell that is required for seed development after fertilization; and (5) genomic imprinting (Brukhin et al. 2005).

An important question that needs to be resolved in this connection concerns the parental conflict and infanticide during embryogenesis (and also during endosperm development), as controlled by maternal genes. There are also evidences to show the presence of gametophytic maternal effect on embryo (and endosperm) as

detailed below, although there are also evidences to indicate that the switch from material to embryonic control of development occurs after fertilization in the zygote itself in angiosperms, i.e. at a very much earlier stage of developing embryo than in animals (Kranz et al. 1999). Two *Arabidopsis* mutants, *emb173* and *mesea* (*mea*) (Grossniklaus et al. 1998; Grossniklaus and Vielle Caldaza 1998), display gametophytic maternal control on seed development, if the genes are inherited through the female gametophyte. The gametophytic maternal effect of *mea* results in aberrant growth regulation during embryogenesis, and the embryos derived from *mea* eggs show excessive growth and die during the desiccation phase of seed. The *MEA* protein forms part of a *Polycomb* group complex that suppresses cell proliferation, not only after fertilization but also in the absence of fertilization. Three other components of the complex are known, all of which share this interesting phenotype (Ohad et al. 1996, 1999; Grossniklaus et al. 1998; Chaudhury et al. 1997; Kinoshita et al. 1999; Yadegari et al. 2000; Köhler et al. 2003; Pischke et al. 2002). Whether the genes encoding other components of the *Polycomb* group complex are also regulated by genomic imprinting or show a maternal effect because they are cytoplasmically stored is currently unknown (Guitton et al. 2004). Nearly half of the gametophytic mutants described by Moore (2002) shows post-fertilization defects at a certain frequency. Outcrossing with wild-type pollen has shown that these effects are indeed under maternal control (Pagnussat et al. 2005). The genes disrupted in these mutants come from diverse families that have been implicated in a wide variety of cellular functions (Pagnussat et al. 2005). There are also genes whose functions are unknown.

18.2.5.2 Non-maternal Genetic Control

The development of embryo is also under non-maternal genetic control; in fact, such a control is more prevalent almost from the beginning of embryo development. Quite a lot of concerted gene action as well as a diverse pattern of gene expression are needed absolutely to build the tissues of the embryo. The immediate consequences of gene expression are the changes in the patterns of protein synthesis and accumulation in the developing embryo. Cells at different loci in the developing embryo come to possess different gene-based commitments. Biochemical changes are triggered at these loci at appropriate times due to sequential expression of specific genes or *creode* (Waddington 1957) which can be analysed by the specific mRNA sequences and proteins synthesized. It is debated whether the zygote has the requisite machinery of its own to synthesize its protein needs or it obtains them from its surroundings. Many believe that the first proteins synthesized by the zygote are coded by the stores of mRNA bequeathed from the egg cell, although there are others who have shown that the zygote is capable of active mRNA/protein synthesis as has been demonstrated in cotton, tomato and tobacco where a new population of ribosomes is reported to be formed. It is likely that both situations may prevail depending on the species. As embryogenesis proceeds with zygote division, a number of proteins (enzymes included) are synthesized, and on an average about 20,000 genes were known, even about 20 years back to be operating during the whole gamut of embryogenesis (Goldberg et al. 1989, 1994). Of these, one fourth to one fifth genes are unique to the embryo, while the rest are shared with other organs of the plant. Of the embryo-specific genes, about 10–12 % (40–50 genes) are believed to be master regulator genes. Some genes have housekeeping role and fulfil structural, metabolic and synthetic activities, while others encode for storage materials as well as for tissue- and organ-specific proteins.

The presence of almost the same total number of different mRNAs in embryos of two morphologically distinct age groups supports the contention that there are no changes in the absolute number of structural genes expressed during the whole embryogenesis. However, new ideas have been obtained regarding the modulation of the appearance of mRNAs and proteins during embryo development in cotton (Dure 1985). There are at least seven separate subsets of mRNAs that are expressed during different times in embryogenesis

and early germination. A similar observation involving three subsets of polypeptides has been made during maize embryogenesis (Boothe and Walden 1990). Embryo-specific expression of cotyledon-abundant and embryo axis-abundant genes isolated from a cDNA library made to poly(A)-RNA of seedlings of *Brassica napus* was also studied. These two sets of genes are active during critical embryogenetic stages with transcripts of the cotyledon-abundant genes reaching maximum levels in embryos about to desiccate (Harada et al. 1988). The expression of numerous developmental genes isolated from the sporophytic organs of plants has been followed in embryos of wild-type and transgenic plants. These genes include genes encoding homebox proteins, CDKs, extensins, histones, chloroplast genes, lipid transfer proteins, phytochromes, actin, tubulin, MADS-Box proteins, etc., during different stages of embryogenesis. Most mRNA sequences transcribed during embryogenesis persist as stored mRNA in seeds and serve as template for the synthesis of proteins needed for germination (Raghavan 2000).

It should be emphasized that gene expression during embryogenesis is more cryptic and appear more fundamental than that determined by cell division planes or cell lineages (Raghavan 2000). Hence, Hence, a good strategy would be to identify genes and characterize their expression in transgenic embryos of transgenic plants by a promoter trap method as this promises to provide markers for restricted cell types of young embryos before spatial patterns of gene expression are activated. Lu et al. (1996) have isolated and characterized a cDNA clone called *ATML1* and this encodes a new homeodomain protein in *Arabidopsis*. After the asymmetric division of the zygote, transcripts of this clone are expressed in the apical cell of the two-celled proembryo and then get concentrated in the cells derived from this apical cell up to the globular embryo stage. At the globular 16-celled stage, the expression of the transcripts of this clone is restricted to the protoderm cells and totally disappears from the inner cells. In the mature embryo, its expression is restricted to the L1 layer of the shoot apical meristem. Thus, the expression of *ATML1* gene is considered to be the first molecular marker of the apicobasal polarity and pattern formation in the developing embryo. A number of mutants that result in abnormal cell lineage patterns have been described in *Arabidopsis*. These affect, like the *ATML1*, apicobasal pattern or radial or other pattern-related aspects of the developing embryo. These are referred to as *pattern genes*. The genes that express specifically in the apical cell of the two-celled proembryo are *MP*, *BDL* and *WOX2*, while those that are expressed in the basal cell are *PIN7*, *WOX8* and *WOX9*. However, it is still not clear whether these genes are already expressed in egg cell or are induced by fertilization. The mutants that lack apicobasal polarity and characteristic pattern include *gurke* (*gr*), *fackel* (*fc*), *pasticcino* (*pas*), *mp*, *gn*, *wus*, *rootless* (*rtl*), etc. (for their and other mutants' phenotypic effects, see Table 18.1). *KT1* gene forms a good marker of polarity in the globular embryos of soybean as the gene gets transcribed in a small number of cells at the micropylar pole of the embryo. *STM* gene products form a marker for the earliest stage of differentiation of the shoot apical meristem in *Arabidopsis*. In the same plant, farnesyltransferase (FTase) β-subunit encoded by the *ERA1-WIGGUM* gene is expressed in the embryo proper throughout embryogenesis and is essential for meristem development (Yalovsky et al. 2000). An ubiquitin-specific protease is known to function in embryo development (Doelling et al. 2001). Histological studies on pattern mutants reveal that anarchic cell divisions in the zygote and/or proembryo cause problems in histogenetic patterns in the embryo (Mayer et al. 1993). A study of these pattern mutants also reveals that the body plan of the embryo is specified by a hierarchy of genes acting at very defined stages of development and that the embryo patterns are not dictated by cell size or shape or orientations of planes of cell division but probably by the relative positions which the cells occupy (positional information). The protein products of these pattern genes probably function as components of a network of ubiquitous proteins that affect common cellular functions: these proteins include among others intracellular transport proteins associated with Golgi bodies, vesicle traffic directing proteins, transcription factors, etc.

Table 18.1 Most important embryo-defective and embryo-pattern mutants and their phenotypic expressions in *Arabidopsis*

S. no	Name of the mutant	Phenotypic expression
1.	*raspberry (ras), suspensor (sus), twin 1 (twn 1)*	Growth of embryo proper inhibited with characteristic suspensor anomalies; suspensor shows embryo-like behaviour
2.	*twin 2 (twn 2)*	Apical cell of two-celled proembryo arrested, but suspensor is proliferated; produce twin seedlings, one from embryo proper and the other from suspensor embryo
3.	*leafy cotyledon (lec)*	Cotyledons become leafy both morphologically and anatomically; embryo maturation features (like desiccation and dormancy) not seen; no accumulation of storage proteins
4.	*cup-shaped cotyledon (cuc)*	Embryo with fused cotyledons
5.	*gurke (gu)*	Embryo lacks both shoot apical meristem and cotyledons
6.	*fackel (fc)*	Cotyledons attached to the root; no hypocotyl present
7.	*pastaccino (pas)*	Embryo with misshaped cotyledons and with a short hypocotyl
8.	*mp*	Embryo without hypocotyl or radicle due to proliferative hypophysis
9.	*gn*	Embryo reduced to a cellular mass; zygote division symmetrical followed by irregular divisions in the apical cell alone
10.	*Wus, stm*	Embryo lacks shoot apical meristem
11.	*rootless (rtl)*	Embryo lacks radicle
12.	*knolle (kn)*	Embryo lacks protoderm; affects radial pattern of the embryo; chaotic divisions in octant embryo to result in large multinucleate cells
13.	*keule (keu)*	Embryo with abnormal epidermal cells; affects radial pattern of the embryo; chaotic divisions in octant embryo to result in large, multinucleate cells
14.	*fist*	Radicle pattern in embryo affected; protoderm not formed
15.	*fs*	Does not affect normal zygotic division, but apical cell irregularly divides several times to produce a radially enlarged embryo and an incipient radicle; embryo shape affected
16–18	*hydra (hyd), knopf (knf), mickey (mic)*	Embryo shape affected; also produce abnormal seedlings

The role of MADS-box genes in controlling embryogenesis should be mentioned at this juncture. Despite the fact that many MIKC^c-type MADS-box genes show detectable expression during embryo development (Lehti Shiu et al. 2003), very little is known on their roles in embryo development. One of the earliest known such genes is AGL15, whose overexpression promotes the production of secondary embryos (Harding et al. 2003).

Some information is available on genes affecting storage protein synthesis and accumulation in embryo cotyledon (and endosperm) (see details in a subsequent section of this chapter). The mRNAs encoding storage proteins of embryos increase from very low levels, become dominant in the embryo cotyledon(s) during peak protein accumulation period and again decrease to a very low level at embryo maturity. Thus, there is a mechanism to limit gene transcription during embryo maturation. Generally storage protein synthesis is regulated at the transcriptional level, but there is also some post-transcriptional storage protein synthesis, as, for example, conglycin. Genes coding for storage proteins are also targets for growth regulators, and hence there is an increased transcription and thus an increased protein accumulation. Cloning and sequencing studies in some legume storage protein transcription regulation indicate the presence of conserved upstream sequences known as 'legumin box' and 'vicilin box' in the genes determining tissue specificity. The legumin box is a 28 bp DNA elements identified in the promoter region of most globulin genes and consists of motifs known as RY-repeats (5′-CATGCAT-3′, 5′-CATGCAC-3′ and 5′-CATGCATG-3′), while

vicilin box is a highly conserved C-rich region of approximately 120 bp from the transcription start of the vicilin genes. These two boxes are absent in cruciferin and napin genes.

18.2.6 Role of Auxins

Cell fate in the developing embryo is shown to depend also on positional signal molecules. It is now generally accepted that auxin is essential for apical cell specification. For the specification of the basal cell, an *Arabidopsis* MAPKK kinase gene, named *YODA*, has been identified. *YODA* promotes extraembryonic cell fates in the basal cell derivatives (Lukowitz et al. 2004), instead developing into suspensor; there is an associated suppression of the apical cell derivatives (see Okamoto and Kranz 2005). Auxin concentration gradient plays an important role in pattern formation in the young embryo (Lyndon 1990; Jürgens 2001). For instance, the apicobasal axis of young *Arabidopsis* embryo is established by efflux-dependent auxin gradients (Friml et al. 2003; Friml and Wisniewska 2005). Vesicle transport mediated by *GNOM/EMB30* (*GN*) actively localized putative auxin transporters of the *PINFORMED* (*PIN*) family in apolar fashion in the cell, suggesting a directional flow of auxin (Busch et al. 1996; Geldner et al. 2001; Shevel et al. 1994; Steinmann et al. 1999). The *Arabidopsis BODENLOS* (*BDL*) gene encodes an auxin response protein that inhibits *MONOPTEROS* (*MP*)-mediated embryo patterning (Hamann et al. 2002). Both are necessary for normal root development. The mutant *monopteros* lacks function of ARF5, a transcription factor of the ARF (auxin response factor) family that activates auxin-responsive target genes (Hardike and Berleth 1998; Ulmasov et al. 1999) and the auxin-insensitive mutant fail to initiate the radicular meristem during early embryogenesis. The *Arabidopsis* ubiquitin-related protein T1R1 functions in auxin response via the COP9 signalosome (Schwechheimer et al. 2001, 2002). The ability to respond to auxin may differ between the apical and basal cells of the two-celled proembryo. In the basal cell of *Arabidopsis*, two-celled proembryo, *PIN7*, encoding a putative auxin efflux carrier protein, is expressed, and the PIN7 protein localizes at the apical region of the cell indicating auxin transport from basal to apical cells (Friml et al. 2003). PIN7 protein-depending high auxin level in the apical region cells continues until eight-celled stage embryo. In the globular embryos, auxin transport becomes basipetal via PIN1, 4 and 7 proteins from apical part of the embryo to hypophysis, and, thereafter, in the cordate embryo, auxin is accumulated at regions which form root meristem, cotyledons and provascular tissue (Aida et al. 2002; Furutani et al. 2004; Baima et al. 1995, 2001).

Among the tissue regions of the embryo, formation of radicular meristems through auxin-dependent molecular mechanisms is the best characterized. ARF5/MP, a transcription factor that activates auxin-responsive genes (Ulmasov et al. 1999) *IAA12/BDL*, a putative inhibitor of MP (Hamann et al. 2002), is probably central to auxin response during embryogenesis. Loss of MP or gain of BDL function interferes with specification of the apical cell and prevents the formation of radicle (Hamann et al. 1999, 2002). MP and BDl are coexpressed throughout embryogenesis and form heterodimers. Auxin-dependent degradation of BDL results in the release of MP and MP-dependent expression of auxin-responsive genes. Auxin facilitates degradation of AUX/IAA proteins which are transcriptional regulators. Degradation of these proteins and auxin-regulated transcription are mediated by an F-box protein, T1R1, which has been shown to be an auxin receptor (Dharmasiri et al. 2005a; Kepinski and Leyser 2005). Besides T1R1, three additional F-box proteins interact with BDL and mediate auxin response during plant development (Dharmasiri et al. 2005b). One of the putative targets of *MP* is *WOX* (*WUSCHEL-RELATED HOMEBOX*) *9*, since *WOX9* expression is altered in *mp* and *bdl* mutant embryos (Haecker et al. 2004). WUSCHEL transcription factor is expressed throughout embryogenesis and is required for embryonic shoot meristem formation (Bowman and Eshed 2000; Sharma et al. 2003). Haecker et al. (2004) had demonstrated that *WOX2* and *WOX8* are expressed in the zygote

and that expression of *WOX2* and *WOX8* is restricted to apical and basal cells of the two-celled proembryos, respectively.

18.2.7 Embryo Suspensor

The embryo suspensor is the product of the basal cell of the two-celled proembryo. Although there are several taxa where a morphologically distinct suspensor is absent, there are an equal number of taxa where the suspensor shows highly impressive morphological formations (see details in Maheshwari 1950; Swamy and Krishnamurthy 1980; Krishnamurthy 2015). In many instances, it functions as a haustorial structure for nutritive purposes, either involving only its free cell (the remaining suspensor cells serving as a channel of transport of nutrients) or a few or all of its cells. The haustorial suspensor cell(s) undergoes conspicuous hypertrophy with concomitant great increase in the size of its nucleus, which becomes polyploidal/polytenic; in some the nucleus becomes multinucleate. The DNA content of nucleus may increase up to 8192C, as in *Phaseolus* species, where polyteny is associated with puffing of chromosomes, probably with selective amplification of some genes and under-replication of some others. The suspensor is also shown, through biochemical studies, to be the locus of synthesis of growth regulators.

As already indicated, the fate of an organized suspensor component of the embryo is decided by genes operating in the basal cell of the two-celled embryo. Once the embryo approaches maturity, the suspensor undergoes PCD.

18.3 Endosperm Development

18.3.1 Introduction

Cells or nuclei derived from the *primary endosperm nucleus* (*PEN*) present in the central cell of the female gametophyte and formed as a result of triple fusion (see Chap. 17 of this volume) constitute the *endosperm*. Most nuclei also sooner or later become enclosed in cells. The endosperm tissue is largely nutritive to the growing embryo but also serves a regulatory role controlling the orderly development of the embryo. All angiosperms, except members of Trapaceae, Podostemaceae and Orchidaceae, have endosperm. In many taxa, the endosperm develops accessory haustorial structures that absorb nutrients from surrounding ovular tissues (or even ovary tissues in some), particularly located in the micropylar and/or chalazal direction. The endosperm may persist to a variable extent in the mature seeds (*endospermous or albuminous seeds*) or may be totally used up without any trace in the seeds (*nonendospermous* or *exalbuminous seeds*). In the former type of seeds, the persisting endosperm also helps in providing the nutritional/regulatory requirements of the germinating embryo. In a number of plants, particularly in cereals, the endosperm constitutes the edible part forming the staple food for humans and/or cattle/poultry.

18.3.2 Types of Endosperm Development

The PEN has varied position in the central cell before it divides: depending on the species, it remains in its place of formation and then moves to the centre of the female gametophyte or seen nearer to the antipodals. Three types of endosperm development are known in the angiosperm (Fig. 18.2): ab initio *cellular*, ab initio *nuclear* and *helobial*. In dicots the first two types alone are known, while all the three are seen in monocots. In cellular type, the division of PEN is promptly followed by a cell wall, so also in all subsequent divisions of the two resultant daughter cells. In nuclear type, the division of PEN, as well as subsequent divisions, is not followed by walls so as to result in several free endosperm nuclei. In helobial type, the PEN is always located near the chalazal pole of the embryo sac and divides there to form a large *micropylar chamber* and a small *chalazal chamber*. Free-nuclear divisions (as in nuclear endosperm) continue in the micropylar camber to result in a large number of nuclei, while the nucleus in the chalazal

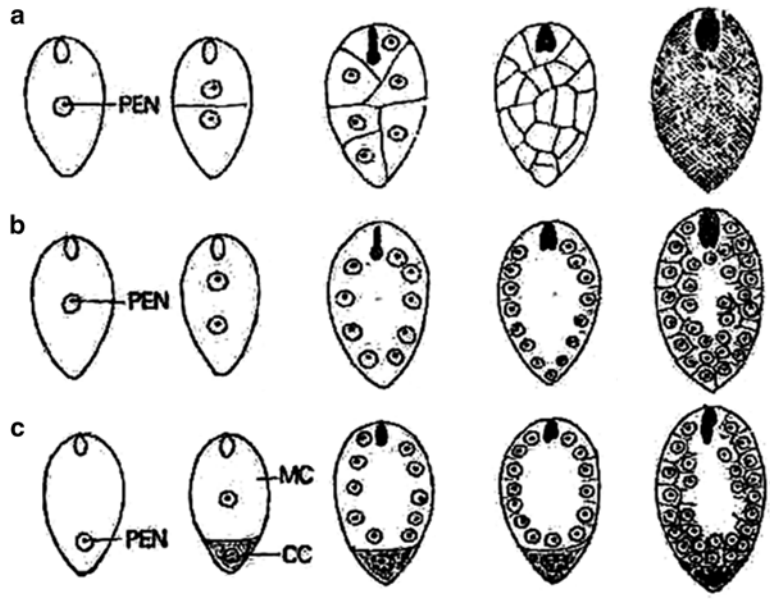


Fig. 18.2 Major types of endosperm development (**a**) cellular, (**b**) nuclear, (**c**) helobial; *CC* chalazal chamber, *MC* micropylar chamber, *PEN* primary endosperm nucleus (Swamy and Krishnamurthy 1980)

chamber may not divide at all, may divide a few times to result in some free nuclei or may undergo endomitosis/polyploidy/polyteny. Cell wall formation may or may not take place in the micropylar chamber or may happen partially (Swamy and Krishnamurthy 1980; Krishnamurthy 2015).

In species with cellular endosperm, the division of PEN is followed by a transverse wall (very early reports of longitudinal division in taxa like *Adoxa* need verification). The subsequent few divisions may also be transverse, especially in species with elongated embryo sacs. However, later divisions may be in diverse planes. In taxa with nuclear endosperm, the free-nuclear division phase is of variable duration, the shortest being in *Coffea* where only four nuclei are formed and the longest being seen in some palms like coconut where thousands of free nuclei are formed. The endosperm remains nuclear in rare instances like *Limnanthes* and *Oxyspora*, but in almost all cases, cell walls are formed in between the free nuclei. The wave of wall formation spreads from periphery to the centre, from micropylar end to chalazal end or from both micropylar and chalazal ends towards the centre. The free nuclei often undergo conspicuous morphological and cytological changes, indicating the intense physiological activity of the endosperm. The most common feature relates to the increase in the size of the nuclei. For example, in maize endosperm some nuclei undergo about 1,000-fold increase in size in 24 days subsequent to pollination. This enlargement is caused by repeated endomitosis. In *Gagea* nuclear enlargement is accompanied by an increase in chromosome number. The increase in nuclear size/ploidy level is very often accompanied by an increase in the number of nucleoli as well. A few days after endosperm initiation, there is neither normal mitosis nor endomitosis, but there is only a selective replication of nucleoli; this perhaps represents the amplification of ribosomal genes involved in the increased protein-synthesis efficiency of endosperm cells. The polyteny due to metaphase mitotic inhibition observed in endosperm nuclei is different from the one which occurs in the salivary glands of insects, where polyteny occurs in the interphase, and it corresponds to what is called *dispersion endomitosis*; however, in some palms like *Borassus*, endosperm nuclei undergo interphase endoreduplication. In some taxa, wall formation is completed around all nuclei, while in some it is incomplete, leaving free nuclei in the central part of the embryo sac or in the chalazal pole. Rarely cells are formed enclosing more than one nucleus

or without any nucleus being enclosed. Cellularization of the endosperm is now shown to be under genetic control, especially due to the loss of *FIS* gene function (Köhler and Makarevich 2006). All these variations are also noticed in the micropylar endosperm chamber of helobial endosperm. The chalazal chamber, often called *basal apparatus*, with a single hypertrophied nucleus (polyploidal/polytenic) or a few nuclei, depending on the taxon, remains haustorial.

One very significant development with reference to the molecular biology of endosperm concerns the levels of DNA methylation of endosperm nuclei. Similar to extraembryonic tissues in mammals (Santos et al. 2002), the endosperm nuclei have reduced levels of DNA methylation compared to the embryo or vegetative tissues (Hsieh et al. 2009; Gehring et al. 2006, 2009). Reduced methylation is brought about by transcriptional repression of the maintenance DNA-methyltransferase MET1 during female gametogenesis (Jullien et al. 2008), along with active DNA methylation by the DNA glycosylase DEMETER (DME) protein (Hsieh et al. 2009; Choi et al. 2002). The global DNA methylation levels differ only slightly between the embryo and endosperm (~6 % for CG methylation), but methylation differences at transposable elements and repeat sequences are significantly more pronounced (Hsieh et al. 2009; Jullien et al. 2008). However, the functional significance of this genome-wide demethylation of the endosperm is not yet understood, but it is likely that DNA methylation might cause transposon activation and generation of small interfering RNAs (siRNA) that might move to egg cell or embryo where siRNA-medicated DNA methylation would lead to more methylation of parasitic genomic sequences (Hsieh et al. 2009; Weinhofer et al. 2010). This idea is supported by the accumulation of 24 nt siRNAs in the embryo sac and endosperm (Mosher et al. 2009). Weinhofer et al. (2010) have discovered that the FIS PCG complex in the endosperm of *Arabidopsis* targets transposable elements that are protected by DNA methylation in vegetative tissues, implicating that DNA methylation and H3K27me3 (trimethylated lysine 27 on histone H3) are alternate repressive marks that may compensate for each other in the repression of a subset of transposable elements.

18.3.3 Endoperm Haustoria

As mentioned in the introductory part of this section (Sect. 18.3.1), the cellular and nuclear endosperms of some taxa develop special accessory structures called haustoria that aid in the nutrition of the embryo. The haustoria may be chalazal (the most common category), micropylar or both chalazal and micropylar, made of a single cell, two cells or many cells, usually intercellular or rarely intracellular and unbranched or branched. The haustorial cells are deeply cytoplasmic, greatly enlarged and with single polyploidal/polytenic nucleus or many nuclei. In *Pedicularis*, for example, the chalazal haustorial nucleus shows 96 ploidy and the micropylar haustorial nucleus 192–384 ploidy. The highest recorded ploidy level is 24576 in *Arum maculatum*. The walls of haustorial cells are demonstrated to have transferred cell morphology in some taxa, thus efficiently aiding in short-distance transport of materials. The haustorial cells are endowed with an array of enzymes, both hydrolytic and otherwise, helping in their penetration through extraembryonic sac tissues and in metabolizing and transferring nutritive materials towards the developing embryo. In helobial endosperm, the basal apparatus itself acts like a haustorium (Krishnamurthy 2015).

18.3.4 Functions of Endosperm

The endosperm may be said to have two major functions: (1) nutritive and (2) regulatory. The first implies that the endosperm is the source of nutrients for the growing embryo up to seed maturity (Lopes and Larkins 1993; Costa et al. 2004) and in many cases (in endospermous seeds) for the germinating embryo until the seedling becomes autonomous and takes care of its nutri-

tional requirements. The second implies that the endosperm regulates and controls the orderly development of the embryo, right from zygote to mature embryo, as well as embryo size, polarity and symmetry (Hong et al. 1996; Krishnamurthy 1988). The regulatory role is not easily realized when compared to the nutritive role of endosperm.

The nutritive role of the endosperm has been very well documented. It is the major source of nutrients for the zygote and growing embryo for most of its nutritional requirements. The endosperm haustoria are additional structures that facilitate embryo nutrition. The transfer of nutritive materials from endosperm to the embryo is proved by the isolation of two genes from maize endosperm that are specifically expressed during its early stages of development: *BET* (for 'basal endosperm transfer cells'), whose expression is restricted to the basal endosperm transfer cells with extensive wall ingrowths (Heuros et al. 1995), and *ESR* (for 'embryo surrounding region'), whose expression is seen in the endosperm at the immediate vicinity of the growing embryo. The latter gene probably plays a direct role in the nutrition of the embryo by exporting gene products into the embryo (Opsahi-Ferstad et al. 1997). The nutritive role of endosperm is also evident from a study of interspecific and intergeneric crosses, where hybrid embryos abort at various stages of development due to absence or faulty endosperm development, as well as from embryo culture experiments.

Midway during embryogenesis, the endosperm, especially of albuminous seeds, gradually becomes committed to the synthesis and accumulation storage products. How the metabolic activity of an endosperm programmed for a modest synthesis of housekeeping proteins changes to a structure with a large-scale synthesis of storage substances like starch, lipids and complex proteins is not clearly known. The main storage proteins are generally classified under *albumins*, *globulins*, *prolamins* and *glutelins*. The principal storage proteins of the endosperm of maize, barley, sorghum and a few other cereals are of the prolamin type, while those of wheat are prolamins and glutelins. In rice glutelins are the major proteins, although prolamins and globulins also form significant percentage. Generally, the cereal endosperm proteins are named after the generic name of the cereal from which they are known, such as zein (prolamins of *Zea mays*), hordein (prolamins of *Hordeum vulgare*) and oryzenin (glutelins of *Oryza sativa*).

The regulatory role of endosperm is evident from various sources. The vacuolar sap of central cell/endosperm chamber is an important reservoir of sugars, amino acids and inorganic salts and that their concentrations in the chalazal and micropylar poles are totally different, thus establishing a physicochemical gradient with different pH at different regions. This gradient appears to be different in the prefertilization stage, immediately after fertilization and much later after fertilization. Naturally this concentration gradient and pH affect the osmoticum of the egg/zygote and embryo. In other words, the chalazal and micropylar regions of the central cell/endosperm have different 'topophysic effects' or positional effects (Evenari 1984), emphasizing the existence of different morphogenetic fields in the embryo sac in pre- and post-fertilization stages (Krishnamurthy 1994). There is growing evidence that the endosperm is a rich source of growth regulators, which are likely to regulate the growth and development of the embryo. IAA from immature corn kernels, nicotinic acids and indole pyruvic acid from maize kernels, auxin-like substances (not IAA) in the endosperm of apple, etc., are some examples of growth regulators present in endosperm. Coconut milk has an 'embryo factor' as well as 1,3-diphenyl urea, both of which have regulatory roles. The contour changes from filamentous to globular to heart-shaped to torpedo phases are most likely to be regulated by the endosperm as has been shown by Krishnamurthy (1988). The transition between these contours is marked by the following specific changes in the endosperm, particularly in nuclear and (micropylar chamber of) helobial endosperms: the free-nuclear phase, the cell wall formation phase and the phase at which storage products are deposited. During the free-nuclear phase, the embryo reaches the

globular stage. At the time of initiation of wall formation, the hypophysis and epiphysis are differentiated, as well as the initiation of cotyledon development. At the time of initiation of deposition of storage products in the endosperm, the rapid development of cotyledon(s) occurs. If wall formation around free endosperm nuclei fails or gets delayed, as in cases of seed failure for various reasons, the globular embryo stops to transform itself into the heart-shaped stage or proceeds to this stage after delay, respectively. This is seen, for example, in colchicine-treated *Argemone mexicana* or in *Citrus* crosses.

18.3.5 Endosperm-Maternal Tissue Balance

The embryo and endosperm develop inside the female gametophyte and are surrounded or adjoined by maternal tissues like the ovular integument, nucellus (in some taxa at least), chalaza and placental tissue. The endosperm-maternal tissue relationship is a very important deciding factor in seed set. The effect of the maternal sporophytic tissue on endosperm (and embryo) is called *sporophytic maternal effect*, a study of which is very important in seed development (Sussex and Dale 1979). The effect of *gametophytic maternal effect* is different from the above (see next section). In an interesting theory called *somatoplastic sterility*, Brink and Cooper (1939) suggested that in a normal balance between endosperm and maternal sporophytic tissue, the endosperm competes, successfully transferring nutrients from the ovule to the immature embryo. If the endosperm lacks its normal vigour because of inbreeding, incongruous interspecific crosses, improper chromosomal balance or chemical treatments, the maternal structures (nucellus, integuments) surrounding the endosperm (and embryo) then becomes hyperplastic at the expense of the endosperm. In other words, in a normal seed, the size of the maternal tissue immediately around the endosperm is merely accommodated to that of the rapidly expanding endosperm to which it is subordinate and that in abnormal cases this nicely balanced relationship is upset very early during seed development.

18.3.6 Genetics of Endosperm Development

Lots of evidence have come to show that endosperm development is under genetic control. One line of evidence for this comes from an analysis of mutants that affect endosperm development. These mutants include female gametophytic mutants which through the operation of maternal gametophytic effects affect very early endosperm development. For the former category of mutants, the following may be cited as the most important: the *fertilization-independent endosperm* (*fie*) mutant and *fertilization-independent seed* (*fis*) mutants like *fis1*, *fis2* and *fis3* in *Arabidopsis* which can form 'endosperm' in the absence of fertilization (Grossniklaus et al. 1998; Ohad et al. 1996; Chaudhury et al. 1997); these can affect indirectly the 'zygote', if formed. In plants heterozygous for *fie* and *fis1, 2, 3* loci, the secondary nucleus starts dividing in the absence of pollination. Thus, these gametophytic gene products are likely to be involved in the activation of reproductive programme in response to fertilization. All four loci are not or very poorly transmitted through the female gametophyte. If fertilized, seeds derived from female gametophytes carrying the mutant alleles abort irrespective of the paternal contribution. The other *Arabidopsis* mutant *medea* (*mea*) (Grossniklaus et al. 1998; Grossniklaus and Vielle Caldaza 1998) also displays gametophytic maternal control on endosperm (and also on embryo). An enlarged chalazal cyst is formed in the endosperm due to genomic imprinting, where only the maternally inherited allele is active after fertilization (Vielle-Calzada et al. 1999). The MEA protein (like FIS and FIE proteins) forms part of a *Polycomb* group complex (PCG) (Hennig and Derkacheva 2009) that suppresses cell proliferation, not only after fertilization but also in the absence of fertilization, preventing fertilization-independent endosperm development. Three other components of the complex are known, all of which share this interesting phenotype (Ohad et al. 1996, 1999; Grossniklaus et al. 1998; Chaudhury et al. 1997; Kinoshita et al. 1999; Yadegari et al. 2000; Köhler et al. 2003; Pischke et al. 2002). Whether the genes encoding other components of the

Polycomb group complex are also regulated by genomic imprinting or show a maternal effect because they are cytoplasmically stored is currently unknown (Guitton et al. 2004).

The known endosperm-specific mutants are cereals, particularly in maize, and these include mutants like *brittle* (*bt*), *floury* (*fl*), *opaque* (*op*), *shrunken* (*sh*), *sugary* (*su*) and *waxy* (*wx*). These control the nature of the storage reserves in the endosperm. The defective kernel (dek) mutants show lesions in endosperm development and result in grains with collapsed or shrunken endosperm with soft and fluid consistency. Studies made on *miniature seed* (*mn*), a dek mutant, have revealed that there is impairment of supply of photosynthates to the endosperm and that this causes a physical destruction of cells at the chalazal pole, often seen as a gap between the pedicel and the endosperm. This has been attributed to the presence of low levels of an *MN* gene encoded, endosperm-specific, cell wall invertase in the mutant endosperm and the resultant osmotic imbalance caused by failure of sucrose mobilization, particularly at the chalazal pole. The *shrunken endosperm expressing xenia* (*sex*) mutations of barley, though similar to dek mutants, show a total lack of aleurone cells to total loss of starchy endosperm traits; sometimes, even cell types that are unknown to wild type are developed.

The role of MADS-box genes on endosperm development has been brought to our attention by some investigators (see Masiero et al. 2011). *AGL80* which is expressed in central cell is also seen during endosperm development. *AGL62*, a close paralogue of *DIANA* (*AGL61*), suppresses premature endosperm cellularization. The down-regulation of *PHERES1* (*PHE1*), *PHE2*, *AGL35*, *AGL36*, *AGL40*, *AGL62* and *AGL90* coincides with the transition of endosperm from the free nuclear to cellularization phase, and this appears to be crucial for endosperm differentiation (Kang et al. 2008).

18.4 Fruit Development

Fruits develop invariably from the ovary part of the gynoecium, and hence fruit development involves the further differentiation of a preexisting organ after fertilization. However, in certain taxa, fruit development involves floral organs/parts other than the ovary such as the receptacle, bract, calyx, corolla, floral tube or pedicel/peduncle. In the fruit, the ovary wall becomes the *pericarp*, which depending upon the taxon may have three zones (outer *epicarp* or *exocarp*, middle *mesocarp* and an inner *endocarp*) or only two zones (epicarp and endocarp). Depending on the fruit type, the pericarp may be fleshy (fully fleshy or partly fleshy) or dry. The latter may dehisce or decay to liberate the seeds, while the former only decays to release its seeds.

In most plants, the early fruit ontogeny can be broadly divided into three stages. The first stage involves the development of the ovary which is dependent on the decision to abort or to proceed with fruit development; this is generally referred to as *fruit set*. The second stage essentially involves cell division. The third stage starts after cell division stops, although fruit growth continues mostly by cell enlargement until the final size of fruit is achieved. This stage is the visible and physiologically the most significant stage as it involves strong sink activity exercised by the expanding cells; in some fruits like avocado, cell division and cell expansion overlap during this stage (Gillespy et al. 1993). A fourth stage is seen in fleshy fruits only and it involves ripening.

18.4.1 Biochemical Factors in Fruit Development

Normal fruit development requires double fertilization as well as additional nutrient and growth regulatory substances produced from endosperm, embryo, other parts of the developing seed, other persisting floral parts, if any, and flag and other vegetative leaves. These are required not only for the ordered development of the fruit but also for preventing abscission and premature fruit drop. Most important among the abovementioned parts is the developing seed, since selective removal of seeds as well as aborting seeds often result in misshapen fruits; the pericarp tissue adjacent to these seeds does not develop to the extent as that of near normally developing seeds. Developing seeds exert their influence on fruit development through chemical substances of hormonal nature,

especially through GA; extracts of developing seeds when applied on the wall of unpollinated/unfertilized ovaries develop as pericarp tissue to variable extent. The active chemical principle contained in seeds appears to be similar in most, if not all, fruits. For instance, extracts of immature corn kernels as well as of the seeds of apple promote the growth of tomato ovary into fruit. Also, the more the number of seeds, the greater is the size of the fruit.

The growth regulators of the seed that affect fruit development belong to the categories of auxins, gibberellins, kinins and growth inhibitors, and these have been identified in the seeds of many taxa. For example, auxins have been isolated from the seeds, but none is present in the receptacle of strawberry. Also, the amount of growth regulators present in the seed fluctuates greatly during the various stages of fruit development. For example, peak gibberellins production in the seeds of *Pharbitis nil* coincides with the period of greatest seed development (i.e. around 13 days after anthesis); similarly, the level of auxin is the maximum midtime between pollination and fruit maturation. Generally, kinins occur in great amounts when there is active cell division in the developing fruit. The role of gibberellins in fruit development is often very critical. There is often a direct correlation between growth rate and the endogenous GA level in the developing fruits of apricot, bean, grape, orange and peach. Particular type of endogenous GA is implicated in any given type of fruit during its growth, while other types of GAs may be important in fruit maturation, yet others in ripening, although their levels may, in fact, be lower during ripening (see a subsequent section for more details). In tomato fruit there is a significant decrease in the transcript levels of the chloroplast gene for RbcL in cells of immature fruits when compared with leaves. The leaves of *RbcS* and *Cab* mRNA are also considerably lower than those of *RbcL* and *psbA* throughout fruit development (Piechulla et al. 1986). The role of *RbcS* gene in pericarp development was found out by assessing the pattern of gene expression of individual members of the gene family, composed of five genes (*RbcS1*, *RbcS2*, *RbcS3A*, *RbcS3B* and *RbcS3C*). Within this domain, only *RbcS1* and *RbcS2* are expressed in the cells of the pericarp. The transcripts of these genes accumulated during early fruit development, but rapidly decreased during fruit maturation.

18.4.2 Physical Factors in Fruit Development

Many fruits show a typical sigmoidal growth curve with reference to change in their size, volume, cell numbers, etc. In apple fruit, for instance, there are about two million cells at the time of anthesis, which increase to 40 million when it is harvested. Certain fruits like figs and grapes show a more complex growth pattern with double or even triple sigmoidal curves due to the presence of two or three rapid growth phases interrupted by one or two periods of little or no growth. In grape, the two rapid growth phases are designated as periods I and III, while the interrupting slow growth phase is designated as period II, and about 40 % of total growth is seen in period III; about 80 % of total growth in peach is in period III.

Studies made on gourd fruits have shown that the differences in fruit morphology in various races are due to differential growth rates in diverse dimensions. In the long and narrow fruits, there is a faster increase in length than in width, but at a relatively constant rate, while in the broader fruits, the reverse is true, again at a relatively constant rate. In bottle gourds with miniature and giant-sized fruit races, the relative growth rate remains the same, and the shape/size of the two kinds of fruits at maturity differs because of a difference in the duration of growth rather than rate. Fruit shape differences are mainly due to the differences in the relative number of cells lying along the different axes, and hence, there must be an involvement of genetic control on the planes of cell division. The differences in fruit size are generally due to variations in both cell number and size. Often, the innermost tissues of a fruit show cessation of cell division earlier than the peripheral fruit tissues, but

their cells experience a more rapid enlargement than the peripheral tissue cells.

There are also differences in rate of growth of different parts of the same fruit. In apple, whose fruit is a product of the combined growth of the fleshy receptacle and the fertilized ovary, intense cell division activity contributes to a substantial diameter increase in the fruit within 5–6 days after pollination. The thick endocarp develops rather fairly early at a more rapid rate than any other part of the fruit. In contrast, the fleshy part continues to grow for a further period of about 2 months, both by cell division and enlargement in the early part but predominantly by cell enlargement at the later part. Its growth stops around 150 days after pollination.

In *Arabidopsis*, fruit differentiation is controlled by antagonistically acting *SHP1*, *2* and *FUL* genes, which are expressed in the fruits valve margins and in the valves, respectively (Ferrandiz et al. 2000; Colombo et al. 2010). The B-sister clade gene *GORDITA* (*GOA*) regulates fruit size in *Arabidopsis* by repressing cell enlargement (Prasad et al. 2010). The close paralogue of *GOA*, *ARABIDOPSIS BSISTER* (*ABS*; *TT16*) controls the development of the innermost layer of the integument (endothelium) during seed ontogeny and, thus, seed maturation (Mizzotti et al. 2012).

18.4.3 Fruit Ripening

Fruit ripening, a characteristic feature of fleshy fruits, involves a series of changes in colour, texture (which depends on the degree of contact between its cells and the nature of cell walls), taste, flavour and aroma (due to production of a variety of volatile compounds) occurring in the fully developed fruit. In this process, the structure and composition of the mature fruit are altered in such a way as to make it acceptable for eating by humans or other animals or to make it easily decayed by microbes so as to expose the seeds inside the fruit. Ripening of fruits usually occurs while they are still attached to the plant, but in fruits like avocado, it starts only after separation from the plant. Ripening is initiated usually by sugar accumulation due to starch hydrolysis in many fruits such as banana and apple, loss of acidity, drying up of latex if the mature fruit is laticiferous, softening of texture, skin colouring (usually a change from green to yellow, red or other colours), etc. (Krishnamurthy 2015). Physiologically, ripening of a number of fruits is usually marked by an increased rate of respiration, called *climacteric respiration*, roughly from two to ten times the levels of the minimum respiration value depending on the fruit, and these fruits attain their optimum eating quality only after the climacteric respiration period. There are some *non-climacteric* fruits such as chilli, citrus, cherry and some varieties of grapes. Ethylene promotes both climacteric respiration and other ripening-associated changes; inhibitors of ethylene biosynthesis retard arrest fruit ripening. Moreover, ethylene is produced by all fruits during as well as just preceding ripening. Peak ethylene production precedes (e.g. banana), coincides with (e.g. mango) or comes immediately after (e.g. apple) the peak in respiration. Non-climacteric fruits do not show any accelerated ethylene production at any particular period in fruit ripening, i.e. ethylene production in these fruits is uniform, although very low.

Ripening fruits show complex metabolic changes, both anabolic and catabolic. These pertain to the production of new pigments and new volatile compounds. A number of changes in the plastids such as chloroplasts becoming transformed into chromoplasts and hydrolysis of starch grains present in amyloplastids have been recorded. Very important and complex cell wall changes take place which help in removing cell-to-cell adhesion and the consequent separation of cells from one another to result in the softening of the fruit. Pectolytic enzymes play an important role in this softening. The other changes reported include cell enlargement, polyploidization of nuclei (up to 64-ploid in some taxa), increasing of water content, accumulation of organic substances such as sugars and organic acids, accumulation of leucoanthocyanins, tannins/phenolic acids in some taxa and accumulation of organic

acids, alcohols, carbonyl compounds, hydrocarbons, terpenoids, phenolics, alkaloids, minerals, etc. The changes, physical, chemical and biological, during ripening of mature fruits have led to a debate on whether ripening is a degradative process or an active metabolic process. In support of the latter contention, the increase in protein content, retention of protein (and RNA) synthesis capacity and prevention of ripening through inhibition of protein and RNA synthesis are often cited. In strawberry, striking changes in mRNA subsets, including the appearance of new messages in ripening fruits, a decrease in other mRNAs with advancing ripening and the total disappearance of yet other mRNAs in over ripened fruits are seen, again indicating the strong metabolic activity during ripening. Others consider ripening as a well-designed PCD where also active physiological and genetic controls are seen.

Some data are available on the genetic control of fruit ripening, particularly relating to chloroplast-chromoplast transition and the cell wall softening process. During the ripening of tomato fruits, the transcript levels of various genes encoding for proteins of photosystem I (PS I) and photosystem II (PS II) and stroma decrease to extreme low/zero levels with the exception of *psbA* transcripts in the chromoplasts, which is at least 20 times more than the transcript level of other photosynthetic genes. The *PSY* gene controlling the enzyme phytoene synthase, which is essential for carotenoid synthesis in tomato, has been shown to be expressed during fruit ripening. Gene expression relating to cellulose, pectin methyl esterase (PME) and polygalacturonase (PG) has been investigated in tomato and avocado fruits based on the levels of translatable mRNA. A nearly 50-fold increase in cellulase transcripts in avocado fruits during ripening has been reported. PME, which is responsible for the dissolution of middle lamella pectins, increases two- to threefold during the beginning of ripening of tomato but decreases during active ripening. The tomato fruit mutants like *never ripe* (*nr*), *non-ripening* (*nor*) and *ripening inhibitor* (*rin*) are good examples where fruits do not undergo normal ripening and the ripening-associated chlorophyll degradation, carotenoid synthesis, ethylene production and appearance of polygalacturonase enzyme activity are negatively regulated to varying degrees. The pattern of expression of *PME* gene in the fruits of *nor* and *nr* mutants is similar to that of wild-type fruits, whereas it is downregulated in *rin* mutant fruits. Fruits of plants transformed with an antisense *PME* gene are found to contain reduced levels of *PME*, but yet they show normal ripening as well as other ripening characteristics (Raghavan 2000).

In tomato during the initiation of fruit ripening, there is an accumulation of PG in the pericarp, and it accounts for 3–5 % of the total soluble proteins in that locus of the fruit; it is not seen in the fruit locules where the seeds are located (Tieman and Handa 1989). The transcripts of PG gene show a more than 2,000-fold increase in mature ripe fruits, and it is significant to note that they are almost absent in immature green fruits. The expression of PG gene appears to be regulated at the transcriptional level, with contributions from post-transcriptional processes such as mRNA stability and/or transport. In the mutants, PG and its gene transcript level are greatly reduced, an observation also noticed in transgenic experiments. Although PG expression in transgenic *rin* fruits results in cell walls pectin degradation, there is no significant effect on fruit softening or colour development. This observation questions the generally held view that cell wall pectin degradation is the primary cause for fruit ripening. The explanation for this may come from the identification of a gene for *expansin* from tomato fruits. The transcripts of expansin gene are abundantly expressed in ripe fruits, particularly during the period of softening and are present only at barely detectable levels in *nor* or *rin* mutants. Hence, it is likely that expansin, through loosening bonds between cellulose and hemicelluloses (especially xyloglucan), may play a much more important role in ripening than PG.

Fruit ripening in tomato is shown to need additional genes called *E8* and *E4*, which are ethylene related. *E8* gene is expressed actively during normal fruit ripening as well as in unripe fruits exposed to ethylene, and its activity is

mediated by a DNA-binding component that specifically interacts with the base-pair sequences flanking the gene. This is based on the observation that DNA-binding activity is low in unripe fruit but increases during ripening. *E4* gene transcripts are also abundant in ripening fruits. As in *E8*, the same DNA-binding protein probably coordinated the *E4* gene. *TAGL1*, a MADS-box gene of tomato and an orthologue of *SHP1*, *2* of *Arabidopsis* control fleshy fruit expansion and the ripening process, while in *Arabidopsis* the gene specifies the replum in the siliqua (fruit). It is remarkable that members of the same subfamilies of genes are involved in different functions in different fruit types such as siliqua (*Arabidopsis*), berry (*Solanum*, *Vaccinium*), *pseudocarp* (strawberry and apple), etc. The strawberry *SEP1*, *2* orthologue *FaMADS9* controls fruit development and ripening; the tomato orthologue of *SEP1*, *2*, *TM29* downregulates parthenocarpic development of fruit, but not involved in fruit ripening. By contrast, the tomato *SEP4* orthologue *RIPENING INHIBITOR* (*RIN*) regulates fruit ripening, climacteric respiration and ethylene biosynthesis (*rin* mutant has the opposite features) (Smaczniak et al. 2012).

Not much information is available on the genetic control of fruit ripening in other taxa. Available data indicate the expression of genes such as those of cytochrome P-450 (from avocado), pectin lyases, endochitinase, β-1,3 glucanase, thaumatin-like protein, ascorbate peroxidase, metallothionein, ACC-oxidase and a senescence-related protein (all from banana), pectin lyases and HSP (from strawberry), chitinase and thaumatin-like protein (from grapes) and endo-β-1,4 glucanase (from *Prunus*).

18.5 Seed Development

18.5.1 Introduction

A seed was defined earlier in this chapter as a fertilized ovule, but such a rigid definition is not often followed in botanical literature. This definition, according to Eames (1961), is inadequate since this term is applied even to immature fertilized ovule. The presence of a mature embryo cannot also be used as a strict criterion for the definition of a seed since many taxa release 'seeds' that contain immature embryos (e.g. orchids). So, a seed can be defined as a fertilized ovule containing a viable embryo at some stage of its development. Since the seed is a dispersal unit or *diaspore* of the spermatophyte, it is very important for the evolutionary survival of the species. Seed formation requires the coordinated development of not only the embryo and endosperm but also of the maternal sporophytic tissue of the ovule. The seed is genetically a *triantic entity* (Evenari 1984; Krishnamurthy 2003, 2015). It is the only structure in a plant that has this distinction and hence makes it unique. Its sporophytic maternal tissues like chalaza, seed coat (s) and nucellus (if present) have 2n genetic makeup, with a maternal (m) and paternal (p) ploidy ratio of 2 m:0p (zero p); the zygote/embryo, resulting from syngamy, also has a 2n genetic makeup, but exhibits 1 m:1p ploidy ratio; and the endosperm, resulting from triple fusion, in most angiosperms, has a 3n genetic makeup, but exhibits 2 m:1p ploidy ratio. If these ratios between m and p are altered in the three components of the seed, its development is disturbed or abnormal to varying degrees. In addition, as already discussed, certain component cells of the endosperm (like haustorial cells), embryo (suspensor) and seed coat (endothelial cells) may exhibit endopolyploidy/polyteny.

18.5.2 Role of Chalaza in Seed Development

The behaviour of the chalaza is a very vital component of seed development. As indicated in Chap. 17 of this volume, chalaza is the epicentre for controlling ovule and seed development, not only from a topographical perspective but also from a functional and regulatory point of view. As mentioned again in that chapter, the topographical relationship between the chalaza, funicle and micropyle varies in the different ovular configurations. This relationship is liable to

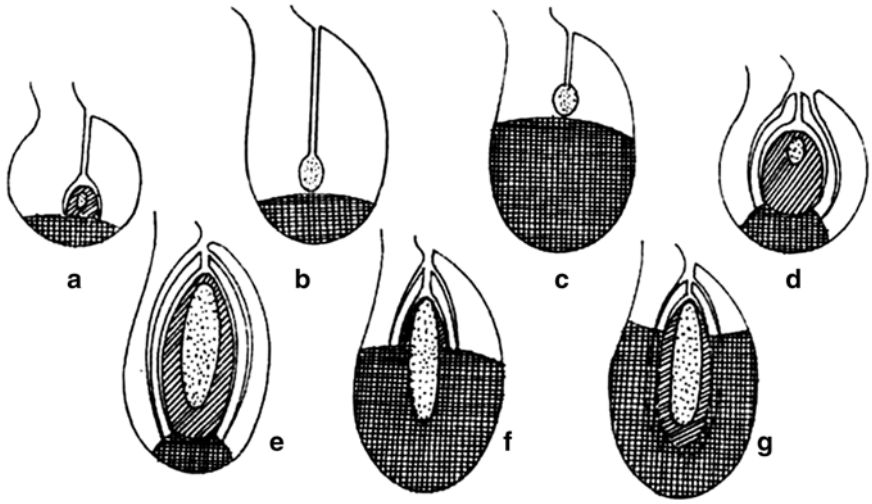


Fig. 18.3 Behaviour of chalaza during seed development. (**a**, **d**) Uni- and bitegmic ovules respectively before fertilization. (**b**, **e**) normal chlazal type. There is no appreciable change in the proportion of the chalaza in the seed as compared to the ovule. (**c**, **f**) Massive chalazal type. Chalaza shows an overall increase of size in seed. (**g**) Perichlazal type where chalaza increases only along a certain direction so that the seed becomes bilaterally symmetrical. The seed is stippled, nucellus cross-hatched and chalaza netted (Periasamy 1962)

further modifications and refinements during seed development. It should be emphasized here that the ovule generally does not undergo a *pari passu* growth during the formation of a seed (Swamy and Krishnamurthy 1980). The relatively small extent of the chalaza in comparison to the remainder of the ovule at its maturity (Fig. 18.3) becomes completely reversed during seed development and maturity. There are three major types of chalazal behaviour during seed development (Periasamy 1962).

1. *Normal chalazal development*: The chalaza continues to occupy a comparatively small portion of the seed so that there is no appreciable alteration of the size relationship that existed between the chalaza and the remainder of ovule prior to fertilization. This type of chalazal behaviour is seen in members of Scrophulariaceae, Passifloraceae, Acanthaceae, etc.
2. *Massive chalazal or pachychalazal development*: The chalaza shows an overall increase in size so that the earlier size relationship is significantly altered in the seed. The major part of the seed is occupied by chalaza-derived tissues. This type of behaviour is seen in species of *Degeneria*, *Myristica* and many Rubiaceae.
3. *Perichalazal development*: The chalaza, instead of showing an overall growth, increases only along a certain direction, resulting in the seed losing the radial symmetry that was characteristic of the ovule from which it is derived; the seed becomes bilaterally symmetrical. This type of chalazal behaviour is seen in the members of Annonaceae, Vitaceae and Menispermaceae.

The chalaza also functions as a regulatory centre of seed development (especially of seed coat, endosperm and embryo) (Krishnamurthy 2003). It is interesting to note that the chalazal control on the embryo and endosperm is noticed in spite of the absence of any direct or symplastic connections between them (Evenari 1984; Thorne 1985). The lack of symplastic connection prevents transmission of viruses from maternal tissues to embryo/endosperm. As indicated in the previous chapter of this book, many genes operate in the region of chalaza-integument complex of the ovule/developing seed. The *SHORT*

INTEGUMENT (*SIN*) mutant gene causes defects in embryo development, and the affected embryo possesses funnel-shaped cotyledons or masses of unorganized tissue. This is an example of a maternal gene affecting embryo development. *AINTEGUMENTA* (*ANT*) and *HUELLENLOS* (*HLL*) mutants lack integuments and the seeds abort. *FBP7* and *FBP11* genes in barley and an ovule-specific MADS gene in *Petunia* also operate in the chalaza-integument domain and affect endosperm development and thus seed development also. The chalaza epicentre also regulates the polarity of the developing embryo along the micropylar-chalazal axis.

The chalaza also serves as a physiological epicentre. It controls the movement of nutrients from the parent plant to the developing seed. Several accommodations have been made in the chalaza for this purpose, especially at the chalaza-endosperm interface. The unloading of materials from phloem terminals takes place in the chalaza, where they get assimilated in the form of simple sugars, amino acids and fatty acids. They, then, are transported from chalaza to the basal part of the endosperm apoplastically, and this is facilitated easily by the transfer cells (with wall ingrowths) of both the basal endosperm cells and also of the upper chalazal cells (Charlton et al. 1995; Howell 1998). Attention was already drawn to the expression of *BET* gene in the basal endosperm cells controlling nutrients transfer into the endosperm. The cDNA *Bet1* serves as a marker for these endosperm cells during the apoplastic transfer between chalaza and endosperm. It codes for a small cell wall protein (probably related to expansins) that is unique to transfer cells and controls the development of cell wall ingrowths. Moreover, water transport into the seed is controlled by chalaza at the time of initiation of seed desiccation and just before dispersal. Data also indicate that the chalazal domain may serve as the signal emanation centre for PCD, especially of endosperm haustoria and seed coat cells. The wave of death of seed coat and endosperm haustoria appears to be from the chalaza towards the micropylar direction.

18.5.3 Role of Other Ovular Structures in Seed Development

The details on the contribution of other ovular structures in seed development are summarized in Kapil and Vasil (1963), Corner (1976) and Bouman (1984), and hence important aspects alone are given here. The seed coat contains only a *testa*, derived from the only integument of unitegmic ovules or from the outer integument of bitegmic ovules or a testa and *tegmen* where the latter is a product of the inner integument of a bitegmic ovule. The seed coat provides the colour, texture and ornamentation of the seed, all of which vary greatly depending on the taxon. It is also very important in controlling the dormancy period of the seed, besides offering protection to the embryo (and endosperm, if present) contained inside it. The relative thickness and the number of constituent cell layers of the seed coat depend not only on the relative contributions of the two integuments or on the contribution of the only integument, and the contribution may be *proliferative* (when the number of cell layers of the integument(s) increase through periclinal divisions), *degenerative* (when the integumental cell layers already present in the ovule degenerates to various degrees) or both proliferative and degenerative (when the number of cell layers increase first followed by degeneration to various extent). The seed coat may be fleshy with living cells, partly with living and partly with dead dry cells or dry with dead cells. Dying cells often get modified into various types of sclereids through PCD.

Many seeds develop local outgrowths, appendages or hairs in the form of glands, hooks/pickles, ancillary pulpy or scaly *aril*-like envelopes, *operculum*, *caruncle*, wings, or long comose or cellulosic hairs. These are developed either to aid in efficient seed dispersal through *anemochory* (wind dispersal), *hydrochory* (water dispersal) or *zoochory* (animal dispersal) or to help in germination. Details are beyond the purview of this chapter and can be obtained from other sources.

References

Aida M, Vernoux T, Furutani M, Traas J, Tasaka M (2002) Roles of PIN-FORMED1 and MONOPTEROS in pattern formation of the apical region of the *Arabidopsis* embryo. Development 129:3965–3974

Baima S, Nobili F, Sessa G, Lucchetti S, Ruberti I, Morelli G (1995) The expression of the Athb-8 homebox gene is restricted to provascular cells in *Arabidopsis thaliana*. Development 121:4171–4182

Baima S, Possenti M, Matteuci A, Wisman E, Altamura MM, Ruberti I, Morelli G (2001) The *Arabidopsis* ATHB-8 HD-ZIP protein acts as a differentiation-promoting transcription factor of the vascular meristem. Plant Physiol 126:643–655

Boothe JG, Walden DB (1990) Gene expression in embryos and seedlings of maize. Maydica 35:187–194

Bouman F (1984) The ovule. In: Johri BM (ed) Embryology of angiosperms. Springer, New York, pp 123–158

Bowman JL, Eshed Y (2000) Formation and maintenance of the shoot apical meristem. Trends Plant Sci 5:110–115

Brink RA, Cooper DC (1939) Somatoplastic sterility in *Medicago sativa*. Science 90:545–546

Brukhin V, Curtis MD, Grossniklaus U (2005) The angiosperm female gametophyte: no longer the forgotten generation. Curr Sci 89:1844–1952

Busch M, Mayer U, Jürgens G (1996) Molecular analysis of the *Arabidopsis* pattern formation gene GNOM: gene structure and intragenic complementation. Mol Gen Genet 250:681–691

Charlton WL, Keen CL, Merriman C, Lynch P, Greenland AJ, Dickinson HG (1995) Endosperm development in *Zea mays*: implication of gametic imprinting and paternal excess of regulation of transfer layer development. Development 121:3089–3097

Chaudhury AM, Ming L, Miller C, Craig S, Dennis ES, Peacock WJ (1997) Fertilization- independent seed development in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 94:4223–4228

Choi Y, Gehring M, Johnson L, Hannon M, Harada JJ, Goldberg RB, Jacobsen SE, Fischer RL (2002) DIMETER, a DNA glycosylase domain protein, is required for endosperm gene imprinting and seed viability in *Arabidopsis*. Cell 110:33–42

Colombo M, Brambilla V, Marcheselli R, Caporali E, Kater MM, Colombo L (2010) A new role for the SHATTERPROOF genes during *Arabidopsis* gynoecium development. Dev Biol 337:294–302

Corner EJH (1976) Seeds of dicotyledons, vol 2. Cambridge University Press, Cambridge

Costa LM, Gutierrez-Marcos JF, Dickinson HG (2004) More than a yolk: the short life and complex times of the plant endosperm. Trends Plant Sci 9:507–514

Créte P (1963) Embryo. In: Maheshwari P (ed) Recent advances in the embryology of angiosperms. International Society of Plant Morphologists, New Delhi, pp 171–220

Dharmasiri N, Dharmasiri S, Estelle M (2005a) The F-box protein TIR1 is an auxin receptor. Nature 435:441–445

Dharmasiri N, Dharmasiri S, Weijers D, Lechner E, Yamada M, Hobbie L, Ehrismann JS, Jürgens G, Estelle M (2005b) Plant development is regulated by a family of auxin receptor F box proteins. Dev Cell 9:109–119

Doelling JH, Yan N, Kurepa J, Walker J, Vierstra RD (2001) The ubiquitin-specific protease UBP14 is essential for early embryo development in *Arabidopsis thaliana*. Plant J 27:393–405

Dure L III (1985) Embryogenesis and gene expression during seed formation. In: Miflin BS (ed) Oxford surveys of plant molecular and cell biology, vol 2. Oxford University Press, Oxford, UK, pp 179–197

Eames AJ (1961) Morphology of the angiosperms. McGraw-Hill Book Company, New York

Esau K (1965) Vascular differentiation in plants. Holt, Rinehart and Winston, New York

Evenari M (1984) Seed physiology: from ovule to maturing seed. Bot Rev 50:143–170

Ferrandiz C, Lilijegrens SJ, Yanofsky MF (2000) Negative regulation of the SHATTERPROOF genes by FRUITFULL during *Arabidopsis* fruit development. Science 289:436–438

Friml J, Wisniewska J (2005) Auxin as an intercellular signal. In: Fleming A (ed) Intercellular communication in plants, vol 16, Ann Plant Rev. Blackwell Publishing, Oxford, UK

Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, Hamann T, Offringa R, Jürgen G (2003) Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. Nature 426:147–153

Furutani M, Vernoux T, Traas J, Kato T, Tasaka M, Aida M (2004) PIN-FORMED1 and PINOID regulate boundary formation and cotyledon development in Arabidopsis embryogenesis. Development 131:5021–5030

Gehring M, Huh JH, Hsieh TF, Penterman J, Choi Y, Harada JJ, Goldberg RB, Fischer RL (2006) DEMETER DNA glycosylase establishes MEDIA polycomb gene self-imprinting by allele-specific demethylation. Cell 124:495–506

Gehring M, Bubb KL, Henikoff S (2009) Extensive demethylation of repetitive elements during seed development underlies gene imprinting. Science 324:1447–1451

Geldner N, Friml J, Stierhof YD, Jürgens G, Palme K (2001) Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. Nature 413:425–428

Gillespy G, Ben David H, Gruissem W (1993) Fruits: a developmental perspective. Plant Cell 5:1439–1451

Goldberg RB, Barker SJ, Perez-Grau L (1989) Regulation of gene expression during plant embryogenesis. Cell 56:149–160

Goldberg RB, de Paiva G, Yadegari R (1994) Plant embryogenesis: zygote to seed. Science 266:605–614

Grimanelli D, Perotti E, Ramirez J, Leblanc O (2005) Timing of the maternal-to-zygotic transition during early seed development in maize. Plant Cell 17:1061–1072

Grossniklaus U, Schneitz K (1998) The molecular and genetic basis of ovule and megagametophyte development. Semin Cell Dev Biol 9:227–238

Grossniklaus U, Vielle Caldaza JP (1998) Response: parental conflict and infanticide during embryogenesis. Trends Plant Sci 3:328

Grossniklaus U, Vielle Calzada JP, Hoeppner MA, Gagliano WB (1998) Maternal control of embryogenesis by *medea*, a Polycomb group gene in *Arabidopsis*. Science 280:446–450

Guitton AE, Page DR, Chambrier P, Lionnet C, Faure JE, Grossniklaus U, Berge F (2004) Identification of new members of fertilization independent seed polycomb group pathway involved in the control of seed development in *Arabidopsis thaliana*. Development 131:2971–2981

Haecker A, Groβ-Hardt R, Geiges B, Sarkar A, Breuninger H, Herrmann M, Laux T (2004) Expression dynamics of *WOX* genes mark cell fate decision during early embryonic patterning in *Arabidopsis thaliana*. Development 131:657–668

Hamann T, Mayer U, Jürgens G (1999) The auxin-insensitive bodenlos mutation affects primary root formation and apical-basal patterning in the *Arabidopsis* embryo. Development 126:1387–1395

Hamann T, Benkova E, Bäurle I, Kientz M, Jürgens G (2002) The *Arabidopsis* BODENLOS gene encodes and auxin response protein inhibiting MONOPTEROS-mediated embryo patterning. Genes Dev 16:1610–1615

Harada JJ, Baden CS, Comai L (1988) Spatially regulated genes expressed during seed germination and post-germinative development are activated during embryogeny. Mol Gen Genet 212:466–473

Hardike CS, Berleth T (1998) The *Arabidopsis* gene *MONOPTEROS* encodes a transcription factor mediating embryo axis formation and vascular development. EMBO J 17:1405–1411

Harding EW, Tang W, Nichols KW, Fernandez DE, Perry SE (2003) Expression and maintenance of embryogenic potential is enhanced through constitutive expression of AGAMOUS-LIKE15. Plant Physiol 133:653–663

Hennig L, Derkacheva M (2009) Diversity of Polycomb group complexes in plants: same rules, different players? Trends Genet 25:414–423

Heuros G, Varotto F, Salamini F, Thompson RD (1995) Molecular characterization of *BET1*, a gene expressed in the endosperm transfer cells of maize. Plant Cell 7:743–757

Hong SK, Kitano H, Satoh H, Nagato Y (1996) How embryo size is genetically regulated in rice? Development 122:2051–2058

Hoshino Y, Scholten S, von Wiegen P, Lörz H, Kranz E (2004) Fertilization-induced changes in the microtubular architecture in the maize egg cell and zygote-an immunocytochemical approach adapted to single cells. Sex Plant Rep 17:89–95

Howell SH (1998) Molecular genetics of plant development. Cambridge University Press, Cambridge

Hsieh TF, Ibarra CA, Silva P, Zemach A, Eshed-Williams L, Fischer RL, Zilberman D (2009) Genome-wide demethylation of *Arabidopsis* endosperm. Science 324:1451–1454

Johansen DA (1950) Plant embryology: embryogeny of the spermatophyta. Chronica Botanica, Waltham

Jullien PE, Mosquna A, Ingouff M, Sakata T, Ohad N, Berger F (2008) Retinoblastoma and its binding partner MSI1 control imprinting in *Arabidopsis*. PLoS Biol 6:e194

Jürgens G (2001) Apical-basal pattern formation in *Arabidopsis* embryogenesis. EMBO J 20:3609–3616

Kang IH, Steffen JG, Portereiko MF, Lloyd A, Drews GN (2008) The AGL62 MADS domain protein regulates cellularization during endosperm development in Arabidopsis. Plant Cell 20:635–647

Kapil RN, Vasil IK (1963) The ovule. In: Maheshwari P (ed) Recent advances in the embryology of angiosperms. International Society of Plant Morphologists, New Delhi, pp 41–67

Kepinski S, Leyser O (2005) The *Arabidopsis* F-box protein TIR1 is an auxin receptor. Nature 435:446–451

Kinoshita T, Yadegari R, Harada JJ, Goldberg RB, Fischer RL (1999) Imprinting of the MEDEA Polycomb gene in the *Arabidopsis* endosperm. Plant Cell 11:1945–1952

Köhler C, Makarevich G (2006) Epigenetic mechanisms governing seed development in plants. EMBO Rep 7:1223–1227

Köhler C, Hennig L, Spillane C, Pien S, Gruissem W, Grossniklaus U (2003) The Polycomb-group protein MEDEA regulates seed development by controlling expression of the MADS-box gene PHERES 1. Genes Dev 17:1540–1553

Köhler C, Page DR, Gagliardini V, Grossniklaus U (2005) The *Arabidopsis thaliana* MEDEA Polycomb group protein controls expression of PHERES1 by parental imprinting. Nat Genet 37:28–30

Kranz E, Lörz H (1993) In vitro fertilization with isolated, single gametes results in zygotic embryogenesis and fertile maize plants. Plant Cell 5:739–746

Kranz E, Bautor J, Lörz H (1991) In vitro fertilization of single, isolated gametes of maize mediated by electrofusion. Sex Plant Reprod 4:12–16

Kranz E, von Wiegena P, Quadera H, Lörz H (1998) Endosperm development after fusion of isolated, single maize sperm and central cells in vitro. Plant Cell 10:511–524

Kranz B, Kumehn J, Dresselhaus T (1999) Fertilization and zygotic embryo development in vitro. In: Cresti M, Cai G, Moscatelli A (eds) Fertilization in higher plants. Springer, Berlin, pp 337–349

Krishnamurthy KV (1988) Endosperm controls symmetry changes in the developing embryos of angiosperms. Proc Indian Acad Sci (Plant Sci) 98:257–259

Krishnamurthy KV (1994) The angiosperm embryo: correlative controls in development, differentiation, and maturation. In: Iqbal M (ed) Growth patterns in vascular plants. Dioscorides Press, Portland, pp 372–404

Krishnamurthy KV (1999) On embryos and embryoids. Curr Sci 76:647–659

Krishnamurthy KV (2003) Certain key issues in seed development. In: Pandey AK, Dhakal MR (eds) Advances in plant reproductive biology. Narendra Publishing House, Delhi, pp 77–88

Krishnamurthy KV (2015) Growth and development in plants. Scientific Publishers, Jodhpur

Lehti Shiu MD, Adamczyk BJ, Fernandez DE, Perry SE (2003) Expression and maintenance of embryogenic potential is enhanced through constitutive expression of AGAMOUS-LIKE15. Plant Physiol 138:653–663

Lindsay K, Topping JE (1993) Embryogenesis: a question of pattern. J Exp Bot 259:359–374

Lopes MA, Larkins BA (1993) Endosperm origin, development and function. Plant Cell 5:1383–1399

Lu P, Porat R, Nadeau JA, O'Neill SD (1996) Identification of a meristem L1 layer-specific gene in *Arabidopsis* that is expressed during embryonic pattern formation and defines a new class of homebox genes. Plant Cell 8:2155–2168

Lukowitz W, Roeder A, Parmenter D, Somerville C (2004) A MAPKK kinase gene regulates extra-embryonic cell fate in Arabidopsis. Cell 116:109–119

Lyndon RF (1990) Plant development. Unwin Hyman, Boston

Maheshwari P (1950) An introduction to the embryology of angiosperms. McGraw-Hill, New York

Mansfield SG, Briarty LG (1990) Early embryogenesis in *Arabidopsis thaliana*. II, The developing embryo. Can J Bot 69:461–467

Masiero S, Colombo L, Grini PE, Schnittger A, Kater MM (2011) The emerging importance of type I MADS box transcription factors for plant reproduction. Plant Cell 23:865–872

Mayer U, Büttner G, Jurgens G (1993) Apical-basal pattern formation in the *Arabidopsis* embryo: studies on the role of the *gnom* gene. Development 117:149–162

Mizzotti C, Mendes MA, Caporali E, Schnitter A, Kater MM, Battaglia R, Colombo L (2012) The MADS-box genes SEEDSTICK and ARABIDOPSIS Bsister play a maternal role in fertilization and seed development. Plant J 70:409–420

Moore JM (2002) Isolation and characterization of gametophytic mutants in Arabidopsis thaliana. PhD thesis, State University of New York at Stony Brook, Stony Brook

Mosher RA, Melnyk CW, Kelly KA, Dunn RM, Studholme DJ, Baulcombe DC (2009) Uniparental expression of Pol IV-dependent siRNAs in developing endosperm of Arabidopsis. Nature 460:283–286

Ohad N, Margossian L, Hsu YC, Williams C, Repetti P, Fischer RL (1996) A mutation that allows endosperm development without fertilization. Proc Natl Acad Sci U S A 93:5319–5324

Ohad N, Yadegari R, Margossian L, Hannon M, Michaeli D, Harada JJ, Goldberg RB, Fischer RL (1999) Mutations in FIE, a WD Polycomb group gene, allow endosperm development without fertilization. Plant Cell 11:407–415

Okamoto T, Kranz E (2005) In vitro fertilization- a tool to dissect cell specification from a higher plant zygote. Curr Sci 89:1861–1869

Okamoto T, Higuchi K, Shinkawa T, Isobe T, Lörz H, Koshiba T, Kranz E (2004) Identification of major proteins in maize egg cells. Plant Cell Physiol 45:1406–1412

Okamoto T, Scholten S, Lörz H, Kranz E (2005) Identification of genes that are up-or down-regulated in the apical or basal cell of maize two-celled embryos and monitoring their expression during zygotic development by a cell manipulation and PCR-based approach. Plant Cell Physiol 46:332–338

Opsahi-Ferstad HG, Le Deunff E, Dumas C, Rogowsky PM (1997) *ZmEar*, a novel endosperm-specific gene expressed in a restricted region around the maize embryo. Plant J 12:235–246

Pagnussat GC, Yu HJ, Ngo QA, Rajani S, Mayalagu S, Johnson CS, Capron A, Xie LF, Ye D, Sundaresan V (2005) Genetic and molecular identification of genes required for female gametophyte development and function in *Arabidopsis*. Development 132:603–614

Periasamy K (1962) The ruminate endosperm: development and types of rumination. In: Plant embryology: a symposium. CSIR, New Delhi, pp 62–74

Periasamy K (1977) A new approach to the classification of angiosperm embryos. Proc Indian Acad Sci 86B:1–13

Periasamy K (1994) Morphogenesis of the angiosperm proembryo. In: Iqbal M (ed) Growth patterns in vascular plants. Dioscorides Press, Oregon, pp 405–417

Piechulla B, Pichersky E, Cashmore AR, Gruissem W (1986) Expression of nuclear and plastid genes for photosynthesis-specific proteins during tomato fruit development and ripening. Plant Mol Biol 7:367–376

Pischke MS, Jones JG, Otsuga D, Fernando DE, Drews GN, Sussman MR (2002) An *Arabidopsis* histidine kinase is essential for megagametogenesis. Proc Natl Acad Sci U S A 99:5800–15805

Prasad K, Zhang X, Tobón E, Ambrose BA (2010) The *Arabidopsis* Bsister MADS-box protein. GORDITA, represses fruit growth and contributes to integument development. Plant J 62:203–214

Pritchard NH (1964) A cytochemical study of embryo development. Am J Bot 51:472–479

Raghavan V (2000) Developmental biology of flowering plants. Springer, New York

Santos F, Hendrich B, Reik W, Dean W (2002) Dynamic reprogramming of DNA methylation in the early mouse embryo. Dev Biol 241:172–182

Schel JHN, Kieft H, van Lammeren AAM (1984) Interactions between embryo and endosperm during early developmental stages of maize caryopses (*Zea mays*). Can J Bot 62:2842–2853

Scheres B, Wolkenfelt H, Willemsenm V, Terlouw MP, Lawson E, Dean C, Weisbeek P (1994) Embryonic origin of the *Arabidopsis* primary root and root meristem initials. Development 120:2475–2487

Scholten S, Lörz H, Kranz E (2002) Parental mRNA and protein synthesis coincides with male chromatin decondensation in maize zygotes. Plant J 32:221–231

Schulz R, Jensen WA (1968) *Capsella* embryogenesis: the egg, zygote and young embryo. Am J Bot 55:807–819

Schwechheimer C, Serino G, Callis J, Crosby WL, Lyapina S, Deshaies RJ, Gray WM, Estelle M, Deng XW (2001) Interactions of the COP9 signalosome with the E3 ubiquitin ligase SCFTIRI in mediating auxin response. Science 292:1379–1382

Schwechheimer C, Serino G, Deng XW (2002) Multiple ubiquitin ligase-mediated processes require COP9 signalosome and AXR1 function. Plant Cell 14:2553–2563

Sharma VK, Carles C, Fletcher C (2003) Maintenance of stem cell populations in plants. Proc Natl Acad Sci U S A 100:11823–11829

Shevel DE, Leu WM, Gilimor CS, Xia G, Feldmann K, Chua NH (1994) EMB30 is essential for normal cell division, cell expansion, and cell adhesion in *Arabidopsis* and encodes a protein that has similarity to Sec7. Cell 77:1051–1062

Smaczniak C, Immink GH, Angnent GC, Karfmann K (2012) Developmental and evolutionary diversity of plant MADS- domain factors: insights from recent studies. Development 139:3081–3098

Souèges R (1937) Les Lois der development. Actualités Scientifiques et Industrielles, Hermann et Cie, Paris

Steinmann T, Geldner N, Grebe M, Mangold S, Jackson CL, Paris S, Gälweiler L, Palme K, Jürgens G (1999) Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF. Science 286:316–318

Street HE (1976) Experimental embryogenesis–the totipotency of cultured plant cells. In: Graham CF, Wareing PF (eds) The developmental biology of plants and animals. Blackwell Science Publication, Oxford, UK, pp 73–90

Sussex JM, Dale RMK (1979) Hormonal control of storage protein synthesis in *Phaseolus vulgaris*. In: Rubenstein I, Phillips RL, Green CE, Gengenbach BG (eds) The plant seed: development, preservation and germination. Academic, New York, pp 129–140

Swamy BGL, Krishnamurthy KV (1975) Certain conceptual aspects of meristems. I. On hypophysis and quiescent centre. Phytomorphology 25:60–65

Swamy BGL, Krishnamurthy KV (1977) Certain conceptual aspects of meristems. II. Epiphysis and shoot apex. Phytomorphology 27:1–8

Swamy BGL, Krishnamurthy KV (1978) Certain conceptual aspects of meristems. III. A model. Phytomorphology 28:1–7

Swamy BGL, Krishnamurthy KV (1980) From flower to fruit——embryology of angiosperms. Tata-McGraw Hill, New Delhi

Swamy BGL, Krishnamurthy KV (1981) On embryos and embryoids. Proc Indian Acad Sci (Plant Sci) 90:401–411

Swamy BGL, Padmanabhan D (1961) Embryogenesis in *Sphenoclea zeylanica*. Proc Indian Acad Sci B 54:169–187

Thorne JH (1985) Phloem unloading of C and N assimilates in developing seeds. Annu Rev Plant Physiol 36:317–343

Tieman DM, Handa AK (1989) Immunocytolocalization of polygalacturonase in ripening tomato fruit. Plant Physiol 90:17–20

Tykarska T (1976) Rape embryogenesis I. The proembryo development. Acta Soc Bot Pol 45:3–16

Tzafrir I, Pena Muralla R, Dickerman A, Berg M, Rogers R, Hutchens S, Sweeney TC, McElver J, Aux G, Patton D, Meinke D (2004) Identification of genes required for embryo development in *Arabidopsis*. Plant Physiol 135:1206–1220

Ulmasov T, Hagen G, Guilfoyl TJ (1999) Activation and repression of transcription by auxin response factors. Proc Natl Acad Sci U S A 96:5844–5849

Vielle-Caldaza JP, Baskar R, Grossniklaus U (2000) Delayed activation of the parental genome during seed development. Nature 404:91–94

Vielle-Calzada JP, Thomas J, Spillane G, Coluccio A, Hoeppner MA, Grossniklaus U (1999) Maintenance of genome imprinting in the *Arabidopsis* medea locus requires zygotic DDM1 activity. Genes Dev 13:2971–2982

Waddington CH (1957) The strategy of the genes. Allen & Unwin, London

Wardlaw CW (1965) Organization and evolution in plants. Longmans, London

Weinhofer I, Hehenberger E, Rozak P, Henning L, Kohler C (2010) H3K27me3 profiling of the endosperm implies exclusion of Polycomb group protein targeting by DNA methylation. PLoS Genet 6:e 1001152

Wolpert L (1970) Positional information and pattern formation. In: Waddington CH (ed) Towards a theoretical biology. Edinburgh University Press, Edinburgh, pp 198–230

Wolpert L (1971) Positional information and pattern formation. In: Mosocona AA, Monroy A (eds) Current topics in developmental biology. Academic, New York, pp 183–224

Wolpert L (1981) Positional information and pattern formation. Phil Trans R Soc Lond B 295:441–450

Yadegari R, Kinoshita T, Lotan O, Cohen G, Katz A, Choi Y, Nakashima K, Harada JJ, Goldberg RB, Fischer RL, Ohad N (2000) Mutations in the FIE and MEA genes that encode interacting Polycomb proteins cause parent-of-origin effects on seed development by distinct mechanisms. Plant Cell 12: 2367–2381

Yalovsky S, Kulukian A, Rodriguez Concepcion M, Young CA, Gruissem W (2000) Functional requirement of plant farnesyl-transferase during development in *Arabidopsis*. Plant Cell 12:1267–1278

Seed Biology and Technology

19

K. Bhanuprakash and Umesha

Abstract

Seeds play a pivotal role in agriculture, serving as the means to propagate plants from one generation to the next, as food for humans and animals and as an important commodity in the global economy. Seed biology and technology is central to our civilisation. Mechanisation of agriculture and adoption of biotechnology are two of the pillars for advancement of seed science in recent years. Understanding the reproductive behaviour and dissecting the function of a flowering plant opened unlimited avenues for a successful seed production with development of new varieties/cultivars and hybrids leading to increased productivity. Application of biotechnology in seed science is undoubtedly emerging as a promising technology to modify the composition of seeds to improve nutrition status, therapeutic value, pre- and postharvest tolerance to biotic and abiotic stresses, etc. More and more stringent seed laws and bills, acts and rights, rules and regulations, legislation and registration matters brought quality seeds into seed supply chain for profitable marketing. This chapter provides a review and serves as a ready reckoner for various aspects of seed science from the beginning to the end.

Keywords

Seed biology • Seed dormancy • Factors causing seed dormancy • Germination • Seed testing • Seed vigour testing • Genetic purity testing • Testing for seed moisture content • Synthetic seed technology

K. Bhanuprakash (✉)
Department of Seed Science and Technology,
Indian Institute of Horticultural Research (IIHR)
Hessaraghatta, Hessaraghatta Lake Post,
Bangalore 560 089, Karnataka, India
e-mail: kbp@iihr.ernet.in

Umesha
Department of Seed Science and Technology,
College of Agriculture, University of Agricultural
Sciences GKVK, Bangalore 560 065,
Karnataka, India

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_19,

19.1 Introduction

The typical life cycle of flowering plants involves several distinct developmental stages, beginning from a seed that undergoes the process of germination to form an established plant that then progresses to a reproductive stage, in which specialised organs for reproduction are produced, ultimately enabling new seed production. Given that plants have adapted to their native environments, the time taken between fertilisation and germination of the mature seed can vary greatly between species (Ma et al. 2006). There is an ample evidence that poor crop stand establishment is a widespread constraint of crop production in developing countries, particularly in the marginal environments farmed by poor people. *Clearly*, *anything that can be done to increase the proportion of seeds that emerge*, *and the rate at which they do so*, *will have a large impact on farmers' livelihoods*.

Every farmer is sensitive to the need for rapid, uniform seedling emergence because it is the foundation on which stand establishment is based and potential yield is determined. Therefore, fundamental knowledge about mechanism underlying seed development, germinability, dormancy and storability is required to improve the performance of seed. Production of high-quality seeds is fundamental to the success of agriculture since crop production relies heavily on high-quality planting seeds. Quality of seed lot is determined by its physical and genetic purity level. The impurity of the pollen source and the mixture of the parental seeds or other cultivar seeds with the hybrids will also lower the genetic purity of the produced hybrids. Low-purity seed would cause big loss for the seed company from the planters' claim. Therefore, it is of critical importance to evaluate the genetic purity in seed production and trade (Garg et al. 2006). For years the method used to check hybrid seed purity has been the grow-out test. This consists of growing a representative sample of the F_1 seed and later classifying it using descriptors of differences as true hybrid seed or off-types. This method is time consuming, space demanding and often does not allow the unequivocal identification of genotypes.

To make the best use of recently evolved varieties for enhanced seed yield, a systematic but effective package of practices are very much needed. Ambient environments in tropical and subtropical regions are generally not good for seed storage, and therefore the maintenance of viability during storage is a great problem. Storing seed at low moisture content in moisture-impervious containers under ambient condition would benefit countries like India where the cost of low-temperature storage is prohibitive. Horticultural crop seeds are known to be sensitive to temperature during drying. The storability of primed, pelleted and coated seeds is also important as the seeds after treatment need to be stored for considerable period as in the case of normal seeds. Hence, storage studies are required to identify problems involved in storage of such seeds and to identify suitable techniques for safe storage.

The success of seed germination and the establishment of a normal seedling are determining features for the propagation of plant species, which are of both economic and ecological importance. Because of its high vulnerability to injury, disease and water/environmental stress, germination is considered to be the most critical phase in the plant life cycle. Seed quality enhancement technologies have expanded over the last 10 years due to the vegetable industry's demands for strong and uniform stand establishment. Precision seeding reduces seed costs per acre, and seed enhancement increases production flexibility and harvest pack-out.

Among the seed enhancement techniques, the technique of seed priming has gained popularity since ages due to its beneficial effects like enhancement in seed germination, advancement of germination, pest and disease resistance, better crop stand establishment and stable yields even under adverse conditions. Seed pelleting and coating is a new technology in India. In Western countries, high-value hybrid vegetable seeds are invariably either coated or pelleted and sold. Most of the coating/pelleting materials are patented and costly too. Hence, it is essential to

identify suitable indigenous pelleting/coating materials and to develop protocol for pelleting and coating along with nutrients and growth regulators to enhance the seed quality.

Plant varieties developed within a country are invaluable national resources. Farmers contribute in conserving, improving and making available plant genetic resources for developing new plant varieties. It is therefore necessary to protect plant varieties as also the rights of farmers and plant breeders to stimulate investment in research and development related to new plant varieties. This makes high-quality seeds and plant material available to farmers and gives a general boost to the growth and development of agriculture in the country. Putting in place an effective system of protection of plant varieties and rights of farmers and plant breeders is also incumbent in India in view of its ratification of the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) under the World Trade Organization (WTO).

Under the Seed Act, 1966 of India, labelling is compulsory and certification is voluntary for the seeds sold in the market. Therefore, the Government of India has prescribed standards so that seeds sold conform to the minimum limits of physical purity, genetic purity and germination, maximum limit of moisture content and status of seed health. These seed quality parameters known as 'seed standards' have been notified for various crops, viz. cereals, millets, pulses, vegetables, etc.

Seed-testing rules are regularly updated by ISTA (International Seed Testing Association, Switzerland) or AOSA (Association of Official Seed Analyst, USA) on the basis of research work done globally and incorporated in ISTA/AOSA Rules for seed testing. The International Rules for Seed Testing (International Seed Testing Association, Switzerland) contain prescription for all kinds of seed-testing activities of a large number of species cultivated all over the world, and it forms the basic and essential reference book for seed testing and seed trade. The changes in orientation of production and other ancillary changes have affected the seed industry as a whole, creating a new global industry, which is guided by large multinational companies.

Progress in biotechnological research during the last two decades has opened up unprecedented opportunities in many areas of basic and applied biological research. Plant tissue culture, which is an important component of plant biotechnology, presents new strategies for the improvement of cereals, legumes, forest trees, plantation crops and ornamental plants. Nowadays, artificial seed technology is one of the most important tools to breeders and scientists of plant tissue culture. It has offered powerful advantages for large-scale mass propagation of elite species.

19.2 Seed Development and Maturation

Seed is defined as a fertilised, matured ovule consisting of an embryonic plant together with a store of food, all surrounded by a protective coat. The pattern of seed formation, development and maturation in plants was well described (Bewley and Black 1994; Black et al. 2006; Copeland and McDonald 2001; Hartmann et al. 2002), and the readers can refer to these for more information (see also the Chap. 18 in this volume). The quality of seed depends on various factors. Seed maturation in relation to dormancy induction and release was studied through ABA biosynthesis pathway, and genes associated with it, viz. *ABA1*, the 9-*cis*epoxycarotenoid dioxygenase, *ABA2*/*GIN1*, etc., were identified.

19.3 Seed Dormancy

A dormant seed does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors that are otherwise favourable for its germination, i.e. after the seed becomes nondormant. A completely nondormant seed has the capacity to germinate over the widest range of normal physical environmental factors possible for the genotype. Many reviews on seed dormancy and germination published (Finch-Savage and Leubner-Metzger 2006; Hilhorst and Toorop

1997) clearly illustrate why dormancy for some period is important in some crops and ways and means of breaking the same.

19.3.1 Factors Causing Seed Dormancy

A wide range of factors alter seed dormancy, e.g. temperature, light, nitrate salts or naturally occurring chemical signals (ABA and four other terpenes) and leachate from litter that covers the seeds in their habitat.

19.3.2 Primary Versus Secondary Seed Dormancy

Seeds that are released from the plant in a dormant state are said to exhibit primary dormancy. Seeds that are released from the plant in a non-dormant state but which become dormant if the conditions for germination are unfavourable exhibit secondary dormancy.

19.3.3 Classification of Seed Dormancy

Seeds with primary dormancy can display exogenous (physical), endogenous (physiological and morphophysiological) or combinational dormancy (physical and physiological).

19.3.3.1 Exogenous Dormancy and Removing the Dormancy

The major type of exogenous dormancy is called physical dormancy and these are often called hard seeds. Physical dormancy is caused by the outer seed coverings preventing the seed from taking up water. In nature, physical dormancy is most often satisfied by exposing the seed to high-temperature conditions. Since this can take many years, scarification is followed alternatively. The three most common ways to scarify seeds include hot water, acid or scratching the seed surface.

Hot-water treatment can be accomplished by placing seeds in water that has just begun to boil. Remove the boiling water container from the heat source and allow the seeds to soak for 1–10 min depending on the seed type. Too long exposure to the hot water can kill the seed. This works for many seeds with physical dormancy, but usually only a small percentage of seeds become able to absorb water.

Acid treatment involves soaking the seeds in concentrated sulphuric acid for various durations, and in most cases it needs to be standardised depending on the thickness of seed coat, e.g. *Malvaceae* and *Leguminosae*. Scratching the seed surface with a small file is the recommended method for scarifying small batches of seeds. Scratching is also done by rubbing against rough surface or by other means that removes the upper layer, e.g. bitter gourd. This allows water to penetrate the seed. In clipping method, a portion of the seed is cut at distal end without damaging the embryo so as to facilitate the entry of water into the seed, e.g. *Bixa* spp. and *Terminalia* spp.

19.3.3.2 Endogenous Dormancy

Physiological and morphophysiological are the two major types of endogenous dormancy. Morphological dormancy is a third type of endogenous dormancy, but it is most often seen in herbaceous plants.

Seeds with physiological dormancy require a period of moist chilling to satisfy dormancy. A moist, chilling period is called stratification. In nature, physiological dormancy is satisfied by having the seeds in moist soil over the winter. The same conditions can be simulated by keeping the seeds in a plastic bag containing a moist substrate (sand or vermiculite) in the refrigerator for several months. The optimum temperature for stratification is between 1 and 5 °C (35 and 50 °F).

Seeds with morphophysiological dormancy have an embryo that is less than one-third the size of the seed. In most cases, the seeds require a period of moist, warm stratification to allow the embryo to continue development. However, once

the embryo completes development, it still has physiological dormancy that requires a period of moist, chilling stratification. In nature, seeds with morphophysiological dormancy can take several years to germinate because they need to be exposed to summer and winter conditions. To get quicker germination, these seeds can be placed moist in a warm place (about 21 °C, 75 °F) for several months before being moved to the cool temperature for several months more.

19.3.3.3 Combinational Dormancy

Combinational dormancy occurs in seeds that have both exogenous (physical) and endogenous (physiological) dormancy. This is not a common form of dormancy, but eastern redbud (*Cercis canadensis*) is a good example of a plant with combinational dormancy. In this case, the physical dormancy must be satisfied before the physiological dormancy can be relieved. These seeds are first scarified (by scratching the seed coat with a file) to allow seeds to absorb water. This is followed by moist, chilling stratification.

19.3.3.4 Embryo Dormancy

A dormant embryo is characterised by a high ABA/GA ratio, high ABA sensitivity and low GA sensitivity. Embryo dormancy release involves remodelling of hormone biosynthesis and degradation towards a low ABA/GA ratio, a decrease in ABA sensitivity and an increase in GA sensitivity. Thus, ABA dominates the embryo dormancy programme and GA the embryo germination programme. A nondormant embryo is characterised by increased growth potential, the ability for cell extension growth and the ability to induce the release of coat dormancy.

19.3.3.5 Endosperm Dormancy

Endosperm weakening can be either part of the coat dormancy release or part of the germination programme. Since the endosperm is in most cases a living tissue, it can actively participate in regulating embryo constraint by influencing both the ABA/GA ratio and sensitivity to these hormones. GA acts by increasing the embryo growth potential and by promoting endosperm weakening which is achieved through ABA-independent and ABA-inhibited mechanisms (http://www.seedbiology.de/dormancy.asp).

19.4 Seed Germination

To the seed analyst, germination is 'the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favourable conditions'. Based on the fate of the cotyledons, two kinds of seed germination occur. When cotyledons remain below soil surface due to rapid elongation of epicotyl, then it is called as hypogeal germination, for example, majority of monocotyledons; some trees like mango, coconut and areca nut; and some large-seeded legumes. When cotyledons pushed above soil surface due to rapid elongation of hypocotyls, then it is called as epigeal germination mostly in horticultural and woody plant species, e.g. cotton, cucumber, guar, gourds, tamarind, castor, sunflower and groundnut.

19.4.1 Requirements for Germination

Water Water is a basic requirement for germination. In their resting state, seeds are characteristically low in moisture and relatively inactive metabolically. Once seeds absorb water, activation of enzyme occurs resulting in breakdown, translocation and utilisation of reserve material by the growing tissues.

Gases Air is composed of about 20 % oxygen, 0.03 % carbon dioxide and about 80 % nitrogen gas. If one provides different proportions of each of these gases under experimental conditions, it soon becomes clear that oxygen is required for germination of most species. Carbon dioxide concentrations higher than 0.03 % retard germination, while nitrogen gas has no influence.

Temperature Seed germination is a complex process involving many individual reactions and phases, each of which is affected by temperature. The effect on germination can be expressed in terms of cardinal temperature: that is, *minimum*, *optimum* and *maximum* temperatures at which germination will occur.

19.5 Seed Testing

Seed testing is done to determine the standards of a seed lot, viz. physical purity, moisture, germination and ODV, and thereby enable the farming community to get quality seeds. The seed-testing laboratory is the hub of seed quality control. Seed-testing services are required from time to time to gain information regarding planting value of seed lots.

19.5.1 Objectives and Importance of Seed Testing

Seed testing is required to achieve the following objectives for minimising the risks of planting low-quality seeds:

- To determine their quality, that is, their suitability for planting
- To determine the need for drying and processing and specific procedures that should be used
- To determine if seed meets established quality standards or labelling specifications
- To establish quality and provide a basis for price and consumer discrimination among lots in the market. The primary aim of the seed testing is to obtain accurate and reproducible results regarding the quality status of the seed samples submitted to the seed-testing laboratories.

19.5.2 Role of Seed-Testing Laboratories

Seed-testing laboratories are essential organisations in seed certification and seed quality control programmes. The main objective is to serve the producer, the consumer and the seed industry by providing information on seed quality. Based on the results, whether the seed lot is to be accepted or not is decided (http://Agritech.tnau.ac.in/seed/Seed_seedtesting.html).

19.5.3 Physical Purity Testing

Physical purity analysis tells us the proportion of pure seed component in the seed lot as well as the proportion of other crop seeds, weed seeds and inert matter by weight in percentage for which seed standards have been prescribed. Thus, it helps in:

1. Improving the plant stand (by increasing the pure seed component)
2. Raising a pure crop (by eliminating other crop seeds and weed seeds)
3. Raising a disease-free crop (by eliminating inert matter)
4. Using of seed drill (by selecting uniform particles)

19.5.3.1 Obtaining Working Sample

The working sample is the whole of the submitted sample or a subsample thereof, on which one of the seed quality tests is made. Boerner or soil-type seed divider should be used to homogenise the submitted sample before reducing it to the size of working sample.

The following guidelines need to be followed:

1. Check the cleanliness of the divider and the container.
2. Pour the entire contents of the submitted sample into the hopper of the divider.
3. Allow the content of the submitted sample to pass through the main body of the divider. In case of ‘soil-type’ seed divider, this can be accomplished by tilting the hopper over the body of the divider, while in the case of ‘Boerner’ divider, by opening the gate valve situated at the base of the hopper.
4. Recombine the contents of both sample receiving pans and again pass it through the divider.
5. Repeat this process twice in order to homogenise the submitted sample.
6. Divide the submitted sample.
7. Set aside the contents of one container.
8. Divide the contents of the other container subsequently till the weight of working sample is obtained.

19.5.3.2 Separation

(a) Clean the work board, sample and purity dishes before starting the separation.
(b) Examine the working sample to determine the use of particular aid such as blower or sieves for making separation.
(c) After preliminary separation with the help of sieves or blower, place and spread the retained or heavier portion on the purity work board.
(d) With the help of spatula or forceps, draw working sample into thin line and examine each particle individually, the criteria used being the external appearance (shape, size, colour, gloss, surface texture) and/or appearance in transmitter light.
(e) Separate out impurities such as other crop seeds, weed seeds and inert matter and place the impurities separately in purity dishes, leaving only the pure seed on the purity board.
(f) Seed enclosed in fruits other than those indicated in pure seed should be separated and the detached empty fruit/appendages classed as inert matter.
(g) Collect the pure seed in the sample pan.
(h) Put the lighter portion of the work board and examine under magnification for further separating into the requisite classes (other crop seeds, weed seeds and inert matter).
(i) After separation, identify the other crop seeds and weed seeds and record their names on the analysis card. The kind of inert matter present in the sample should also be identified and recorded.
(j) Weigh each component, pure seed and other crop seeds, weed seeds and inert matter in grams to the number of decimal places shown below:

SL. no.	Wt. of working sample (g)	No. of decimal place required	Example
1.	Less than 1	4	0.9025
2.	1–9.990	3	9.025
3.	10–99.99	2	90.25
4.	100–999.99	1	902.5
5.	1,000 or more	0	1,025

(k) Calculate the percentage by weight of each component to one decimal place only, basing the percentage on the sum of the weight of all the four components. If any component is less than 0.05 %, record it as 'Trace'. Component of 0.05–0.1 % is reported as 0′, 1 %.

19.5.3.3 Reporting the Results

The results of purity test must be given to one decimal place only, and the percentage of all components must total 100. If the result for a component is nil, this must be shown as 0.0 % in the appropriate space of the report form. The report should also include the kind of inert matter and the Latin names of the crop seed and weed seed found in the sample (Seednet.ap.nic.in/stl/htmlpages/physicalpuritytesting.htm).

19.5.4 Seed Germination Test

The purpose of laboratory testing of seed germination is to assess seed quality or viability and to predict performance of the seed and seedling in the field. A notified laboratory under the Seeds Act or qualified laboratory of ISTA for testing seeds must test seed processed for sale. The ultimate aim of testing the germination in seed-testing laboratory is to obtain information about the planting value of the seed sample and by inference the quality of the seed lot. In addition, the laboratory germination results are also required for comparing the performance potential or superiority of the different seed lots. In general, the farmers, seedsmen and public agencies use the germination results for the following purposes:

1. Sowing purposes, with a view to decide the seed rate to achieve desired field establishment
2. Labelling purposes
3. Seed certification purposes
4. Seed Act and law enforcement purposes

In seed testing, germination has been defined as 'the emergence and development from the seed embryo of those essential structures which, for the kind of seed tested, indicate its ability to develop into a normal plant under favourable conditions in soil'. The seedlings devoid of an essential structure, showing weak or unbalanced development, decay

or damage affecting the normal development of seedling, are not considered in calculating the germination percentage. Factors that can affect the performance of seed in germination tests include diseased seed, old seed, mechanically damaged seed, seed stored under high moisture and excessive heating of seed during storage or drying.

19.5.4.1 Laboratory Procedures

The working sample or germination test consists of 400 pure seeds randomly drawn either manually or with the help of counting devices. The seed for germination test must be drawn as follows in accordance with the following two situations:

1. When both purity and germination tests are required:
   (a) Seeds for germination tests must be taken from the pure seed fraction after conducting the physical purity analysis.
   (b) The counting of the seed must be made without discrimination as to the size and appearance.
2. Only germination test is required:
   (a) If the percentage of pure seed is estimated or determined to be above 98 %, the pure seed for germination test shall be taken indiscriminately from a representative portion of the submitted sample.
   (b) If the pure seed is found to be less than 98 %, the seeds for germination test must be obtained by separating the sample into two components, namely:
      (i) The pure seed
      (ii) Seeds of other species and inert matter

For this purpose, at least one-fourth of the quantity required for regular purity analysis must be used after proper mixing and dividing the submitted sample.

19.5.4.2 Number of Replication

Four replications of 100 seeds or a minimum of 3 replications of 100 seeds may be used under unavoidable situations or 8 or 6 replications of 50 seeds or 16/12 replication of 25 seeds according to the kind and size of containers.

19.5.4.3 Substrata for Germination Testing

After placing the seeds on the prescribed substrata, the test should be transferred to the prescribed controlled temperature condition maintained in the cabinet or walk-in germinator for a prescribed period, which varies according to the species (ISTA Seed Testing Rules). In the rules for seed testing, two kinds of temperature conditions are provided. A single numerical indicates the constant temperature where as numericals separated by a dash (-) indicates an alternating temperature. The daily alternation of temperature is brought out manually either by transferring the test from one germinator to another or by changing the temperature of the chamber as in the case of automatic seed germinator.

19.5.4.4 Evaluation of Germination Test

The germination tests need to be evaluated on the expiry of the germination period, which varies according to the kind of seed. However, the seed analyst may terminate the germination test on or before the final count day or extend the test beyond the period depending on the situation. First and second counts are usually taken in the case of top of paper (TP) and between paper (BP) media. At the first and subsequent counts, only normal and dead seeds (which are source of infection) are removed and recorded.

While evaluating the germination test, the seedlings and seeds are categorised into normal seedlings, abnormal seedlings, dead seeds and fresh ungerminated and hard seeds. It may also be necessary to remove the seed coat and separate the cotyledons in order to examine the plumule in species where essential structures are still enclosed at the end of the test.

19.5.4.5 Calculation and Expression of Result

Results are expressed as percentage by number.

$$\text{Germination}(\%) = [\text{number of seeds germinated / number seeds kept}] \times 100$$

When four 100-seed replicates of a test are within the maximum tolerated range, the average represents the percentage germination to be reported on the analysis certificate. The average percentage is calculated to the nearest whole number. The total % of all the category of seeds (normal, abnormal, dead hard, fresh ungerminated) should be 100.

19.5.4.6 Retesting

If the results of a test are considered unsatisfactory, it will not be reported, and a second test will be made by the same method or by an alternative method under the following circumstances:

- Replicate performance is out of tolerance.
- Results being inaccurate due to wrong evaluation of seedlings or counting or errors in test conditions.
- Dormancy persistence or phytotoxicity or spread of fungi or bacteria. The average of the two tests shall be reported.

19.5.4.7 Use of Tolerances

The result of a germination test can be relied upon only if the difference between the highest and the lowest replicates is within accepted tolerances. To decide if two test results of the same sample are compatible again, the tolerance table is used (Agrawal and Dadlani 1995).

19.5.5 Seed Vigour Testing

Vigour testing does not only measure the percentage of viable seed in a sample, it also reflects the ability of those seeds to produce normal seedlings under less than optimum or adverse growing conditions similar to those which may occur in the field. Seeds may be classified as viable in a germination test which provides optimum temperature, moisture and light conditions to the growing seedlings; however, they may not be capable of continuing growth and completing their life cycle under a wide range of field conditions. Generally, seeds start to lose vigour before they lose their ability to germinate; therefore vigour testing is an important practice in seed production programmes.

Testing for vigour becomes more important for carryover seeds, especially if seeds were stored under unknown conditions or under unfavourable storage conditions. Seed vigour testing is also used as indicator of the storage potential of a seed lot and in ranking various seed lots with different qualities.

19.5.5.1 Uses of Seed Vigour Test

1. Vigour tests are commonly used by seed production companies to establish 'in-house' seed quality standards and to monitor seed quality during the various phases of seed production and processing.
2. Seed store managers may use vigour test results to make better informed decisions about the suitability of seed lots for storage, the possible length of storage time and the storage conditions required.
3. Seed exporters can use vigour information to decide which seed lots can withstand the rigours of transport and thus be expected to arrive in the importing country with quality unimpaired.
4. For the ultimate consumer, the farmer, it would be advantageous to know the vigour status of each high-germinating seed lot before making any decision as to which one to buy.
5. Breeding programmes can employ vigour tests to develop cultivars with improved seed performance.

19.5.5.1.1 Hiltner Test

Hiltner test was initially developed to detect seed-borne infection by *Fusarium* spp. It was subsequently developed as a vigour test when it was shown to reflect defects that prevent normal seedlings other than those resulting from seed-borne infection. Seeds having low vigour cannot withstand physical stress during germination. Hence, seeds are sown within layers of sterile brick grit or course sand with a particle size of 2–3 mm. This provides a mechanical barrier through which seeds must penetrate in order to emerge. The emergence of normal seedlings is considered to indicate seed vigour status.

19.5.5.1.2 Cold Test

The cold test simulates early spring field conditions by germinating the seeds in wet soils (70 % water holding capacity) and incubating them at 5–10 °C/41–51 °F for a specified period. At the end of the cold period, the test is transferred to a favourable temperature for germination (e.g. 25 °C/77 °F in the case of sweetcorn). The percentage of normal seedlings is considered as an indication of seed vigour. Vigorous seeds germinate better under cold environments.

19.5.5.1.3 Accelerated Ageing Test (AAT)

The principle of this test is to stress seeds with high temperatures of (40–45 °C/130–139 °F) and near 100 % relative humidity (RH) for varying lengths of time, depending on the kind of seeds, after which a germination test is made. High-vigour seeds are expected to tolerate high temperatures and humidity and retain their capability to produce normal seedlings in the germination test.

19.5.5.1.4 Electrical Conductivity Test

This test measures the integrity of cell membranes, which is correlated with seed vigour. It is well established that this test is useful for garden beans and peas. It has been also reported that the conductivity test results are significantly correlated with field emergence for corn and soybean. As seeds lose vigour, nutrients exude from their membranes, and so low-quality seeds leak electrolytes such as amino acids and organic acids, while high-quality seeds contain their nutrients within well-structured membranes. Therefore, seeds with higher conductivity measurement are an indication of low-quality seeds and vice versa.

19.5.5.1.5 Seedling Vigour Classification Test (SVCT)

This vigour test is an expansion of the standard germination test (SGT). The normal seedlings obtained from the SGT results are further classified into 'strong' and 'weak' categories. This test has been used for corn, garden beans, soybean, cotton, peanuts and other crops.

Seedlings have four significant morphological sites for evaluating vigour:

A. Root system
B. Hypocotyl (the embryonic axis between cotyledons and root)
C. Cotyledons (storage tissue of reserve food for seedling development)
D. Epicotyl (the embryonic axis above the cotyledons)

In this test, seedlings are classified as 'strong' if the above four areas are well developed and free from defects, which is an indication of satisfactory performance over a wide range of field conditions. On the other hand, normal seedlings with some deficiencies such as missing part of the root, one cotyledon missing, hypocotyl with breaks, lesions, necrosis, twisting or curling are classified as 'weak'.

19.5.5.1.6 Paper Piercing Test

The principle of paper piercing test is similar to that of brick gravel test. High-vigour seed lots are expected to produce strong seedlings which can pierce a particular type of paper, while seedlings of poor vigour lots may not be able to pierce the paper. Therefore, the seedlings which emerge by piercing the paper are more vigorous than those which are not able to emerge through the paper.

19.5.5.1.7 RQ Test

During the first few hours of imbibition of water, respiration rate increases which is highly associated with subsequent seedling growth rate (maize, wheat, rice, lima bean, etc.). During respiration, the ratio of volume of CO_2 evolved to the volume of O_2 consumed per unit time is termed as RQ and it is related to seed vigour. The rate of gas exchange is measured in a respirometer called 'Warburg respirometer'.

19.5.6 Genetic Purity Testing

19.5.6.1 Grow-Out Test

A seed is said to be genetically pure if it possesses all the genetic qualities that the breeder has placed in the variety. With any deterioration

in the genetic make-up of the variety during seed multiplication and distribution cycle, there would definitely be proportionate decrease in its performance, e.g. yield, disease/pest resistance, etc. Genetic purity of a seed lot is determined on the basis of distinct morphological characters of the variety expressed at seed, seedling and plant level by comparing its submitted sample with authentic sample under identical environmental situation (Li-Wang Liu et al. 2007; Dongre and Parkhi 2005).

19.5.6.2 Chemical Tests for Species and Cultivar Identification

The chemical tests are spot tests and useful in identification by change in seed colour as well as solution due to added chemicals. The chemical tests, viz. phenol test, peroxidase test, NaOH and KOH test, ferrous sulphate test and seedling response to various chemicals, have been proved to be quite useful in detecting varietal mixtures and grouping of large number of genotypes into distinct classes. These chemical tests are very quick, easy and reproducible, and these tests provide supportive evidence for morphological evaluation of seeds (Agrawal and Dadlani 1995). Protein gel electrophoresis method is the most commonly employed biochemical test for species and cultivar identification. Among the biochemical techniques, SDS-PAGE is an economical, simple and extensively used biochemical technique for describing the seed protein diversity of crop germplasm (Fufa et al. 2005).

Advantages

1. These tests can be easily done by a laboratory technician on large scale in a seed-testing laboratory in the shortest period.
2. They are relatively inexpensive.
3. The test permits detection of percentage admixture of other types.

19.5.6.3 Molecular Tests for Species and Cultivar Identification

Repeatability and accuracy of results using biochemical markers are subject to question, leading to use of DNA molecular markers (RAPD), particularly the codominant markers (SSRs). Simple sequence repeat (SSR) markers are of great importance for rapid assessment of hybrid and parental line seed purity (Sundaram et al. 2006; Pallavi et al. 2011).

19.5.7 Testing for Seed Moisture Content

The seed moisture content (mc) is the amount of water in the seed. It is usually expressed as a percentage on wet weight basis in any seed-testing laboratory. The seed moisture content is the most vital parameter, which influences the seed quality and storage life of the seed. Seed moisture content is closely associated with several aspects of physiological seed quality. For example, it is related to seed maturity, optimum harvest time, mechanical damage, economics of artificial seed drying, seed longevity and insect and pathogen infestation. The objective is to determine the moisture content of seed by methods suitable for routine use. The optimum method for moisture testing depends upon chemical composition of seed, seed structure, moisture content level, degree of accuracy and precision required, constraints of time, technical expertise and cost.

The ideal method could be that it is adapted to all seeds, measures moisture content from 0 % to100 %, is reproducible, requires less training and is low in cost. It is impossible to combine all these. However, in order to measure the moisture content of seeds, methods can be broadly grouped in two categories: (a) direct method and (b) indirect method.

19.5.7.1 Direct Method

Under this category, the seed moisture content is measured directly by loss or gain in seed weight. These are desiccation method, phosphorus pentoxide method, oven-drying method, vacuum-drying method, distillation method, Karl Fischer's method, direct weighing balance and microwave oven method.

19.5.7.2 Indirect Method

These are not so accurate; estimation is approximate, but convenient and quick in use. These are frequently used at seed processing plants. These measure other physical parameters like electrical conductivity or electrical resistance of the moisture present in the seed. Values are measured with the help of seed moisture metres, and these values are transformed into seed moisture content with the help of calibration charts, for each species, against standard air-oven method or basic reference method.

Above all, Karl Fischer's method has been considered as the most accurate and the basic reference method for standardising other methods of seed moisture determination. The constant temperature oven-drying method is the only practical method approved by International Seed Testing Association (ISTA) and other organisations to be used for routine seed moisture determination in a seed-testing laboratory.

19.5.7.3 Constant Temperature Oven-Drying Method

The constant temperature oven-drying method is broadly grouped into two categories:

1. Low constant temperature oven method
2. High constant temperature oven method

Low constant temperature oven method: This method has been recommended for seed of the species rich in oil content or volatile substances. In this method, the pre-weighed moisture bottles along with seed material are placed in an oven maintaining a temperature of 103 °C. Seeds are dried at this temperature for 17 ± 1 h. The relative humidity of the ambient air in the laboratory must be less than 70 % when the moisture determination is carried out.

High constant temperature oven method: The procedure is the same as above except that the oven is maintained at a temperature of 130–133 °C. The sample is dried to a period of 4 h for *Zea mays*, 2 h for other cereals and 1 h for other species. In this method there is no special requirement pertaining to the relative humidity of the ambient air in the laboratory during moisture determination.

19.5.7.4 Seed Moisture Testing Procedure

1. Seed moisture determination be carried out in duplicate on two independently drawn working samples.
2. Weigh each bottle with an accuracy of 1 or 0.1 mg.
3. First, weigh the empty bottle/container with its cover.
4. Grind the seed material evenly using any grinder/grinding mill that does not cause heating and/or loss of moisture content.
5. Mix thoroughly the submitted sample, using spoon, and transfer small portions (4–5 g) of seed samples directly into weighing bottles/containers by even distribution on bottom of the containers.
6. After weighing, remove the cover or lid of the weighing bottles/containers.
7. Place the weighing bottles/containers in an oven, already heated to or maintained in the desired temperature, for the recommended period.
8. At the end of seed drying period, weighing bottles/containers must be closed with its lid/cover.
9. Transfer the weighing bottles/containers to the desiccators having silica gel (self-indicating blue) to cool down for 40–45 min.
10. Weigh again the cooled weighing bottles/containers.
11. Calculate the seed moisture content.

The moisture content as a percentage by weight (fresh weight basis) is calculated to one decimal place, by using of the formulae:

$$\text{Percentage seed moisture content (m.c)} = \left[M_2 - M_3 / M_2 - M_1\right] \times 100$$

where

M_1 = weight of the weighing bottle/container with cover in gm

M_2 = weight of the weighing bottle/container with cover and seeds before drying

M_3 = weight of the weighing bottle/container with cover and seeds after drying

{Note: The seed moisture determination must be done in two replicates, with precise weighing (i.e. up to three decimal places) using lightweight weighing bottles/containers.}

If the seed is pre-dried or dried in two steps, the seed moisture content is calculated from the results obtained in the first (pre-dried) and second stages of seed drying, using the following formula, and expressed as percentages, as under:

$$\text{Percentage seed moisture content}\,(\text{mc}) = \frac{\left[(S_1 + S_2) - (S_1 * S_2)\right]}{100}$$

where
S_1 = is the moisture loss in the first stage
S_2 = is the moisture loss in the second stage

19.5.7.5 Reporting of Results

Seed moisture content must be reported to the nearest 0.1 % on ISTA analysis certificate. If the seed moisture content is determined using any moisture metre, the brand name and type of the equipment must be mentioned on the analysis certificate, under the column 'other determinations'; reporting of range for which the moisture metre is calibrated is another requirement on seed analysis certificate.

19.6 Seed Production System in India

The Indian seed programme largely adheres to the limited generation system for seed multiplication in a phased manner. Generation system of seed multiplication is nothing but the production of a particular class of seed from specific class of seed up to certified seed stage. The choice of a proper seed multiplication model is the key to further success of a seed programme. This basically depends upon (a) the rate of genetic deterioration, (b) seed multiplication ratio and (c) total seed demand (seed replacement rate). The system recognises three generations, namely, breeder, foundation and certified seeds, and provides adequate safeguards for quality assurance in the seed multiplication chain to maintain the purity of the variety as it flows from the breeder to the farmer.

19.6.1 Nucleus Seed

The initial handful of seeds are obtained from selected individual plants of a particular variety, for the purpose of purifying and maintaining that variety, by the originating plant breeder.

19.6.2 Breeder Seed

Breeder seed is the progeny of nucleus seed of a variety and is produced by the originating breeder or by a sponsored breeder. This provides for initial and recurring increase of foundation seed. Breeder seed is monitored by a joint inspection team of scientists and officials of certification agency and National Seed Corporation (NSC). The genetic purity of breeder seed crop should be maintained at 100 %. Breeder seed production is the mandate of the Indian Council of Agricultural Research (ICAR) and is being undertaken with the help of (1) ICAR research institutions, national research centres and All India Coordinated Research Project of different crops, (2) state agricultural universities (SAUs), (3) sponsored breeders recognised by selected state seed corporations and (4) non-governmental organisations.

19.6.3 Foundation Seed

Foundation seed is the progeny of breeder seed and is required to be produced from breeder seed or from foundation seed which can be clearly traced to breeder seed. The responsibility for production of foundation seed has been entrusted to the NSC, State Farm Corporation of India (SFCI), state seed corporation, state departments of agriculture and private seed producers, who have the necessary infrastructure facilities. Foundation seed is required to meet the standards of seed certification prescribed in the Indian Minimum Seed Certification Standards, both at the field and laboratory testing.

19.6.4 Certified Seed

Certified seed is the progeny of foundation seed and must meet the standards of seed certification prescribed in the Indian Minimum Seed Certification Standards, 1988. In the case of self-pollinated crops, certified seeds can also be produced from certified seeds provided it does not go beyond three generations from foundation seed stage-I.

The production and distribution of quality/certified seeds is primarily the responsibility of the state governments. Certified seed production is organised through state seed corporation, departmental agricultural farms, cooperatives, etc. The distribution of seeds is undertaken through a number of channels, i.e. departmental outlets at block and village level, cooperatives, outlets of seed corporations, private dealers, etc. NSC markets its seeds through its own marketing network and also through its dealer network. SFCI markets its seeds mainly through the state departments of agriculture and the state seed corporations. The production of certified seed by NSC and state seed corporations is mainly organised through contract growing arrangements with progressive farmers. SFCI undertakes seed production on its own farms. The private sector has also started to play an important role in the supply of quality seeds of vegetables and crops like hybrid maize, sorghum, bajra, cotton, castor, sunflower, paddy, etc.

19.7 Seed Certification

In general, seed certification is a process designed to maintain and make available to the general public continuous supply of high-quality seeds and propagating materials of notified kinds and varieties of crops so grown and distributed to ensure the physical identity and genetic purity. Seed certification is a legally sanctioned system for quality control of seed multiplication and production.

19.7.1 Objectives of Seed Certification

The main objective of the seed certification is to ensure the acceptable standards of seed viability, vigour, purity and seed health. A well-organised seed certification should help in accomplishing the following three primary objectives.

- The systematic increase of superior varieties
- The identification of new varieties and their rapid increase under appropriate and generally accepted names
- Provision for continuous supply of comparable material by careful maintenance

19.7.2 Eligibility Requirements for Certification

Any variety to become eligible for seed certification should meet the following requirements:

1. General requirements
2. Field standards
3. Specific requirements
4. Seed standards

The variety selected for certified seed production should be a notified variety under Section 5 of the Indian Seed Act, 1966, and it should be in the production chain and its pedigree should be traceable.

In a seed quality control programme through seed certification, the minimum seed certification standards, in fact, are the minimum standard conditions which must be met. The minimum seed certification standards thus are the standards required for the certification of seeds by the certification agencies. The certification standards in force in India are called the *Indian Minimum Seed Certification Standards*. These were published by the Central Seed Certification Board. As a general principle, these standards have been kept at the level, which demand scrupulous attention of the certified seed growers, but at the same time are practical enough that these can be met also. The minimum

seed certification standards can be broadly grouped into two groups:

1. The general seed certification standard aims at outlining the general requirements for the production of genetically pure good-quality seed. These standards prescribed the procedure for certified seed production so that maximum genetic purity and good quality of the seed is ensured.
2. Specific crop standard consists of field standards and seed standards. The field standards consist of:
   (a) The minimum preceding crop requirement specified to minimise genetic contamination from the diseases volunteer plants.
   (b) The minimum isolation requirement specified to minimise seed-borne diseases.
   (c) The number of field inspection and specified stage of crop described to ensure verification of genetic purity and other quality factors.

19.7.3 Seed Standard

Seed standard consists of:

(a) The minimum percentage of pure seeds and maximum permissible limits for inert matter, other crop seeds have been prescribed.
(b) The maximum permissible limits for objectionable weeds, seeds infected by seed-borne diseases have been prescribed to ensure good seed health.
(c) The maximum permissible limits for moisture content have been prescribed for the safe storage of seeds.

The two combined sets of standards constitute the minimum seed certification standards for seed certification.

19.7.4 Seed Certification Agencies

Seeds Act, 1966, provides for the establishment of seed certification agencies in each state. Seed certification agency should function on the following broad principles:

- Seed certification agency should be an autonomous body.
- Seed certification agency should not involve itself in the production and marketing of seeds.
- The seed certification standards and procedures adapted by seed certification agency should be uniform throughout the country.
- Seed certification agency should have close linkage with the technical and other related institutions.
- Its long-term objective should be to operate on no-profit, no-loss basis.
- Adequate staff trained in seed certification should be maintained by the certification agency.
- It should have provision for creating adequate facilities for ensuring timely and thorough inspections.
- It should serve the interests of seed producers and farmers/users.

19.7.5 Seed Certification Control Measures

Seed source verification is the first step in seed certification programme. Unless the seed is from approved source and of designated class, certification agency will not accept the seed field for certification, thereby ensuring the use of high quality true to type seed for sowing of seed crops.

19.7.5.1 Field Inspection

In the evaluation of the growing crop in the field for varietal purity, isolation of seed crop is to prevent outcross, physical admixtures and disease dissemination and also ensure crop condition as regards the spread of designated diseases and the presence of objectionable weed plants.

19.7.5.2 Sample Inspection

Assessing the planting value of the seeds by laboratory tests. Certification agency draws representative samples from the seeds produced under

certification programme and subject them to germination and other purity tests required for conforming varietal purity.

19.7.5.3 Bulk Inspection

Under certification programme provision has been made for bulk inspection. Hence, the evaluation of the lot for the purpose of checking homogeneity of the bulk seed produced as compared with the standard sample is carried out. This gives an idea about the genuinity of lot and sample.

19.7.5.4 Control Plot Testing

Here the samples drawn from the source and final seed produced are grown side by side along with the standard samples of the variety in question. By comparison it can be determined whether the varietal purity and health of the produced seed are equal to the results based on field inspection.

19.7.5.5 Grow-Out Test

Evaluation of the seeds for their genuineness to species or varieties or seed-borne infection. Here the samples drawn from the lots are grown in the field along with the standard checks. Growing plants are observed for the varietal purity. Grow-out test helps in the elimination of the substandard seed lots of the seed crop in the field to verify its conformity to the prescribed field standards (http://seednet.gov.in/material/IndianSeedSector.htm#Seed Certification System in India).

19.8 Seed Legislation

Development of improved crop varieties is vital for sustained increase in agriculture production and productivity. Timely supply of quality seed is equally significant since the contribution of quality seed alone is estimated to be 15–20 % of the total crop production. India with a population of more than one billion and an arable area of 168 million hectares has one of the largest potential seed market in the world. The total Indian seed market valued around $500 million 5 years back (Gadwal 2003), but it values $1 billion presently with large portion of seed trade involving local exchanges of established varieties or farmer-bred seeds. The total amount of certified seeds produced is only 8 % (Gadwal 2003) of total seed sown each year. Therefore, it is imperative to increase the production and distribution of quality seeds. Seed quality attains more significance in view of emerging biotic and abiotic stresses, issues related to quality and phytosanitary measures, competition in domestic and international markets and emerging food needs.

Measures of seed legislation with respect to quantity and quality were initiated in the country by establishment of National Seed Corporation during 1963 under Ministry of Agriculture. The seed sector in India during the period was dominated by the public sector. The National Seed Corporation was the central body to produce seeds of superior dwarf varieties in rice and wheat and superior hybrids in maize, pearl millet and sorghum. This was followed by various seed legislations enacted by the Government of India, the details of which have been enumerated in the following pages. Further, AICRP-National Seed Project during 1979 (NSP) was undertaken by the Indian Government. The project resulted in achieving breeder seed production surpassing the indents in all major crops. Recently, the Government's decision to embrace biotechnology as a means of achieving food security has made seed quality an important aspect in R&D and business sector in India such as 'approval for commercial cultivation of Bt cotton' in the year 2002. Several leading multinational seed companies have entered the seed market, and at present the composition of the seed industry by volume of turnover has reportedly reached a ratio of 60:40 between the private and public sectors.

Since most of the farming community is illiterate or semi-literate, it is the responsibility of the Government to frame rules that govern the production and distribution of quality seeds to the farming community. Though the Seed Act had been implemented in European countries at the end of eighteenth century, India didn't have an act to designate seed quality parameters. This void was fulfilled during 1966, when the Seed Act was formed and followed by Seed Rules in 1968. Both were adopted during 1969 for the

whole of India. Amendments were made subsequently for the Seed Act during the years 1972, 1973, 1974 and 1981. With new varieties coming into the agricultural scenario, the Seed Control Order was formed insisting on compulsory licensing of the dealer. This was made even more stringent by bringing the seeds under the Essential Commodity Act, 1955. To help multinational corporation in utilising the manpower and knowledge base of our country, the Plants, Varieties and Fruits Order was passed during 1989 and amended subsequently during 1998, 2000 and 2001. Finally the order was revised by another order, Plant Quarantine (Regulation of Import into India) Order in 2003. Signing of WTO in 1995 paved the way for private research and development of varieties. In order to regulate such varieties, the protection of Plant Varieties and Farmers' Right Act was passed in 2001 which was followed by National Seed Policy, 2002, and Seeds Bill, 2004.

19.8.1 Protection of Plant Varieties and Farmers Right Act, 2001

Global realisation on the role of plant genetic resources in development of superior crop varieties and use of many traditionally grown plants in development of medicines and various industrial applications raised concerns for Conservation of Biological Diversity (CBD) which came into force in the year 1993. The Government of India felt the need to provide protection to plant varieties which have tremendous commercial value after India became signatory to the Trade-Related Aspects of Intellectual Property Rights Agreement (TRIPS) in the year 1994. The TRIPS agreement required the member countries to provide for protection of plant varieties either by a patent or by an effective sui generis system or by any combination thereof. The sui generis system for protection of plant varieties was developed by India, integrating the rights of breeders, farmers and village communities. The Protection of Plant Varieties and Farmers Right Act was thus formulated in the year 2001 (Pratibha Brahmi et al. 2003; Ramamoorthy et al. 2006).

19.8.1.1 The Main Objectives of the Act

- To recognise and protect the rights of farmers for their contribution made at any time in conserving, improving and making available plant genetic resources for the development of new plant varieties
- To encourage the development of new varieties of plants for accelerated agricultural development
- To accelerate the agricultural development in the country and protect Plant Breeders' Rights (PBR) and to stimulate investment in research and development (R&D) both in the public and private sectors for breeding new plant varieties
- To facilitate the growth of the seed industry, which will ensure the availability of good-quality seed and plant material to farmers

19.8.1.2 Salient Features of the Act

The registration of a plant variety under the PPVFR Act is a legal process, and as per the act it is compulsory. This process establishes the Plant Breeders' Rights (PBR) on the plant variety in favour of the applicant(s). PBR is a legal ownership right granted on a plant variety. Ownership of PBR is not permanent but only for a specific period (15–18 years). PBR can be inheritable by succession, transferable and saleable. The act prescribes the following:

1. Who are eligible for registration of plant variety.
2. The crop varieties that can be registered: these include new varieties that are novel, distinct, uniform and stable.
3. Extant varieties that were in existence before the act.
4. Farmers' varieties: those that are traditionally cultivated and evolved by the farmers in their fields or a wild relative or land race or a variety about which the farmers possess knowledge. The act also specifies varieties that are to be excluded from registration.

19.8.1.2.1 Process of Varietal Registration

1. Submission of application to the registrar in the prescribed proforma

2. Deposition of seed to National Gene Bank for conducting DUS test
3. Advertisement of application to call for any opposition
4. Issue of certification of registration
5. Publication of list of registered varieties
6. Breeder to deposit the seeds/propagating material of registered varieties to National Gene Bank
7. Registration to confer PBR

A National Register of Plant Varieties shall be maintained at the Head Office of the Plant Variety Registry in the Authority. The register will contain the name of registered plant variety with the name, addresses and rights of their breeders and particulars of the denominations of the registered variety.

19.8.1.2.2 Breeders' Rights

Breeder of a registered variety shall have an exclusive right to produce, sell, market, distribute, import or export the variety. In the case of an extant variety, unless a breeder or his successor establishes his or her right, the central government and, in cases where such extant variety is notified for a state or for any area thereof under Section 5 of the Seeds Act, 1966 (54 of 1966), the state government shall be deemed to be the owner of such rights.

19.8.1.2.3 Researchers' Rights

(a) The use of any variety registered under this Act by any person using such variety for conducting experiment or research
(b) The use of a variety by any person as an initial source of variety for the purpose of creating other varieties

Provided that the authorisation of the breeder of a registered variety is required where the repeated use of such variety as a parental line is necessary for commercial production of such other newly developed variety.

19.8.1.2.4 Establishment of 'National Gene Fund' (NGF)

A National Gene Fund (NGF) shall be established under this act, receipts of which include benefit shares, registration fee, compensation payments and other grants from national and international organisations. The NGF will be utilised for promotion of on-farm and ex situ conservation by individuals, communities, panchayats and institutions, for rewarding and recognising conservation undertaken by individuals and communities and for disbursing the pronounced benefit shares and compensations.

19.8.1.2.5 Benefit Sharing

On registration of the variety, any person or group of persons may submit his or her claim of benefit sharing in the prescribed form, and with prescribed fee to the authority if his or her material has been used in the development of that variety, the authority shall take the decision on the matter after considering the following points:

(a) The extent and nature of use of the genetic material of the claimant in the development of the variety relating to which the benefit sharing has been claimed
(b) The commercial utility and demand in the market of the variety relating to which the benefit sharing has been claimed

19.8.1.2.6 Gene Bank

A National Gene Bank will be established by the authority to maintain the seed samples of the registered varieties for the entire period of protection under the act. The applicant of the registered variety shall rejuvenate the seed if so desired by the registrar.

19.8.2 National Seed Policy, 2002

Indian agriculture has made enormous strides in the past 50 years, raising food grain production from 50 million tonnes to over 230 million tonnes. In the process, the country has progressed from a situation of food shortages and imports to one of surpluses and exports. Having achieved food sufficiency, the aim now is to achieve food and nutritional security at the household level. The seed sector has made impressive progress over the last three decades. The Seeds Act, 1966, and Seed Control Order (1983) promulgated thereunder and the New Policy on Seeds

Development (1988) form the basis of promotion and regulation of the seed industry. Far-reaching changes, however, have taken place in the national economic and agricultural scenario and in the international environment since the enactment of the existing seed legislation and the announcement of the 1988 policy.

19.8.2.1 Aims and Objectives

It has become evident that in order to achieve the food production targets for the future, a major effort will be required to enhance the seed replacement rates of various crops. This would require a major increase in the production of quality seeds, in which the private sector is expected to play a major role. At the same time, private and public sector seed organisations at both central and state levels will be expected to adopt economic pricing policies which would seek to realise the true cost of production. The creation of a facilitative climate for growth of a competitive and localised seed industry, encouragement of import of useful germplasm and boosting of exports are core elements of the agricultural strategy of the new millennium.

Biotechnology will be a key factor in agricultural development in the coming decades. Genetic engineering/modification techniques hold enormous promise in developing crop varieties with a higher level of tolerance to biotic and abiotic stresses. A conducive atmosphere for application of frontier sciences in varietal development and for enhanced investments in research and development is a pressing requirement. At the same time, concerns relating to possible harm to human and animal health and biosafety, as well as interests of farmers, must be addressed.

Globalisation and economic liberalisation have opened up new opportunities as well as challenges. The main objectives of the National Seeds Policy (2002), therefore, are the provision of an appropriate climate for the seed industry to utilise available and prospective opportunities, safeguarding of the interests of Indian farmers and the conservation of agro-biodiversity. While unnecessary regulation needs to be dismantled, it must be ensured that gullible farmers are not exploited by unscrupulous elements. A regulatory system of a new genre is, therefore, needed, which will encompass quality assurance mechanisms coupled with facilitation of a vibrant and responsible seed industry. The National Seed Policy, 2002, covers ten thrust areas which are as follows (http://Agricoop.nic.in/seedpolicy.htm):

- Varietal development and plant variety protection
- Seed production
- Quality assurance
- Seed distribution and marketing
- Infrastructure facilities
- Transgenic plant varieties
- Import of seed and planting material
- Export of seeds
- Promotion of domestic seed industry
- Strengthening of monitoring system

19.9 Seed Storage

The ability of seed to tolerate moisture loss allows the seed to maintain the viability in dry state. Storage starts in the mother plant itself when it attains physiological maturity. After harvesting the seeds are either stored in warehouses or in transit or in retail shops. During the old age days, the farmers used to save seeds in little quantities, but introduction of high-yielding varieties and hybrids and modernisation of agriculture necessitated the development of storage techniques to preserve the seeds.

The main purpose of traditional seed storage is to secure the supply of good-quality seed for a planting programme whenever needed. If sowing time follows immediately after seed collection and processing, seeds can go directly from the processing unit to the nursery, and storage is not needed. This is, however, rarely the case. In seasonal climates with a relatively short planting season, sowing time is normally determined by the wish to have plantable size seedlings at the beginning of the planting season. Hence, seeds must often be stored during the period from harvest to sowing, that is, short-term storage of less than a year.

Many species produce seed (or good seed crops) at long intervals, ranging from a few years

to many years. To assure seed supply during the period between two good seed crops, a seed stock should be established (Wang 1975). Even where fruiting is regular and abundant every year, it may be more cost efficient to collect surplus seed to cover several years' supply rather than to undertake collection every year.

Hence, a seed store serves as a buffer between demand and production and has a regular turnover. Seeds are stored during periods of seed availability and shipped to nurseries or other recipients when required to raise plants. A new type of seed store has arisen during the last few decades, viz. stores for conservation of genetic resources. In these so-called gene banks, seeds (and sometimes other propagation material) are stored for long periods at very low moisture content and temperature (cryopreservation). The techniques applied for storage at ultralow temperatures are quite different from conventional seed storage.

The biochemistry and molecular biology of loss in seed viability during seed storage was investigated in various crops, and many indicators as biomarkers were developed to estimate the viability status of seeds during seed storage. Membrane degradation was shown to be the earliest event to occur during seed deterioration. Membrane reorganisation is slower or may be prevented as a consequence of ageing and death, and perturbations in energy synthesis pathways and respiration which are vital to a viable seed are shown to be associated with early events during seed deterioration. All biochemical pathways require enzymes to catalyse reactions within cell, and their degradation appears a major cause for loss of viability during seed storage. Studies suggest that the first changes in seed deterioration occur at the DNA/RNA level leading to poor cell replication and impaired translation. Identification of changes in storage protein profiles, isozymes and protein nativity through electrophoresis application are considered as best markers for estimating the quality and viability of a seed lot. At molecular level, loss in DNA integrity, DNA fragmentation and downregulation of vigour-related genes were identified as suitable indicators for quality checking.

19.9.1 Stages of Seed Storage

The seeds are considered to be in storage from the moment they reach physiological maturity until they germinate or until they are thrown away because they are dead or otherwise worthless. The entire storage period can be conveniently divided into the following stages: storage on plants (physiological maturity until harvest), harvest, until processed and stored in a warehouse, in storage (warehouses), in transit (railway wagons, trucks, carts, railway sheds, etc.), in retail stores and on the user's farm.

19.9.2 Classification of Seed Storage Behaviour

A large variation in storability is encountered between species. In seed handling terminology, seeds have traditionally been grouped into two main groups according to their physiological storage potential, viz. recalcitrant and orthodox seed (Roberts 1973). Orthodox seed encompasses seed that can be dried to low (2–5 %) moisture content and can, with low moisture content, be stored at low temperature. Viability is prolonged in a predictable manner by such moisture reduction and reduction in storage temperature. Seeds of recalcitrant species maintain high moisture content at maturity (often >30–50 %) and are sensitive to desiccation below 12–30 %, depending on species. They have a short storage potential and rapidly lose viability under any kind of storage conditions.

Although the terms 'orthodox' and 'recalcitrant' are relatively well established, storage physiology of seeds seems to cover a more or less continuous spectrum, ranging from extremely recalcitrant, which lose viability in few days, to extremely orthodox, the viability of which under optimal conditions counts in decades or centuries (Farent et al. 1988). Recalcitrant seeds vary with regard to temperature; tropical recalcitrant seeds are normally sensitive to low temperature, whereas temperate recalcitrant seeds can be stored at temperatures slightly above freezing. Recalcitrant seeds vary with regard to temperature; tropical

recalcitrant seeds are normally sensitive to low temperature, whereas temperate recalcitrant seeds can be stored at temperatures slightly above freezing. This climatic distinction is, however, not always valid. For example, in Kenya recalcitrant species of *Cordia* and *Vitex* tolerate storage temperatures of +2 °C (Schaefer 1991). A group of species which can be dried to a moisture content low enough to qualify as orthodox but are sensitive to low temperatures typical for orthodox seeds has recently been termed 'intermediate' (Ellis et al. 1990). An example of such a species is *Swietenia macrophylla*. Further transition groups within the main classes, sometimes termed sub-orthodox and sub-recalcitrant, demonstrate the continuum in the range of storage behaviour. For example, orthodox seeds generally respond to reduced moisture content with extended viability (within the normal range of moisture content) with an approximately doubled storage life for every 1 % reduction of moisture content (Harrington 1972). That holds for moisture reduction down to 4–5 %, depending on species. Further desiccation does not increase storability, but the seeds are generally not adversely affected by lower moisture content as long as they are humified before imbibition. However, some orthodox species do not tolerate moisture content below a certain minimum, regardless of storage temperature.

19.9.3 Factors Affecting Seed Storage

The period seeds will remain viable in store, their longevity as determined by their genetic and physiological storage potential and by any deteriorating events or damage prior to or during storage, as well as by the interaction between individual factors.

19.9.3.1 Genetic Factors

The seed storage potential is influenced by the genetic make-up of the seed. Storage potential is heritable. Species and sometimes genera typically show inherited storage behaviour, which may be either orthodox or recalcitrant. Accordingly, each species is likely to respond identically to a given set of storage conditions (Bonner et al. 1994). Large genetic variation may, however, occur within species, sometimes ranging from orthodox to recalcitrant, more often expressed as different longevities of seeds from different provenances, individuals or clones when stored under similar conditions. Genetic variation within species may occur on different levels, e.g. land races, provenances, individuals and clones.

Genetic influence on storability may be directly related to progressive ageing, or it may be indirect, ascribed to different susceptibility to factors, which may ultimately lead to loss of viability. For example, inherited variation in seedcoat morphology may cause variation in susceptibility to physical damage during processing, which in turn may influence storability.

19.9.3.2 Initial Seed Quality

Seed lots with high initial viability also have a higher longevity in storage than seed with low initial viability. Seeds of high initial viability are much more resistant to unfavourable storage environmental conditions than low viable seed. Once seed starts to deteriorate, it proceeds rapidly. The seed which is injured mechanically suffered a lot and loses its viability and vigour very quickly. Generally small seeds escape injury, whereas large seeds are more likely to be extensively damaged (e.g. bean, lima bean and soybean). Spherical seeds usually give more protection than flat or irregularly shaped seeds.

The progression of natural ageing with resultant loss of viability is not linear over time but typically follows a sigmoid pattern. Loss of viability is initially slow, followed by a period of rapid decline. The higher the viability when the seed lot enters into storage, the longer the seed will keep viable under a given storage environment. For example, a seed lot with an initial viability of 100 % may take several years to lose 50 % of its viability in storage, while the same seed lot having deteriorated during a few weeks of suboptimal conditions to say 80 % may reach

50 % viability in much shorter time. The different rate of loss of viability during the storage period emphasises the importance of storage at the best conditions available as soon as possible after collection. That becomes especially important for species that rapidly lose viability at, e.g. ambient temperature but respond greatly to improved (e.g. cold) storage conditions.

19.9.3.3 Seed Moisture Content

The amount of moisture in the seeds is the most important factor influencing seed viability during storage. Generally if the seed moisture content increases, storage life decreases. If seeds are kept at high moisture content, the losses could be very rapid due to mould growth. Very low moisture content below 4 % may also damage seeds due to extreme desiccation or cause hard seededness in some crops. Since the life of a seed largely revolves around its moisture content, it is necessary to dry seeds to safe moisture contents. The safe moisture content however depends upon storage length, type of storage structure and kind/variety of seed type of packing material used. For cereals in ordinary storage conditions for 12–18 months, seed drying up to 10 % moisture content appears quite satisfactory. However, for storage in sealed containers, drying up to 5–8 % moisture content depending upon particular kind may be necessary.

19.9.3.4 Relative Humidity

Relative humidity is the amount of H_2O present in the air at a given temperature in proportion to its maximum water holding capacity. Relative humidity and temperature are the most important factors determining the storage life of seeds. Seeds attain specific and characteristic moisture content when subjected to given levels of atmospheric humidities. This characteristic moisture content is called equilibrium moisture content. Equilibrium moisture content for a particular kind of seed at a given relative humidity tends to increase as temperature decreases. Thus the maintenance of seed moisture content during storage is a function of relative humidity and to a lesser extent of temperature. At equilibrium moisture content, there is no net gain or loss in seed moisture content.

19.9.3.5 Temperature

Temperature also plays an important role in life of seed. Insects and moulds increase as temperature increases. The higher the moisture content of the seeds, the more they are adversely affected by temperature. Decreasing temperature and seed moisture is an effective means of maintaining seed quality in storage. The following thumb rules by Harrington are useful measures for assessing the effect of moisture and temperature on seed storage. These rules are as follows:

1. For every decrease of 1 % seed moisture content, the life of the seed doubles. This rule is applicable between moisture content of 5–14 %.
2. For every decrease of 5 °C in storage temperature, the life of the seed doubles. This rule applies between 0 and 50 °C.
3. Good seed storage is achieved when the percentage of relative humidity in storage environment and the storage temperature in degrees Fahrenheit add up to 100, but the contribution from temperature should not exceed 50 °F.

19.9.3.6 Microflora and Insects

Loss of viability during storage can be caused instantly by insect or fungal attack or by progressive natural deterioration (ageing). Any of these events are influenced by the storage environment. Temperature and humidity are the most important factors in seed storage. Nondormant seeds may germinate if their moisture content is above 30 %. Rapid deterioration by microorganisms can occur if moisture content is 18–30 %, and seeds with moisture content above 18–20 % respire and metabolise actively. Metabolising seeds may be damaged by accumulation of toxic metabolites or heat if improperly ventilated. Certain seed insects are active at a moisture content of less than 10 %, and damage by fungi may occur down to 4–5 % (Bewley and Black 1994).

19.9.4 Types of Storage

19.9.4.1 Storage at Ambient Temperature and Humidity

Seeds can be stored in piles, single layers, sacks or open containers, under shelter against rain, well ventilated and protected from rodents, and store at least for several months.

19.9.4.2 Dry Storage with Control of Moisture Content but Not Temperature

Orthodox seeds will retain viability longer when dried to low moisture content (4–8 %) and then stored in a sealed container or in a room in which humidity is controlled than when stored in equilibrium with ambient air humidity. Cool condition is especially favourable.

19.9.4.3 Dry Storage with Control of Both Moisture Content and Temperature

This is recommended for many orthodox species which have periodicity of seeding but which are planted annually in large-scale afforestation projects. A combination of 4–8 % moisture content and 0–5 °C temperature will maintain viability for 5 years or more.

19.9.4.4 Dry Storage for Long-Term Gene Conservation

Long-term conservation for orthodox agricultural seeds is −18 °C temperature and 5 ± 1 % moisture content.

19.9.4.5 Moist Storage Without Control of Moisture Content and Temperature

Suitable for storage of recalcitrant seeds, for a few months over winter. Seeds may be stored in heaps on the ground, in shallow pits, in well-drained soils or in layers in well-ventilated sheds, often covered or mixed with leaves, moist sand, peat or other porous materials. The aim is to maintain moist and cool conditions, with good aeration to avoid overheating which may result from the relatively high rates of respiration associated with moist storage. This may be accomplished by regular turning of the heaps.

19.9.4.6 Moist Cold Storage, with Control of Temperature

This method implies controlled low temperature just above freezing or, less commonly, just below freezing. Moisture can be controlled within approximate limits by adding moist media, e.g. sand, peat or a mixture of both to the seed, in proportions of one part media to 1 part seed by volume, and remoistening periodically or more accurately by controlling the relative humidity of the store. This method is much applicable to temperate recalcitrant genera.

19.9.4.7 Cryopreservation

It is also called as cryogenic storage. Seeds are placed in liquid nitrogen at −196 °C. Seeds are actually placed into the gaseous phase of the liquid nitrogen for easy handling and safety. Metabolic reactions come to a virtual standstill at the temperature of liquid nitrogen, and the cells will remain in an unaltered state until the tissues are removed from the liquid nitrogen and defrosted. Therefore, little detrimental physiological activity takes place at these temperatures, which prolongs the storage life of seeds. It is not practical for commercial seed storage, but is useful to store the valuable germplasm.

19.10 Seed Quality Enhancement

19.10.1 Seed Priming

Priming is a pre-sowing treatment that involves exposure of seeds to a low external water potential that limits hydration (controlled hydration of seed) to a level that permits pre-germinative metabolic activity to proceed, but prevents actual emergence of the radical. This will ensure better field emergence and disease resistance under various adverse conditions. The purpose of priming is to reduce the germination time and improve stand and percentage germination under adverse environmental conditions. Primed seeds are used immediately, but may be dried and stored for short time for later use. Basic objective of seed priming is to ensure rapid seed germination and faster growth and to achieve successful and uniform stand establishment in the field. A seed

priming treatment can be pre-sowing or prestorage (or mid-storage) treatment. Many priming methods are in practice for seed quality enhancement (Bhanuprakash et al. 2010).

19.10.1.1 Methods of Priming

19.10.1.1.1 Hydro-priming

This technique implies soaking the seeds in water for about specific duration. This terminology is currently used both in the sense of steeping (imbibition in water for a short period) and in the sense of 'continuous or staged addition of a limited amount of water'. Hydro-priming methods have practical advantages of minimal waste material produced when compared to osmo- and matrix priming.

19.10.1.1.2 Hydro-priming: 'Steeping'

This is one of the simplest methods and it is being practised over many centuries. On-farm steeping was advocated in many parts of the world as a pragmatic, low-cost/low-risk method for improved crop establishment. Steeping can also remove residual amounts of water-soluble germination inhibitors from seed coats. It can also be used to infiltrate crop protection chemicals for the control of deep-seated seed-borne diseases. This type of seed treatment usually involves immersion or percolation (up to 30 °C for several h), followed by draining and drying back to near original moisture content. Short 'hot-water steeps' (thermotherapy), typically <50 °C for 10–30 min, are used to disinfect or eradicate certain seed-borne fungal, bacterial or viral pathogens. However, extreme care and precision are needed to avoid loss of seed quality.

19.10.1.1.3 Halo- and Osmo-priming

In halo-priming, seeds will be soaked in various solutions of inorganic salts such as KCl, KNO_3, $CaCl_2$, Ca $(No_3)_2$, KH_2PO_4, etc. This method is practised for higher germination and plant emergence in salt-affected soils. In the case of osmo-priming, substances like polyethylene glycol (PEG), sugars, glycerol, sorbitol, mannitol, etc., are used as osmotic solutes to develop lower water potential. As this process, unlike hydro-priming, regulates water movement in much controlled fashion for longer period, this method is preferred in those crops where soaking in treatment solutions, even for shorter period, leads to germination (e.g. onion, beans, etc.).

19.10.1.1.4 Matrix Priming

Solid matrix priming is done using solid carriers with low matrix potentials, e.g. vermiculite, peat moss, sand, celite, etc., for slow imbibition process. In this case, seeds slowly imbibe and reach an equilibrium hydration level. After priming, the moist matrix material is removed by sieving or screening or may be partially incorporated into a coating. This process mimics the natural uptake of water by the seed from soil. Seeds are generally mixed into carrier at matric potentials from −0.4 to −1.5 MPa at 15–20 °C for 1–14 days.

19.10.1.1.5 Thermo-priming

It is a kind of pre-soaking seed treatment with high and low temperature to improve germination and emergence under different environmental (low- and high-temperature) conditions. This process enables seeds to germinate at temperatures lower or higher than those at which they would haven't been able to germinate if untreated.

19.10.1.1.6 Bio-priming

Treating the seed with some of microbial agents like *Rhizobium*, *Azospirillum*, *Pseudomonas aureofaciens*, *Bacillus*, *Trichoderma*, *Gliocladium*, etc., is practised in this method for improving seed viability or vigour. Beneficial microbes are included in the priming process, either as a technique for colonising seeds or to control pathogen proliferation during priming. Compatibility with existing crop protection seed treatments and other biological need to be looked into while practising this method. Costs of registration and other factors currently limit the commercial use of bio-priming. Bio-priming as seed treatment that integrates the biological and physiological aspects of disease control was recently used as alternative method for controlling many seed and soil-borne pathogens.

19.10.1.1.7 Drum Priming

Seeds are hydrated in a tumbling drum using a precise volume of water. The amount of water is limited so that it is less than the amount needed for natural imbibition and seed germination to occur. In this method, the seeds are evenly and slowly hydrated to a predetermined moisture content (typically <25–30 % fresh weight basis) by misting, condensation or dribbling. Drum priming enhances seed performance without the loss of additional materials associated with the conventional osmotic priming technique.

19.10.1.1.8 Priming Using Growth Regulators

In this method, seeds are primed using solutions containing minute quantities of plant growth regulators like gibberellic acid, indole acetic acid, benzyl adenine, methyl jasmonate, 1-aminocyclopropane-1-carboxylic acid, etc. This method is usually followed to address seed dormancy problems or to enhance seed germination under adverse soil conditions or to reactivate impaired metabolism of aged and deteriorated seeds. Soaking papaya seeds in GA_3 250 ppm enhanced seedling emergence even at low-temperature conditions (Bhanuprakash et al. 2010).

19.10.1.1.9 Seedling Dipping

In this method, a suspension was prepared by mixing culture in desired quantity of water. Seedlings will be dipped in the suspension for 15–20 min and transplanted immediately. Generally 1:10 ratio of inoculant and water will be considered. Dipping in nutrient solutions (starter solutions/formulations) is also practised in this method for healthy and vigorous seedlings.

19.10.1.1.10 On-Farm Seed Priming

Farmers can prime their own seed if they know the safe limits. These safe limits are calculated for each variety so that germination will not continue once seeds are removed from the water. Primed seed will only germinate if it takes up additional moisture from the soil after sowing. It is important to note this distinction between priming and pre-germination – sowing pre-germinated seed under dry land conditions can be disastrous. In most cases seed can be primed overnight and is simply surface-dried and sown the same day. Occasionally, sowing may be unavoidably delayed – by heavy rain, for example. If primed seed is surface-dried and kept dry, it can be stored for several days and then sown as usual, and this still performs better than non-primed seed. Farmers can prime their own seeds if they know the maximum length of time for which their seeds can be soaked before seed or seedling damage occurs. After the seeds have been soaked for the appropriate length of time, the water is drained off and the seeds are surface-dried by placing them on a cloth or plastic sheet on the ground for 15–30 min or, for small amounts of seeds, rolled gently in a dry cloth so that they do not stick together (Harris 2006).

19.10.1.2 Advantages of Seed Priming

1. Increases germination rate
2. Early and uniform emergence
3. Germination under broader environment (drought and high salt)
4. Improves performance of low-vigour seeds
5. Improves vigour of immature seeds
6. Breaks seed dormancy
7. Permits germination in suboptimal temperature
8. Reverses seed deterioration effect
9. Increases enzyme activity, protein content and ATP level
10. Synchronisation in flowering among hybrid and parental lines

The beneficial effect of priming is associated with an increase in respiration activity as well as increase in synthesis of proteins and of RNA and DNA. Synthesis of new proteins related to vigour, germ-related proteins, LEA proteins, β-tubulin expression, HSP synthesis and disappearance and expression genes related to vigour are noticed in relation to quality enhancement through seed priming.

19.10.2 Seed Pelleting

Seed pelleting is the mechanism of applying needed materials in such a way that they influ-

Table 19.1 Types of seed pelleting and its constituents

Types of seed pelleting	Ingredients	Filler materials	Adhesives
Nutrient and seed pelleting	Coating with macro- and micronutrients, e.g. DAP, $ZnSO_4$, $FeSO_4$, $CuSO_4$, KCl, Borax, etc.	Gypsum or charcoal or chalk powder	Rice gruel, wheat gruel, gum arabic, carboxyl methyl cellulose (CMC), gelatin, plastic resin, polyvinyl acetate 200–300 ml/kg of seeds
Organic seed pelleting	Leaf powders, e.g. *Albizia amara*, *Pongamia*, Neem, *Prosopis*, *Moringa*, rhizome, *Curcuma*, *Acorus*	The leaf powder itself acts as a filler	
Hydrophilic seed coating	Starch polymers, magnesium carbonate, peroxides of Zn and Ca	Gypsum or charcoal or chalk powder	
Protecting pelleting	Biocontrol agents – *Bacillus* sp., *Streptomyces* sp., pesticides		
Inoculants coating	Biofertilisers – *Rhizobia*, *Azospirillum*, *Azotobacter*, VAM		
Oxygen supplier coating	Peroxides of zinc and calcium		

ence the seed or soil and the seed-soil interface. The main objective was to build small irregularly shaped seeds into spheres facilitating precision drilling in order to achieve optimum plant stand and thereby reduce the need for gap filling. Seed pelleting also serves as a mechanism of applying needed materials in such a way that they affect the seed or soil at the seed-soil interface. The three basic steps involved in pelleting are stated as stamping, coating and rolling. First in the sequence fungicide is to be directly coated on to the seed to improve its efficiency followed by filler materials before coating the nutrients. It is essentially required to avoid direct contact of nutrient chemicals to the seed. Otherwise this may result in scorching of the seed and developing seedlings, as final sequence seeds can be coated with filler materials followed by bioinoculant and biofertilisers (Table 19.1).

19.10.3 Seed Colouring

The practice of providing an exogenous colour coating to seed is only of recent interest. But it is very much prevalent in developed countries for the last decade. Modern seed technology provides a wide selection of enhancements that can be aimed to translate varieties of genetic potential into improved harvest yield and quality. Colouring of seeds along with pelleting/film coating is adapted in private sector seed companies in the USA, Canada and Europe; these enable the seeds to be sown in defined pattern besides modifying germination. Seeds of many cultivated crops have naturally irregular proportions and surfaces and wide size ranges and are sticky or very small. These features can make it hard to handle and efficiently sow the seed. So seed colouring helps in sealing the cracks on the seed coat, and it will improve the physical appearance of seeds. However, it gives the seed a distinct and attractive look to reduce dust and promote environment safety as well as worker safety in the field. Hence, 'pelleting', 'colouring' and coating (Table 19.2) are a family of treatments that are used to make seeds sown easily by altering their shape, weight and textures besides modifying its germination.

Table 19.2 Methods of seed coating technologies

Seed film coating	Seed colouring	Seed pelleting
Seed coating polymer + active ingredients (fungicide + insecticide)	Colouring materials (natural or synthetic dyes)	Adhesive + filler material
		Nutrients + adhesive
		Adhesive + bioinoculants
		Biofertilisers + pelleted seed

19.11 Synthetic Seed Technology

The successful demonstration of encapsulation of tissue culture-derived propagules in a nutrient gel has initiated a new line of research on synthetic

seeds. Synthetic seed can be defined as the artificial encapsulation of somatic embryo, shoot buds or aggregates of cell or any tissues which has the ability to form a plant in in vitro or ex vivo condition. Synthetic seeds can be stored for a long time in appropriate condition.

Recently, production of synthetic seeds by encapsulating somatic embryos has been reported in few species. One prerequisite for the application of synthetic seed technology in micropropagation is the production of high-quality, vigorous somatic embryos that can produce plants with frequencies comparable to natural seeds. Inability to recover such embryos is often a major limitation in the development of synthetic seeds. Synthetic seed technology requires the inexpensive production of large numbers of high-quality somatic embryos with synchronous maturation. The overall quality of the somatic embryos is critical for achieving high conversion frequencies. Encapsulation and coating systems, though important for delivery of somatic embryos, are not the limiting factors for development of synthetic seeds.

19.11.1 Types of Synthetic Seeds

Desiccated Synthetic Seeds Desiccated synthetic seeds are produced from somatic embryos either naked or encapsulated in polyoxyethylene glycol. This type of synthetic seeds is produced in desiccation-tolerant species of plant.

Hydrated Synthetic Seeds Hydrated synthetic seeds are produced by encapsulating the somatic embryos in hydrogels like sodium alginate, potassium alginate, carrageenan, sodium pectate or sodium alginate with gelatin. Alginate hydrogel is frequently selected as a matrix for synthetic seed because of its moderate viscosity and low spinnability of solution, low toxicity for somatic embryos and quick gelation, low cost and biocompatibility characteristics.

19.11.2 Importance of Synthetic/ Artificial Seeds

The artificial seeds can be used for specific purposes, notably multiplication of non-seed-producing plants and ornamental hybrids (currently propagated by cuttings) or the propagation of polyploid plants with elite traits. The artificial seed system can also be employed in the propagation of male or female sterile plants for hybrid seed production. Cryopreserved artificial seeds may also be used for germplasm preservation, particularly in recalcitrant species (such as mango, cocoa and coconut), as these seeds will not undergo desiccation. Furthermore, transgenic plants, which require separate growth facilities to maintain original genotypes, may also be preserved using somatic embryos. Somatic embryogenesis is a potential tool in the genetic engineering of plants. Potentially, a single gene can be inserted into a somatic cell. In plants that are regenerated by somatic embryos from a single transgenic cell, the progeny will not be chimeric. Multiplication of elite plants selected in plant breeding programmes via somatic embryos avoids the genetic recombination and therefore does not warrant continued selection inherent in conventional plant breeding, saving considerable amount of time and other resources. Artificial seeds produced in tissue culture are free of pathogens. Thus, another advantage is the transport of pathogen-free propagules across the international borders avoiding bulk transportation of plants, quarantine and spread of diseases.

19.11.3 Advantages of Synthetic Seeds

1. Easy propagation of hybrid plants.
2. Easy propagation of genetically modified crops.
3. Easy propagation of endangered species.
4. Elite genotype can be preserved and propagated using synthetic seeds.

19.11.4 Uses of Synthetic Seeds

1. Synthetic seed production is cost effective.
2. Genetical uniformity is maintained by using synthetic seed technology.
3. Synthetic seeds are small; therefore, they are easy to handle and transport.
4. Provides protection against pest and diseases.

19.11.5 Limitations of Synthetic Seeds

1. Limited production of viable micropropagules in synthetic seed production and asynchronous development of somatic embryos
2. Improper maturation of somatic embryos that makes them inefficient for germination and conversion into normal plants
3. Lack of dormancy and stress tolerance in somatic embryos that limit storage of synthetic seeds
4. Poor conversion of even apparently normally matured somatic embryos and other micropropagules into plantlets

Acknowledgments The author are grateful to Prof Bir Bahadur, Prof KV Krishnamurthy and Dr. Leela Sahijram for their help in various ways and constructive guidance and criticism in the writing of their review.

References

Agrawal PK, Dadlani M (1995) Techniques in seed science and technology, 2nd edn. South Asian Publishers, New Delhi, pp 109–113, International Book Company Absecon Highlands

Bewley JD, Black M (1994) Seeds: physiology of development and germination, 2nd edn. Plenum Press, New York

Bhanuprakash K, Yogeesha HS, Arun MN (2010) Physiological and biochemical changes in relation to seed quality in ageing bell pepper seeds. Indian J Agric Sci 80(9):10–13

Black M, Bewley JD, Halmer P (2006) The encyclopedia of seeds: science, technology and uses. CAB International, Wallingford

Bonner FT, Vozzo JA, Elam WW, Land SB Jr (1994) Tree seed technology, training course. Instructors manual. Southern Forest Exp. Station, U.S. Dept. Agric, New Orleans. General Technical Report SO-106

Brahmi P, Saxena S, Dhillon BS (2003) The protection of plant varieties and farmers rights act of India. Curr Sci 86(3):392–398

Copeland LO, McDonald MB (2001) Principles of seed science and technology, 4th edn. Kluwer Academic Publishers, Boston

Dongre A, Parkhi V (2005) Identification of cotton hybrids through the combination of PCR based RAPD, ISSR, and microsatellite markers. J Plant Biochem Biotechnol 14:53–55

Ellis RH, Hong TD, Roberts EH (1990) An intermediate category of seed storage behaviour. J Exp Bot 41:1167–1174

Farent JM, Pammenter NW, Berjak P (1988) Recalcitrance – a current assessment. Seed Sci Technol 16:155–166

Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytol 171:501–523

Fufa H, Baenziger PS, Beecher BS, Dweikat I, Graybosch RA, Esk Ridge KM (2005) Comparison of phenotypic and molecular marker based classification of hard red winter wheat cultivars. Euphytica 145:133–146

Gadwal VR (2003) The Indian seed industry: its history, current status and future. Curr Sci 84(3):399–406

Garg A, Singh AK, Prabhu KV, Mohapatra T, Tyagi NK, Nandakumar N, Singh R, Zaman FU (2006) Utility of a fertility restorer gene linked marker for testing genetic purity of hybrid seeds in rice (*Oryza sativa* L.). Seed Sci Technol 34:9–18

Harrington JF (1972) Seed storage and longevity. In: Kozlowski TT (ed) Seed Biol 3:145–245

Harris D (2006) Development and testing of "on-farm" seed priming. Adv Agron 90:129–178

Hartmann HT, Kester DE, Davies FT, Geneve RL (2002) Plant propagation: principles and practices, 7th edn. Prentice Hall, New Delhi Englewood Cliffs

Hilhorst H, Toorop PE (1997) Review on dormancy, germinability, and germination in crop and weed seeds. Adv Agron 61:111–165

http://Agricoop.nic.in/seedpolicy.htm

http://Agritech.tnau.ac.in/seed/Seed_seedtesting.html

http://seednet.gov.in/material/IndianSeedSector.htm#Seed. Certification System in India

Liu L-W, Wang Y, Gong Y-Q, Zhao T-M, Liu G, Li X-Y, Fan-Min Y (2007) Assessment of genetic purity of tomato (*Lycopersicon esculentum* L.) hybrid using molecular markers. Sci Hortic 115:7–12

Ma L, Chen B, Gao G, Xu K, Li X (2006) Detection of DNA methylation changes during seed germination in rapeseed (*Brassica napus*). Chin Sci Bull 51(2):182–190

Pallavi HM, Gowda R, Vishwanath K, Shadakshari YG, Bhanuprakash K (2011) Identification of SSR markers for hybridity and seed genetic purity testing in

sunflower (*Helianthus annuus* L.). Seed Sci Technol 39:259–264
Ramamoorthy K, Sivasubramaniam K, Kannan A (eds) (2006) Seed legislation in India. Agrobios, Jodhpur
Roberts EH (1973) Predicting storage life of seeds. Seed Sci Technol 1:499–514
Schaefer C (1991) Storage of tree seeds in Kenya, recommendations and problems. In: Proceedings 1st National Tree Seed Workshop, 1–5 Jul 1991, Nairobi, pp 99–113. Kenya Forestry Seed Centre/Kenya Forestry Research Institute
Sundaram RM, Naveen kB, Biradar SK, Balachandran SM, Mishra B, Ilyas AM, Virakatamath BC, Yasuhiri M, Masato T, Massahi H (2006) An SSR based linkage map of *Capsicum annum*. Mol Breed 18:157–169
Wang BSP (1975) Tree and shrub seeds. In: Bradnock WT (ed) Advances in research and technology of seeds, part 1: 34–43. PUDOC, Wageningen. www.seedbiology.de/dormancy.asp

Mineral Nutrition of Plants

20

Renu Pandey

Abstract

In this chapter, a brief overview of the history of plant mineral nutrition is provided. Soil serves as the source of nutrient elements, and so the availability of nutrients is governed by soil properties. The term 'essential mineral element' has been defined, and these elements are grouped according to their biochemical behaviour and physiological functions. In addition to these elements, another group of elements called beneficial elements has been discussed. The pathway of movement of elements (as ions) through roots and different mechanisms for absorption and transport of nutrients has been outlined in this chapter. Besides the inorganic nutrient elements, the use of biofertilisers in agriculture has been discussed. These biofertilisers associate with the plants either symbiotically or non-symbiotically, to help them enhance absorption of nutrient elements from soil and improve growth.

Keywords

Plants • Mineral nutrition • Macro- and microelements • Essential mineral element • Transport and absorption of nutrients • Biofertilisers

20.1 Introduction

Inorganic minerals present in the Earth's crust are used for nutrition by plants by extracting them from soil or the aquatic environment. These mineral elements are formed by the complex interaction involving weathering of rock minerals, decay of organic matter, animals and microbes. Among nutrient elements, nitrogen is exceptional as its primary source is gaseous nitrogen of the atmosphere and little occurs in minerals. Roots absorb

R. Pandey (✉)
Mineral Nutrition Laboratory, Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi 110012, India
e-mail: renu.pandey.iari@gmail.com

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_20,

mineral nutrients in the form of their salts dissolved in soil water. The study of absorption of inorganic mineral elements and their assimilation by plants is called mineral nutrition. Once the elements are absorbed by roots, they are translocated to various parts of the plant where they are involved in carrying out important biological functions resulting in normal growth and development.

In agriculture, the addition of mineral elements to soil to improve plant growth dates back to more than 2000 years. About 150 years ago, the function of mineral nutrients in plant growth was a topic of scientific debate. However, it was Justus von Liebig (1803–1873) who collected, compiled and summarised the scattered information pertaining to the importance of mineral elements for plant growth. This established the mineral nutrition of plant as a scientific discipline. These achievements led to a rapid increase in the use of mineral fertilisers in agriculture. By the end of the nineteenth century, particularly in Europe, large amount of potash, superphosphate (phosphorus) and, later, inorganic nitrogen was used in agriculture and horticulture to improve plant growth. It was concluded based solely on observation and speculation rather than by precise experimentation that mineral elements such as nitrogen, sulphur, phosphorus, potassium, calcium, magnesium, silicon, sodium and iron are essential for plant growth.

By the end of nineteenth century, a large number of studies were undertaken to establish the 'mineral element theory'. From the extensive investigation carried out on mineral composition of different plant species growing on different types of soils, it was concluded that neither the presence nor the concentration of mineral element in a plant is the criteria for essentiality. Plants have a limited capability to absorb selectively those mineral nutrients that are essential for their growth. They also take up mineral elements that are not essential for growth and may be even toxic. Therefore, it was evident that the mineral composition of plants growing in soils cannot be used as a criterion to judge the essentiality of a mineral element. Once this fact was established, both water and sand culture experiments were carried out in which particular elements were omitted. The technique of growing plants in soilless culture media was termed as hydroponics. These techniques were used to characterise the essentiality of individual mineral elements more precisely and led to a better understanding of their role in plant metabolism. Discovery of essentiality of micronutrients by various workers using hydroponics is presented in Table 20.1.

Table 20.1 Discovery of essentiality of micronutrients for higher plants (Marschner 1995)

Element	Year	Discovered by
Iron	1860	J. Sachs
Manganese	1922	J. S. McHargue
Boron	1923	K. Warington
Zinc	1926	A. L. Sommer and C. B. Lipman
Copper	1931	C. B. Lipman and G. Mackinney
Molybdenum	1938	D. I. Arnon and P. R. Stout
Chlorine	1954	T. C. Broyer et al.
Nickel	1987	P. H. Brown et al.

20.2 Soil as Source of Nutrient

Soil is a complex physical, chemical and biological substrate which acts as a matrix for various organic and inorganic nutrients. Soil contains all the three phases, viz., solid, liquid and gaseous, which interact with mineral elements. The solid phase provides a reservoir of both inorganic nutrients (potassium, calcium, magnesium and iron) as well as organic compounds which provide nitrogen, phosphorus and sulphur among other elements. The liquid phase or the soil solution is very important from the view point of absorption of nutrients by roots because it contains dissolved nutrient ions and facilitates the movement of ions towards root surface. The airspaces between soil particles which are occupied with gases such as oxygen, carbon dioxide and nitrogen constitute the gaseous phase of soil. From the biological perspective, soil constitutes a diverse ecosystem in which plant roots and microorganisms compete strongly for mineral nutrients. In spite of this competition, roots and

microorganisms can form associations for their mutual benefit (nitrogen-fixing bacteria, arbuscular mycorrhiza).

Based on the bioavailability of inorganic nutrient elements to the plant roots, the soil may be considered as fertile or non-fertile. The term fertility refers to the inherent capacity of soil to supply nutrients to plants in adequate amounts and appropriate proportions. In non-fertile soils, in order to increase the availability of nutrients in a balanced proportion, inorganic elements in the form of fertilisers are added externally. Most fertilisers are formulated to overcome the deficiencies of mineral elements in soil.

20.2.1 Soil Properties Affecting Nutrient Availability

The chemical (surface charge, pH) and physical (soil texture and structure) properties of soil determines the availability of nutrients on the root surface. The soil particles, both organic and inorganic, are negatively charged. This negative charge is due to the fact that many inorganic soil particles are crystal lattices which are arranged in a tetrahedral form of aluminium and silicon (Al^{3+} and Si^{4+}). These tetrahedral arrangements are covalently bound to oxygen atoms forming aluminates and silicates. These tetrahedral structures undergo isomorphic substitution wherein another cation of lesser charge replaces the Al^{3+} or Si^{4+} thus making them negatively charged particles. Microbial decomposition of dead plants, animals and microorganisms leads to the formation of organic soil particles. The dissociation of hydrogen ions from carboxylic acid and phenolic groups present in organic soil particles provides negative surface charges.

Charge on the surface of soil particles has an important role in plant nutrition. Mineral cations such as ammonium (NH_4^+) and potassium (K^+) adsorb to the negatively charged surface of the organic and inorganic soil particles. This adsorption is an important factor in governing soil fertility. The major advantage of adsorption is that the cations are not easily lost from the surface of soil particles when the soil is drained with water (leaching loss). However, these adsorbed nutrients can be replaced by other cations in a process known as cation exchange. The capacity of a soil to adsorb ions and exchange it with other ions is termed as cation exchange capacity (CEC) and is highly dependent on soil types. Soils with small particles like clays have a high ratio of surface area to volume resulting in a higher CEC. A soil with higher CEC generally has larger reserves of mineral nutrients. On the other hand, mineral anions such as nitrate (NO_3^-), chloride (Cl^-) and phosphates (PO_4^{3-}) do not get adsorbed because of repulsion due to the negative charge on the surface of soil particles and thus remain dissolved in the soil solution. These anions are highly prone to leaching loss.

Another important chemical property affecting nutrient availability is pH or the hydrogen ion concentration of soil solution. The reaction of soil solution, whether it is neutral, alkaline or acidic, has a distinct effect on the availability of mineral elements to plant roots. The range of pH for most crops lies approximately between 5.5 and 6.5 at which the greatest average levels of all essential plant nutrients become available (Fig. 20.1). This pH range also favours the root growth. Extreme fluctuations of soil pH on either side can cause nutrient imbalances in plants resulting in deficiency or toxicity symptoms.

The physical property of soil includes different sizes of soil particles. The relative proportion of various soil particle types is referred to as soil texture. The arrangement of soil particles, that is, sand, silt and clay in soil, is known as soil structure. The soil particles are named as gravel, coarse sand, fine sand, silt and clay based on two systems as shown in Table 20.2. Practically, all soils are mixtures of sand, silt and clay. Soils with 10–25 % clay and the rest about equal parts of sand and silt are called loams. The soil texture governs the fertility of soil.

Soil aeration is another important physical property affecting the availability of some of the nutrient elements. Soils rich in clay and humus can hold more water expelling air from the space between the particles. Such soil lacks oxygen and is not ideal for plant growth. In these waterlogged (reduced) soils, the availability of reduced elements

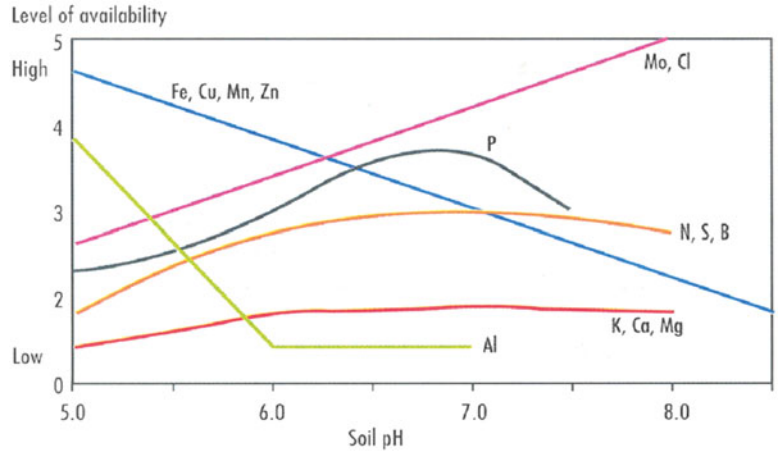


Fig. 20.1 Relationship between level of availability of different elements and soil pH (Source: Goedert et al. 1997)

Table 20.2 Classification of soil particles by diameter (Salisbury and Ross 1985)

Particle	USDA system (mm)	World system (mm)
Coarse sand		2.0–0.2
Fine sand	2.0–0.05	0.2–0.02
Silt	0.05–0.002	0.02–0.002
Clay	<0.002	<0.002

such as Fe^{3+} increases. The microorganism present in waterlogged soil utilises Fe^{2+} as an electron acceptor and thus Fe is reduced. This reduced form of Fe^{2+} is absorbed in excess by some plants adapted to wetland such as paddy and suffer from iron toxicity (bronzing). The best agricultural soil from the point of view of good plant growth and nutrient availability are sandy loams and clay loams.

20.3 Criteria for Essentiality of Elements

The nutrient elements essential for healthy growth of plants are called essential nutrients or essential mineral elements. Till date, only 17 elements (nickel being the recently enlisted) are considered as essential. Arnon and Stout in the year 1939 proposed the term 'essential mineral element'. They established that the following three criteria must be met for an element to be considered essential.

1. A plant must be unable to complete its life cycle in the absence of the mineral element.
2. The function of the element must not be replaceable by another mineral element.
3. The element must be directly involved in plant metabolism, for example, as a component of an essential plant constituent such as an enzyme or it must be required for a distinct metabolic step such as an enzymatic reaction.

The second criterion has some exceptions; it cannot be followed as such because there are some elements that can be replaced by others without causing any adverse effect on the plant. For example, the monovalent cation K^+ can be replaced by Na^+; both these ions play important roles in osmoregulation. On the other hand, this strict definition of essentiality excludes those mineral elements that compensate for toxic effects of other elements or replace mineral nutrients involved in some specific function. Such elements are not essential but perform certain important functions and are classified as beneficial elements. By definition, beneficial elements are those that stimulate growth but are not essential or might be essential for certain plant species under specific conditions. The optimum genetic potential of crop plants cannot be achieved if the beneficial elements are excluded from the agricultural production system.

Based on the essentiality criteria, mineral elements have specific and essential functions in plant metabolism. Therefore, depending on the requirement of a nutrient element to produce

optimum plant growth, the nutrient is referred to as either macronutrient or micronutrient. The macronutrients are required in larger quantities and are present in plant tissues in amounts ranging between 0.2 and 4.0 % (on a dry weight basis), while the concentration of micronutrients in plant tissue ranges from 5 to 200 ppm or less than 0.02 %.

The macronutrients are further divided based on their requirements into primary macronutrients consisting of nitrogen (N), phosphorus (P) and potassium (K) and secondary macronutrients including calcium (Ca), sulphur (S) and magnesium (Mg) (Table 20.3). Another classification based on physiochemical properties divides nutrients into metals (K, Ca, Mg, Fe, Mn, Zn, Cu, Mo, Ni) and non-metals (N, S, P, B, Cl). However, the most widely used classification is based on the quantity of requirement of mineral element rather than the physiochemical properties.

Table 20.3 Essentiality of mineral elements for higher plants

Classification	Element	Higher plants
Macronutrient	N, P, S, K, Mg, Ca	+
Micronutrient	Fe, Mn, Zn, Cu, B, Mo, Cl, Ni	+
Beneficial elements	Na, Si, Co, Al	±

20.3.1 Quantitative Requirements of Nutrient and Tissue Analysis

The concentrations of essential elements required by plants for maintaining optimum growth and preventing any deficiency symptoms are given in Table 20.4. Such values serve as useful guide to plant physiologists and farmers because concentration of elements in plant tissue is more reliable than in soil indicating whether the plant will grow faster if more of a given nutrient is applied. However, for determining the optimum growth of a plant, a farmer should take into consideration the soil analysis of his field. Soil analysis is the chemical determination of the nutrient content and their quantification in soil sample. The soil

Table 20.4 The form of nutrient taken up by plant and its average concentration in shoot dry matter required for adequate growth

Element	Abbreviation	Form taken by plants	Concentration in dry tissue (ppm)	Concentration in dry tissue (%)	Relative no. of atoms
Molybdenum	Mo	MoO_4^-	0.1	0.00001	1
Nickel	Ni	Ni^{2+}	~0.1	0.00001	1
Copper	Cu	Cu^+, **Cu^{2+}**	6	0.0006	100
Zinc	Zn	Zn^{2+}	20	0.002	300
Manganese	Mn	Mn^{2+}	50	0.005	1,000
Iron	Fe	Fe^{3+}, **Fe^{2+}**	100	0.01	2,000
Boron	B	H_3BO_3	20	0.002	2,000
Chlorine	Cl	Cl^-	100	0.01	3,000
Sulphur	S	SO_4^{2-}	1,000	0.1	30,000
Phosphorus	P	**$H_2PO_4^-$**, HPO_4^{2-}	2,000	0.2	60,000
Magnesium	Mg	Mg^{2+}	2,000	0.2	80,000
Calcium	Ca	Ca^{2+}	5,000	0.5	125,000
Potassium	K	K^+	10,000	1.0	250,000
Nitrogen	N	**NO_3^-**, NH_4^+	15,000	1.5	1,000,000
Oxygen	O	O_2, H_2O	450,000	45.0	30,000,000
Carbon	C	CO_2	450,000	45.0	35,000,000
Hydrogen	H	H_2O	60,000	6.0	60,000,000

Source: Modified after Salisbury and Ross (1985)

Between one of the two forms of element, bold-faced type is the preferred form by plants

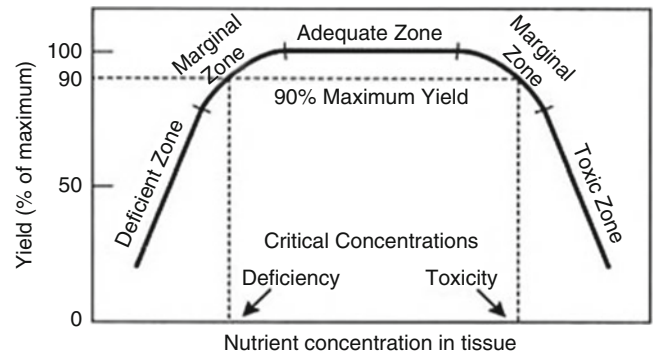


Fig. 20.2 Generalised plot of growth rate as a function of the concentration of a nutrient element in plant tissue

analysis reflects overall levels of nutrients potentially available to plant roots. However, it fails to evaluate the uptake conditions and amount of nutrients actually absorbed by the plants. This additional information can be obtained by plant tissue analysis.

Proper use of plant tissue analysis requires an understanding of the relationship between plant growth and the mineral content of plant tissue samples. Figure 20.2 shows an idealised plot of growth rate as a function of the concentration of any given nutrient element in the plant. When the nutrient content in tissue sample is low, growth is reduced called the deficient zone. In this zone, an increase in tissue mineral content is directly related to an increase in growth or yield. As the nutrient content in tissue sample increases further, a point is reached at which additional increases in tissue mineral content do not appreciably affect growth or yield. This region of the curve is called adequate zone. The transition between deficiency and adequate zone of the curve represents the critical concentration of the nutrient in question, which may be defined as the minimum tissue concentration of the nutrient that is sufficient to give maximal growth or yield. The adequate zone also represents the luxury consumption of elements during which there is no increase in growth and the nutrient taken up in excess is stored in vacuoles. This occurs for a few elements like K, otherwise increases in tissue concentration beyond the adequate zone results in toxicity limiting the growth or yield. Plant analysis and soil analysis data are useful in establishing fertiliser schedules that sustain yield and ensure the food quality of many crops.

The distribution of mineral nutrients between different types of cells within a given tissue (e.g. epidermis, guard cells, mesophyll cells of leaf) also provides important information about function of mineral nutrients. This is particularly true for the distribution of ions in different cellular compartments. In the last decade, much progress has been made in this respect by applying techniques, such as X-ray microanalysis, nuclear magnetic resonance (NMR), ion-selective microelectrodes or fluorescent dyes, for studies on ion distribution in cytoplasm and the organelles contained within it (e.g. chloroplast) and the vacuole. New insight into the functions of mineral nutrients, for example, of calcium as a secondary messenger, is based on these studies of cellular compartmentation. David E. Salt and his group (2008) have proposed the concept of 'ionome'. They defined 'ionome' as the entire mineral nutrient and trace element composition of an organism representing the inorganic component of cellular and organismal systems. Hence, ionome includes both essential and non-essential elements. Ionomics is the study of the ionome, involving quantitative and simultaneous measurement of the elemental composition of living organisms and changes in this composition in response to physiological stimuli, developmental cues and genetic modifications. This study requires application of high-throughput elemental analysis technologies such as inductively coupled plasma-optical emission spectroscopy (ICP-OES) or ICP-mass spectroscopy (ICP-MS)

and their integration with both bioinformatics and genetic tools. The information about functional state of an organism under different conditions brought about by the genetic and developmental differences and by biotic and abiotic factors can be captured by ionomics.

The progress towards a better understanding of the function of mineral nutrients helps in comparing genotypes or mutants within a given plant species. The advanced techniques and concept in mineral nutrition like ionome analysis provides a powerful approach to not only analyse the functions of genes and gene networks directly controlling the ionome but also the extended gene networks that control physiological and developmental processes which indirectly affects the ionome.

20.4 Importance of Macro and Microelements

For easy understanding, both the macro- and micronutrient elements have been classified into four groups as given below (Malik and Srivastava 1982):

Group 1: N and S – present in reduced form and are covalently bonded constituents of organic matter

Group 2: P, B and Si – occurs as oxyanions such as phosphate, borate or silicate

Group 3: K, Na, Mg, Ca and Cl – involved in osmoregulation and ionic balance and have specific functions of enzyme conformation and catalysis (e.g. metalloprotein complexes)

Group 4: Fe, Cu, Mo and Zn – present as structural chelates or metalloproteins; also involved in oxidation-reduction (redox) reactions (first three elements)

The following paragraphs describe the occurrence and physiological functions of macro- and micronutrients in detail with recent developments in the field of mineral nutrition. A summary of physiological functions and the deficiency symptoms of mineral elements in plants are presented in Table 20.5 (modified after Malik and Srivastava 1982).

20.4.1 Physiological Functions of Macronutrients

20.4.1.1 Nitrogen

The Earth's atmosphere consists of about 80 % N, but the extremely stable form of atomic N (dinitrogen, N_2) is not available to plants. However, the microorganisms both free-living and symbiotic can fix atmospheric N_2 to ammoniacal form (NH_4^+) which is then directly taken up by the plants or converted into nitrate (NO_3^-) by nitrifying bacteria. The preferred form in which N is taken up by plants depends on soil conditions and plant species. Plants adapted to low pH and waterlogged soil conditions tend to take up NH_4^+, for example, paddy. In aerobic soils with higher pH, NO_3^- is the predominant form preferred by most of the plants. Also, organic N compounds such as amino acids are found in soil, and there is growing evidence that these can also form important N sources.

20.4.1.1.1 N Uptake and Distribution

The uptake of nitrate or ammonium into plants from soil via the roots involves two basic categories of transport system, high-affinity transport system (HATS) and low-affinity transport system (LATS), identified by kinetic studies of N uptake. The HATS operates under conditions of low external nitrate concentration (*Km* <200 μM), and LATS operates under higher external concentrations even as high as 50 mM without saturation. Based on molecular, physiological and biochemical studies, the transport systems were further subdivided into inducible and constitutive depending on substrate induction process. The HATS is comprised of inducible and constitutive HATS (iHATS and cHATS) and are encoded by the members of *NRT2* gene family (Williams and Miller 2001). The constitutive system (cHATS) is available when plant has been previously starved for nitrate, and the inducible system (iHATS) is stimulated by supplying nitrate. Similarly, LATS encoded by members of *NRT1* gene family is also comprised of both constitutive and inducible elements, evidence of which was shown in *Arabidopsis thaliana.* First inducible LATS gene

Table 20.5 Summary of physiological functions and the deficiency symptoms of mineral elements in plants

Elements	Primary function	Specific deficiency symptoms
Oxygen	Final electron acceptor in aerobic respiration; element in carbohydrates, nucleic acids and many other organic compounds	
Carbon	Building block of all organic compounds in the plants' body	
Hydrogen	Component of water and all organic compounds	
Nitrogen	Constituent of amino acids, proteins, chlorophyll, nucleic acid and some coenzymes	'V' shape chlorosis starting from the tip in lower leaves, plant becomes pale green, poor growth
Potassium	Involved in enzyme activation, protein metabolism, cell membranes, ionic balance, cell extension growth, opening and closing of stomata, cell turgor	Chlorosis of older leaves later turns into necrotic lesions on leaf tip, rolling of leaves, shortening of internodes leading to stunted growth
Calcium	Constituent of cell wall, middle lamella, enzyme cofactor, controls cell permeability and cell wall configuration, involved in cell signalling	Twisting and deformation of growing tips and youngest leaves, later necrosis occurs at the leaf margin
Phosphorus	Involved in photosynthesis, constituent of high-energy intermediaries like ATP, nucleic acids, phospholipids in membranes	Stunted growth, foliage turns dark green, high root/shoot ratio, delayed maturity of plants
Magnesium	Central element of chlorophyll molecule and an activator of several enzymes	Interveinal chlorosis of older leaves, purple coloration with necrotic spots, leaves become stiff and intercostal veins twist
Sulphur	Component of some amino acids (cysteine and methionine) and coenzymes	Symptoms appear in younger most formed leaves, a general chlorosis of entire leaf including vascular bundles
Iron	Involved in chlorophyll biosynthesis, structural component of cytochromes and ferredoxin	Symptoms appear first in younger growing organs, interveinal chlorosis; in severe cases, leaves become white which later dries out
Chlorine	Stomatal regulation in some plants (e.g. onion), stimulation of proton pumping ATPase located at tonoplast, involved in photosynthetic oxygen evolution, osmoregulation	Symptoms appear first in younger leaf, reduction in leaf surface area, wilting of leaf margins, interveinal chlorosis of mature leaves, highly branched root system
Copper	Activator of some enzymes like Cu-Zn SOD	Young leaves turn dark-green colour, twisted with necrotic spots, depressed internode growth, bushy appearance, stunted growth, reduction in panicle formation
Manganese	Activator of some enzymes (Mn-SOD), involved in PS I as water-splitting complex	Small yellow spots and interveinal chlorosis on younger leaves
Zinc	Activator of some enzymes, involved in synthesis of auxin	Stunted growth due to shortening of internode called 'rosette', drastic decrease in leaf size
Molybdenum	Cofactor of enzymes involved in nitrogen metabolism	Chlorosis and stunted growth in younger leaves, drastic reduction in size and whiptail appearance of leaf blade
Nickel	Metal cofactor of urease enzyme	Symptoms closely related to Fe deficiency

isolated was *Chl1* from *A. thaliana* (renamed as *AtNRT1.1*) primarily expressed in roots, and its induction was caused by high external nitrate concentrations up to 25 mM. Later, kinetic studies revealed that *AtNRT1.1* possessed both low- and high-affinity components and therefore classified as dual-affinity transporter (DATS) (Wang and Crawford 1996; Wang et al. 1998; Liu et al. 1999). Another exception of DATS was reported in *Medicago truncatula*, *MtNRT1.3* (Morère-Le

Paven et al. 2011). Very recently, the molecular mechanism of switching affinity of NRT1.1 from low to high revealed that it is controlled by phosphorylation and dephosphorylation of a key amino acid residue, threonine, at position 101 (Parker and Newstead 2014). In Arabidopsis, the entire family of *NRT1* and *NRT2* genes have been characterised comprising of nine and seven members, respectively, that control the flux of nitrate from soil into root tissues and throughout the plant body. These transport systems are induced by NO_3^- concentration in soil solution as well as the N status of the plant. Besides nitrate transporters, a large number of high- and low-affinity ammonium transporters are encoded by the AMT family in plants grown under reduced soil conditions (Howitt and Udvardi 2000). Organic N forms are also transported throughout the plant by proton-dependent oligopeptide transporters of the POT/PTR family.

20.4.1.1.2 Assimilation and Biological Functions of N

Plants cannot use inorganic N as such so it has to be reduced. Two important enzymes of N assimilation, nitrate reductase and nitrite reductase, are involved in reducing the oxidised form of N, that is, NO_3^- to NH_4^+. Other enzymes of N assimilation pathway include glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrogenase (GDH), aspartate aminotransferase (AspAT) and asparagine synthetase (AS). These enzymes are responsible for the incorporation of NH_4^+ into amino acids such as glutamine, glutamate, asparagine and aspartate. The primary function of N is to provide amino groups in amino acid constituent of bases in nucleotides of purine and pyrimidine. Besides these, N is an essential constituent of many nonprotein compounds such as coenzymes, photosynthetic pigments, secondary metabolites and polyamines and vitamins. When N is in ample supply, the NO_3^- form is stored in the vacuole where it contributes to generation of turgor pressure.

20.4.1.1.3 Symptoms of Deficiency and Excess N

Deficiency symptom is seen as a general chlorosis starting in the lower leaves due to loss of chlorophyll. Typical symptom of N deficiency is the formation of 'V' shape of chlorosis starting from the tip of the leaf (Fig. 20.3). This yellowing later can be seen in younger leaves, and in case of severe N deficiency, the older leaves fall off. The plant becomes pale green and the leaf petiole and veins become purple due to anthocyanin pigment synthesis. The overall plant growth is poor and stunted due to low protein synthesis. The symptom appears later in the young leaves because N is highly mobile in plant, and so it is translocated from older leaves to the young growing points.

Plants grown with excessive N usually have dark-green-coloured leaves, produce excess foliage but have a poorly developed root system and, thus, a low root/shoot ratio. For example, potato plants supplied with excess N produce profuse foliage with a few small tubers and poor root growth; perhaps sugar translocation to tubers and root is affected due to hormonal imbalance. Flowering and seed development in several agricultural and horticultural crops are reduced; the flowering is delayed due to excessive vegetative growth. However, short-day plants given abundant N flower faster. Excess N also causes tomato fruits to split as they ripen.

20.4.1.2 Phosphorus

Almost 90 % of P is fixed in soil in the form of aluminium/iron phosphates or calcium/magnesium phosphates depending on soil pH. Plants cannot use these fixed or non-labile forms of P. Another part of insoluble P, called the labile fraction, exchanges with the soil solution. The inorganic P released from the labile fraction into the soil solution, called solution P, is extremely slow and can take a few years. This is the only form of P accessible to plants for uptake. Therefore, P deficiency is a widespread phenomenon. Since phosphatic fertilisers are made from rock phosphates, P is considered as a non-renewable resource which is expected to be exhausted within the next 50–60 years. The form in which P is found in soil solution is pH dependent (Fig. 20.1), but at typical soil solution pH, P occurs exclusively as $H_2PO_4^-$, the preferred form of inorganic P (Pi) taken up by plants.

Fig. 20.3 Deficiency symptoms of macronutrients created in maize seedlings (15 days old) grown in hydroponics. The element in question was omitted from the nutrient solution thus producing severe deficiency symptoms. –N leaf showing characteristic 'V' shape chlorosis starting from tip of leaf, stubby root growth without laterals. –P shoot growth more depressed than root growth, dark-green leaves. –K leaves with marginal necrosis, poor root growth without lateral roots. –Ca characteristic symptom is death of growing point, bushy roots. –Mg interveinal chlorosis in older leaves, purple coloration due to anthocyanin. –S interveinal chlorosis in younger leaves (Symptoms developed by Pandey R.)

20.4.1.2.1 P Uptake and Distribution

In response to persistent Pi deficiency, plants have developed many adaptive morphological, physiological and molecular mechanisms to cope with low Pi. These includes changes in root growth (increased root surface area, fine root hairs, root length) and architecture, induction of high-affinity Pi transporters, increased secretion of acid phosphatase enzyme and low-molecular-weight organic acids, symbiotic associations with mycorrhizal fungi and changes in the activity of several key photosynthetic enzymes (reviewed by Lopez-Arredondo et al. 2014). Several plant species (e.g. Proteaceae) form dense clusters of fine lateral roots called proteoid roots under P deficiency. These clusters release large amounts of organic acids that act as chelators and help in bringing sparingly soluble calcium phosphates into soil solution.

A wide variety of Pi transporter family has been identified in plants recently based on genome sequence analysis and experimental evidences.

These transporters are involved in the uptake of Pi, movement within the cell and around the plant body. Based on their protein sequence, structure, localisation in membrane and functions, they have been grouped into different families. These include Pht1 (plasma membrane), Pht2 (plastid inner envelope), Pht3 (mitochondrial inner membrane), Pht4 (chloroplasts, heterotrophic plastids and Golgi) and pPT (plastid inner envelope). Among the Pi transporters, Pht1 family is most widely studied which belongs to high-affinity Pi transporter family involved in Pi uptake by roots. High-affinity Pi transporter operates at low external Pi concentrations (l–10 μM), whereas the low-affinity Pi transporter, which is a Pi/H^+ symporter, has an apparent K_m of 0.4 mM (Rausch and Bucher 2002). The Pht1 families from Arabidopsis and rice contain 9 and 13 members, respectively, and have been well characterised (Mudge et al. 2002). The presence of mycorrhizal associations also influences the expression of specific transporters. Vacuolar sequestration of P occurs during seed development where large amounts of P and other minerals (Ca, Mg, Zn, Fe) are stored in seeds forming a complex inositol-hexaphosphate called phytate.

20.4.1.2.2 Assimilation and Biological Functions of P

Unlike N and S, P remains in its highly oxidised form once it enters the plant. The inorganic P is either found as soluble Pi (orthophosphate) or as PPi (pyrophosphate). Organic P is mainly bound to hydroxyl groups to a carbon chain (C-O-P) as a simple phosphate ester (e.g. sugar phosphate) or attached to another phosphate by the energy-rich pyrophosphate bond (P~P), such as in ATP. Another type of phosphate bond is the diester state (C-P-C) with relatively high stability. In this association, phosphate forms a bridging group between connecting units resulting in more complex macromolecular structures. P as a structural element is a constituent of nucleic acids (DNA, RNA) (responsible for their strongly acidic nature) and phospholipids of biomembranes forming phosphatidylcholine (lecithin). In membranes, Pi acts as a link between glycerol-fatty acid (lipophilic part) and the choline (hydrophilic) part of the lipid. Choline is strongly hydrophilic because of the negative charge on phosphate group, and this helps in proper orientation in the membrane. Further, P has a major role in energy transfer reactions. This involves formation and disruption of pyrophosphate bond to maintain energy homeostasis in cellular processes. Hydrolysis of one mole of ATP releases 30 kJ of energy. ATP is the basis of many synthetic pathways, and other similar energy-rich phosphonucleotides (UTP, CTP and GTP) play a central role in nucleic acid metabolism. UTP is involved in synthesis of sucrose, starch and cellulose, while CTP provides energy during phospholipid biosynthesis.

The energy-rich phosphates like ATP, GTP or ADP modulate enzyme activities by reversible phosphorylation (Fig. 20.4). This regulatory

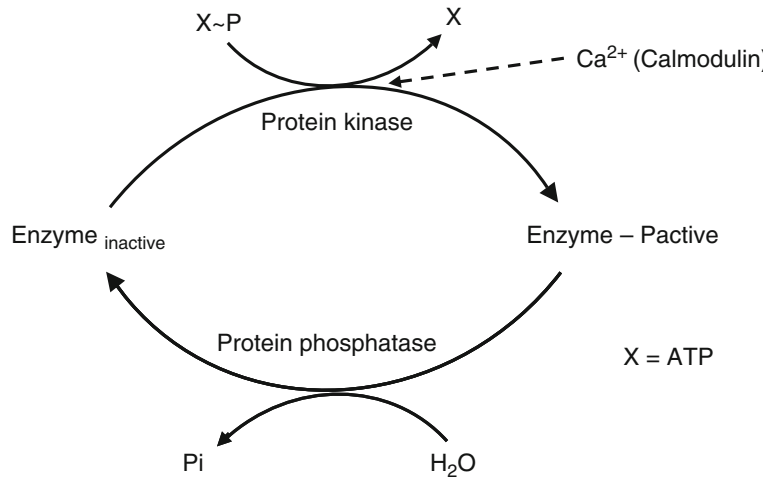


Fig. 20.4 Modulation of enzyme activity by reversible protein phosphorylation

phosphorylation is mediated by protein kinases and can result in activation, inactivation and/or changes in the allosteric properties of the target molecule. Protein kinase phosphorylates serine or threonine residues of proteins and the enzyme becomes activated, while dephosphorylation is carried out by phosphatases that release Pi, hence inactivating the enzyme protein. Protein phosphorylation also has an important role in signal transduction.

The large amounts of P stored in seeds as phytic acid facilitate development of embryo, seed germination and seedling growth. The concentration of Pi has a very important role in the process of photosynthesis. Generally photosynthesis is limited by Rubisco activity or the capacity to regenerate ribulose 1,5-*bis*phosphate (RuBP). In light, for optimal photosynthesis, a Pi concentration in the range of 2.0–2.5 mM is required in the chloroplast; however, photosynthesis is inhibited if the Pi concentration falls below 1.4–1.0 mM (Marschner and Marschner 2012). This may be due to many of the intermediary steps involving sugar phosphates during carbon fixation. Therefore, the demand for Pi is higher in chloroplast which is achieved by the Pi/triose phosphate (Pi/TP) translocator present in the chloroplast envelope. The synthesis of starch in chloroplast is regulated by Pi/TP ratio. A high Pi/TP ratio in chloroplast inhibits the key enzyme ADP-glucose pyrophosphorylase, thus decreasing starch synthesis. However, a low Pi/TP ratio activates this enzyme, and TPs are diverted towards sucrose synthesis in the cytoplasm.

20.4.1.2.3 Symptoms of Deficiency and Excess P

Deficiency of P causes stunted growth of plants and foliage is often dark green. The dark-green colour of leaves is because of the accumulation of starch and sugars in the leaves. Under P deficiency, shoot growth is much more repressed than root growth leading to a higher root/shoot ratio (Fig. 20.3). In severe cases, the roots also develop purple coloration (observed in hydroponically grown maize plants). The leaf expansion is affected due to reduced cell division and enlargement thereby producing smaller leaves. Because P is highly mobile inside the growing tissues in plants, the older leaves are first to show chlorosis. Plant maturity is also delayed.

Excess P application leads to P toxicity in plants leading to delayed formation of reproductive organs. This may be because P and N have synergistic effect, meaning presence of P will lead to more uptake of N.

20.4.1.3 Potassium

The Earth's crust contains around 2.3 % K. Mostly K is bound in primary minerals or present in secondary clay minerals making clayey soils rich in K. Examples of some of the minerals containing K as K_2O are alkali feldspar (4–15 %), muscovite or K mica (7–11 %), biotite or Mg mica (6–10 %) and illite (4–7 %). A typical concentration of K in soil solution varies between 0.1 and 1 mM K^+. Soil K occurs in three forms: K present in soil solution (readily available to plant), K adsorbed in exchangeable form to soil colloids such as clay minerals and K as a structural element of soil minerals. Generally, K deficiency is a rare occurrence, but plant growth is usually stimulated by additional K supply.

20.4.1.3.1 K Uptake and Distribution

Plants take up K as monovalent cation, K^+. Uptake of K in plant tissues occurs at high rates due to relatively high permeability of plant membranes to K. This high permeability of membranes to K results from ionophores located in membrane that enables facilitated diffusion. Besides the passive uptake, K also enters plant roots via high- and low-affinity transporters (Giertha and Maser 2007; Wang and Wu 2013). Studies employing electrophysiology techniques indicated that passive transport of K occurs through ion channels with millimolar K_m denoting low-affinity and active transport through H^+-cotransporters with micromolar K_m denoting high-affinity transporters. Once inside the plant, K is highly mobile at all levels, i.e. within individual cells, tissues and in long-distance transport via the xylem and phloem. The bulk of K is taken up during vegetative phase. A large flux of K from shoot to root is maintained through phloem, which is crucial to maintain K homeo-

stasis and to provide a constant supply of cations to accompany anions like NO_3^- for their movement towards the shoot.

20.4.1.3.2 Biological Functions of K

Unlike other elements, K is not metabolised in the plant and it forms only weak complexes in which it is readily exchangeable. K has an exceptional role in plant-water relations. Besides maintaining turgor, it is required for activating an array of enzymes in metabolic reactions. It is maintained in the range of 100–200 mM in the cytosol, and almost similar concentration is found in chloroplast. This storage pool is called 'metabolic pool', which is not replaceable by other inorganic cations such as Na^+. However, K concentration in vacuole may vary between 10 and 200 mM or sometimes may even reach 500 mM, and this storage pool is called 'non-metabolic pool', frequently replaced by other cations.

In cytosol, K is involved in enzyme activation; specific enzymes include vacuolar pyrophosphatases (PPases) that accumulate protons into the vacuolar lumen and are strictly dependent on K. Besides this, many enzymes involved in carbon metabolism such as pyruvate kinase, phosphofructokinase and ADP-glucose starch synthase are also activated by K. It is essential for chlorophyll development and catalyses normal carbohydrate breakdown during respiration. Protein synthesis mediated by ribosomes is another key process that requires high concentrations of K. The metabolic enzymes involved in transcriptional and post-transcriptional regulation are also affected by K status of the plants, thereby influencing the metabolism. K is involved in the up-regulation of malic enzyme and assimilation of nitrate via the GS/GOGAT pathway, while the uptake of nitrate and its reduction are downregulated (Armengaud et al. 2009). It plays an important role in photosynthesis since it is the dominant counterion to the light-induced proton flux across the thylakoid membrane and for establishing the transmembrane pH gradient required for ATP synthesis (photophosphorylation). The dominant role of K is in turgor maintenance and water homeostasis termed as osmoregulation. The turgor pressure-driven solute transport causes cell extension, stomatal movements and other photonastic and seismonastic movements. Moreover, the loading and unloading of sucrose in phloem are also dependent on K concentration in the sieve tubes.

20.4.1.3.3 Symptoms of Deficiency and Excess K

As with N and P, K is also easily redistributed in plant tissue, so the deficiency symptoms first appear on the older leaves. The typical symptom of K deficiency is the development of chlorosis which later turns into necrotic lesions on the leaf tip spreading downwards on the margins. Under severe K deficiency, the younger leaves also become chlorotic (Fig. 20.3). In maize, root development is poor without lateral roots and the stalks are weak. Lignification of vascular bundle is generally impaired making the plant prone to lodging. Other characters of K deficiency are rolling of leaves and shortening of internodes leading to stunted growth. Severe K deficiency causes accumulation of reducing sugars and depletion of organic acids and synthesis of toxic amines such as putrescine and agmatine by the decarboxylation of arginine.

High K content in the growing medium does not usually produce any toxic symptoms in plant. This may be because uptake of K in excess represents 'luxury consumption' and it is stored in the vacuole without any increment in growth.

20.4.1.4 Sulphur

Soils contain inorganic and organic forms of S, but the major soil S reservoir is organically bound S. The organic S is mostly present in the form of phenolic and choline sulphates as well as lipids and amino acids, and the C/N/S ratio in soil organic matter is approximately 125:10:1.2. In saline and sodic soils, inorganic salts are predominant. Under aerobic soil condition, inorganic S is present primarily as sulphate (SO_4^{2-}): the preferred form for uptake by plants. Under waterlogged conditions, inorganic S occurs in reduced forms such as FeS, FeS_2 and H_2S. Sulphate reduction under waterlogged (anaerobic) condition is carried out by bacteria belonging to genus *Desulfovibrio* leading to formation of H_2S. The

H_2S undergoes oxidation to elemental S by chemotrophic S bacteria such as *Beggiatoa* and *Thiothrix*. The total S content in soil varies in the range from 0.005 to 0.04 %.

20.4.1.4.1 S Uptake, Distribution and Assimilation

In addition to S uptake by roots as SO_4^{2-}, plants can also absorb S from the atmosphere in the form of SO_2 through stomatal openings. Uptake of SO_4^{2-} is an active process as it is absorbed via plasma membrane sulphate transporters energised by H^+ gradient. The proton-coupled cotransport occurs with $3H^+/SO_4^{2-}$ stoichiometry. Transcription of this type of mechanism is induced when S becomes deficient. Analysis of genome sequences of Arabidopsis and rice has led to the identification of 14 putative sulphate transporter genes in each genome. These genes are subdivided into 4 closely related groups and all having 12 membrane-spanning domains and a STAS domain at their carboxy-terminus (Aravind and Koonin 2000). The fifth group is more diverse but related with two smaller proteins lacking the STAS domain (Hawkesford 2003). However, only groups 1 and 2 transporters have sulphate transport activity. In roots, the genes belonging to *Sultr* family are involved in sulphate uptake that are located in epidermal and cortical plasma membranes. The high-affinity sulphate transporters which belong to group 1 operate at low sulphur concentration (K_m: 1.5–10 μM). However, group 2 sulphate transporters belong to low-affinity transport activity and are constitutively expressed under high sulphate concentration (K_m: 99.2 μM and 1.2 mM). Group 2 transporters, *Sultr2;1* (Km 0.41 mM) and *Sultr2;2* (Km 1.2 mM), have specific functions in the vascular movement of sulphate. *Sultr2;1* is expressed in the xylem parenchyma and phloem cells in leaves, but *Sultr2;2* is localised specifically in phloem of roots and vascular bundle sheath cells of leaves (Buchner et al. 2004).

Inside plants, SO_4^{2-} is highly mobile resulting in its rapid transport from root to shoot tissues through xylem. Its translocation is mainly in an upward (acropetal) direction. Once sulphate is taken up, bulk of it is reduced in shoot chloroplasts in the presence of light, while some may be reduced in root plastids. Surplus S is deposited in vacuoles as SO_4^{2-}. When SO_2 reacts with water in cells forms bisulphite (HSO_3^-), and in this form, it inhibits photosynthesis and causes chlorophyll degradation.

The S assimilation involves activation of SO_4^{2-} ions by ATP forming adenosine phosphosulphate (APS). The APS then serves as a substrate for the synthesis of sulphate esters (sulpholipids), polysaccharides or secondary metabolite (glucosinolates). Through another pathway, APS is reduced and incorporated into amino acid cysteine, the first stable product of a series of reactions involving glutathione, reduced ferredoxin and acetyl serine. The enzymes of assimilatory SO_4^{2-} reduction are localised in the chloroplasts and to some extent also found in roots.

20.4.1.4.2 Biological Functions of S

Most S is found in reduced form in the amino acids cysteine and methionine and hence a constituent of protein. Both of these amino acids are precursors of other S-containing compounds such as coenzymes (CoA), vitamins (biotin, thiamine) and secondary metabolite (glucosinolates, alliins). S is a structural constituent of these compounds (e.g. R^1-C-S-C-R^2) or acts as a functional group, sulfhydryl (R-SH or thiol), that can undergo reversible oxidation. The formation and disruption of these S bridges affect the tertiary and quaternary structure of protein, thereby influencing protein activity. The production of glutathione, a tripeptide having –SH group, serves many functions in plant. Glutathione is readily water soluble and acts as a powerful antioxidant, present mostly in chloroplasts. Glutathione may function as a transient storage pool of reduced S and also a precursor of phytochelatins or 'class III metallothioneins'. These phytochelatins have a general structure of (Glu-Cys)*n*-Gly, where *n* is between two and more than ten and functions in detoxifying heavy metals. Research in the direction of increasing phytochelatin production is being carried out in order to make (crop) plants more tolerant to pollutants such as arsenic and Cd^{2+} and to improve the phytoremediation potential. Another important family of thiols in plants is thioredoxins, which functions as regulatory

protein in carbon metabolism. In reduced form, thioredoxin activates several enzymes of Calvin cycle.

The biomembranes contain S as sulpholipids; in chloroplast thylakoids, they are essential for the stabilisation of photosystem components. Sulpholipids are also involved in the regulation of ion transport across biomembranes. High levels of sulpholipids in roots provide tolerance to plants against salt. The S as in glucosinolates is stored in vacuoles, and their hydrolysis catalysed by enzyme myrosinase releases sulphate that can be recycled under S-deficient condition (Grubb and Abel 2006).

20.4.1.4.3 Symptoms of Deficiency and Excess S

Unlike nitrogen, S deficiency symptom first appears in the younger most leaf (Fig. 20.3). Chlorosis occurs throughout the entire leaf including vascular bundles (veins). Under severe S deficiency, inhibition of protein synthesis takes place which also drastically decreases chlorophyll content of leaves. In S-deficient plants, there is an accumulation of soluble organic nitrogen and nitrate.

Plants are comparatively insensitive to high SO_4^{2-} concentrations in the nutrient media. However, if the SO_4^{2-} concentration increases beyond 50 mM as in some saline soils, plant growth is adversely affected. The symptom of excessive S is a reduction in growth rate and dark-green leaves. High SO_2 in the environment is toxic to plants as it causes bleaching of chlorophyll characterised by necrotic symptoms in leaves.

20.4.1.5 Calcium

Calcium is abundant in the lithosphere, constituting about 3.64 % of the Earth's crust. The Ca-containing minerals are Al-silicates (feldspars and amphiboles), Ca phosphates and Ca carbonates. Severely weathered soils followed by leaching lead to Ca-deficient soil, which is accelerated by low soil pH. The Ca^{2+} adsorbed on soil colloids may be exchanged with the soil solution resulting in 'free' Ca^{2+} that forms insoluble compounds with elements such as P, thus making it less available. Most soils contain enough levels of Ca^{2+} in soil solution, and their exchange sites are well enough saturated with Ca^{2+} to adequately meet the crop demand.

20.4.1.5.1 Ca^{2+} Uptake and Distribution

Plants absorb calcium as a divalent cation, Ca^{2+}. The uptake potential of Ca is lower than other cations such as K^+ even though the Ca concentration of soil solution is ten times higher than K. This is because Ca absorption takes place only through young root tips in which the endodermis cell walls are still unsuberised. Uptake of Ca from soil solution by roots is mainly a passive process, and even within the plant tissues, Ca translocation occurs passively. The upward translocation of Ca in xylem sap occurs with the transpiration stream. Since Ca precipitates as Ca-phosphate in the phloem sap, the downward translocation of Ca is very slow. Ca enters the root through Ca^{2+}-permeable channels. Some of these channels are Ca^{2+} selective, but others are 'nonselective' ion channels. The identity of the specific protein(s) that mediates Ca^{2+} uptake is still unknown (Demidchik and Maathuis 2007). Within the plant, the major Ca^{2+} transporter at the plasma membrane and also at endoplasmic reticulum is a Ca-pumping ATPase (Ca^{2+}/H^+ antiporter). However, inside the plant, Ca is rather immobile; the ions have a tendency to be sequestered in the large vacuole of mature cells. No transporters have been identified that are responsible for loading Ca^{2+} into xylem vessels. A proportion of xylem Ca^{2+} is contributed by the apoplast. However, Ca^{2+} mobility is low in the vascular system.

20.4.1.5.2 Biological Functions of Ca

Cellular functions of Ca^{2+} are mainly concerned with structure and as a secondary messenger. It is a constituent of middle lamella in the form of calcium pectate and helps to cement the wall of cells together. The proportion of calcium pectate in the cell walls is of particular importance as it is responsible for the susceptibility of tissue to fungal and bacterial infections and also for fruit ripening. Ca is also present as insoluble crystals of Ca-oxalate called raphides and sphaeraphides in the vacuoles of some plant species (e.g. in *Colocasia*, raphides are responsible for itching

sensation). Ca is essential for the formation of cell membranes and lipid structures. Most of the Ca present in plant tissues is localised in the apoplast and in vacuoles. The Ca^{2+} concentration of cytoplasm is low ranging from 10^{-6} to 10^{-8} M. Such a low cytosolic concentration is of vital importance because higher Ca^{2+} concentration inhibits various enzymes located in cytoplasm as well as in chloroplasts. However, Ca concentration is much higher in the mitochondria. In cytosol, Ca is reversibly bound to a small protein, a polypeptide of 148 amino acids, called calmodulin (*cal*cium-*modul*ated prote*in*). Calmodulin then activates downstream events in the signalling cascade by phosphorylation of soluble and membrane-bound proteins. Ca in small amounts is essential for normal mitosis and is associated with chromatin or mitotic spindle organisation.

The formation of secretory vesicles and their fusion with plasma membrane leading to exocytosis require Ca. This helps in the formation of cell wall from the precursor cellulose as well as the formation of mucilage and callose. In root-caps, secretion of mucilage depends on extracellular (apoplasmic) Ca concentration. One of the vital functions performed by Ca is cell membrane stabilisation which is brought about by bridging phosphate and carboxylate groups of phospholipids and protein at membrane surfaces. Ca present in vacuole contributes to cation-anion balance by acting as a counterion for inorganic and organic anions. Free Ca at a very low cytosolic concentration (0.1–0.2 μM) acts as secondary messenger. A wide range of stimuli evokes rapid changes in free cytosolic Ca^{2+} in plants including responses to abiotic and biotic stresses, stomatal regulation and physical damage.

20.4.1.5.3 Symptoms of Ca Deficiency

The characteristic symptom of severe Ca deficiency is the twisting and deformation of growing tips and youngest leaves (Fig. 20.3). At a more advanced stage, necrosis occurs at the leaf margin. Ca deficiency also causes disintegration of cell wall and collapse of the affected tissues such as the petioles and upper part of the stems. In fast-growing tissues, Ca^{2+} levels may fall below a critical level leading to development of diseases such as 'blossom-end rot' in tomatoes, 'black heart' in celery and 'bitter pit' in apples. Ca influences the permeability of plasma membrane, thus its deficiency makes the membrane leaky. Ca also mediates starch hydrolysis to sugar, hence its deficiency results in the accumulation of starch in leaves. The number of mitochondria in wheat roots reduces under Ca deficiency.

20.4.1.6 Magnesium

Magnesium is derived from the Greek word '*Magnesia*'. The Mg content of soil varies between 0.05 and 0.5 %. Easily weatherable ferromagnesian minerals such as biotite, serpentine, hornblende and olivine and other secondary clay minerals add Mg to the soil. In soil solution just like Ca^{2+}, Mg^{2+} is also present in fairly high concentration between 2 and 5 mM. The size of hydrated ion is small (0.428 nm radius), due to which Mg adsorption to soil particles is relatively weak resulting in high leaching loss to the tune of 2–30 kg per hectare. Such losses lead to recurring Mg deficiency in crops.

20.4.1.6.1 Mg Uptake and Distribution

In plants, the free cytoplasmic Mg^{2+} remains at a concentration of about 0.5 mM (Yazaki et al. 1998), but total Mg^{2+} levels may vary from 0.3 to 1.0 %. Transport of Mg^{2+} is passive, mediated by ionophores in which Mg^{2+} moves down against the electrochemical gradient. In this transport system, Mg^{2+} faces cation competition; therefore, if an excess of other cations such as K^+ or NH_4^+ is present in the medium, the uptake of Mg^{2+} is adversely affected. Mg^{2+} is very mobile in the phloem and so can be translocated from older to younger leaves or the meristem. Since Mg^{2+} has a primary role in photosynthesis, the highest tissue Mg^{2+} concentration is usually measured in shoots. Most of the Mg^{2+} taken up by plants is stored in the vacuole where it contributes to turgor generation and charge balancing of anions.

20.4.1.6.2 Biological Functions of Mg

Mg occupies the central position in the porphyrin structure of chlorophyll molecule where it coordinates covalently with four N atoms. Insertion of

Mg^{2+} atom in the porphyrin structure during chlorophyll biosynthesis is catalysed by the enzyme Mg^{2+}-chelatase (Sirijovski et al. 2008). Mg plays a crucial role in photosynthesis, particularly in promoting the light reactions in the stroma of chloroplast. Carbohydrate partitioning is also regulated by Mg^{2+} concentration in the tissues. The loading of sucrose into the phloem in source leaf requires H^+-pumping ATPase, and for the optimal activity of this enzyme, about 2 mM Mg^{2+} is essential. The majority of cellular Mg^{2+} functions as enzyme cofactors in the stabilisation of nucleotides and nucleic acids. Some important enzymes activated by Mg^{2+} are phosphokinases, dehydrogenases and enolases. Activation of ribulose 1,5 *bis*phosphate carboxylase during light reaction occurs due to Mg^{2+} influx into the thylakoid. The most prominent enzyme reactions where Mg^{2+} is indispensable are those associated with energy transfer and phosphorylation/dephosphorylation. In these reactions, Mg^{2+} forms a bridge between the pyrophosphate structure of ATP or ADP and the enzyme molecule (Fig. 20.5). Mg plays an important role in gene transcription and translation as it readily binds to nucleic acids. As a result, DNA-melting temperatures remain considerably higher in the presence of Mg^{2+}. In RNA also, Mg^{2+} has similar roles and helps in maintaining secondary structure. Mg stabilises the ribosomal subunits necessary for protein synthesis and also has a similar stabilising effect in the matrix of the nucleus. Further, the transfer of aminoacyls from aminoacyl tRNA to the polypeptide chain is also activated by Mg^{2+} (Fig. 20.5).

20.4.1.6.3 Symptoms of Mg Deficiency

The first symptom of Mg deficiency is interveinal chlorosis of older leaves. In maize seedlings, leaves develop purple coloration along with necrotic spots when Mg was completely omitted from the growth medium (Fig. 20.3). In dicotyledonous plants including grapes, beans, potatoes and sugar beet, Mg^{2+}-deficient leaves become stiff and brittle and the intercostal veins twist in addition to chlorosis. Protein synthesis is also adversely affected resulting in poor growth of plants. Other ultrastructural changes in Mg^{2+}-deficient leaves include irregular shape of grana with reduction in their numbers, accumulation of starch grains in chloroplast, deformation of the lamellar structure, underdeveloped cristae of mitochondria and decrease in chlorophyll and carotenoid contents. These symptoms of ultrastructural disorganisation lead to visual Mg deficiency symptoms. In maize roots, increased suberisation of endodermis and hypodermis occurs due to Mg deficiency.

20.4.2 Physiological Functions of Micronutrients

20.4.2.1 Iron

Iron makes up about 5 % by weight of Earth's crust and is present in almost all soils. The primary minerals of Fe are ferromagnesian silicates such as olivine, augite, hornblende and biotite. The concentration of plant available Fe in soils is extremely low. The Fe solubility in soil is largely controlled by pH of soil solution. At higher pH (7.4–8.5), the solubility is at minimum, while at low pH or acid, soils Fe availability is very high. Further, in aerated soils, maintained at physiological pH range, the concentrations of Fe^{2+} and Fe^{3+} are below 10^{-15} M. Under waterlogged or reduced soil condition, Fe^{3+} is reduced to F^{2+} and this reduction is brought about by anaerobic bacteria which uses Fe oxides as electron acceptors in respiration.

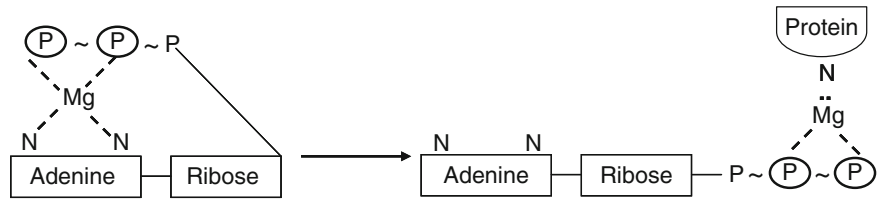


Fig. 20.5 Magnesium acts as bridging molecule between nitrogen and phosphoryl groups in ATP (Source: Marschner 1995)

20.4.2.1.1 Uptake and Distribution

The preferred uptake form of Fe by plants is the reduced cation Fe^{2+}. Fe cannot be taken up in ionic form as such; it has to chelate with other compounds that facilitate Fe uptake. Plant species differ in their ability to utilise sparingly soluble inorganic Fe and Fe chelates. Plants grown under Fe stress show physiological and morphological changes that help in Fe uptake. The mechanism of Fe uptake in plants via roots is based on two strategies: *Strategy I* involves reduction of Fe^{3+} to Fe^{2+} carried out by membrane-bound enzyme ferric-chelate reductase (FCR). The reduced form of iron is transported into root cells through the metal transporter protein called *i*ron-*r*egulated *t*ransporter (IRT). This mechanism operates in dicotyledonous plants. *Strategy II*, a chelation-based mechanism occurs in Poaceae (grass) family where roots release phytosiderophores (PS) which form stable complexes with Fe^{3+} and is taken up by plant as Fe^{3+}-PS chelate (Romheld and Marschner 1986). The gene encoding FCR enzyme belongs to the FRO family and IRT belongs to ZIP family (Guerinot 2000; Wu et al. 2005; Mukherjee et al. 2006; Jeong et al. 2008). In Arabidopsis, *FRO2* gene identified in root has been found to encode for Fe^{3+}-chelate reductase (Mukherjee et al. 2006). The mechanism of Fe uptake by roots is strongly regulated by a complex system, which involves transcription factor, bHLH, of which PYE and FIT/FER play a central role (Ivanov et al. 2012). Besides IRT, Fe uptake takes place through other transporters present in the leaf cells. These include several members of the YSL (yellow stripe-like) family expressed in leaves (such as AtYSL1, AtYSL3 and AtYSL2 in *A thaliana* and OsYSL2 and OsYSL15 in rice), but their expression is usually confined to the vascular tissue (Curie et al. 2009).

In rapidly growing plants, about 80 % of Fe is stored in chloroplasts. It is localised in the plastid stroma as phytoferritin. Some amount of phytoferritin is also detected in xylem, phloem and seeds. It also acts as storage for Fe in nodules of legumes. The major form of Fe transported through the xylem is ferric citrate.

20.4.2.1.2 Biological Functions of Fe

Being a redox-active metal, Fe plays a role in photosynthesis, mitochondrial respiration, assimilation of nitrogen, synthesis of chlorophyll and hormone (ethylene, gibberellic acid, jasmonic acid), osmoprotection, pathogen defence and production and scavenging of reactive oxygen species. Based on the type of iron ligand, there are three groups of Fe-containing proteins:

1. *Proteins with Fe-sulphur clusters (Fe-S)*: The Fe-S clusters are synthesised from inorganic Fe and sulphide (Fig. 20.6). These nonheme-proteins with Fe-S clusters have a key role in electron transfer. They constitute part of substrate-binding sites in enzymes, form iron storage moieties, are involved in transcriptional or translational regulation, control protein structure in the vicinity of the cluster and are also involved in disulphide reduction and sulphur donation (e.g. in thioredoxins). Therefore, Fe-S proteins serve as enzymes, as electron carriers (e.g. ferredoxin) and as regulatory proteins (e.g. aconitase).
2. *Fe-containing hemeproteins*: The well-characterised hemeproteins are the cytochromes involved in electron transfer reactions of photosynthetic and respiratory systems,

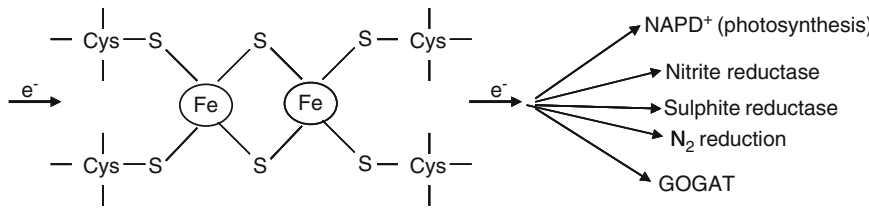


Fig. 20.6 Role of ferredoxin as an electron carrier in a number of basic metabolic processes (Source: Marschner 1995)

which contain a heme Fe-porphyrin complex. The oxidative enzymes like catalase, peroxidase, and NADPH oxidase involved in the production and scavenging of free radicals contain hemeprotein. Another very large group of enzymes called cytochrome P450 also contains hemeprotein. Globins such as leghemoglobin in nodules of legumes are involved in oxygen binding and transport. Nitrite reductase and sulphite reductase enzymes involved in N and S assimilation contain a siroheme and a Fe-S cluster in the enzyme. Hemeproteins are distributed all over the locations in subcellular organelles, for example, cytochrome P450 is localised in endoplasmic reticulum, catalase in peroxisomes and other enzymes in the cytoplasm.

3. *Other Fe proteins*: These proteins are grouped as nonhemeproteins, which bind Fe ions directly. Ferritins or phytoferritins are most common among this group of proteins. Ferritins control the interaction between Fe homeostasis and oxidative stress in plants. These are high-molecular-weight 24-mer proteins that store up to 4,500 Fe atoms in soluble and bioavailable form. Mostly nongreen plastids such as etioplasts and amyloplasts contain ferritins, but they are not found in mature chloroplasts (Briat and Lobreaux 1998).

20.4.2.1.3 Symptoms of Deficiency and Excess Fe

Since Fe is relatively immobile inside the plant tissue, the visual deficiency symptoms first appear on young growing organs. The young leaf shows interveinal chlorosis, and in severe cases, the leaf becomes white (due to loss of chlorophyll) which later dries out. In contrast, the mature leaves may not show chlorosis at all. Lack of Fe inhibits protein synthesis.

Excess Fe uptake leads to toxicity, which is a major problem in wetland rice-cropping systems. In rice, symptoms of Fe toxicity involve development of tiny brown spots that later spreads into uniform brown colour, called bronzing. The Fe concentration in rice leaves remains excessively high in the range of 300–1,000 μg Fe per g dry weight.

20.4.2.2 Copper

The soil contains Cu in divalent cation form, Cu^{2+}, in the range of 5–50 ppm. However, the concentration of Cu in soil solution is very low 10^{-8} to 60×10^{-8} M. More than 98 % of Cu is bound to organic matter in soil, which is an important factor regulating Cu mobility in soil. Cu is absorbed both as divalent cupric (Cu^{2+}) ion in aerated soils or monovalent cuprous (Cu^{1+}) ions in waterlogged soils.

20.4.2.2.1 Uptake and Distribution

Plants take up Cu in very little quantities, so the Cu content in plant tissues varies from 2 to 20 ppm. Cu uptake is an active process requiring metabolic energy. Under physiological pH, Cu exists in two oxidation states Cu^{1+} and Cu^{2+} and can interchange between these forms (monovalent copper is unstable). This property of Cu is responsible for its redox function in biochemical reactions. Cu has high affinity for N atom of amino groups, sulfhydryl groups, carboxylic groups and phenolic groups. More than 98–99 % of Cu is present in these complexed forms in soil solution and in xylem and phloem sap. Cu ions can catalyse the production of free radicals, which is potentially toxic leading to the damage of proteins, DNA and other biomolecules. Therefore, immediately following Cu uptake, the metal-scavenging proteins like metallothionein bind majority of Cu ions, thus preventing it from accumulating at a toxic level.

20.4.2.2.2 Biological Functions of Cu

Cu is of utmost importance for life. It is essential for photosynthesis and mitochondrial respiration, carbon and nitrogen metabolism, oxidative stress protection and cell wall synthesis. Three different types of Cu-protein exist in plants in which Cu is the metal component. These are:

Type 1: *Blue Cu-proteins*, without oxidase activity that functions in one-electron transfer. Common example of this is plastocyanin; more than 50 % of Cu localised in chloroplast remains in this form. Plastocyanin is a component of electron transport chain of photosystem I in photosynthesis.

Type 2: *Non-blue Cu-proteins,* which are peroxide-producing oxidases and oxidise monophenols to diphenols.

Type 3: *Multi Cu-proteins*, contains at least four Cu atoms per molecule which act as oxidases. Multi-Cu enzymes contain all three types of Cu, e.g. ascorbate peroxidase and diphenol oxidase. Cytochrome oxidase is a mixed Cu-Fe protein catalysing the terminal oxidation in mitochondria.

Recent studies have revealed that more than 100 Arabidopsis proteins are predicted to be complexed with Cu (Kramer and Clemens 2005). The high affinity of Cu to dioxygen molecules explains the role of Cu as a catalytic metal in many oxidases. Cu metabolism is also intimately linked to Fe metabolism. Depending on the bioavailability of Cu and Fe, plants possess enzymes for the alternative use of Cu and Fe, thus catalysing the same biochemical reaction with completely different apoproteins. Such reactions include Cu-nitrite versus heme-nitrite reductase, Cu/Zn-superoxide dismutase versus Fe-superoxide dismutase and cytochrome oxidase versus di-iron oxidase. Cu has also been reported to be a part of the ethylene receptor and is involved in molybdenum cofactor biosynthesis (Rodriguez et al. 1999; Kuper et al. 2004).

20.4.2.2.3 Symptoms of Deficiency and Excess Cu

Visible symptoms of Cu deficiency appear in young leaves that often become dark green in colour and are twisted exhibiting necrotic spots. However, in cereals, the leaf tip becomes white and the leaves narrow and twisted. The growth of internodes is depressed leading to bushy appearance and stunted growth. Pollen grain viability is affected resulting in reduction in panicle formation. Lignin synthesis is impaired due to lack of two important Cu-containing enzymes, phenolase and laccase. Of the total Cu concentration in plants, almost half is found in the chloroplasts where it plays an important role in photosynthetic reactions. Cu deficiency symptom in citrus is called 'die back' because young leaves die out.

The critical toxicity level of Cu in the leaves is above 20–30 µg Cu per g dry weight. The ability of Cu to displace other metal ions like Fe from physiologically active sites is associated with Cu toxicity symptoms. Thus, the Cu toxicity symptom in plants will manifest as Fe deficiency symptom. Other symptoms of toxicity include inhibition of root growth more than shoot growth. There are some Cu-tolerant species called metallophytes, which can tolerate Cu content as high as 1,000 µg Cu per g dry weight.

20.4.2.3 Zinc

The Zn content of the lithosphere is about 80 ppm and it is usually present in soil in the range of 10–300 ppm. The ionic radius of Zn^{2+} is similar to that of Fe^{2+} and Mg^{2+} due to which Zn^{2+} may replace these elements by isomorphous substitution in the mineral structure. Zn interacts with organic matter in soil and forms both soluble and insoluble Zn-organic complexes. The soluble Zn-organic complexes are mainly associated with amino, organic and fulvic acids, while the inorganic complexes are derived from humic acids.

20.4.2.3.1 Uptake and Distribution

Plants absorb Zn in the form of divalent cation. Uptake of Zn is metabolically controlled, that is, Zn uptake is an active process requiring energy. The form in which Zn is translocated from roots to shoots is not clear. Mobility of Zn inside the plant organs is relatively less, so there is accumulation in root tissues particularly when Zn concentration in the media is high. In older leaves, Zn can become very immobile. Much of the Zn absorbed is localised in the seeds and grains in the protein bodies in the form of globoid crystals. These globoids consist mainly of phytate or salts of phytic acid.

Zn transport via roots takes place through transporters belonging to ZIP family (*Z*RT, *IRT-like p*rotein). ZRT1 and ZRT2 (zinc-regulated transporter) are designated as the high- and low-affinity Zn transporters, respectively. Till now, over 25 ZIP family members have been identified which is subdivided into two subfamilies (reviewed by Guerinot 2000).

20.4.2.3.2 Biological Functions of Zn

Zn has a strong tendency to form tetrahedral complexes with N-, O- and S- ligands, which

help in regulating metabolic functions. Therefore, Zn plays a catalytic (functional) and structural role in enzyme reactions. Since it exists only as Zn(II), so it does not take part in oxidation-reduction reactions. Zn plays a crucial role in many biological processes. It is an integral component of many enzymes, alcohol dehydrogenase, carbonic anhydrase, Cu-Zn-superoxide dismutase, alkaline phosphatase, phospholipase, carboxypeptidase and RNA polymerase, to name a few. Large number of enzymes requires Zn for activation such as dehydrogenases, aldolases, isomerases and transphosphorylases. Zinc is important in DNA and RNA metabolism and protein synthesis and maintains the structural integrity of biomembranes. More than 1,200 protein molecules (Zn metalloprotein) have been identified including a large number of 'zinc-finger'-containing proteins and transcription factors, oxidoreductases and hydrolytic enzymes such as metalloproteases. Zn is a structural component of ribosomes thus essential for their structural integrity. It plays a major role in carbohydrate metabolism by regulating key enzymes, fructose 1,6-*bis*phosphatase and aldolase. Synthesis of auxin, indole acetic acid, is particularly impaired under Zn deficiency. Further, Zn also has a role in signal transduction through mitogen-activated protein kinases (MAPK).

20.4.2.3.3 Symptoms of Deficiency and Excess Zn

The most striking symptoms of Zn deficiency in dicotyledonous plants are stunted growth due to shortening of internodes called 'rosette' and a drastic decrease in leaf size termed as 'little leaf'. When the Zn deficiency is severe, the growing shoot apex shows 'die back' symptom. Further, these symptoms combined with chlorosis produce 'mottled leaf'.

The critical toxicity levels of Zn in the leaves ranges between 100 to 300 μg or more per gram dry weight. Symptoms of Zn toxicity are inhibition of root elongation, chlorosis in young leaves and inhibition of photosynthesis.

20.4.2.4 Boron

The soil contains B in the range of 20–200 ppm. The primary mineral tourmaline contains about 3–4 % B. Only the monomeric species $B(OH)_3$ and $B(OH)_4^-$ are present in soil solution depending on soil pH.

20.4.2.4.1 Uptake and Distribution

Uptake of B is a non-metabolic process and its distribution in the plants is also governed by transpiration stream. Boric acid channels, which are major intrinsic proteins, facilitate B transport across membranes. Nodulin26-like intrinsic protein 5;1 (NIP5;1), identified from Arabidopsis, is involved in efficient uptake of B into root cells under B-stress condition. Another channel protein, AtNIP6;1, helps in preferential distribution of B in young shoot tissues (Takano et al. 2006). Besides boric acid channels, transporters have also been identified from Arabidopsis, viz., BOR1, BOR2 and BOR4 (Takano et al. 2002). BOR1 is the first B transporter identified by analysis of Arabidopsis *bor1-1* mutant which requires high levels of B for normal leaf expansion (30 mM) and fertility (100 mM). BOR1 encodes an efflux-type B transporter which is located in the plasma membrane and is expressed in root cells including endodermis (Noguchi et al. 1997). Thus, BOR1 is required for effective xylem loading under B-limited conditions, and it is also involved in the preferential distribution of B to young leaves. BOR2 encodes a B-efflux transporter located in plasma membrane and is strongly expressed in epidermis of elongation zones of roots and lateral rootcaps. At toxic B concentrations, BOR4 is stably accumulated in plasma membrane and confers high B tolerance to plants suggesting its involvement in B tolerance by exporting the mineral out of symplast (refer to Miwa and Fujiwara 2010). Certain plant organs such as anthers, stigma and ovary contain high concentrations of B, which is twice as high as in stem.

20.4.2.4.2 Biological Functions of B

B is involved in a wide range of biological functions but the exact metabolic functions are not exactly understood. These important physiological processes include protein synthesis, transport of sugars, respiration and the metabolism of plant hormones (indole acetic acid), RNA and carbo-

hydrate. Other functions of B are related to cell wall synthesis and lignification, cell wall structure maintenance by cross-linking of polysaccharides and regulation of the structural integrity of biomembranes. B activates enzymes like plasmalemma ATPase thereby increasing the transport of chlorine and phosphorus. Stimulation of H^+ pumping by B causes hyperpolarisation of membrane potential. Since the wall-associated kinase in the plasma membrane has an extracellular matrix in connection with the pectin molecule, the membrane cell wall connection is B dependent. B promotes structural integrity of biomembranes and formation of lipid rafts. It also influences pollen germination, pollen tube growth and subsequently fertilisation.

20.4.2.4.3 Symptoms of Deficiency and Excess B

Death of the root and shoot tips occurs due to B deficiency resulting in stunted (rosette) plant growth. Leaves develop a thick coppery texture and become curled and brittle. B also affects flower retention, pollen formation, pollen tube growth or germination, N fixation and nitrate assimilation. Root elongation is inhibited and root tips become swollen and discoloured. The fleshy tissue in fruits disintegrate causing disorders like 'heart rot' in sugar beet, 'water core' in turnip and 'browning' of cauliflower.

Typical B toxicity symptoms appear on mature leaves producing marginal or tip chlorosis and necrosis. The critical B toxic content in tissue varies depending on the plant species; however, it ranges from 100 to 1,000 mg per kg dry weight.

20.4.2.5 Manganese

The primary rock minerals containing Mn are pyrolusite (MnO_2) and manganite [MnO (OH)]. Total Mn levels of soil vary between 200 and 3,000 ppm. In biological systems, Mn occurs in oxidation states II, III and IV, with Mn(II) and Mn(IV) being fairly stable and Mn(III) unstable. In plants, Mn(II) is the dominant form, but it can readily undergo oxidation reaction and form Mn(III) and Mn(IV). This property makes it possible for Mn to play a crucial role in redox reactions. The preferred form of uptake by plants is the divalent cation, Mn^{2+}. Under waterlogged condition, Mn availability increases just like Fe. Mn^{2+} can replace other divalent cations like Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+} from their active uptake site.

20.4.2.5.1 Uptake and Distribution

Mn uptake is an active process involving metabolic energy. It is relatively immobile in the plants. It is preferentially translocated to meristematic tissues; therefore, young plant organs are rich in Mn. The genes involved in transport of transition metal in plants have been identified which are also responsible for Mn^{2+} transport. The gene families associated with Mn^{2+} transport include cation/H^+ antiporters, natural resistance-associated macrophage protein (NRAMP) transporters, ZIP transporters, cation diffusion facilitator (CDF) transporter family and P-type ATPases. These transporters are responsible for accumulation of Mn into the cell and its release from various organelles. The active sequestration of Mn into endomembrane compartments, particularly the vacuole and endoplasmic reticulum, also takes place by these transporters (reviewed by Pittman 2005).

20.4.2.5.2 Biological Functions of Mn

Mn is essential for plant metabolism and approximately 35 enzymes of a plant cell contain Mn in three oxidation states, II, III and IV. Mn can fulfil two functions in protein – it serves as catalytically active metal and it exerts an activating role on enzymes. Enzymes in which Mn has catalytic role are Mn-containing superoxide dismutase which protects the cell from damaging effects of free radicals, oxalate oxidase and Mn-containing water-splitting system of PS II (Barber 2003). Some of the Mn-activated enzymes are PEP carboxykinase, isocitrate dehydrogenase, malic enzyme and phenylalanine ammonia lyase (PAL). Manganese activation was seen in enzymes of nitrogen metabolism (glutamine synthetase, arginase), gibberellic acid biosynthesis, RNA polymerase activation and fatty acid biosynthesis.

20.4.2.5.3 Symptoms of Deficiency and Excess Mn

Visible symptoms of Mn deficiency first appear on younger leaves in the form of small yellow

spots and interveinal chlorosis. At ultrastructural level, Mn deficiency leads to disorganisation of chloroplast and lamellar system. The cell volume is reduced, cell walls dominate and the inter-epidermal tissue shrinks. 'Grey speck' in oats and 'marsh spot' in cotyledons of pea are the symptoms of Mn deficiency.

Mn toxicity symptoms are characterised by brown spots in older leaves surrounded by chlorotic areas. Excess Mn can also induce deficiency of other mineral nutrients such as Fe, Mg and Ca. The critical toxicity level of Mn varies from 200 (in maize) to 5,300 ppm (in sunflower) (Foy et al. 1988).

20.4.2.6 Molybdenum

Most soils contain Mo between 0.6 and 3.5 ppm. It occurs in soil as molybdate oxyanion, MoO_4^{2-}. A fraction of soil Mo also occurs in organic form.

20.4.2.6.1 Uptake and Distribution

Plants absorb Mo as molybdate ion (MoO_4^{2-}). However, the uptake may be reduced due to the competition by other anions like SO_4^{2-}, whereas ions such as PO_4^{2-} are known to enhance the uptake of Mo. The uptake of Mo in plant cells has been discovered recently (Tejada-Jimenez et al. 2007; Tomatsu et al. 2007; Baxter et al. 2008). Mo is cotransported through various mechanisms such as phosphate uptake system (Heuwinkel et al. 1992), P-type ATPases, heavy metal transporters (Palmgren and Harper 1999), sulphate transporters (Tweedi and Segel 1970) and nonspecific anion transporters (Mendel and Hansch 2002). The chemical properties indicate that it is transported as molybdate ion (MoO_4^{2-}), similar to sulphate (SO_4^{2-}), phosphate (PO_4^{2-}), tungstate (WO_4^{2-}) and vanadate (VO_4^{2-}). A putative sulphate transporter, *AtSultr5;2*, has been identified as molybdate transporter (MOT1) in Arabidopsis (Tomatsu et al. 2007). MOT1 is classified as high-affinity transporter (K_m <21 nM) expressed in both roots and shoots and located in plasma membrane and vesicles. In plants, Mo is localised mainly in the phloem and vascular parenchyma and is readily translocated throughout the system (Gupta 1997). Unlike other elements, Mo can be taken up in excess by the plants without resulting in any toxic effects.

20.4.2.6.2 Biological Functions of Mo

Mo is an essential component (cofactor) of a few important enzymes in higher plants. In these enzymes, Mo has both structural and catalytic functions and is involved directly in redox reactions. These enzymes are nitrate reductase, nitrogenase, xanthine oxidase/dehydrogenase and sulphite reductase. The physiological processes controlled by these enzymes are N assimilation, S metabolism, phytohormone biosynthesis and stress reactions. The final step of abscisic acid biosynthesis is catalysed by Mo-enzyme aldehyde oxidase, while sulphite oxidase protects the plant against toxic levels of sulphite. A novel Mo-enzyme, mitochondrial amidoxime reducing component (mARC), has been reported on the envelope of mammalian mitochondria. Here it is associated with detoxification and catalyses the reduction of N-hydroxylated amidines acting jointly with cytochrome *b*5 and cytochrome *b*5 reductase. The homologues of this new enzyme were found in plants and eubacteria.

20.4.2.6.3 Symptoms of Deficiency and Excess Mo

In Mo-deficient plants, particularly legumes, symptoms of N deficiency are common, producing chlorosis in younger leaves and stunted growth. In some dicotyledonous species, e.g. cauliflower, there is a drastic reduction in size and irregularities in the formation of leaf blade. Under such severe Mo deficiency, only the midrib of leaf is present giving it a whip appearance called 'whiptail'. Local chlorosis and necrosis along the main veins of mature leaves called 'yellow spots' are common in citrus.

Mo toxicity generally does not occur because there is a wide gap between the critical concentrations for deficiency and toxicity levels, which vary by a factor of 10^4 times (0.1–1,000 μg Mo per gram dry weight). However, if at all it is taken up beyond the toxic levels, malformation of leaves and a golden-yellow discoloration of shoot tissue occur.

20.4.2.7 Chlorine

In nature, chlorine is abundantly present since it is available in various sources such as soil

reserves, irrigation water, rain, fertilisers and air pollution. Therefore, in crop production, chloride toxicity is of major concern than its deficiency. It is present in aqueous form in soil as monovalent anion, Cl^-. It is not adsorbed to soil particles and is mobile thus highly prone to leaching.

20.4.2.7.1 Uptake and Distribution

Chlorine uptake is an active process and uptake occurs very rapidly in considerable amounts. However, its uptake also takes place against an electrochemical gradient by carrier proteins present in plasma membrane. In green tissue, under light Cl^- uptake is enhanced as ATP formation during photosynthetic phosphorylation provides energy source for active uptake. Uptake of chloride through transporters was confirmed after the discovery of chloride channel (CLC) family protein in *A. thaliana* and *Nicotiana tabacum* (Hechenberger et al. 1996, Lurin et al. 1996). Seven CLC genes each have been identified in *A. thaliana* (*AtClCa–AtClCg*; Marmagne et al. 2007) and rice (*Oryza sativa*, *OsClC1–OsClC7*; Diédhiou and Golldack 2005). These CLC proteins are involved in anion transport across plant membranes (reviewed by De Angeli et al. 2009).

Uptake of Cl^- faces competition with NO_3^- and SO_4^{2-}. Besides root, foliar absorption of Cl^- as chlorine gas also occurs. Chloride content in plant tissue is usually in the range of 50–500 μmol per kilogram of dry weight. In the cell, it is preferentially stored in the vacuole.

20.4.2.7.2 Biological Functions of Cl

Cl^- is known to be associated with more than 130 organic compounds in plants. Cl^- being a mobile anion, majority of its function is associated with electrical charge balance. It plays an important role in photosynthesis. In photosystem II (P_{680}) of oxygen evolving complex, there are three extrinsic polypeptides which contain Mn. Cl^- acts as a bridging ligand and stabilises the oxidised state of Mn in these associated polypeptides. H^+-pumping ATPase present on tonoplast is specifically stimulated by Cl^-. In guard cells of some plants, e.g. onion, Cl^- accumulation regulates opening and closing of stomata. Therefore, Cl^- indirectly affects plant growth by stomatal regulation. The seismonastic leaf movement of *Mimosa pudica* is directly controlled by this ion. The 'osmotic motor' for the leaf movement is powered by a plasma membrane H^+-ATPase which drives KCl and water fluxes.

20.4.2.7.3 Symptoms of Deficiency and Excess Cl

Cl^- deficiency symptoms include reduction in leaf surface area, wilting of leaf at margins, interveinal chlorosis of mature leaves and restricted and highly branched root systems. Under severe Cl^- deficiency, curling of the youngest leaf followed by shrivelling and necrosis takes place. In palm trees, besides wilting and premature senescence of leaves, frond fracture and stem cracking are typical Cl^- deficiency symptoms.

Toxicity of Cl^- is a more serious problem in agriculture. Cultivation on salt-affected soils shows Cl^- toxicity symptoms in plants. Typical symptoms of Cl^- toxicity are bronzing of leaf tips and margins, premature yellowing and abscission of leaves. Some crops such as sugar beet, barley, maize, spinach and tomato are highly tolerant, while tobacco, beans, citrus, potatoes and legumes are highly susceptible to Cl^- toxicity.

20.4.2.8 Nickel

Chemically Ni is related to Fe and cobalt. It occurs not only in Ni(II) oxidation states but also in I and III states in biological systems and forms stable complexes with cysteine, citrate and Ni enzymes. Ni forms chelates and can readily replace other heavy metals (like Fe) from physiologically important sites. Ni content of soil is usually less than 100 ppm. It is derived from the weathering of ultrabasic igneous rocks (serpentine mineral).

20.4.2.8.1 Biological Functions of Ni

Nickel was the 17th mineral element to be regarded as essential trace (micronutrient) element for plant growth. It was found to be a metal component in enzymes such as urease, dehydrogenases, hydrogenases and methyl reductases in a large number of bacteria. Earlier reports showed that low Ni concentration stimulates germination and growth of many crop species. The first evidence of Ni as a constituent of urease enzyme in higher plants (jack bean) was provided by Dixon et al. (1975). Later,

Ni requirement for legumes and nonlegumes was also established (Freyermuth et al. 2000). Some functions of Ni are now clearly defined and therefore it was included in the list of essential micronutrients (Eskew et al. 1983, 1984).

Nickel is taken up as divalent cation, Ni^{2+}. It is mobile in xylem and phloem, so after uptake, considerable amount of it is transferred to the seeds and fruits. Ni has an important role in N metabolism. The urease enzyme present in plants is involved in breakdown of urea to CO_2 and ammonia via the ornithine cycle. Accumulation of urea in the absence of urease would be toxic to the plant's cellular machinery. Moreover, recent findings suggest that in addition to urea metabolism, plant ureases have a protective role against phytopathogens (Follmer 2008).

20.4.2.8.2 Symptoms of Deficiency and Excess Ni

In crop plants, Ni toxicity is a major problem rather than deficiency. Under Ni deficiency, accumulation of toxic levels of urea might occur. However, the Ni toxicity symptoms closely related to Fe deficiency symptoms. Acute Ni toxicity gives rise to chlorosis. In cereals, pale yellow stripes along the length of the leaf occur. The whole leaf may turn white and necrosis occurs at leaf margins. In dicots, the Ni toxicity causes chlorotic patches between leaf veins, similar to Mn deficiency.

20.4.3 Beneficial Elements

Summary of beneficial elements regarding plant concentrations, beneficial effect, physiological mechanisms and deficiency or toxicity symptoms of nutrients (Pilon-Smits et al. 2009) is presented in Table 20.6.

20.5 Ion Absorption

The anions and cations freely dissolved in the soil solution are the most readily available forms to be absorbed by the roots, even though per se their concentration may be low. These nutrients reach the vicinity of roots in three ways: (1) diffusion of ions through the soil solution, (2) the passively charged ions carried along as water moves by bulk flow into the roots and (3) extending growth of roots towards the dissolved ions. In both higher and lower plants, ion uptake is characterised by three principles:

1. *Selectivity*: Plants preferentially take up certain mineral elements, while others are discriminated against or almost excluded. In soil solution, some mineral elements may be present in higher concentration, but they are not entirely required for plant growth. So, the plant has mechanism to exclude or selectively take up only those elements required for its growth.
2. *Accumulation*: The concentration of mineral elements can be much higher in the plant cell sap than in the external solution. It has been seen that the ion concentration in the root cell sap is generally much higher than that in the nutrient solution. This is evident in the case of potassium, nitrate and phosphate, which are accumulated at higher concentration in the root cells.
3. *Genotype*: Distinct differences among the plant species exist in terms of ion uptake. This difference depends on the condition of the growing media. For example, in alga species, *Nitella* grown in pond water had higher concentration of potassium, sodium, calcium and chloride in the cell sap, while in *Valonia* grown in highly saline sea water, only potassium remained at higher concentration in the cell sap, whereas the sodium and calcium concentrations remain at a lower level than in sea water.

The accumulation of ions in plant tissues in quantities more than the circumambient solution indicates that the ions diffuse through the cell against a concentration gradient. This type of ion uptake requires expenditure of metabolic energy, and therefore, it is dependent on the metabolic status of absorbing cells or tissues in plants. This phenomenon of ion or salt absorption called active absorption, and is most common in rapidly growing tissues such as meristematic cells, and it decreases with the increase in maturity of cells. There are some evidences which prove that the active absorption of solute requires metabolic

Table 20.6 Plant concentrations, beneficial effect, physiological mechanisms and deficiency or toxicity symptoms of nutrients categorised as beneficial elements

Element	Form	Plant concentration	Beneficial effects	Mechanisms hypothesised	Symptoms	Plant species/hyperaccumulators
Aluminium	Al^{3+}, $Al(OH)^{2+}$ $Al(OH)_2^{+}$	<0.1 % non-accumulators ≥0.1 % Al accumulators	Increases root and shoot growth Resistance to herbivore	Increases antioxidant activity Increases P availability Decreases Fe toxicity	Toxicity: inhibition of root growth, alteration in root architecture, disruption of root elongation, symptoms similar to P deficiency	*Miscanthus sinensis* (maiden grass), *Camellia sinensis* (tea, *Melastoma malabathricum*
Cobalt	Co^{2+} Co^{3+}	10^{-6} – 0.001 % non-accumulators ≥0.1 % Co accumulators	Increases growth, nodule number and weight, plant nutrient levels, as well as seedpod yield and seed quality in legumes Retardation of leaf senescence Enhancement of drought resistance in seeds Resistance to herbivore	Cobalamin (vit B_{12})-dependent enzyme systems in *Rhizobium*: methionine synthase, ribonucleotide reductase, methylmalonyl-coenzyme A mutase, inhibition of ethylene biosynthesis	Deficiency: affects nodule development, yellowing of leaves similar to N deficiency symptoms, lower plant N content, accumulation of Co in root nodules Toxicity: symptom resembles Mn deficiency	Families of Lamiaceae, Scrophulariaceae, Asteraceae and Fabaceae examples: *Pisum sativum* (Pea), *Lotus japonicus* (Lotus) *Crotalaria cobalticola* (indicator plant)
Sodium	Na^{+}	<0.05 % non-halophytes >0.25 % in halophytes	Increases plant growth, leaf area, number of stomata per unit leaf area Essential for C_4 and CAM plants Can facilitate nitrate uptake Improves water balance of plants	Cell expansion Stomatal regulation by substituting K and maintaining higher relative water content Regeneration of phosphoenolpyruvate	Enzymes activated by K are inhibited, less starch content in tissue, accumulation of sucrose	*Atriplex vesicaria*, *Zea mays* (maize), *Sorghum bicolor* (sorghum), members of Chenopodiaceae

Selenium	Se_2^-, SeO_3^{2-}, **SeO_4^{2-}**	≤0.01 % non –Se accumulators 0.01– 0.1 % Se accumulators ≥0.1 % Se hyperaccumulators	Increases plant growth Resistance to pathogen and herbivore attack Structural component of specific selenoproteins and seleno-tRNAs	Increases antioxidant activity Prevents P toxicity Replaces SO_4^{2-} and forms amino acids selenocysteine (SeCys) and selenomethionine (SeMet) Volatile Se (dimethylselenide) deters herbivores, Accumulated Se is toxic to herbivores and pathogens	Toxic levels in plants lead to disorders in animals feeding on them; acute toxicity leading to death, chronic blindness and paralyses, chronic alkali disease leading to lameness Deficiency of Se in produces symptoms of P toxicity in plants	*Astragalus bisulcatus* (milkvetch), species of *Xylorrhiza*, *Stanleyea*, *Brassica oleracea* (broccoli) Ryegrass, lettuce, potato, duckweed
Silicon	$Si(OH)_4$	<0.5 % most species 10–15 % horsetails	Maintains plant sturdiness thus preventing lodging, leaf erectness for better light interception Resistance to pathogen and herbivore attack Resistance to abiotic stress (heavy metals, salinity, drought, UV radiation, extreme temperature)	Strengthens cell walls Activation or synthesis of stress-related molecules Systemic acquired resistance, SAR-like bioactivity Regulation of metal transport (Mn and Fe) Increases antioxidant activity Sodium exclusion from roots	In animals, fed on high Si content feed leads to abrasion of rumen wall, secondary deposition in kidney	*Equisetum arvense* (horsetail millet), *Oryza sativa* (paddy rice), *Saccharum officinarum* (sugarcane)

energy: (1) higher rate of respiration increases salt accumulation inside the cell, (2) respiratory inhibitors check the process of salt uptake and (3) by decreasing oxygen content in the medium, the salt absorption also decreases. These evidences suggest that absorption of salt is directly coupled with respiratory rate and energy level in the plant body.

20.5.1 Pathway of Solute from External Solution into the Cells

The water and dissolved ions in the external solution move into the xylem cells of roots via three possible pathways: (1) through the cell walls or apoplast of epidermal and cortical cells; (2) through the cytoplasmic or symplast system, moving from cell to cell; and (3) from vacuole to vacuole of the living root cells where the cytosol of each cell forms part of the pathway. In the following paragraphs, this pathway is discussed in detail.

Mineral salts dissolved in water are absorbed by roots and taken up through the xylem vessel from where it is distributed to all parts of the plant body. The dissolved cations and anions along with water moves from cell to cell through spaces between cell wall by diffusion, called *apoplastic pathway*. On the other hand, ions entering cell wall of the epidermis move across the cell wall of cortex, cytoplasm of endodermis and cell walls of pericycle and finally reach the stele. This pathway involving the living (cytoplasm) part of the cell is referred to as *symplastic pathway*. In this pathway, nutrient elements entering the cytoplasm of the epidermis move across the cytoplasm of the cortex and endodermis of pericycle through plasmodesmata and finally reach the xylem vessels.

After the nutrients are absorbed by roots, it is translocated to other plant parts by the transpiration stream moving through the xylem. As water is continuously lost by aerial parts of the plant called *transpiration*, it creates a transpirational pull. This driving force keeps the water moving up along the plant with mineral salts in the xylem vessel. This transpirational pull is so strong that water and nutrients move in upward direction to several feet in the tallest trees growing in nature. Stout and Hoagland have proved that mineral nutrients absorbed by the roots are translocated through the xylem vessel.

Nutrient uptake by roots is a well-regulated process and is also specific and selective. Subsequent translocation of ions across cortex to reach the stele (xylem loading) is also determined by several interrelated factors. The pathway of ion or solute movement across the root faces some special constraints because of the anatomy of roots. All plant cells are separated by cell walls, and ions can move through channels in the cell wall spaces without ever entering a living cell. This continuum of cell wall, which allows extracellular ion movement, is called the free space or apoplast. Besides cell wall forming a continuous phase, the cytoplasms of neighbouring cells are also connected to each other and form a continuum, collectively termed as the *symplast*. The cytoplasmic bridges interconnecting the neighbouring cytoplasm are called *plasmodesmata*. These are cylindrical pores of 20–60 nm in diameter. Each plasmodesmata (singular) is lined with a plasma membrane and contains a narrow tubule, called *desmotubule* which is the continuation of endoplasmic reticulum. The cells near the root tips where most nutrient absorption occurs has a high density of plasmodesmata.

The anatomy of root cell and the pathway of solute or ion movement in root cells are depicted in Fig. 20.7. The apoplast forms a continuous phase from the root surface through the cortex. There is a layer of specialised cells called endodermis at the boundary between vascular cylinder and the cortex. The endodermis contains a layer or strip of suberised cells known as Casparian strip, which functions as a barrier to the entry of water and mineral ions into the stele through the apoplast. This Casparian strip has hydrophobic properties and completely surrounds each endodermis cell. When a solute enters the root, it may enter the symplast immediately by crossing an epidermal cell plasma membrane, or it may diffuse between the epidermal cells through the cell

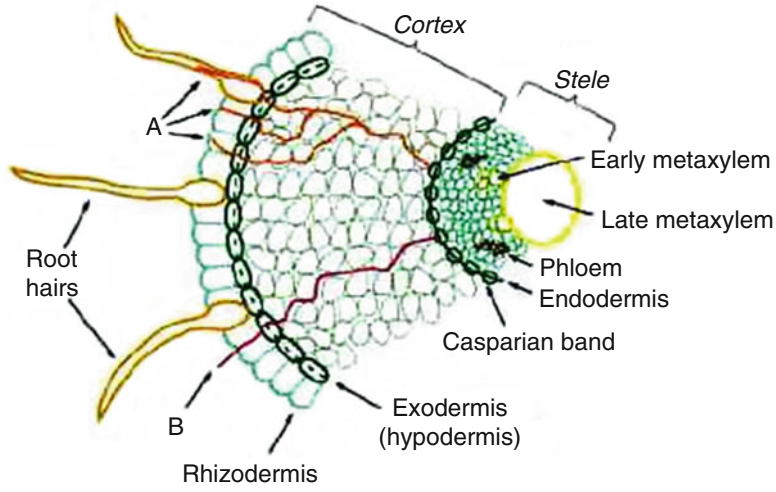


Fig. 20.7 Pathway of ion transport across the root (*A*) symplastic (*B*) apoplastic

walls. From the apoplast of cortex, ions/solutes may cross the plasma membrane of cortical cell, thus taking the symplastic route, or may radially diffuse all the way to endodermis through the apoplastic route. In either case, the ion/solute must first enter the symplast before entering into the stele due to the presence of Casparian strip.

Once ions enter the symplast of root at epidermis or cortex, they are loaded into the tracheid or vessel element of stele to be translocated to the shoot. Since xylem treachery elements are dead cells, the ions exit symplast by crossing a plasma membrane for the second time. 'Xylem loading', as the name suggests, is the movement of ions from symplast into the conducting cells of xylem. Here again, the Casparian strip prevents back-diffusion of ions through apoplast. Though this strip acts as a barrier to water and solute movement, the major advantage is that it helps the plant to maintain an ionic concentration in xylem higher than the soil water surrounding roots.

There is a possibility that ions could enter tracheid and vessel element of xylem by passive diffusion. But in this case, the movement of ions from root surface to the xylem would take only a single step requiring metabolic energy. This single energy-dependent step of ion uptake occurs at the plasma membrane surfaces of root epidermal, cortical and endodermal cells. According to the passive diffusion model, ions move passively into the stele via symplast through a concentration gradient and then leak out of the living cells of stele (possibly because of lower oxygen availability interior of root) into the nonliving conducting cells of xylem.

The electrochemical potential of various ions across the roots could be measured by using ion-specific microelectrodes. From various studies, it was indicated that K^+, Cl^-, Na^+, SO_4^{2-} and NO_3^- were all taken up actively by epidermal and cortical cells and maintained in xylem against an electrochemical potential gradient when compared with the external medium. However, none of these ions is at a higher electrochemical potential in xylem than in cortex or living portion of stele. Therefore, the final movement of ions into xylem could be due to passive diffusion. However, other studies have shown that this final step of xylem loading may also involve active process within the stele. By using treatments with inhibitors and other plant hormones, investigators have shown that ion uptake by cortex and ion loading in xylem operates independently. Treatments with protein synthesis inhibitor like cycloheximide or with cytokinin (benzyladenine) inhibit xylem loading without affecting uptake by cortex. This result indicated that efflux from the stelar cells is regulated independently from uptake by cortical cells.

Recent biochemical studies have indicated that the xylem parenchyma cells have a role in xylem loading. The plasma membranes of xylem

parenchyma cells contain H^+ pumps, water channels and a variety of ion channels specialised for influx or efflux. Absorption of ions by roots is pronounced in the root hair as compared to meristem or elongation zones. Presence of root hairs greatly increases the surface area available for ion absorption.

20.5.2 Mechanisms of Ion Absorption

According to Fick's law, the movement of molecules or ions by diffusion always takes place spontaneously from a place of high concentration to a low concentration, that is, down a concentration or chemical gradient until equilibrium is reached. This movement of molecules by diffusion without the involvement of metabolic energy is termed as passive transport. Once the equilibrium is reached, no further net movement of solute will occur without the application of a driving force. On the other hand, movement of substances after the equilibrium is reached or against a concentration gradient by involving metabolic energy termed as active transport.

20.5.3 Passive Absorption

The concept of passive absorption is based on 'outer space' also called as 'free space' or 'diffusion space'. If only a portion of tissue volume is open to free diffusion, the ions will move freely in or out of the tissue. After sometime, the part of tissue undergoing free diffusion will reach equilibrium with external solution resulting in same ion concentration within the tissue as that of the external solution. The part of a cell or tissue that allows free diffusion of ions is called free space or outer space. Usually the volume of root tissue accessible for free space is only a small fraction ~5 % of total root volume. The extent of solute flux into free space for a given volume of free space depends on several factors such as transpiration rate, solute concentration and root hair formation. However, the presence of such a free space enables cortex cells to take up solutes directly from the external solution.

The cell walls contain negative charge because of polygalacturonic acid [carboxylic groups (R. $CHOO^-$)] present in the middle lamella. These negative charges in apoplasm act as cation exchangers resulting in accumulation of cations, whereas the anions are repelled. Therefore, the entry of charged solutes is restricted in the free space. Hope and Stevens (1952) introduced the term apparent free space (AFS) to describe 'free space'. AFS comprises of water free space (WFS) which is freely accessible to ions, charged and uncharged molecules. Another term, Donnan free space (DFS), was introduced where cation exchange and anion repulsion take place (Fig. 20.8). The various theories of passive absorption are discussed below:

20.5.3.1 Mass Flow Hypothesis

According to this theory, ions are absorbed by root along with mass flow of water under the influence of transpirational pull. In detopped tomato plants, an increase in transpiration increased salt absorption by replacing ions once they were released into the xylem duct. The dilution thus caused enhanced ion absorption. This resulted in accumulation of a small proportion of total salt uptake by plants through passive absorption. However, this salt accumulation by passive process could be explained as follows: (1) free diffusion of ions along concentration gradient into the apparent free space of a tissue, (2) accumulation of ions against concentration gradient due to ion exchange or Donnan equilibrium and (3) mass flow of ions through roots due to transpirational 'pull' may also occur. All these processes do not require expenditure of metabolic energy.

20.5.3.2 Ion Exchange Theory

Both cations and anions have a tendency to get adsorbed on the surfaces of cell walls or membranes of cells and exchange with ions present in soil solution. During the absorption of a positively charged ion such as K^+, either a positively charged ion such as H^+ is displaced from the cell (ion exchange) or a negatively charged ion enters the cell. Similarly, anions can exchange with free

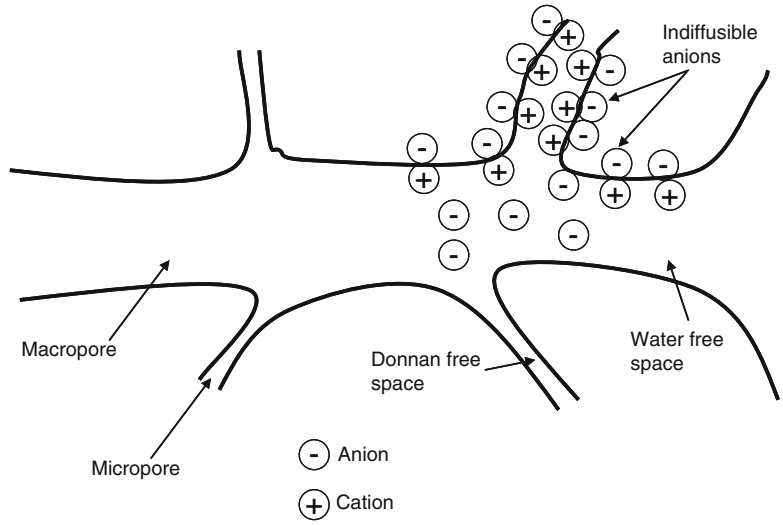


Fig. 20.8 Schematic representation of the pore system of the apparent free space (Source: Marschner 1995)

hydroxyl (OH^-) ions. This process of exchange between the adsorbed ions and ions in the solution is known as ion exchange. Sometimes the uptake of nutrient cations from a soil solution into the roots exceeds the anion uptake and vice versa. In each case, the neutrality is maintained. In a case where anion uptake exceeds cation uptake, the OH^- and bicarbonate (HCO_3^-) ions are transported outwardly from inside the cells into the free space. Likewise, if cation absorption exceeds anion uptake, cells exchange some hydrogen ions. The ionic exchange process is explained as follows:

1. *Carbonic acid exchange theory*

   The roots respire continuously giving out CO_2 in the rhizosphere. This combines with water and forms carbonic acid (H_2CO_3), which dissociates into H^+ and HCO_3^- ions. A zone of carbonic acid is developed in root tips because of high respiratory activity. The H^+ ions exchange with cations absorbed on the clay micelle, and the cations come into soil solution where it diffuses onto the root surface.

2. *Contact exchange theory*

   A similar ion exchange takes place between root and soil colloids at the point where they are in direct contact with each other without being first dissociated in the soil solution.

3. *Donnan equilibrium*

   This theory takes into account the effect of fixed or nondiffusible ions. The cell contents are separated from external solution by a differentially permeable membrane. This membrane is impermeable to anion concentration present in cell. A negative nondiffusing charge on one side will create a potential gradient across the membrane through which the ions will diffuse. This results in diffusion of equal number of cation and anion through the membrane until electrochemical equilibrium is reached. However, because of the 'fixed' negative (anion) charge on the inner side of the membrane, additional cations will be required to balance this charge. Therefore, the cation concentration will be greater in internal solution (inside the cell) than the external solution. Also, the concentration of anions in external solution will be less than that of the external one because of the excess of negative charges due to 'fixed' anions. Therefore, Donnan equilibrium, a term proposed by F.G. Donnan, is attained if the product of anions and cations in the internal solution becomes equal to the product of anions and cations in the external solution.

$$\text{Donnan equilibrium} = \frac{(\text{Positive ions inside})}{(\text{Positive ions outside})} = \frac{(\text{Negative ions inside})}{(\text{Negative ions outside})}$$

20.5.3.3 Facilitated Diffusion

The passive transport can also take place by carrier proteins localised in the plasma membrane where it can transport a much wider range of possible substances. This passive transport mediated by a carrier is called facilitated diffusion. However, it resembles diffusion only in that it transports substances down their gradient of electrochemical potential without any additional input of energy.

20.5.4 Active Absorption

As discussed in the previous section, active absorption involves transport of solutes against the concentration gradient. Carriers, pumps and channels located in the membrane are involved in active transport of ions/solutes (Fig. 20.9). It involves electrogenic and cytochrome pumps to be discussed in the following sections. Active transport is further divided into two categories: primary active transport and secondary active transport.

20.5.4.1 Primary Active Transport

When the transport of ions is directly coupled to expenditure of metabolic energy, such as ATP hydrolysis, an oxidation-reduction reaction (the electron transport chain of mitochondria and chloroplast), or absorption of light by the carrier protein (in halobacteria, bacteriorhodopsin), it is termed as primary active transport. Membrane proteins involved in primary active transport are called pumps. Most pumps transport energy ions, such as H^+ or Ca^{2+}.

The pumps can be further characterised as either electrogenic or electroneutral. Electrogenic transport refers to ion transport involving the net movement of charge across the membrane,

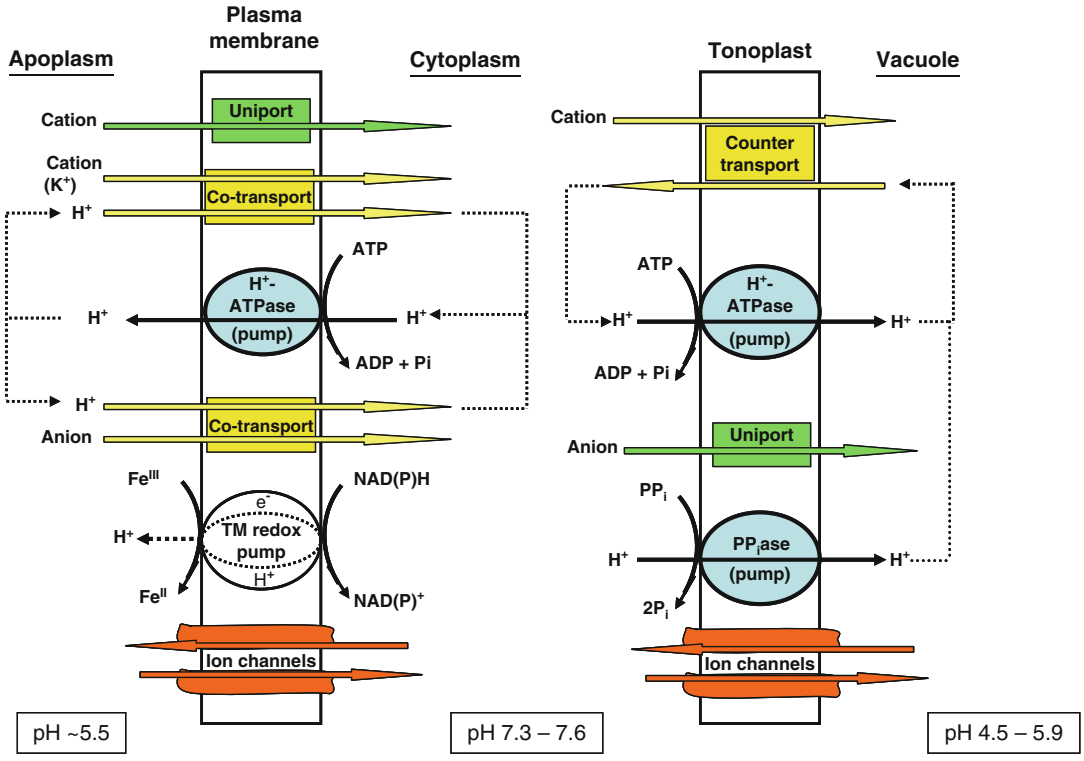


Fig. 20.9 Model for the localisation and functioning of electrogenic proton pumps (H+-ATPase, PPiase), transmembrane redox pump (NAD(P) oxidase), ion channels and transport of cations and anions across the plasma membrane and tonoplast (Source: Marschner 1995)

whereas in electroneutral transport, no net movement of charge is involved. The pumps are driven by energy released during hydrolysis of ATP carried out by ATPase located at plasma membrane and tonoplast. This enzyme is considered as '*master enzyme*' because of its key role in regulation of cytoplasmic pH and the driving force for cation and anion uptake. For example, the Na^+/K^+-ATPase of animal cell pumps out three Na^+ for every two K^+ ions taken in, resulting in net outward movement with one positive charge. The Na^+/K^+-ATPase is therefore an electrogenic ion pump. In contrast, H^+/K^+-ATPase of the animal gastric mucosa pumps one H^+ out of the cell for every one K^+ taken in, so there is no net movement of charge across the membrane. Therefore, the H^+/K^+-ATPase is an electroneutral pump.

In general, H^+ is the principal ion which is electrogenically pumped across the cell membranes of plants, fungi and bacteria. The plant H^+-ATPases exhibit a regulatory role in creating the electrochemical potential gradient across the plasma membrane, while the vacuolar H^+-ATPase (V-ATPase) and the H^+ pyrophosphatase (H^+-ppase) pump proton into the lumen of vacuole and Golgi cisternae.

20.5.4.2 Secondary Active Transport

The other important mechanism by which solutes can be transported across membrane against the gradient of electrochemical potential is coupling of 'uphill' transport of one ion/solute to the 'downhill' transport of another. Carrier proteins located in the membranes carry out such cotransport of ions/solutes, which is also called 'secondary active transport'. These carriers are indirectly driven by the action of pumps. In carrier-mediated ion transport, the substance being transported is initially bound to a specific site on the carrier protein. This binding causes a conformational change in the protein, which exposes the substance to solution on the other side of the membrane. Transport completes when substance dissociates from the carrier's binding site. Rate of transport by a carrier is about 10^6 times slower than transport through a channel.

Secondary active transport utilises the energy stored in the proton-motive force (PMF) or Δp, created when protons are extruded from the cytosol by the electrogenic H^+-ATPases. This takes place both at the plasma membrane and at the vacuolar membrane where a membrane potential and pH gradient develop at the expense of ATP hydrolysis. The PMF represents stored free energy or the H^+ gradient that is used to drive the transport of other substances against their chemical potential gradient. The secondary active transport may be either symport (two ions/solutes moving in the same direction) or antiport (downhill proton movement drives active (uphill) transport of solute in the opposite direction). Transport by carriers may be 100–1,000 ions or molecules per second.

The presence of ion channels in plant cell membranes has also been established. These ion channels have a unique ability to regulate or 'gate' ion flux subject to the physical and chemical environment of the channel protein. Transport through the channel may or may not involve transient binding of solute to the channel protein. In any case, as long as the channel pore is open, solute that can penetrate the pore diffuses through it extremely rapidly. Ion transport through channels is always passive. The pore size and density of charges on interior lining determine the transport specificity; channel transport may be mainly limited to ions or water. The region in the channel that determines specificity is called 'selectivity filter'. However, ion channels remain closed most of the time and their number per cell is also meagre.

A class of relatively abundant membrane proteins that form water channels, are named as 'aquaporins'. Aquaporins are common to plant and animal membranes; their expression and activity in response to water availability are regulated by several mechanisms including protein phosphorylation.

20.5.4.3 Cytochrome Pump

H. Lundegardh in 1954 proposed this theory when he observed a quantitative relationship between anion absorption and respiration, whereas no such correlation with cation absorption was found. It was also noticed that salt respiration and anion absorption were inhibited by

cyanide or even carbon monoxide. He, therefore, suggested that anions could be transported across the membrane by cytochrome system and absorption of anion is independent of cation. Energy is supplied by direct oxidation of respiratory intermediates. The rate of respiration solely determined by anion absorption is called 'anion respiration' or 'salt respiration'. The respiration rate (excluding anion respiration) observed in distilled water is called 'ground respiration'.

$$\text{Total respiration}\left(R_t\right) = \text{Ground respiration}\left(R_g\right) + \text{Salt or anion respiration}\left(R_a\right)$$

20.5.5 Passive Absorption

The absorption of minerals without expenditure of metabolic energy is termed 'passive absorption'. Passive ion absorption in the root system was demonstrated by Briggs and Robertson (1957). The distinguishing characteristics of passive absorption include:

1. Mineral salt absorption not affected by temperature or metabolic inhibitors
2. Rapid uptake of ions when plants are transferred from a medium of low to high concentration

20.6 Biofertilisers

Biofertilisers are natural fertilisers consisting of microbial inoculants of bacteria, fungi and algae, either alone or in combination resulting in enhanced growth of plants mediated by increased availability of nutrients. Biofertilisers can be applied to seed, plant surfaces or soil, where they colonise the rhizosphere or the interior of plant and increase the supply of primary nutrients to the host plant. These microorganisms, either through symbiotic or non-symbiotic association, help the plants to fix N from atmosphere, solubilise and mobilise fixed P; translocate minor elements like Zn and Cu to the plants; produce plant growth-promoting hormones, vitamins and amino acids; and control plant pathogenic fungi. Thus, biofertilisers are expected to reduce the use of chemical fertilisers and pesticides. Besides microorganisms, biofertilisers include organic fertilisers such as farmyard manure (FYM) obtained by decomposing farm wastes.

The use of biofertilisers is both economical and environment friendly. These microorganisms are abundant in the natural environment. Use of biofertilisers leads to enhanced crop productivity and reduction in the usage of chemical fertilisers resulting in improvement of soil texture and mitigating other harmful environmental effects. As a matter of fact, the system of integrated nutrient management (INM) subscribes to the use of a combination of inorganic and organic/biofertilisers. Biofertilisers include the following groups:

1. Symbiotic N fixers, e.g. *Rhizobium* spp. for legumes
2. Non-symbiotic free-living N fixers, e.g. *Azotobacter*, *Azospirillum*
3. Algae biofertilisers (blue-green algae (BGA) in association with *Azolla*)
4. Phosphate solubilising bacteria (PSBs)
5. Mycorrhiza
6. Organic fertilisers

20.6.1 Symbiotic Nitrogen Fixers and Algal Biofertilisers

Bacteria such as *Rhizobium* spp. exhibit symbiotic relationship with host plants especially legumes. Blue-green algae (BGA) or cyanobacteria have been used in agriculture since long. The bacterial biofertilisers fix about 50–150 kg N/ha or more per year. The root-nodulating bacteria, such as *Rhizobium*, are common in nature. However, there are a few other genera that produce stem nodules. For example, *Azorhizobium* spp. produce stem nodules on *Sesbania rostrata*. Rhizobium is classified based on the specific legume species they nodulate. For example, *R. leguminosarum* nodulates pea (*Pisum sativum*), khesari (*Lathyrus sativus*) and lentil (*Lens culinaris*); *R. phaseoli* produces nodules on French bean (*Phaseolus vulgaris*) and bean (*Phaseolus*

multiflorus); *R. meliloti* nodulates lucerne (*Medicago sativa*) and fenugreek (*Trifolium foenumgraecum*); *R. trifoli* nodulates clover (*Trifolium* sp); and *R. lupini* nodulates white lupins (*Lupinus alba*).

Cyanobacteria or BGA are photosynthetic prokaryotic organisms that can fix atmospheric N in association with a fresh water fern called *Azolla*. Cyanobacteria belong to the genera *Nostoc, Anabaena, Plectonema, Aulosira* and *Tolypothrix. Azolla* cultures are inoculated in paddy fields under both aerobic and lowland conditions. However, the effectiveness of the inoculum is higher under lowland condition, wherein about 20–30 kg N is fixed per ha per crop season. The enzyme nitrogenase is present in cyanobacteria and N fixation occurs in specialised cells called 'heterocysts'. These heterocysts have *nif* gene (involved in N fixation) and also act as oxygen-proof compartment, protecting nitrogenase from oxygen inactivation. *Azolla* owes its N-fixing capacity to *Anabaena,* while the fern supplies food material to the alga. In addition to N fixation, BGA decomposition contributes to the ecosystem biomass, thereby enhancing soil physical properties.

20.6.2 Non-symbiotic Free-Living N Fixers

Azotobacter and *Azospirillum* are free-living bacterial genera which fix atmospheric N. *Azotobacter* can be used to inoculate crops like maize, wheat, mustard, cotton, potato and other vegetable crops. By utilising soil organic matter, it fixes about 30 kg N per ha per year. *Azospirillum* inoculants are recommended especially for cultivation of wheat, maize, sugarcane, millets and sorghum.

20.6.3 Phosphate Solubilising Microorganisms

Phosphate solubilising microorganisms capable of solubilising Pi from insoluble sources enhance P bioavailability. These microorganisms belong to the bacterial genera *Thiobacillus*, *Bacillus*, etc. These bacteria, known as phosphate solubilising bacteria (PSBs), produce substances like pseudobactin (also called 'siderophores') that chelate Fe.

Availability of P is low both in alkaline and acidic soils since it is fixed as insoluble oxides. The inoculants of PSBs when applied to either soil or seed can be useful to mobilise fixed P. Rock phosphate when used with PSBs can reduce the phosphatic fertiliser requirement by 50 %. Mere seed inoculation with PSBs results in crop yield responses equivalent to that obtained by application of 30 kg P_2O_5 per ha in the form of chemical fertilisers.

20.6.4 Mycorrhizae

Mycorrhizae (*mycor* fungus; *rhiza* root) are a symbiotic (intimate) and mutualistic (mutually beneficial) association between a nonpathogenic or weakly pathogenic fungus and living root cells of plant, particularly cortical and epidermal cells. The fungi receive organic nutrients from the plant and, in turn, improve the mineral element and water-absorbing properties of roots. The roots of most soil grown plants are mycorrhizal. About 83 % of dicot and 79 % of monocot plants and all gymnosperms are mycorrhizal worldwide. Generally, the fungus infects tender young roots because, on older parts, the epidermis and cortex are lost and a protective layer of suberin develops in cork cells. Root hair production either slows or ceases upon infection, so mycorrhizae often have few such hairs. This reduction in root hairs greatly decreases the absorbing surface area; however, the slender fungal hyphae extending from mycorrhizae explore greater soil volume.

Two main groups of mycorrhiza are present in nature: (1) ectomycorrhiza and (2) endomycorrhiza. However, there is a rare group with intermediate properties called the ectendotrophic, which is also sometimes found. In ectomycorrhizae (ECM), the fungal hyphae form a mantle outside the root and also inside the root in the intercellular spaces of epidermis and cortex. No intracellular penetration into epidermal or corti-

cal cells occurs, but an extensive network called the Hartig net is formed between these cells. Ectomycorrhizae are commonly found on the roots of trees including members of Pinaceae family (pine, fir, spruce, larch, hemlock), Fagaceae (oak, beech, chestnut), Betulaceae (Birch, alder), Salicaceae (willow, poplar) and a few other families.

In endomycorrhiza, fungi live inside the cortical cells and also grow in the intercellular spaces. Endomycorrhiza consists of three subgroups, namely, the vesicular arbuscular mycorrhizae (VAM) the most common, the ericoid and the orchidaceous mycorrhizae. The VAM belongs to the family Endogonacae. They produce a branched haustorial structures called arbuscules between the cortical cells and a mycelium that extends out in the soil. These extraradical mycelium or hyphae are involved in absorbing mineral salts and water. The arbuscules are short lived, about 10–12 days, and are the main sites of solute exchange with the host. There are mainly four genera of VAM fungi, namely, *Acaulospora*, *Gigaspora*, *Glomus* and *Sclerocystis*. Out of these genera, *Glomus* is present abundantly in soil. It has been found that not all endomycorrhizal fungi produce vesicles as lipid-rich storage organs, so the VAM has been renamed as AM (arbuscular mycorrhiza).

The host plants secrete root exudates containing flavanoids (e.g. β-estradiol), which attract these fungi, and the root infection and colonisation occur. In mycorrhizal roots, for fungal growth, a substantial proportion of carbons fixed during photosynthesis are required. In AM plants, root respiration may be 20–30 % higher than in non-mycorrhizal plants, and 87 % of higher respiration is attributed to the fungus. The mycorrhizal colonisation affects root and shoot growth differently. Its effect is more pronounced in nutrient-poor soils rather than nutrient-rich soils. Mycorrhizal colonisation helps the plant to acquire nutrients which are less mobile in soil, particularly P and also N, Zn, Cu and S. Besides these, the mycorrhizal association also helps in increasing the hormonal (IAA, cytokinin, ABA) content of plants, improves plant-water relations (increase drought tolerance capacity) and suppresses soil-borne fungal and bacterial root pathogens (e.g. *Pseudomonas syringae* in tomato plant).

20.6.5 Organic Fertilisers

Organic fertilisers are the plant and animal wastes which after decomposition release nutrients essential for plant growth. These include farmyard manures (decomposed mixture of dung, urine and litter of farm animals), compost (rotted wastes of farm like sugarcane trash, paddy straw, etc.), sewage and sludge, vermicompost (decomposition of organic matter by earthworms), green manures (undecomposed plant material) and other livestock manures (poultry, sheep and goat sweepings). These organic manures contain small percentage of nutrient elements and they are applied in large quantities. The practice of growing crops with only organic fertilisers and without the use of chemical pesticide or herbicide is called organic farming. Besides supplying nutrients, the organic fertilisers improve soil physical properties, increase availability of other nutrients and control plant parasitic nematodes and fungi.

20.6.6 Advantages and Limitation of Biofertilisers

The relevance of biofertilisers is increasing rapidly. This is because it has been realised that application of chemical fertilisers causes serious harmful effects on the environment and human health. Further, they are costly and in short supply. Therefore, the advantages of using biofertilisers in general are as follows:

1. They add nutrients to the soil and/or increase their availability to the crops.
2. They secrete certain growth-promoting substances such as indole acetic acid (IAA).
3. Under certain conditions, they exhibit antifungal activities, thereby protecting the plants from pathogenic fungi.

4. They are harmless and eco-friendly low-cost agro-input supplementary to chemical fertilisers.
5. They improve soil structure (porosity) and water-holding capacity.
6. They enhance seed germination.
7. They increase soil fertility and fertiliser use efficiency and ultimately increase yield by 15–20 % in general.

However, the limitation in the use of biofertilisers lies in the fact that its acceptability by the farming community is low, the reason being organic fertilisers do not produce quick and spectacular responses. Moreover, the quantity of nutrients provided by biofertilisers is not sufficient to adequately meet the crop demand for high yields.

20.7 Hydroponics: History and Scope in Indian Agriculture

Hydroponics (Greek words 'hydro' water; 'ponos' labour) is a method of growing plants using mineral nutrient solutions without soil. It is also called "controlled environment agriculture" (CEA) since raising plants hydroponically requires control of environmental factors such as light intensity and duration, temperature, humidity, pH of the solution/medium and mineral nutrients.

The study of crop nutrition began thousands of years ago. The classic work on growing terrestrial plants without soil was published by Sir Francis Bacon in 1627, the book named 'Sylva Sylvarum'. After Bacon's work, water culture became a popular research technique. John Woodward (1699) published his work on water culture experiments with spearmint where he mentioned that 'plants grew better in less pure water sources than plants in distilled water'. German botanists, Julius von Sachs and Wilhelm Knop (1859–1865), developed the techniques of soilless cultivation. It was Professor William Frederick Gericke (1937) who finally introduced the term hydroponics and wrote the book named *Complete Guide to Soilless Gardening*. Two other plant nutritionists, Dennis R. Hoagland and Daniel I. Arnon, at the University of California, wrote a classic in 1938 in agricultural bulletin 'The Water Culture Method for Growing Plants without Soil'. They developed several formulations for mineral nutrient solutions, known as Hoagland solutions and the modified Hoagland solutions that are still in use today. This technique was used to establish the criteria of essentiality for different nutrient elements.

There are primarily two types of hydroponics, viz., (1) solution culture and (2) medium culture. In solution culture, there is no solid medium for the roots to support, just the nutrient solution, while the medium culture has a solid medium for the roots and is named after the type of medium being used. Again, solution culture is divided into three main methods of growing plants:

(a) Static solution culture – plants are grown in containers such as plastic buckets, tubs or tanks with nutrient solution. The solution may or may not be aerated, but usually gentle aeration is required.
(b) Continuous flow solution culture – the nutrient solution constantly flowing past the roots, e.g. nutrient film technique.
(c) Aeroponics – no substrate is required, and the roots are suspended in the air in a closed chamber and are sprayed intermittently with fine mist or aerosol of nutrient solution. Aeroponics is widely used in laboratory studies of plant physiology. National Aeronautics and Space Administration (NASA) has done extensive hydroponic research for their 'Controlled Ecological Life Support System' (CELSS). Special attention has been given to this technique since a mist is easier to handle in a zero-gravity environment as compared to a liquid.

In medium culture, there are variations for each media, i.e. passive subirrigation, flood and drain subirrigation, top irrigation and deep-water culture. The media used for growing plants may be expanded clay, rock wool, coir, perlite, vermiculite, sand, gravel/quartz and brick shards.

Plant nutrients used in hydroponics are dissolved in water and are mostly in inorganic and ionic forms. All the 17 elements that are essential

for plant growth are supplied using different chemical combinations. Chelating agents such as EDTA are used to keep Fe in soluble form. The pH of nutrient solution ranges from 5.6 to 6.0 depending on the crop. Once the plants are grown in nutrient solution, the composition of solution is altered due to depletion of specific nutrients more rapidly than others, absorption of water from the solution and alteration of pH by excretion of either acidity or alkalinity. Utmost care should be taken while deciding the optimum salt concentration to be used in solution, so as to avoid toxicity, early nutrient depletion or pH changes which may together attribute to inadvertent effects on plant growth and development.

In India, hydroponics was not a popular practice until 1946. The Government of Bengal initiated Experimental Farm at Kalimpong, Darjeeling. After a careful appraisal of salient problems, the Bengal System of Hydroponics was developed in 1946–1947 representing the efforts to meet Indian requirements. Recently, in 2008, a few farmers in southern districts of Gujarat adopted this technology for cultivating varieties of exotic hybrid tea roses. Further, the hydroponic system was installed on 12 ha of land in Kuch village of Navsari district. Exotic crops such as strawberry, green garlic and tomatoes are also cultivated using this technology. The second biggest hydroponics project in Gujarat is 'Landmark Agrotech'. In India, wastelands with poor-quality soil and sufficient water can be brought under hydroponics for sustainable land use and increase crop productivity.

References

Aravind L, Koonin EV (2000) The STAS domain: a link between anion transporters and antisigma-factor antagonists. Curr Biol 10:53–55

Armengaud P, Sulpice R, Miller AJ, Stitt M, Amtmann A, Gibon Y (2009) Multilevel analysis of primary metabolism provides new insights into the role of potassium nutrition for glycolysis and nitrogen assimilation in Arabidopsis roots. Plant Physiol 150:772–785

Arnon DI, Stout PR (1939) The essentiality of certain elements in minute quantity for plant with special reference to copper. Plant Physiol 14:371–375

Barber J (2003) Photosystem II: the engine of life. Q Rev Biophys 36:71–89

Baxter I, Muthukumar B, Park HC, Buchner P, Lahner B et al (2008) Variation in molybdenum content across broadly distributed populations of *Arabidopsis thaliana* is controlled by a mitochondrial molybdenum transporter (*mot1*). PLoS Genet 4(2):e1000004

Briat JF, Lobreaux S (1998) Iron storage and ferritin in plants. Met Ions Biol Syst 35:563–584

Briggs GE, Robertson RN (1957) Apparent free space. Annu Rev Plant Physiol Plant Mol Biol 8:11–30

Buchner P, Takahashi H, Hawkesford MJ (2004) Plant sulphate transporters: co-ordination of uptake, intracellular and long-distance transport. J Exp Bot 55(404):1765–1773

Curie C, Cassin G, Couch D, Divol F, Higuchi K et al (2009) Metal movement within the plant: contribution of nicotianamine and yellow stripe 1-like transporters. Ann Bot 103:1–11

De Angeli A, Monachello D, Ephritikhine G, Frachisse J-M, Thomine S et al (2009) CLC-mediated anion transport in plant cells. Philos Trans R Soc B 364:195–201

Demidchik V, Maathuis FJM (2007) Physiological roles of non-selective cation channels in plants: from salt stress to signalling and development. New Phytol 175:387–404

Diédhiou CJ, Golldack D (2005) Salt-dependent regulation of chloride channel transcripts in rice. Plant Sci 170:793–800

Dixon NE, Gazzola C, Blakely RL, Zerner B (1975) Jackbean urease. A metalloenzyme. A simple biological role for nickel? J Am Chem Soc 97:4131

Eskew DL, Welch RM, Cary EE (1983) Nickel: an essential micronutrient for legumes and possibly all higher plants. Science 222:621–623

Eskew DL, Welch RM, Norvell WA (1984) Nickel in higher plants: further evidence for an essential role. Plant Physiol 76:691–693

Follmer C (2008) Insights into the role and structure of plant ureases. Phytochemistry 69:18–28

Foy CD, Scott BJ, Fisher JA (1988) Genetic differences in plant tolerance to manganese toxicity. In: Graham RD, Hannam RJ, Uren NC (eds) Manganese in soils and plants. Kluwer Academic Publishers, Dordrecht, pp 293–307

Freyermuth SK, Bacanamwo M, Polacco JC (2000) The soybean Eu3 gene encodes a Ni-binding protein necessary for urease activity. Plant J 21:53–60

Giertha M, Maser P (2007) Potassium transporters in plants – involvement in K^+ acquisition, redistribution and homeostasis. FEBS Lett 581:2348–2356

Goedert WJ, Lobato E, Lourenco S (1997) Nutrient use efficiency in Brazilian acid soils: nutrient management and plant efficiency. In: Moniz AC et al (eds) Plant-soil interactions at low pH. Brazilian Soil Science Society, Campinas, pp 97–104

Grubb C, Abel S (2006) Glucosinolate metabolism and its control. Trends Plant Sci 11:89–100

Guerinot ML (2000) The ZIP family of metal transporters. Biochim Biophys Acta Biomembr 1465:190–198
Gupta U (1997) Introduction to molybdenum in agriculture. Molybdenum in agriculture. Cambridge University Press, Cambridge, UK
Hawkesford MJ (2003) Transporter gene families in plants: the sulphate transporter gene family: redundancy or specialization? Physiol Plant 117:115–163
Hechenberger M, Schwappach B, Fischer WN, Frommer WB, Jentsch TJ, Steinmeyer K (1996) A family of putative chloride channels from *Arabidopsis* and functional complementation of a yeast strain with a CLC gene disruption. J Biol Chem 271:632–633
Heuwinkel H, Kirkby E, Le Bot J, Marschner H (1992) Phosphorus deficiency enhances molybdenum uptake by tomato plants. J Plant Nutr 15:549–568
Hope AB, Stevens PG (1952) Electric potential differences in bean roots and their relation to salt uptake. Aus J Sci Res 5:335–343
Howitt SM, Udvardi MK (2000) Structure, function and regulation of ammonium transporters in plants. Biochim Biophys Acta 1465:152–170
Ivanov R, Brumbarova T, Bauer P (2012) Fitting into the harsh reality: regulation of iron-deficiency responses in dicotyledonous plants. Mol Plant 5:27–42
Jeong J, Cohu C, Kerkeb L, Pilon M, Connolly EL, Guerinot ML (2008) Chloroplast Fe(III) chelate reductase activity is essential for seedling viability under iron limiting conditions. Proc Natl Acad Sci U S A 105:10619–10624
Kramer U, Clemens S (2005) Function and homeostasis of zinc, copper, and nickel in plants. Top Curr Genet 14:215–271
Kuper J, Llamas A, Hecht HJ, Mendel RR, Schwarz G (2004) Structure of the molybdopterin-bound Cnx1G domain links molybdenum and copper metabolism. Nature 430:803–806
Liu KH, Huang CY, Tsay YF (1999) *CHL1* is a dual-affinity nitrate transporter of *Arabidopsis* involved in multiple phases of nitrate uptake. Plant Cell 11:865–874
Lopez-Arredondo DL, Leyva-Gonzalez MA, Gonzalez-Morales SI, Lopez-Bucio J, Herrera-Estrella L (2014) Phosphate nutrition: improving low-phosphate tolerance in crops. Annu Rev Plant Biol 65:95–123
Lurin C, Geelen D, Barbier-Brygoo H, Guern J, Maurel C (1996) Cloning and functional expression of a plant voltage-dependent chloride channel. Plant Cell 8:701–711
Malik CP, Srivastava AK (1982) Text book of plant physiology. Kalyani Publishers, New Delhi
Marmagne A et al (2007) Two members of the Arabidopsis CLC (chloride channel) family, AtCLCe and AtCLCf, are associated with thylakoid and Golgi membranes, respectively. J Exp Bot 58:3385–3393
Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London
Marschner H, Marschner P (2012) Marschner's mineral nutrition of higher plants, 3rd edn. Elsevier/Academic Press, London
Mendel RR, Hansch R (2002) Molybdoenzymes and molybdenum cofactors in plants. J Exp Bot 53:1689–1698
Miwa K, Fujiwara T (2010) Boron transport in plants: coordinated regulation of transporters. Ann Bot 105:1103–1108
Morère-Le Paven M-C, Viau L, Hamon A, Vandecasteele C, Pellizzaro A, Bourdin C et al (2011) Characterization of a dual-affinity nitrate transporter MtNRT1.3 in the model legume Medicago truncatula. J Exp Bot 62:5595–5605
Mudge SR, Rae AL, Diatloff E, Smith FW (2002) Expression analysis suggests novel roles for members of the Pht1 family of phosphate transporters in Arabidopsis. Plant J 31:341–353
Mukherjee I, Campbell NH, Ash JS, Connolly EL (2006) Expression profiling of the Arabidopsis ferric chelate reductase (FRO) gene family reveals differential regulation by iron and copper. Planta 223: 1178–1190
Noguchi K, Yasumori M, Imai T et al (1997) bor1-1, an *Arabidopsis thaliana* mutant that requires a high level of boron. Plant Physiol 115:901–906
Palmgren M, Harper J (1999) Pumping with plant P-type ATPases. J Exp Bot 50:883–893
Parker JL, Newstead S (2014) Molecular basis of nitrate uptake by the plant nitrate transporter NRT1.1. Nature 507:68–72
Pilon-Smits EAH, Quinn CF, Tapken W, Malagoli M, Schiavon M (2009) Physiological functions of beneficial elements. Curr Opin Plant Biol 12:267–274
Pittman JK (2005) Managing the manganese: molecular mechanisms of manganese transport and homeostasis. New Phytol 167:733–742
Rausch C, Bucher M (2002) Molecular mechanisms of phosphate transport in plants. Planta 216:23–37
Rodriguez FI, Esch JJ, Hall AE, Binder BM, Schaller GE, Bleecker AB (1999) A copper cofactor for the ethylene receptor ETR1 from Arabidopsis. Science 283:996–998
Römheld V, Marschner H (1986) Evidence for a specific uptake system for iron phytosiderophores in roots of grasses. Plant Physiol 80:1175–1180
Salisbury FB, Ross CW (1985) Plant physiology, 3rd edn. Wadsworth Publishing Company, University of Minnesota, USA
Salt DE, Baxter I, Lahner B (2008) Ionomics and the study of the plant ionome. Annu Rev Plant Biol 59:709–733
Sirijovski N, Lundqvist J, Rosenback M, Elmlund H, AlKaradaghi S et al (2008) Substrate-binding model of the chlorophyll biosynthetic magnesium chelatase BchH subunit. J Biol Chem 283:11652–11660
Takano J, Noguchi K, Yasumori M et al (2002) Arabidopsis boron transporter for xylem loading. Nature 420:337–340
Takano J, Wada M, Ludewig U, Schaaf G, von Wiren N, Fujiwara T (2006) The Arabidopsis major intrinsic protein NIP5;1 is essential for efficient boron uptake

and plant development under boron limitation. Plant Cell 18:1498–1509

Tejada-Jimenez M, Llamas A, Sanz-Laque E, Galvan A, Fernandez E (2007) A high-affinity molybdate transporter in eukaryotes. Proc Natl Acad Sci USA 104:20126–20130

Tomatsu H, Takano J, Takahashi H, Wantanabe-Takahashi A, Shibagaki N, Fijiwara T (2007) An Arabidopsis thaliana high-affinity molybdate transporter required for efficient uptake of molybdate from soil. Proc Natl Acad Sci U S A 104:18807–18812

Tweedi JW, Segel (1970) Specificity of transport process for sulfur, selenium and molybdenum anions by filamentous fungi. Biochim Biophys Acta 196:95–106

Wang RC, Crawford NM (1996) Genetic identification of a gene involved in constitutive, high-affinity nitrate transport in higher plants. Proc Natl Acad Sci U S A 93:9297–9301

Wang Y, Wu W-H (2013) Potassium transport and signaling in higher plants. Annu Rev Plant Biol 64:451–476

Wang R, Liu D, Crawford NM (1998) The Arabidopsis CHL1 protein plays a major role in high-affinity nitrate uptake. Proc Natl Acad Sci U S A 95:15134–15139

Williams LE, Miller AJ (2001) Transporters responsible for the uptake and partitioning of nitrogenous solutes. Annu Rev Plant Physiol Plant Mol Biol 52:659–668

Woodward J (1699) Some thoughts and experiments concerning vegetation. Philos Trans R Soc B 21:93–227

Wu H, Li L, Du J, Yuan Y, Cheng X, Ling HQ (2005) Molecular and biochemical characterization of the Fe(III) chelate reductase gene family in *Arabidopsis thaliana*. Plant Cell Physiol 46:1505–1514

Yazaki K, Tanaka S, Matsuoka H, Sato F (1998) Stable transformation of Lithospermum erythrorhizon with Agrobacterium rhizogenes and shikonin production of the transformants. Plant Cell Rep 18: 214–219

General Overview of Plant Secondary Metabolism

21

Francesc Viladomat and Jaume Bastida

Abstract

The aim of this chapter is to ask and answer basic questions about plant secondary metabolism. It attempts to justify its chemical diversity and looks at where and how secondary metabolites are synthesized and stored, at the level of the cell and the whole plant, taking into account the variations in space and time. The question of why plants synthesize secondary metabolites is approached from three points of view: specific physiological functions and intra- and interspecific interactions. The interspecific relations can be plant-plant (allelopathy), plant-microorganisms/fungi and plant-animal. In the overview of the biosynthetic processes, plant secondary metabolites are classified into three basic groups: glucide (glucides, glycosides and the shikimate-phenylpropanoid pathway), lipid (acetate malonate pathway and terpenes) and nitrogenated metabolites (non-proteinogenic amino acids, secondary peptides, cyanogenic compounds, glucosinolates and alkaloids).

Keywords

Plant secondary metabolism • Biodiversity • Biosynthesis • Storage • Functions

21.1 What Is Secondary Metabolism?

Living organisms synthesize a number of common compounds (nucleotides, amino acids, fatty acids, etc.) with similar metabolism and functions (basic or primary metabolism). They also present a wide array of metabolic pathways not essential for life, which often differ from one species to another and can be considered as the

F. Viladomat • J. Bastida (✉)
Department of Natural Products, Plant Biology and Soil Science, The School of Pharmacy, University of Barcelona,
Av. Diagonal 643, 08028 Barcelona, Spain
e-mail: jaumebastida@ub.edu

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_21,

expression of the chemical individuality of each organism. These reactions are grouped under the term of secondary metabolism, and their products are secondary compounds.

Here follow some of the most significant features of secondary metabolites:

(a) They are present in microorganisms, plants and animals, although in this chapter we will focus primarily on plant secondary metabolites.
(b) Their taxonomic distribution is restricted (each secondary metabolite is limited to certain organisms).
(c) Their structural diversity is vast, taking into account the huge range of plant types (biodiversity) and the ability of each plant to synthesize unique metabolites and that secondary metabolism is a distinguishing factor among species.
(d) Individual plants can be taxonomically defined by their differing chemical composition (chemotaxonomy), as each species has a unique range (qualitative and/or quantitative) of secondary metabolites (chemical individuality).
(e) Secondary metabolites exhibit variability in space and time and are not necessarily synthesized by all the plant cells or consistently through time. The expression of secondary metabolism is a differential aspect of cell specialization, integrated in programs of differentiation and development.
(f) They are not essential for the growth and development of the plant (*not necessary for life*). They may vary qualitatively and quantitatively, or even disappear entirely, without disastrous consequences for the organism that synthesizes them.
(g) However, they are essential for the survival of the producing organisms (*not necessary for life*, *but crucial for survival*). Thus, secondary metabolites, while not necessary for growth and development, play a key role in plant interactions with the environment. They therefore have a *high degree of chemical freedom*, which allows them to adapt to the environment and evolve over time.

21.2 How Can the Chemical Diversity of Secondary Metabolism Be Explained?

Secondary metabolites originate from precursors of primary metabolism, and the genetic material encoding secondary metabolism has evolved from the genetic material of primary metabolism.

The high degree of chemical freedom of secondary metabolism is the basic mechanism that favours the variations caused by mutations in the genes responsible for a particular pathway. However, metabolite viability depends on an ability to resist the selective pressure of a competitive and continuously changing environment. This degree of freedom implies a diversification that follows the patterns of phylogenetic relationships. Thus, organisms with a close phylogenetic relationship often produce similar compounds.

The most significant features of reactions responsible for secondary metabolite diversity are as follows:

(a) A huge range of reactions. The modification of a single enzyme of primary metabolism can lead to a great variety of secondary metabolism enzymes. Enzymes such as dehydrogenases, decarboxylases, glycosyltransferases and hydroxylases have probably evolved from enzymes of primary metabolism, only altering the substrate specificity. Other more specific enzymes, such as phenol oxidases, prenyltransferase, etc., have also evolved from enzymes of primary metabolism but probably by altering the reaction specificity or, at the same time, the reaction substrate specificity (Bernhardt 2006; Usera and O'Connor 2009; Tonfack et al. 2011).
(b) Basic pathways – key intermediates – metabolic networks. Basic secondary metabolic pathways normally lead to key intermediates (more than one in a major pathway). Key intermediates are versatile products and, depending on the enzymes acting on them, can give rise to several pathways, allowing a great diversification. Multidimensional

metabolic networks are quite common in secondary metabolism, so that different options are available between one point in the pathway and another (usually the same reactions are used but in a different order), which favours an increase in the number and diversity of intermediates.

(c) Polymerization reactions. Many secondary compounds, such as the polyketides or isoprenoids, are formed by metabolic pathways originating in polymerization reactions of simple compounds. Isoprenoids, for example, are formed from a single precursor, isopentenyl pyrophosphate, and, according to the degree of polymerization and type of subsequent cyclization, thousands of different compounds may be generated.

(d) Combination of different precursors. Many secondary products are the result of the convergence of two or more metabolic pathways (e.g. reserpine, ergotamine, tubocurarine, etc.). Moreover, many secondary metabolites are glycosylated (cyanogenic glycosides, saponins, cardiotonic glycosides, etc.) or prenylated (different kinds of aromatic compounds, such as quinones and coumarins). This allows multiple combinations that lead to greater diversification.

(e) Analogy. In secondary metabolism different paths characteristically lead to similar structures. The piperidine alkaloids, for example, can be formed from L-lysine or by way of polyketides.

21.3 Where Are Secondary Metabolites Synthesized and Stored?

21.3.1 At the Cellular Level

Like primary metabolism, secondary metabolism constitutes an integration of metabolic pathways and cellular substructures. Secondary metabolism does not involve the formation of new compartments, demonstrating the flexibility of cell spatial organization (Kutchan 2005):

(a) The compartmentalization of precursors and intermediates. The fact that secondary metabolism derives from precursors of primary metabolism implies competition for common substrates. Such competition is regulated not only by a selective control of enzyme quantity and activity but also by pools of intracellular precursors associated with vectorial transfer of substrates across membranes. In the case of intermediates, it should be noted that several metabolic pathways require more than one compartment and that different metabolic pathways can occur simultaneously within the same compartment. In fact, the highly complex compartmentalization of secondary metabolic pathways can require different types of cells (Kutchan 2005; Mahroug et al. 2007).

(b) Metabolic channelling (microcompartments). In a microenvironment associated with membrane surfaces, metabolic channelling helps determine the directionality of pathways within a compartment (Jorgensen et al. 2005; Winkel 2004). An ordered sequence of catalytic sites with intermediates bonded covalently to the enzyme avoids diffusion, enhances catalytic efficiency and protects unstable intermediates. Included in this concept are multifunctional enzymes and multienzyme complexes. An example of a multifunctional enzyme is 6-methylsalicylate synthase, which is located in the polyketide pathway.

21.3.1.1 How and Where Are Secondary Metabolites Stored at the Cellular Level?

To accumulate a metabolite, a plant must not only be able to synthesize it but also maintain a sufficiently low rate of degradation (or transformation) of the product. Only those metabolites that accumulate in a particular proportion can be stored.

21.3.1.2 Why Do Certain Metabolites Accumulate?

Their functionality depends on their being available in significant quantities. However, this

accumulation may be associated with toxicity, obliging the plant to store the metabolites in an appropriate form and location that also allows a controlled usage.

21.3.1.3 Where Do the Metabolites Accumulate?

Secondary metabolites are preferentially stored in separate compartments of the cytosol via membranes, particularly in vacuoles (Iglesias and Meins 2000; Wink 2010). Lipid metabolites can be stored as membrane constituents or as lipid droplets, in both the stroma of the chloroplast and cytoplasm. Many metabolites are discharged into extracellular space, where they can be stored.

21.3.1.4 What Are the Most Important Transport Mechanisms?

Secondary metabolites are transported from the cytosol to the place of storage by various membrane transport mechanisms involving transport proteins with high substrate specificity. The most common mechanisms are:

(a) Proton antiports (secondary active transport). The action of the proton ATPase and pyrophosphatase acidifies the interior of the compartments (vacuoles and others) and the extracellular space, thus creating an electrochemical gradient that acts as a driving force. Notably, this couplet transport is stereoselective.

(b) Uniports. This passive transport mechanism uses a carrier protein with high substrate specificity. It has been postulated that through this mechanism, the metabolites do not accumulate in another compartment until they have undergone transformation reactions.

(c) GS-X pumps (glutathione-conjugated pumps). These specific ATPases use energy directly from the ATP (primary active transport mechanism) by actively transporting glutathione conjugates (GS-X) across the vacuolar membrane (Dixon et al. 2010). The vacuolar transport of the GS-X is linked to the role of the tripeptide glutathione (GSH, γ-Glu-Cys-Gly) and the glutathione-S-transferases (GSTs). They are similar to pumps in the liver of mammals, "multidrug resistance-associated proteins" (MDR), a subfamily of the "ATP-binding cassette (ABC) transporters".

(d) Golgi vesicles. Some secondary metabolites are introduced into the endoplasmic reticulum or in Golgi dictyosomes, where they are transformed. The products are then distributed to the extracellular space or to the vacuoles by fusion of the Golgi vesicles to the corresponding membranes (plasmalemma or tonoplast, respectively).

21.3.1.5 Once Secondary Metabolites Have Crossed the Membranes, How Are They Stored?

There are a number of reactions that facilitate their storage in significant amounts and/or in a stable and non-toxic form:

(a) Transformation reactions. A molecule within the vacuole (or other compartment) is transformed into another, acquiring different characteristics that facilitate its accumulation. This mechanism allows metabolites to be stored even with passive transport systems (uniport), assuming that the transformed molecule is removed from the equilibrium.

(b) Retention of ions (salt formation). This may be significant in the case of nitrogen-bearing compounds such as alkaloids, which capture protons in the acid medium of the vacuoles and cannot cross membranes as cations. Some products can also be ionized in the vacuoles by intravacuolar reactions, such as the conversion of ajmalicine into serpentine. Many alkaloids can accumulate in large amounts within the vacuoles because the high concentration of cations is offset by a significant accumulation of anions (salt formation). For example, in the vacuoles of *Papaver somniferum* latex, meconic acid concentration can reach 250 mM. This mechanism is important for the accumulation and retention of morphine, codeine, papaverine and other alkaloids.

(c) Conjugation. Many secondary metabolites are stored as conjugates (covalent bonding)

with different types of molecules. The main types of conjugation involve the formation of esters and glycosides. For example, tropane and pyrrolizidine alkaloids are found as esters of organic acids. However, glycosidation is the most widespread type of conjugation (saponins, cardenolides, anthocyanins, flavonoids, cyanogenic and steroidal alkaloids, among others, are found as glycosides) (Gachon et al. 2005; Jones and Vogt 2001). After glycosidation, toxic and reactive products become stable and unreactive and more soluble in water. The solubility, detoxification and stabilization of labile and/or reactive molecules are crucial for vacuolar storage. These three properties can be observed in cyanogenic glycosides, which act as prototoxins.

A good example of a molecule that undergoes simultaneous transformation and conjugation as it crosses the endoplasmic reticulum membrane (using a multienzyme complex linked to the membrane) is quercetin.

21.3.2 Where Are Secondary Metabolites Synthesized and Stored in Plants?

Secondary metabolites are synthesized in particular concentrations (quantity) to be stored in the right place (space) at the right moment (time) so they can fulfil their functions. Thus, the expression of secondary metabolism is regulated in space (in a specific organ or tissue) and time (temporally restricted), integrated into the programs of differentiation and development of plants (limited in most cases to specialized cells and specific stages of development), which are also conditioned by environmental factors (environment).

21.3.2.1 Variations in Space

Most plants concentrate their secondary products in certain organs or parts of the plant, for example, seeds (*Strychnos*), roots (*Aconitum*), leaves (*Atropa*), fruits (*Capsicum*) and bark (*Cinchona*). We can therefore say that each plant has a particular distribution of its secondary products. However, the places of maximum metabolite accumulation are not necessarily those where synthesis takes place. In this case, the expression of secondary metabolism is related to translocation mechanisms, involving short-distance or long-distance transport (Kutchan 2005).

21.3.2.1.1 Synthesis and Accumulation in the Same Organ

Although synthesis and accumulation can take place in the same organ or tissue, the process can still be complex. For instance, are all the cells of a particular organ able to synthesize the product? Not necessarily. Do the synthesizing cells also store the product? Not always. The quinolizidine alkaloids (QA) of the genus *Lupinus* are an illustrative example. Following a diurnal rhythm, these lysine-derived alkaloids are biosynthesized in the mesophyll chloroplasts of leaves. Once synthesized, the alkaloids are exported to the vacuole of the epidermal cells of the upper surface of the leaves, where the QA concentration can reach 200 mM. So in this case, the alkaloids are synthesized in one cell type and stored in another while remaining in the same leaf.

21.3.2.1.2 Accumulation in Specialized Cells

So far we have considered product accumulation within the vacuoles of normal cells (e.g. epidermal in the case of *Lupinus*). However, there are species where, depending on the metabolite, storage occurs in the vacuoles of specialized cells, differentiated for this purpose. The most typical example is the laticifers, found in Apocynaceae, Asclepiadaceae, Euphorbiaceae, Moraceae and Papaveraceae (Hagel et al. 2008; Pickard 2008). The laticifers, depending on the degree of differentiation, can be divided into idioblasts (*Sanguinaria*, *Corydalis*, *Ruta*), which are isolated cells, non-articulated laticifers (*Catharanthus*), whose cells are fused but without forming defined ducts, and articulated laticifers (*Papaver*, *Chelidonium*), whose cells fuse to form ducts. A lack of cell differentiation means that callus cultures of these species cannot store products. Specialized storage cells also have particular physical and chemical characteristics, e.g. in *Catharanthus roseus* they

may have a vacuolar pH two units more acidic than normal cells.

21.3.2.1.3 Accumulation Outside the Cells

Secondary metabolites do not always accumulate within cells. When excreted, where do they end up?

(a) Accumulation in defined extracellular compartments. Many products, mainly lipophilic, can accumulate in glandular trichomes (e.g. peppermint monoterpenes) in resin ducts (e.g. pine oleoresin) or in special secretory cavities (e.g. essences in orange peel) (Lange and Turner 2013; Glas et al. 2012; Tissier 2012). These compartments are formed by cavities lined with specialized epithelial cells (secreting cells) that synthesize the compounds and excrete them into the extracellular space, where they accumulate. In glandular trichomes, the cavity is delimited by the cuticle and the secreting cells are in the base of the cavity (Pickard 2008; Zulak and Bohlmann 2010).

(b) Excretion from cells without compartmentation. Three models have been observed: vaporization, solubilization in the cuticle of the epidermis and exudation through the roots. Vaporization occurs outwards in the aerial part of the plant with compounds that evaporate at room temperature (e.g. certain monoterpenes). Solubilization of lipid substances (a mixture of waxes and cutins) in the cuticle of epidermal cells is common in many plant species (e.g. oleanane-type triterpenes in *Catharanthus roseus* leaves and phlorizin in apples) (Koch and Barthlott 2006). Root exudation plays a very important role in plant defence against fungal infections in a wet environment (e.g. α-tomatine in tomato roots).

So far we have considered a number of cases that involve short-distance transport (within the same organ). In fact, there is growing evidence that these products concentrate on or near the surface of the plant, as part of a defence strategy or with a signalling function.

21.3.2.1.4 Synthesis in One Organ and Accumulation in Another

When biosynthesis and accumulation take place in different locations, one can assume that long-distance transport is involved, which can be accomplished through the phloem or xylem. Pyrrolizidine alkaloids synthesized in the roots of *Senecio* plants are translocated through the phloem to the inflorescences (Ober and Kaltenegger 2009; Trigo 2011). Solanaceae alkaloids (e.g. nicotine in *Nicotiana*) are synthesized in roots and accumulate in leaves, with translocation taking place in xylem (Cai et al. 2013).

21.3.2.2 Variations in Time

Each plant has a specific distribution of its secondary products, but this may vary over time:

(a) Ontogenetic fluctuations are said to occur, in both perennial and annual plants, when the changes follow an ontogenetic-type pattern. Among annual plants, the alkaloid content of *Atropa belladonna* leaves reaches a peak when leaves have fully emerged, the level decreasing thereafter to negligible values prior to senescence. Another example is the tomato, where the concentration of glycoalkaloids decreases as the fruit matures (similar processes occur in most fruits). Among the perennials, tannin levels in oak leaves (*Quercus robur* L.) increase in spring to protect the tree from defoliation by caterpillars of the oak leaf roller moth (*Tortrix viridana*), which stop feeding in mid-June. The oaks are thus able to increase the level of tannins when required (Shukla and Singh 2001).

(b) Daily fluctuations. In many plants, besides ontogenetic variations, metabolite production undergoes fluctuations during the day. The best-known example is *Papaver somniferum*, which shows higher levels of alkaloids around midday; before the increase in morphine production during the

morning, codeine and thebaine levels decrease (morphine always being predominant).

(c) Spontaneous variations. Changes may also occur in response to circumstantial pressure from the environment. Thus, levels of QA (lupanine) in the epidermal cells of *Lupinus* leaves rise significantly in defence against predators shortly after a wound is inflicted. The goal is to increase these compounds when the plant is most vulnerable in the parts most at risk. Spontaneous variations are associated with the concept of induced secondary metabolite production, as opposed to their constitutive formation.

21.4 Why Do Plants Synthesize Secondary Metabolites?

A significant portion of the carbon assimilated by plants is used in the formation of secondary metabolites (e.g. in *Nicotiana tabacum* L. 10 % of the assimilated carbon goes to the formation of alkaloids). If these products were synthesized without any purpose, this would imply an unsustainable waste of resources and energy, which in a competitive environment would condemn the plant to extinction. All secondary metabolites must therefore have a function, although in many cases it remains unknown. We nevertheless now have enough basic knowledge to outline some general patterns of activity (Neilson et al. 2013).

Before defining the main functions of secondary products, two important features should be emphasized: (1) different products may have the same function in different organisms, and (2) some products may have more than one function in the same organism. These two features alone denote a complexity and functional diversity.

The basic functions of secondary products can be grouped into three sections: specific physiological functions and intraspecific and interspecific relations.

21.4.1 Specific Physiological Functions

Relationships within an organism and with its physical environment (abiotic) are defined by specific physiological functions. In marked contrast with the uniformity of primary metabolism, secondary metabolites fulfil their physiological functions only in restricted groups of organisms and are replaced by different compounds in other organisms. These functions are too numerous to list, and only a few examples will be discussed here, including membrane stability (carotenoids), protection against radiation (flavonoids, chlorogenic acid), regulation of hormone metabolism (flavonoids, auxins), antioxidant activity (polyphenols), etc. (Buer et al. 2010; Dinkova-Kostova 2008; Jordan 2004; Peer and Murphy 2007).

21.4.2 Intraspecific Relationships

This section focuses on pheromones, which can be defined as chemical signals released into the environment by an organism that affect the physiology or behaviour of other individuals of the same species. They are characterized by their complexity (ranging from single compounds to mixtures of several), diversity (changes in composition and proportion can result in highly specific messages) and effectiveness (small amounts can be effective over long distances). Pheromones have been extensively studied in animals, where they may have a variety of signalling functions (associated with sex, danger, territory, aggregation, etc.). In plants, where the range of functions is more restricted, pheromones are particularly associated with systemic acquired resistance (SAR).

It is worth noting that in animals many pheromone constituents are derived from the plants they eat, either directly or after a variable degree of modification (Mueller and Buchbauer 2011). This indicates a direct relationship between animals and the specific plants that provide them with these compounds; for example, pyrrolizidine alkaloids are obtained by *Danaus* butterflies from *Senecio*

plants (Seybold et al. 2006; Trigo 2011; Blomquist et al. 2010). Additionally, evidence suggests that knowledge of pheromone composition in insects can be applied to control their populations and behaviour in an economically sustainable way.

21.4.3 Interspecific Interactions

The chemical relationships between organisms of different species are defined by interspecific interactions. Plant and animal organisms live in a world of chemical signals that control and delicately balance mutual relationships. How has this situation come about? It can be explained by the theory of coevolution, in which dynamic interactions have led to a proliferation of species and a complex network of relationships between organisms (Ferreira et al. 2006). Among the consequences of this process is the great diversity of plants, predators and secondary metabolites; the presence of highly specialized predators able to consume only one or a few plant species while others are generalist feeders; substances that are toxic for a given species but harmless for another; and the fact that no plant, regardless of its toxicity, is without a predator.

To facilitate the study of interspecific signals, the following interactions are described separately.

21.4.3.1 Plant-Plant Interactions (Allelopathy)

The term allelopathy, introduced by Molish in 1937, defines the chemical interactions among plants. Chemicals released into the environment by a plant can directly influence the growth and development of other plants (Weston and Mathesius 2013; Razavi 2011; Einhellig 2002, 2004). Plants can release the products to the environment in four ways:

(a) Leaching. Non-volatile products accumulate on the surface of plant leaves, where they are leached away by rainwater or dew and deposited in the soil. For example, the California walnut (*Juglans nigra*) inhibits the growth of many species growing in its proximity by the action of juglone. *Poa pratensis*, however, has developed a detoxification mechanism and remains unaffected by this product. It should be noted that there is always one species able to overcome inhibiting agents.
(b) Volatilization from leaves. Volatile allelochemicals released by the plant into the atmosphere interfere with the metabolic processes of neighbouring plants. For example, cineole, camphor and other monoterpenes are synthesized by *Salvia leucophylla* and *Artemisia californica* in the California chaparral.
(c) Exudation from roots. Products released directly to the soil through roots act on the roots of neighbouring plants, conditioning their growth and development. Thus, the continuous production of cinnamic acids by *Parthenium argentatum* (Compositae), a typical plant of desert areas, inhibits the germination and growth of other plants in its vicinity (Kato-Noguchi and Peters 2013; Bertin et al. 2003).
(d) Decomposition products are released when leaves, fruits, twigs or roots decompose in soil. For example, the soil absorbs acids from the decomposition of oleoresin in pine needles.

The potential application of allelopathy in agriculture has been the subject of considerable research directed to the use of secondary metabolites as regulators of growth and/or natural herbicides to promote sustainable agriculture (Chon and Nelson 2010; Macias et al. 2007; Weston and Duke 2003).

21.4.3.2 Interactions Between Plants and Microorganisms/Fungi

These interactions may occur at three levels: plant defence, response to infectious agents and plant products that influence the gene expression of microorganisms/fungi (Ferreira et al. 2006; Dixon 2001).

21.4.3.2.1 Plant Defence

Plants produce defence products that act preventively (avoiding attack or infection) or curatively (after the attack). Preventive barriers can be mechanical or chemical. Mechanical barriers are formed by polysaccharides, waxes, cutins, lignins

and other constituents that can delay or impede the invasion and, in many cases, give the plant time to react. Invasive microorganisms need to break this barrier through specialized exoenzymes (Lazniewska et al. 2012).

Chemical barriers consist of products excreted from the cells (see previous sections). These products, which inhibit the germination of spores and the growth of microorganisms, may be released to the atmosphere (vaporization), deposited on the surface of the plant (many are solubilized in the cuticle of the epidermis) or exuded by the roots (Ferreira et al. 2006). The release of essential oils such as thymol is an example of vaporization (very high concentrations are found near the surface of the plant). Many products deposited on the surface of the plant, such as triterpenoids, tannins and alkaloids, inhibit spore germination. Exudation by roots is very important in plant defence against fungal infections, for example, α-tomatine in *Lycopersicon esculentum* and solanine in *Solanum tuberosum*.

When infectious agents overcome preventive barriers, the plant needs other resources to prevent the disease from progressing. In this context, plants dispose of two types of defence, prototoxins and phytoalexins, which differ in the mechanisms of formation, accumulation and action.

Prototoxins are stored in an inactive, non-toxic form in a particular compartment (see previous section on conjugation) and are activated when the aggression of an invader brings them in contact with specific enzymes found in another compartment (constitutive defence). Examples include cyanogenic glycosides (e.g. linamarin, dhurrin), glucosinolates in Brassicaceae or saponins in *Hedera helix* (Soenderby et al. 2010; Mueller 2009; Vetter 2000).

Phytoalexins are toxic for the pathogen and are synthesized in the plant de novo in response to a stimulus or signal (elicitor) (induced defence). The structurally varied phytoalexins include sesquiterpenes (Solanaceae), isoflavones (Fabaceae) and stilbenes (grapes), as well as many types of polyphenols and alkaloids (Jeandet et al. 2013; Ahuja et al. 2012; Pedras et al. 2011).

Elicitors are substances secreted by the pathogen or formed in the plant cell wall when the plant is injured or exposed to an infection, parasite or predator. Specific receptors in the plasma membrane of the plant are capable of recognizing elicitors (signals). After the process of signal transduction, activated genes trigger a set of responses, including the biosynthesis of phytoalexins, as well as lignins (to reinforce the cell walls), hydrolytic enzymes (to attack the walls of the pathogen) and enzyme inhibitors (to inhibit the pathogen enzymes that degrade the plant cell wall), as well as a hypersensitive response and systemic acquired response.

The hypersensitive response involves the rapid death of cells surrounding the site of infection (programmed cell death – apoptosis) in order to prevent the disease from spreading (Mandadi and Scholthof 2013). A strongly oxidizing environment is generated with a high proportion of ROS (reactive oxygen species). Systemic acquired response involves transmitting a signal (e.g. salicylic acid) from the point of infection to other parts of the plant through the vascular system, generating increased resistance to pathogens (Hammerschmidt 2009; Shah 2003; Cameron 2000). These signals (e.g. in the form of methyl salicylate) can also be transmitted by air to neighbouring plants, preparing them for possible infection and acting as pheromones.

Elicitors can be usefully applied to stimulate or increase the production of a desired product in a plant culture (Verpoorte et al. 2002).

21.4.3.2.1.1 Response of Infectious Agents

To successfully attack a plant, a pathogen depends on its ability to overcome the aforementioned barriers. It must be able to deal with toxic substances in the plant and at the same time attack with cell wall-degrading hydrolytic enzymes and phytotoxins such as fusaric acid (*Fusarium oxysporum*) and destruxins (*Alternaria brassicae*) (Maor and Shirasu 2005; Ferreira et al. 2006; Walton 2006).

21.4.3.2.1.2 Plant Products That Influence Gene Expression in Microorganisms/Fungi

In the process of colonization, infectious agents can establish a symbiotic relationship with plants. The best-known example is that of the microorganisms of the genus *Rhizobium* and

plants of the Fabaceae family. In this case, certain flavones and flavonoids released by the plant roots induce transcription of the *nod* genes of *Rhizobium*. There are also many examples of symbiosis between plants and fungi (Hassan and Mathesius 2012; Narula et al. 2009; Cárdenas and Quinto 2009).

Another model involves the use of plants by microorganisms such as *Agrobacterium tumefaciens*. In this case, acetosyringone and other related phenolic compounds present in the exudates of the injured plant activate the expression of *Agrobacterium vir* genes as well as the transfer and integration of T-DNA into the genome of plant cells.

21.4.3.3 Interactions Between Plants and Animals

As with infectious agents, there are three levels of interaction: plant defence, animal response and the use of animal activity for the benefit of plants (Mithoefer and Boland 2012; Walling 2009).

21.4.3.3.1 Plant Defence

This essentially involves the aforementioned tactics of mechanical and chemical preventive barriers and, as a second line of defence, toxic repellents, both constitutive and induced. In fact, some of these products are toxic but not repellent, or only repellent, while others combine both features. They include toxic and bitter alkaloids and cardenolides and astringent tannins. The repellent effect, which may have different sensory goals (taste, visual, olfactory, irritant), acts as a warning signal to the predator (Rasmann and Agrawal 2009).

Plant substances vary in levels of toxicity, ranging from tannins, which require high concentrations to be effective and are not very specific, to highly toxic alkaloids and cardenolides, which are extremely specific and effective at low concentrations. Some effects can be long term, such as those produced by teratogens (e.g. α-solanine in *Solanum tuberosum* and cyclopamine in *Veratrum californicum*) and hormonal agents, whose purpose is to control predator populations (Hovhannisyan et al. 2009; Gaffield 2000). Plants interfere with insect metamorphosis by using phytoecdysones (which have a similar activity to the insect moulting hormone, ecdysone) or juvenile hormones (Bathori et al. 2008; Dinan 2001; Bede and Tobe 2000). In the case of vertebrates, plants use phytoestrogens such as genistein, formononetin, coumestrol and miroestrol for their contraceptive effect (Michel et al. 2013; Bucar 2013; Dixon 2004).

21.4.3.3.2 Response of Animals

Animals must be able to tolerate the toxic substances in plants they feed on. In fact, every secondary metabolite is tolerated by at least one species, which creates ecological niches where competition is minimalized for specialist feeders. In the most extreme scenario, an animal is adapted to subsist on only one plant species. Consequently, the toxin can serve as an orientative signal for the specialist predator or even be used for defence (protection) or to produce pheromones or other specific products, including hormones (Saporito et al. 2012; Hartmann and Ober 2000).

21.4.3.3.3 Use of Animal Activity by Plants

Plants can take advantage of animal activity for pollination, seed dispersal, defence and food. Pollinators visit flowers to obtain nectar and pollen, so both parties benefit. This interaction is regulated by the smell, colour and shape of flowers and the composition of nectar. Secondary metabolites are instrumental in the selection of specific pollinators, limiting the number of plant species a pollinator will visit, which in extreme cases is restricted to only one (Ayasse et al. 2011; Harborne 2007; Galliot et al. 2006; Pacini et al. 2003). Seed dispersion depends on the colour, smell and flavour of mature fruit (chemical composition). Animals attracted by these signals eat the fruit and disperse the seeds in their droppings (Barry 2010). Some plants have evolved extrafloral nectaries to gain the protection of predatory insects (Oliveira and Freitas 2004). Carnivorous plants are able to paralyze their insect prey by using products like coniine (Mithoefer 2011).

21.5 How Are Secondary Metabolites Biosynthesized?

Do many primary metabolites act as precursors of secondary metabolism? Actually, no. Secondary metabolites originate from a small number of primary precursors, following a few basic metabolic pathways that are subsequently diversified. Depending on the nature of their precursors, secondary products can be classified into three basic groups based on glucides, lipids and nitrogen. However, it should be borne in mind that the division between primary and secondary metabolism is not always clear. In fact, some metabolic pathways can simultaneously lead to the production of both primary and secondary metabolites (e.g. the shikimate pathway). There are also families of secondary products produced by the combination of more than one metabolic pathway (e.g. flavonoids and stilbenoids), while others, including some alkaloids and many phenolic compounds, can be synthesized by more than one route (Wink 2010).

21.5.1 Glucide Group

These products originate from simple monosaccharides derived directly from photosynthetic processes. They can be classified into two groups: secondary glucides and glycosides on the one hand and products derived from the shikimate pathway and phenylpropanoids on the other hand.

21.5.1.1 Secondary Glucides and Glycosides

These include secondary monosaccharides and oligosaccharides and glycosides:

(a) Secondary monosaccharides include unusual monosaccharides such as D-apiose, D-Aldgarose, D-digitoxose, L-streptose, etc., which differ from primary monosaccharides in their unique configuration arising from the presence of unusual groups or branched carbon skeletons.
(b) Secondary oligosaccharides are formed exclusively from secondary monosaccharides and mixtures of primary and secondary monosaccharides or from primary monosaccharides with unique bonding arrangements. Examples include antibiotics (neomycin, kanamycin, streptomycin) or branched oligosaccharides with elicitor activity.
(c) Glycosides include numerous secondary products (e.g. cyanogenic glycosides, saponins, cardiotonic glycosides, flavonoids) that undergo glycosylation with mono- or oligosaccharides to form the corresponding glycosides. Glycoside formation stabilizes and increases the water solubility of many aglycones or genins, which is often a prerequisite for accumulation or detoxification (Yu et al. 2012).

21.5.1.2 The Shikimate Pathway

This pathway leads to aromatic products from carbohydrate precursors. It can produce primary metabolites (e.g. the aromatic amino acids phenylalanine, tryptophan and tyrosine) as well as secondary products and represents a link between the two types of metabolism. Notably, this pathway is found only in microorganisms and plants, making aromatic amino acids essential in animal nutrition. In higher plants, the shikimate pathway occurs in chloroplasts and originates from phosphoenolpyruvate (PEP) and erythrose-4P (E-4P). A series of reactions (seven in total) leads to chorismate, which is the last common precursor of this pathway and a key intermediate (the centre of diversification) for the biosynthesis of numerous aromatic compounds (Dev et al. 2012; Maeda and Dudareva 2012).

21.5.1.2.1 Chorismate Precursors

The chorismate precursors are also the starting point for the synthesis of secondary compounds, notably quinic acid and its derivatives (esters of cinnamic acids) and gallic acid and its derivatives (hydrolysable tannins). Among the esters of cinnamic acids, we can cite chlorogenic acid (see below), which can act as an antioxidant, phytoalexin and radiation shield.

Hydrolyzable tannins possess a central molecule, usually a monosaccharide (typically glucose), whose hydroxyl groups are completely or partially esterified with gallic acid or its

derivatives (products of oxidation, dimerization and polymerization). Broadly speaking, we may speak of gallotannins (when hydrolysis leads to glucose and gallic acid) and ellagitannins (when hydrolysis additionally leads to the artefact ellagic acid or its derivatives). Ellagitannins, formed from gallotannins, are more abundant and can lead to quite complex structures (Okuda and Ito 2011).

The tannins are a group of water-soluble polyphenols capable of forming bonds with proteins and other macromolecules and hence can inhibit digestive enzymes and bacterial flora. They can accumulate in large amounts (quantitative defence) but when hydrolyzed lose their activity (as occurs in ripening fruits). Humans are quite tolerant of tannins due to the proline-rich glycoproteins secreted by the parotid glands and can appreciate the astringency of tannins in beverages and foods of plant origin. Ingestion of small amounts of tannins may be beneficial to health due to antioxidant, antitumor and immunomodulation activities, among others (Koleckar et al. 2008; Buzzini et al. 2008).

21.5.1.2.2 Chorismate Derivatives

The key intermediate chorismate is the starting point of many metabolic pathways, depending on the enzyme acting on it. For example, *p-hydroxybenzoate* is formed by the action of *p*-hydroxybenzoate synthase (only in microorganisms, not in higher plants; see below), isochorismate stems from the action of isochorismate synthase, anthranilate from anthranilate synthase and prephenate from chorismate mutase. Chloramphenicol, a typical *Streptomyces* antibiotic, is a derivative of chorismate.

Isochorismate is the precursor of naphthoquinones, anthraquinones and salicylate. The synthesis of naphthoquinone also requires an α-ketoglutarate derivative and that of anthraquinones a hemiterpene. Salicylate derivatives are formed by this pathway only in microorganisms, as in higher plants they are synthesized from phenylpropanoids or polyketides (tetraketides) (Chen et al. 2009; Wildermuth 2006). This biosynthetic pathway to naphthoquinones and anthraquinones is only found in a small group of plant families (e.g. Rubiaceae, Juglandaceae). Most naphthoquinones (hexaketides) and anthraquinones (octaketides) are synthesized by the polyketide pathway (Han et al. 2001). Juglone is an example of a naphthoquinone synthesized from isochorismate.

Anthranilate is the precursor of tryptophan (L-Trp), while phenylalanine (L-Phe) and tyrosine (L-Tyr) are formed from prephenate (Tzin and Galili 2010; Maeda and Dudareva 2012). Of the three aromatic amino acids, L-Tyr and L-Trp are essential for alkaloid biosynthesis, while L-Phe is the starting point of the phenylpropanoid pathway.

21.5.1.2.3 Phenylpropanoid Pathway

When the PAL enzyme (phenylalanine ammonia lyase) acts on L-Phe, a stereospecific oxidative deamination reaction takes place, leading to cinnamic acid (with a *trans* double bond in the side chain) (MacDonald and D'Cunha 2007). Cinnamic acid is the first phenylpropanoid (C_6-C3) synthesized and is converted by the action of hydroxylases and *O*-methyltransferases into the other members of the family (e.g. *p*-coumaric, caffeic, ferulic and sinapic acids). This group, known generically as cinnamic acids, is the starting point of numerous metabolic pathways leading to the formation of a wide variety of secondary products, grouped under the name of phenylpropanoid derivatives, which include cinnamoyl esters, cinnamoyl amides, coumarins, dihydrocinnamic acids, cinnamic alcohol derivatives (lignins and lignans), styrenes, benzoic acid derivatives, acetophenone, xanthone, styrylpyrones, stilbenes and flavonoids (Vogt 2010; Petersen et al. 2010; Ferrer et al. 2008):

(a) Esters and amides of cinnamic acids include chlorogenic acid, formed by the esterification of quinic acid with caffeic acid (Campa et al. 2008). Hordatine, formed from *p-coumaric* acid and agmatine, is an example of a cinnamic acid amide (Seo et al. 2011; Spasova et al. 2007).

(b) Coumarins. At the beginning of the coumarin pathway, cinnamic acids are hydroxylated at the *ortho* position by a cinnamate-2-hydroxylase. Subsequently, the corresponding *ortho*-coumaric acid derivatives are cyclized to form a lactone, which requires the previous

action of a glycosyltransferase, a *cis-trans* isomerase (requiring the substrate to be glycosylated) and a β-glycosidase. Glycosylated *ortho*-coumaric acid derivatives (including mixtures of the two isomers) can accumulate in plant vacuoles (Bourgaud et al. 2006). Certain conditions (e.g. injuries) may trigger the action of β-glycosidase (accumulated in another compartment), which, by releasing glucose, allows derivatives at the *cis* position to be cyclized to give coumarins (e.g. prototoxin). Simple coumarins with a hydroxyl group at position 7 are precursors of two prenylated coumarins, furanocoumarins and pyranocoumarins, both either linear or angular (Gnonlonfin et al. 2012; Gliszczynska and Brodelius 2012; Curini et al. 2006). Coumarins are widely distributed, particularly in the Apiaceae, and provide significant protection against insects, in many cases causing phototoxic effects (Razavi 2011).

(c) Cinnamic alcohols and derivatives. Their biosynthesis is initiated by a cinnamoyl-CoA ligase, which activates cinnamic acids in the form of cinnamoyl-CoA derivatives. Besides cinnamic alcohols and their derivatives, other groups of phenylpropanoid derivatives (benzoic acids and derivatives, flavonoids, stilbenoids, etc.) also need to be synthesized via a cinnamoyl-CoA intermediate. The biosynthesis of cinnamic alcohols from cinnamoyl-CoA derivatives involves two reduction processes catalyzed by a cinnamoyl-CoA reductase and cinnamoyl alcohol dehydrogenase. The former reaction produces cinnamic aldehydes (*p*-coumaryl aldehyde, coniferyl aldehyde, sinapyl aldehyde). The cinnamic alcohols (*p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol) are precursors of lignins, lignans and the phenylallyl derivatives (eugenol) and phenylpropenes (isoeugenol) (Chaubet et al. 2003).

(d) Lignins are constituents of the secondary cell wall of higher plants and are the most abundant phenolic compounds in nature. These complex polymers of cinnamic alcohols (*p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol) are formed by phenolic oxidative coupling mechanisms catalyzed by phenol oxidase present in the cell walls; other related compounds may also be involved (Novo-Uzal et al. 2012). Coniferyl alcohol is the main component of lignins in gymnosperms. Dicotyledons contain coniferyl alcohol and sinapyl alcohol in similar proportions, while monocotyledons also have *p*-coumaryl alcohol (Liu et al. 2011; Amthor 2003). The amount of lignins and the proportion of sinapyl/coniferyl alcohols increase progressively with age. Both these increases in concentration and higher level of methoxylation have a negative effect on the digestibility of fodder. In the paper industry, the treatments to remove lignins increase the cost of processing as well as the rate of pollution.

(e) Lignans are dimers formed from cinnamic alcohol and/or its derivatives (eugenol, isoeugenol, etc.) by phenolic oxidative coupling reactions, as are lignins. Extra *O-heterocyclic* rings are often formed after dimerization (Satake et al. 2013; Suzuki and Umezawa 2007). Lignans are widely distributed in plants, acting as predator repellents and/or antifeedants, primarily in plant-insect interactions (Apers et al. 2004). They are also responsible for many physiological effects: antioxidant, antiviral, antitumor (podophyllotoxin), cytotoxic, insecticide, antifungal, ichthyotoxic, germination inhibiting, etc. *Aegilops* lignans are cytotoxic and irritant. Plicatic acid is a protective agent naturally found in thuja and cypress resin (ancient trees). Sesamin, found in sesame seeds, has antioxidant properties that stabilize the oil (Dar and Arumugam 2013; Ionkova 2010; Liu et al. 2007).

(f) Benzoic acid derivatives represent a gateway to the metabolism of C_6-C_1 products. First, benzoyl-CoA derivatives are synthesized from cinnamoyl-CoA derivatives by a β-oxidation process catalyzed by a benzoate synthase (similar to the β-oxidation of fatty acids). This complex enzyme allows an acetyl-CoA group to be eliminated, transforming a C_6-C_3 compound into a C_6-C_1 compound (Chen et al. 2009; Wildermuth 2006). Benzoyl-CoA derivatives give rise to a wide variety of products, including benzoic acids

(benzoic acid, *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid, syringic acid, salicylic acid), benzaldehydes (vanillin), benzyl alcohols, methylated derivatives (acetosyringone), benzoyl conjugates (cocaine), hydroquinones and ubiquinones (Kaur and Chakraborty 2013; Walton et al. 2003). It should be remembered that benzoic acid derivatives can also be formed from chorismate precursors via the shikimate pathway (gallic acid, protocatechuic acid), from chorismate (*p*-hydroxybenzoic acid and salicylic acid, in microorganisms) and by the polyketide pathway.

21.5.2 Lipid Group

The products of this branch of secondary metabolism are synthesized by three main pathways: those of acetate malonate, acetate mevalonate and methylerythritol phosphate (MEP) (Schmid and Ohlrogge 2008). The first two arise from acetyl-CoA, whereas the MEP pathway originates from pyruvate and glyceraldehyde-3P. The acetate mevalonate and MEP pathways lead to the formation of terpenes.

21.5.2.1 The Acetate Malonate Pathway

The acetate malonate pathway leads to the secondary fatty acids and polyketides (including those of mixed biosynthesis, such as flavonoids and stilbenoids). The precursors of this pathway are acetyl-CoA and malonyl-CoA. The synthesis of malonyl-CoA from acetyl-CoA is catalyzed by acetyl-CoA carboxylase, an enzyme found in both the cytosol and plastids (Sasaki and Nagano 2004). When located inside plastids, it supplies the malonyl-CoA required for fatty acid synthesis, while cytosolic malonyl-CoA is used to form polyketides and related products. Other precursors less significant for the biosynthesis of fatty acids and polyketides are propionyl-CoA and derivatives, such as methylmalonyl-CoA.

21.5.2.1.1 Secondary Fatty Acids

The synthesis of fatty acids in plants begins in the plastids by a condensation mechanism catalyzed by fatty acid synthase (FAS), a multifunctional enzyme (Harwood 2005). Modifications and elongations take place at the endoplasmic reticulum. Secondary fatty acids differ from primary ones in chain length, an uneven number of C (valeric acid), and also by the presence of additional functional groups or other structural elements, such as alcohol functions (ricinoleic acid), branches, triple bonds (tariric acid, ximeninic acid) and the presence of additional rings (sterculic acid, chaulmoogric acid, vernolic acid) (McKeon and Lin 2002; Fatope et al. 2000).

21.5.2.1.2 Fatty Acid Derivatives

A wide variety of secondary products are derived from fatty acids. Alkanes and alkenes are formed by decarboxylation of fatty acids. Fatty aldehydes and fatty alcohols are formed from the acyl-CoA derivatives of fatty acids by a mechanism similar to the conversion of cinnamic acids into cinnamic aldehydes and alcohols. Other products include fatty acid esters, such as triglycerides (oils), cyanolipids, waxes, cutins and suberins; jasmonate and its derivatives, which originate from linolenic acid (18:3); and macrolides (Marsh and Waugh 2013).

Among the fatty acid esters, triglycerides (oils) containing secondary fatty acids (e.g. castor oil, chaulmoogra oil, sandalwood oil) have unique properties. Cyanolipids (present in Sapindaceae seeds) are similar to cyanogenic glycosides but instead of being conjugated with a carbohydrate molecule are esterified with a fatty acid (Miller and Tuck 2013). Waxes are complex mixtures formed by alkanes, alkenes, fatty aldehydes, fatty alcohols and mostly esters of long-chain fatty alcohols with long-chain fatty acids (Sturaro and Motto 2006). Cutins and suberins are polymeric substances and derivatives of hydroxy fatty acids, which are esterified to each other and phenolic compounds (Samuels et al. 2008).

21.5.2.1.3 Polyketides

As with fatty acids, the synthesis of polyketides involves multifunctional enzymes (e.g. 6-methylsalicylate synthase, chalcone synthase) that require a starter molecule to which various malonyl-CoA molecules are added. With each addition of a malonyl-CoA molecule, the length of the final product is increased by two carbons. According to the number of monomers used, polyketides are classified as triketides (1 starter-CoA + 2 malonyl-CoA), tetraketides (1 starter-CoA + 3 malonyl-CoA), pentaketides, hexaketides, heptaketides, octaketides, nonaketides and decaketides (1 starter-CoA + 9 malonyl-CoA). Starter molecules include acetyl-CoA, propionyl-CoA, malonyl-CoA, malonamoyl-CoA, anthranilyl-CoA, cinnamoyl-CoA, *p*-coumaroyl-CoA and *p-hydroxybenzoyl-CoA*. However, the most commonly used is acetyl-CoA. As a starter molecule, *p-coumaroyl-CoA* plays an important role in the synthesis of polyketides of mixed biosynthesis (e.g. flavonoids and stilbenoids) (Abe 2010; Fujii 2010; Peiru et al. 2009).

Although all the steps involved in chain elongation are common to all polyketides, multifunctional proteins are specific and lead to the formation of specific products, depending on the additional steps (reduction and dehydration in the case of 6-methylsalicylate synthase). Similarly, at the end of the process, intermediates are cyclized to form aromatic or heterocyclic systems (Musiol and Weber 2012; Bringmann et al. 2009; Zhang and Tang 2009). The aromatic structures can be formed by aldol condensation (6-methylsalicylate) or by Claisen condensation (phloroglucinol). The formation of *O*-heterocycles involves a lactonization process (asperline), and the *N*-heterocycles are formed by a Schiff base (coniine) (Teixeira da Silva et al. 2013).

The most significant triketides are phloroglucinol and its derivatives. Phloroglucinol is the starting point for the synthesis of humulone and other bitter principles of *Humulus lupulus* (Faivre et al. 2007). The tetraketides are a very diverse and numerous group that include simple phenols (6-methylsalicylate), *O*-heterocycles, *N*-heterocycles (coniine) and also metabolites of mixed biosynthesis (flavonoids and stilbenoids). Naphthoquinones can derive from hexaketides and anthraquinones from octaketides (Bringmann and Irmer 2008), while tetracyclines (synthesized by *Streptomyces*) are nonaketides and aflatoxins (*Aspergillus* mycotoxins) are decaketides (Abrar et al. 2013). At this point, it should be recalled that naphthoquinones and anthraquinones are also formed by the shikimate pathway from chorismate.

21.5.2.1.4 Flavonoids

Flavonoids are a complex family of phenolic compounds found in most higher plants. They are tetraketides with *p*-coumaroyl-CoA acting as the starter molecule. *p*-Coumaroyl-CoA is formed from Phe and requires both the shikimate and phenylpropanoid pathways. Flavonoids are formed by two aromatic rings linked by a C-3 unit (C_6-C_3-C_6 system) and according to the degree of oxidation of the C-3 unit are classified mainly as chalcones, flavanones, aurones, flavones, isoflavones, flavonols dihydroflavonols, proanthocyanidins, anthocyanins and condensed tannins.

Chalcone synthase (CHS) is a multifunctional enzyme that gives access to the whole flavonoid family (Dao et al. 2011; Jez et al. 2001). Its catalytic action joins three molecules of malonyl-CoA to one of *p*-coumaroyl-CoA, and the resulting intermediate undergoes cyclization (Claisen condensation) to give naringenin chalcone (yellow), which is a precursor molecule for all the flavonoids (Bukhari et al. 2013). Other chalcones, formed from naringenin chalcone, include the dihydrochalcone phloridzin (phloretin-2′-*O*-glucoside), which is a prototoxin with fungitoxic properties characteristic of the genus *Malus* (Gosch et al. 2010).

Chalcones can be transformed into aurones or flavanones (Veitch and Grayer 2006). The formation of aurones, responsible for the yellow colour of flowers of some species of the genera *Antirrhinum* or *Cosmos*, is catalyzed by a chalcone dehydrogenase (Nakayama 2002). The majority of chalcones are converted into flavanones by the chalcone-flavanone isomerase (CHI), a cytoplasmic and reversible enzyme,

which maintains a balance between chalcones and 2(*S*)-flavanones. CHS and CHI are frequently found together in an enzyme complex that ensures the directionality and stereochemical specificity required by subsequent enzymes in the metabolic pathway (Fowler and Koffas 2009). Flavanones such as naringenin (colourless), liquiritigenin and eriodictyol are intermediates in the synthesis of other flavonoids: flavones (flavone synthase), dihydroflavonols (flavanone 3-hydroxylase) and isoflavones (isoflavone synthase).

The flavones (e.g. apigenin, luteolin) are synthesized by an oxidation process (creation of an unsaturation at the 2,3 position) by a flavone synthase. Most flavones undergo glycosylation. The dihydroflavonols (e.g. dihydrokaempferol, dihydroquercetin) are formed by the action of (2*S*)-flavanone 3-hydroxylase and usually have a β-hydroxyl substituent at position 3 (Grayer and Veitch 2006). The dihydroflavonols are the precursors of flavonols, anthocyanins and condensed tannins.

The flavonols (e.g. kaempferol, quercetin) are formed from dihydroflavonols by an oxidation process (unsaturation at position 2,3) involving a flavonol synthase (parallel to the action of the flavone synthase). By the action of dihydroflavonol-4-reductase (DFR), the dihydroflavonols are reduced to leucoanthocyanidins (also known as proanthocyanidins), which usually have a 2,3-*trans*-3,4-*cis* configuration (Tian et al. 2008). The leucoanthocyanidins are precursors of anthocyanins (before the formation of anthocyanidins) and condensed tannins (directly or after the formation of catechins) (He et al. 2008; Xie and Dixon 2005).

Anthocyanidins (pelargonidin, cyanidin, delphinidin) are formed through the initial oxidation and dehydration of leucoanthocyanidins (proanthocyanidin) by an anthocyanidin synthase (Pourcel et al. 2012; Ozeki et al. 2011; He et al. 2010). The glycosylation of the anthocyanidins (anthocyanin glycosyltransferase) leads to the corresponding anthocyanins. Mixtures of the differently coloured anthocyanins are found in virtually all plant organs and are responsible for the colour of fruits.

Condensed tannins are structurally more complex than hydrolysable tannins. They are produced by the polymerization of four to eight units of catechins or proanthocyanidins, or a mixture of both, and are very widespread, representing the largest group of flavonoid polymers (Koleckar et al. 2008; Tanner 2004).

Isoflavones give rise to the whole family of isoflavonoids, which, unlike other flavonoids, have a limited distribution, being found primarily in the Fabaceae (Lapcik 2007). Isoflavones (e.g. genistein, daidzein) are formed by the action of the isoflavone synthase complex (IFS) on flavanones (Ozeki et al. 2011; Wang 2010). The IFS complex consists of two enzymes, 2-hydroxyisoflavanone synthase and 2-hydroxyisoflavanone dehydratase.

Some isoflavones (genistein, formononetin and daidzein) have estrogenic activity (Bucar 2013). Luteone, a prenylated isoflavone, inhibits the growth of phytotoxic fungi (a preinfection barrier). In general, greater lipid solubility is more effective for the antifungal activity. Among the pterocarpans, which derive from isoflavones by the formation of an *O*-heterocyclic ring, there are the phytoalexins medicarpin and pisatin and the estrogenic coumestrol.

Most flavonoids are glycosylated (Veitch and Grayer 2011). These *O*-glycosides (e.g. rutin, naringin) usually accumulate in vacuoles or hydrophilic secretions. Methylations, alkylations, ester formation, etc., make them more lipophilic, favouring their presence in the waxy layer of the cell wall or as products of epidermal glandular trichomes. Some highly methylated flavonoids are toxic both to man and other mammals.

Flavonoids perform a variety of functions in plants (Pollastri and Tattini 2011). They act as visual cues for animals to encourage pollination and seed dispersal (anthocyanins); provide protection against radiation (flavones, flavonols); take part in plant defence (condensed tannins, phytoalexins, phytoestrogens), symbiotic interactions (Fabaceae-*Rhizobium*) and plant hormonal metabolism (auxin degradation); and act as antioxidants (Subramanian et al. 2007).

21.5.2.1.5 Stilbenoids and Other Polyketides of Mixed Biosynthesis

Besides flavonoids, other polyketide families use phenylpropanoid derivatives as starter molecules, e.g. benzalacetones and arylpyrones (diketides), styrylpyrones (triketides) and stilbenoids (tetraketides) (Beerhues and Liu 2009; Brand et al. 2006).

Stilbenoids are ethylene derivatives with two phenyl groups, usually with a *trans* configuration (C_6-C_2-C_6 system) (Chong et al. 2009). They are formed by the action of a multifunctional stilbene synthase (STS), with coumaroyl-CoA as the starter molecule and the addition of three molecules of malonyl-CoA. Cyclization is produced by aldol condensation. Chalcone and stilbene synthases share many similarities. There are a variety of stilbene synthases, each specific to a stilbenoid, e.g. pinosylvin synthase (pinosylvin) and resveratrol synthase (resveratrol).

As there are only two carbons in the central part of the stilbenoid molecule, the third ring cannot be formed, which limits the size of this polyketide family. It nevertheless includes some very important metabolites, such as pinosylvin (a pre-infectious toxin), resveratrol (a phytoalexin) and combretastatin (Pattillo 2011; Singh and Kaur 2009).

Resveratrol is synthesized and located chiefly in the epidermis of black grapes (*Vitis vinifera*) but can also be found in other plants in lesser amounts (Nakata et al. 2012). It has proven antioxidant capacity, being the most significant known antifungal agent (e.g. against *Botrytis cinerea*). Resveratrol is used to treat cardiovascular and liver diseases and also has anti-inflammatory, antioxidant and anti-tumour activity. It can explain certain aspects of the so-called French paradox, since a high dietary ingestion of resveratrol (e.g. in red wine) is thought to have a significant lowering effect on blood lipid and cholesterol levels as well as on platelet aggregation, thus reducing the risk of cardiovascular disease (Murtaza et al. 2013). As an antioxidant, it prevents the formation of free radicals and protects cells from lipid peroxidation. As an antitumor agent, it reduces the risk of cancer, acting at several levels. Many of these functions are interrelated and linked to the activity of the cyclooxyganase-2 (COX-2). Resveratrol blocks the COX-2 gene and inactivates the enzyme (Latruffe and Rifler 2013; Goswami and Das 2009).

21.5.2.2 Terpenes (or Isoprenoids)

Present in all living organisms, from bacteria to higher animals, vascular plants included, terpenes are an extensive group, with a great structural and functional diversity. Terpenes are classified as a single group of secondary metabolites due to their biosynthetic origin. Theoretically, they are regarded as polymers of isoprene (2-methyl-1,3-butadiene), although, in fact, their true molecular base is isopentenyl pyrophosphate (IPP), which can be formed by two different ways, the acetate mevalonate or the methylerythritol phosphate (MEP) pathway. Based on the number of 5-carbon monomers in their structure, the main terpene groups are hemiterpenes (5C), monoterpenes (10C), sesquiterpenes (15C), diterpenes (20C), triterpenes (30C), tetraterpenes (40C) and polyterpenes (>40C) (Keeling and Bohlmann 2012):

(a) Formation of IPP by the acetate mevalonate pathway. This takes place in the cytosol from three molecules of acetyl-CoA. The first three enzymes are associated with the endoplasmic reticulum, while the rest are cytosolic. The hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase) is the regulatory enzyme in the process. The formation of an IPP molecule requires three acetyl-CoA, 3 ATP and 2 NADPH (Vranova et al. 2013; Okada 2011; Kuzuyama 2002).

(b) Formation of IPP by the methylerythritol phosphate (MEP) pathway. Initially it was believed that only the acetate mevalonate pathway existed, until it was observed that mevinolin, a highly specific inhibitor of the HMG-CoA reductase, inhibits the formation of ubiquinones and sterols, but not that of phytol, carotenoids and plastoquinones, which are formed in the chloroplasts. Subsequently, another source of IPP was discovered, the methylerythritol

phosphate (MEP) pathway, located in the chloroplasts, with glyceraldehyde-3-phosphate (GA-3P) and pyruvate (PYR) as the starting molecules and 1-deoxy-D-xylulose 5-phosphate (DOXP) and MEP as intermediates (Graewert et al. 2011; Boronat 2010; Hunter 2007).

(c) Formation of dimethylallyl pyrophosphate (DMAPP). The starter molecule in terpene synthesis, DMAPP, is formed from IPP by the action of isopentenyl pyrophosphate isomerase, which keeps levels of IPP and DMAPP balanced. All isoprenoids can be generated from these two compounds, but it is first necessary to synthesize all the lead compounds.

(d) Formation of the lead compounds. The starter molecule DMAPP is always linked to IPP in a head-to-tail fashion, as are the resulting molecules, which in each case involves a condensation reaction of a homoallylic unit (IPP) on an allylic unit. In these head-to-tail linkages, since the H_R or H_S of the IPP molecule is lost, the process leads to the formation of a *trans* or *cis* double bond, respectively. The *trans* double bonds are far more prevalent, found in the precursors of monoterpenes (geranyl pyrophosphate – GGPP), sesquiterpenes (farnesyl pyrophosphate – FPP) and diterpenes (geranylgeranyl pyrophosphate – GGPP). However, there are some polyterpenes, such as rubber, in which all the double bonds are *cis* (Oldfield and Lin 2012; Okada 2011; Akhila 2007).

Each lead compound is formed by the participation of a specific multifunctional enzyme, which has a particular subcellular location, and starts and ends the product metabolism. According to its location, the enzyme will access substrates from the acetate mevalonate pathway or the MEP pathway, which is why the GPP synthase (1 DMAPP + 2 IPP) and GGPP synthase (1 DMAPP + 3 IPP), both located in the chloroplast, obtain IPP from the MEP pathway, while the FPP synthase (1 DMAPP + 2 IPP) and rubber synthase (1 DMAPP + n IPP), located in the cytosol or associated with the membrane of the endoplasmatic reticulum, use IPP from the mevalonate pathway.

So far, we have mentioned precursors that use *head-to-tail* terpene synthases. In contrast, the formation of precursors of triterpenes (squalene) and tetraterpenes (phytoene) involves *head-to-head* linkage between two units of FPP or GGPP, respectively, mediated by *head-to-head* terpene synthases. The binding of two FPP molecules by squalene synthase, an enzyme located in the membrane of the endoplasmic reticulum, leads to squalene, the starting point of all triterpenes and steroids. Starting from two molecules of GGPP, the phytoene synthase in the chloroplast forms *cis*-phytoene (in higher plants). Phytoene is the basic molecule for all tetraterpenes and carotenoids.

21.5.2.2.1 Hemiterpenes

Containing five carbons and formed from IPP or DMAPP, hermiterpenes are found as prenyl groups associated with a diverse range of molecules, including humulone (triketide of *Humulus lupulus*), lysergic acid and anthraquinones.

21.5.2.2.2 Monoterpenes

These are cyclic or aliphatic C_{10} compounds arising from GPP through a series of reactions catalyzed by enzymes generically known as monoterpene synthases, including isomerizations and cyclizations (monoterpene cyclases). GPP originates in the chloroplasts, and the first steps of diversification are catalyzed by chloroplastic enzymes, which are encoded in the cell nucleus. Simple products such as geraniol, myrcene and limonene are synthesized within the chloroplasts. Subsequent transformations, hydroxylation and oxidation (to form thymol, menthol or camphor), are carried out in the cytosol and are normally associated with the microsomal fraction (Ashour et al. 2010; Yu and Utsumi 2009).

Monoterpenes mainly belong to the group of essential oils. These may be linear (geraniol, myrcene), cyclic (limonene, menthol) or bicyclic (camphor, α-pinene). Some are liquid at room temperature (geraniol, limonene), while others are solid (menthol, camphor). They are usually

synthesized in the epithelial cells that surround the cavities or ducts where they remain sequestered (Woronuk et al. 2011; Erasto and Viljoen 2008; Croteau et al. 2005).

Iridoids constitute an independent group of monoterpenes with particular characteristics (Jensen et al. 2002). They are formed from GPP by a peculiar type of cyclization that eventually leads to loganine and secologanin. These two compounds are the starting point of the two large groups of iridoids (loganine and secologanin type). Most iridoids are found as glycosides and function as bitter predator repellents, although some may attract specialized insects. Some iridoids may acquire an *N*-heterocycle and be converted into monoterpene alkaloids with a pyridine ring (gentianine, actinidine). It should be noted that pyridine alkaloids usually originate from L-Asp. Many alkaloids also have a component derived directly from monoterpene iridoids, e.g. monoterpene indole alkaloids such as reserpine (derived from L-Trp and loganine), an antihypertensive agent isolated from *Rauwolfia serpentina* (Apocynaceae), or tetrahydroisoquinoline monoterpene alkaloids such as emetine (derived from L-Tyr and Secologanin), an antiprotozoal isolated from *Cephaelis ipecacuanha* (Rubiaceae) (Oudin et al. 2007; El-Sayed and Verpoorte 2007).

21.5.2.2.3 Sesquiterpenes

C_{15} isoprenoids synthesized in the cytosol from FPP by a series of reactions very similar to those of monoterpenes (Ashour et al. 2010; Yu and Utsumi 2009) and sesquiterpenes constitute one of the largest and most diverse families of natural products. Excluding the acyclic forms (β-farnesene), they can be classified according to four basic types of cyclization (humulene, germacrene, carotol and bisabolene). They include essential oils (farnesol, bisabolol, cadinene), phytoalexins (capsidiol, risitin), phytotoxins (HS-toxin A) and sesquiterpene lactones (Drew et al. 2009).

Sesquiterpene lactones are an independent group with particular characteristics and a large number of components (Abad-Martínez et al. 2012). They are found mainly in the Asteraceae but have also been isolated from other plant families. They are located mostly in the trichomes of leaves and stems, although in some cases they have also been isolated in roots, e.g. costunolide (*Saussurea lappa*) and helenin (*Inula helenium*). Due to the characteristically bitter taste of sesquiterpene lactones (feeding deterrent), *Artemisia absinthium* and *Cnicus benedictus* have been used in folk medicine and the food industry (cnicine is a very important ingredient in bitter liqueurs). Sesquiterpene lactones are also intermediates in the formation of sesquiterpene alkaloids such as dendrobine (*Dendrobium nobile*). Artemisinin (*Artemisia annua*) is used in the treatment of malaria, while other sesquiterpene lactones have cytotoxic antitumoral and antifungal activities, among others (Brown 2010; Ryden and Kayser 2007).

21.5.2.2.4 Diterpenes

C_{20} compounds with a wide distribution in the plant kingdom (Ashour et al. 2010), diterpenes are formed in chloroplasts from GGPP. Diterpene synthases, like monoterpene synthases, are encoded with an *N*-terminal signal peptide, which is required for crossing chloroplast membranes. They may have a linear shape (geranylgeraniol, phytol), but most are cyclic (macrocyclic, bicyclic, polycyclic) (Wefer et al. 2013; Toyomasu 2008). Phytol (a constituent of chlorophyll) is the most common diterpene.

Macrocyclic diterpenes are a significant group of three basic types: cembrenes (cembrene), casbenes (casbene) and taxanes (taxol). The latex of many plants in the Euphorbiaceae family contains highly irritating and toxic substances that derive from casbene (e.g. phorbol and its esters). Taxol, isolated from the bark of *Taxus brevifolia*, is an antimitotic used in cancer chemotherapy, since it stabilizes microtubules and consequently interferes with cell division (Miller et al. 2008; Makkar et al. 2007).

The majority of diterpenes are bicyclic and polycyclic and are derived from copalyl-PP (CPP), a bicyclic structure catalyzed from GGPP by copalyl-PP synthase. (−)-CPP leads to (−)-kaurene, the precursor of the gibberellins.

(+)-CPP is the precursor of manool (bicyclic structure) and abietadiene and rosadiene (tricyclic structures). Abietadiene is eventually transformed into abietic acid and other resin acids found in the oleoresin of conifers. Oleoresin is a viscous and complex mixture, composed of volatile turpentine (monoterpenes and sesquiterpenes) and non-volatile rosin (diterpenes), which acts as a defensive secretion (physical and chemical) against insects and pathogens (Zulak and Bohlmann 2010; Keeling and Bohlmann 2006). Diterpene alkaloids are also kaurene derivatives and are found in the Ranunculaceae (*Aconitum* and *Delphinium*) and other plant families. They are divided into two basic structural types: those with a C_{20} skeleton, which are relatively non-toxic, and those bearing a C_{19} skeleton, such as aconitine, which are more toxic and heavily substituted (Reina and González-Coloma 2007; Wang and Liang 2002).

21.5.2.2.5 Triterpenes

Triterpenes are derived from squalene (30C), which is synthesized in the cytosol from two FPP molecules by squalene synthase. Triterpene cyclases catalyze a broad range of cyclization reactions to form polycyclic triterpenes (Phillips et al. 2006). Those that convert squalene to hopene and related compounds (pentacyclic triterpenes) are named squalene-hopene cyclases (SHC), while those that convert 2,3-oxidosqualene (or epoxysqualene) are oxidosqualene cyclases (OSC) (Hammer et al. 2013; Siedenburg and Jendrossek 2011). (*S*)-2,3-epoxysqualene is an intermediate in the biosynthesis of pentacyclic and tetracyclic triterpenes, both of which tend to be glycosylated (Abe 2007).

Most pentacyclic triterpenes are saponins and can be divided into many subgroups, such as gammaceranes, hopanes, lupanes, oleananes and ursanes, based on their carbon skeleton. Typical examples are the glycosylated derivatives of glycyrrhetinic acid in *Glycyrrhiza glabra* roots and hederosaponins of *Hedera helix* (James and Dubery 2009; Vincken et al. 2007; Patocka 2003).

The most significant group of tetracyclic triterpenes are the steroids (characterized by a cyclopentanoperhydrophenanthrene ring system) (Kreis and Mueller-Uri 2010; Ohnishi et al. 2009). In plants, the majority of steroids are formed from cycloartenol, which is synthesized from (*S*)-2,3-epoxysqualene by cycloartenol synthase (a microsomal enzyme). Phytosterols (campesterol, sitosterol, stigmasterol) are formed from cycloartenol and, besides stabilizing membranes, act as precursors of other plant steroids, including steroidal saponins (diosgenin, yamogenin), steroidal alkaloids (solanidine, tomatidine, cyclopamine), cardiotonic steroids (cardenolides such as digitoxin and bufadienolides such as proscillaridin), brassinosteroids and phytoecdysteroids (Kutschera and Wang 2012; Hartmann 2004; Dinan 2001).

21.5.2.2.6 Tetraterpenes

Tetraterpenes derive from *cis*-phytoene (40C), formed in the chloroplasts from two molecules of GGPP by phytoene synthase. The most ubiquitous tetraterpenoids are carotenoids, which are characterized by the presence of conjugated double bonds (*trans*) that give rise to yellow or red colours (carotenoid-protein complexes produce other tones) (Lichtenthaler 2012; Rodríguez-Concepción 2010).

In plants, at least four enzymes are required for the conversion of *cis*-phytoene to lycopene: phytoene desaturase and zeta-carotene desaturase, which produce poly-*cis*-compounds that undergo *trans* isomerization by zeta-carotene isomerase and carotenoid isomerase to produce lycopene.

Carotenoids with cyclic groups are generated from lycopene by lycopene cyclases, which can form different types of ionone rings (ε, β or γ). For example, β-carotene has a β-ring at both ends, while α-carotene has a β-ring at one end and an ε-ring at the other. Carotenoids lacking oxygen, such as those mentioned so far, are named carotenes, while those that are oxygenated are xanthophylls. Xanthophylls are formed from carotenes by chloroplast monooxygenases, for example, lutein is derived from α-carotene, and zeaxanthin and violaxanthin from β-carotene (Cazzonelli 2011; Ruban and Johnson 2010; Taylor and Ramsay 2005).

Carotenoids are of great importance as photosynthetic pigments, radiation shields, stabilizers of

thylakoid membranes and pigments in flowers and fruits (lycopene in tomato, capsanthin in paprika). Violaxanthin is the precursor of abscisic acid and carotenes with a β-ring are precursors of vitamin A (von Lintig and Vogt 2004; Milborrow 2001).

21.5.2.2.7 Polyterpenes

Among the polyterpenes, which have more than 40C, the most significant is rubber, obtained mainly from *Hevea brasiliensis* latex (Euphorbiaceae). The rubber molecule is a high-molecular-weight polymer with all-*cis* double bonds. The elasticity of rubber has resulted in its application in many products, such as tyres, gloves, balloons and balls for sports. The enzyme that catalyzes rubber formation has been identified as rubber transferase. IPP is incorporated in rubber on the surface of rubber particles suspended in the latex (Gronover et al. 2011; Swiezewska and Danikiewicz 2005).

21.5.3 Nitrogenated Metabolites

Secondary metabolites with nitrogen in their structure can be formed from amino acids, nitrogenous bases (purine, pyrimidine or nucleosides) and/or other substrates such as porphyrins. Xanthines such as caffeine, theobromine and theophylline (components of coffee, tea and chocolate) are derivatives of purine bases (Ashihara et al. 2008; Ashihara et al. 2013). Cordycepin (3′-desoxyadenosine) is a nucleoside derivative (Tuli et al. 2013). Examples of amino acid derivatives are the non-proteinogenic amino acids, secondary peptides, cyanogenic compounds, glucosinolates and alkaloids.

21.5.3.1 Non-proteinogenic Amino Acids

In addition to the 22 standard amino acids, there is a numerous and diverse group of non-proteinogenic or non-standard amino acids not found in proteins (Selmar 2010; Bell 2003). Their structure is normally derived directly from the proteinogenic amino acids or their biosynthetic pathway, although some of those found in plants of the genus *Lathyrus* (Fabaceae), such as the pyrimidinyl amino acid lathyrine, are formed from uracil. Due to their structural similarity, many of them act as antagonists (competitors) of protein amino acids in the biosynthetic process, resulting in the formation of erroneous proteins. Examples include L-canavanine, which is an analogue of L-arginine, and azetidine-2-carboxylic acid, which interferes with L-proline (Bach and Takagi 2013; Bence and Crooks 2003).

21.5.3.2 Secondary Peptides

These mainly belong to the group of nonribosomal peptides and are synthesized by nonribosomal peptide synthases, which are independent of messenger RNA. Each nonribosomal peptide synthase can synthesize only one type of peptide, which often has a cyclic and/or branched structure, and can contain non-proteinogenic amino acids, including D-amino acids. These products are ubiquitous in bacteria and fungi (Grunewald and Marahiel 2013; Koglin and Walsh 2009). α-Amanitin and phalloidin are bicyclic peptides of eight amino acids belonging to a family of toxins isolated from the deadly *Amanita* genus of mushrooms, and unlike other known fungal peptides, these amatoxins are synthesized on ribosomes (Walton et al. 2010).

21.5.3.3 Cyanogenic Compounds

These typical prototoxins are of two basic types: cyanogenic glycosides and cyanolipids. Cyanogenic glycosides, which have a relatively wide distribution in the plant kingdom, are composed of an α-hydroxynitrile-type aglycone and a sugar moiety (usually D-glucose) (Selmar 2010). The production of HCN depends on both the biosynthesis of cyanogenic glycosides and on the presence (or absence) of their degrading enzymes. The tissue compartmentalization of cyanogenic glycosides and their hydrolyzing enzymes prevents large-scale hydrolysis in intact plant tissue. Their biosynthetic precursors are different L-amino acids that upon hydroxylation are converted to aldoximes and then nitriles, which undergo subsequent hydroxylation and glycosylation. A typical cyanogenic glycoside is dhurrin (Nielsen and Møller 2000).

21.5.3.4 Glucosinolates

Another common example of prototoxins is glucosinolates which contain sulphur and nitrogen and are derived from glucose and an amino acid (Selmar 2010). Glucosinolates vary in their side groups, resulting in a variety of biological activities. They are responsible for the pungency of plants such as mustard, cabbage and horseradish, which is due to mustard oils produced from glucosinolates when the plant suffers damage. Glucosinolates are hydrolyzed by either glucosinolase or thioglucosidase into glucose, HSO_4^- and one of the following aglycone derivatives: isothiocynates, thiocyanates, nitriles or related compounds. These natural chemicals contribute to plant defence against pests and diseases but are also enjoyed in small amounts by humans and are believed to contribute to the health-promoting properties of cabbages and related vegetables (Baskar et al. 2012; Mithen et al. 2010; Yan and Chen 2007).

21.5.3.5 Alkaloids

Unlike other groups of natural products, alkaloids are chemically and biologically heterogeneous. These sophisticated and numerous compounds are difficult to define, although they might be loosely and imprecisely described as basic, structurally complex secondary metabolites bearing one or more usually heterocyclic N atoms. They are typically biosynthesized by plants from amino acids, and their pharmacological activity is characterized by a relative toxicity and action on the nervous system. The ambiguity of this definition is due to the existence of compounds considered as alkaloids that do not meet all the above criteria.

Alkaloids are widely distributed among higher plants and fungi but also in animals (insects, toads, frogs, beavers). They are mainly found in angiosperms, above all in dicotyledons, tending to be concentrated in the most primitive families (Ranunculaceae, Menispermaceae, Papaveraceae) as well as the most evolved (Loganiaceae, Apocynaceae, Rubiaceae, Solanaceae) (Saporito et al. 2012; Daly et al. 2005). They have a lesser and more irregular presence in the intermediate families. In terms of geographical distribution, the percentage of plants with alkaloids is inversely proportional with latitude. This latitudinal gradient is likely a response to environmental pressures, thus corroborating the defensive role of alkaloids (Griffin and Lin 2000).

From the biogenetic point of view, alkaloids can be classified into three groups: true alkaloids, protoalkaloids and pseudoalkaloids. True alkaloids have at least one heterocyclic nitrogen, which originates from an amine formed by the decarboxylation of an amino acid. Protoalkaloids also derive from amino acids but differ from true alkaloids in that the nitrogen from the amino group of the amino acid is part of an aliphatic chain. Pseudoalkaloids have *N*-heterocyclic systems, but do not originate from amino acids.

The biosynthesis of alkaloids is very diverse as it can have many different starting points (Glenn et al. 2013; Roberts et al. 2010; Facchini 2001, 2006). Some are derived from the amino acid L-ornithine (tropane, pyrrolidine and pyrrolizidine alkaloids), L-aspartic acid (pyridine and isoquinuclidine alkaloids), L-lysine (piperidine and quinilizidine alkaloids), L-tyrosine (isoquinoline and tetrahydroisoquinoline alkaloids), L-tryptophan (indole, quinuclidine and quinoline alkaloids), L-histidine (imidazole alkaloids) and anthranilic acid (quinazoline and other minor alkaloids) (Langel et al. 2011). Pseudoalkaloids can arise from the pathway of terpenes (monoterpene, sesquiterpene, diterpene and steroidal alkaloids) or polyketides (polyketide alkaloids). To complicate matters a little further, some alkaloids have two cores from different sources (nicotine), while others are of mixed biosynthetic origin (monoterpene indole alkaloids). The largest groups among the true alkaloids are derivatives of aromatic amino acids L-Tyr and L-Trp (Salim and De Luca 2013; O'Connor and McCoy 2006).

Despite their considerable diversity, the biosynthesis of all alkaloids follows a certain pattern, involving the following steps: preparation of precursors, primary cyclization, preparation of intermediates, secondary cyclizations and/or dimerizations and, finally, diversification and rearrangement. All these steps are not required for the synthesis of all alkaloids, but the arrival at

any particular step entails having passed through all those leading up to it:

(a) Preparation of precursors involves the action of enzymes such as decarboxylases, transaminases, aminooxidases, hydroxylases (cytochrome P_{450} monooxygenases) and methyltransferases (*N*-methyltransferases, *O*-methyltransferases), which act on the initial substrate to obtain a product with amine groups and/or aldehyde that can serve as a substrate in the next step. Examples are the formation of dopamine and 4-hydroxyphenylacetaldehyde from tyrosine and 5-aminopentanal from lysine. The biosynthesis of alkaloids such as mescaline (a hallucinogenic protoalkaloid found in *Lophophora williamsii*) is completed in this step (Sato et al. 2007).

(b) Mechanisms of primary cyclization involve processes such as Schiff base formation or Mannich condensation. The generation of the *N*-methyl-Δ^1-pyrrolinium cation (precursor of tropane and pyrrolizidine alkaloids) from 4-*N*-methylbutanal (formed from ornithine) and the biosynthesis of *O*-methylnorbelladine in the Amaryllidaceae alkaloids are examples of Schiff base formation. Examples of Mannich condensation include the synthesis of (*S*)-norcoclaurine from dopamine and 4-hydroxyphenylacetaldehyde, using (*S*)-norcoclaurine synthase, and the formation of strictosidine (precursor of monoterpene indole alkaloids) from tryptamine (formed from tryptophan) and secologanin (monoterpene) by strictosidine synthase (Bastida et al. 2006; Bonamore et al. 2010; Stoeckigt et al. 2008).

(c) Preparation of intermediates. After the primary cyclizations, alkaloids are prepared to undergo secondary cyclizations by hydroxylases and methyltransferases. This step essentially defines the positions of the free phenol groups in each molecule, which condition the type and direction of cyclization. The formation of (*S*)-reticuline, (*S*)-protosinomenine, (*S*)-orientaline, (*S*)-isoorientaline and (*S*)-*N*-methylcoclaurine from (*S*)-norcoclaurine is an example of this step. Papaverine (isoquinoline alkaloid), which lacks free phenol groups, is an example of an alkaloid whose biosynthetic process ends at this step (Ziegler et al. 2009; Liscombe and Facchini 2008).

(d) Secondary cyclizations and/or dimerizations are of two basic types: the more common phenol oxidative coupling and the formation of the berberine bridge. In the oxidative coupling reaction of phenols, there are specific phenol oxidase enzymes that remove a proton from two phenol groups, creating two mesomeric and highly reactive phenolate radicals. These radicals rapidly disappear by coupling or dimerization, forming a stable covalent bond between the two electrons of the radicals. The *O*-methylations occurring before the phenol oxidative coupling are of great importance in determining the coupling direction: *ortho-ortho*, *para-para*, *ortho-para*, etc. For example, isoboldine (an aporphine alkaloid) is formed from reticuline by an *o-p′* intramolecular coupling, while salutaridine synthase converts (*R*)-reticuline into salutaridine (the precursor of all morphine alkaloids in *Papaver somniferum*) by a *p-o′* intramolecular coupling. Intermolecular oxidative phenol couplings may form dimeric alkaloids like tubocurarine (Ziegler et al. 2009). The formation of the berberine bridge is an oxidative cyclization mechanism performed by the berberine bridge enzyme (BBE), which catalyzes the conversion of (*S*)-reticuline to (*S*)-scoulerine by the formation of a carbon-carbon bond between the *N*-methyl group and the phenolic ring. BBE is a highly selective enzyme that can only act on (*S*)-reticuline. (*S*)-scoulerine is the precursor of all alkaloids derived from the berberine core (protoberberine, protopine, phthalideisoquinoline, benzophenanthridine and rhoeadine alkaloids) (Hagel and Facchini 2013; Fraaije and Mattevi 2008).

(e) Diversification. Once the secondary cyclizations are completed, many alkaloids enter a process of diversification through hydroxylation (hydroxylases), methylation (methyltransferases), demethylation (monooxygenases) or formation of methylenedioxy bridges

(oxidative cyclization mechanism). It should be borne in mind that many of the phenol groups have fulfilled their function after the secondary cyclizations. An example of this step is the conversion of (*S*)-scoulerine into (*S*)-stylopine, which involves the formation of two methylenedioxy bridges (Grycova et al. 2007).

(f) Bond cleavage and rearrangement. Prior to rearrangement, there are two basic types of bond cleavage involving either CC or CN bonds. An example of the former is the formation of *Erythrina* alkaloids. A neoproaporphine-type intermediate is generated from (*S*)-norprotosinomenine by a *p*-*p*′ phenol oxidative coupling mechanism, which after a CC bond cleavage and subsequent rearrangement leads to erysodienone, the first *Erythrina*-type alkaloid (Reimann 2007). Examples of the cleavage of CN bonds include the formation of protopine or sanguinarine (benzophenanthridine alkaloid) from (*S*)-stylopine (Hagel and Facchini 2013).

References

Abad-Martínez MJ, Bedoya-Del Olmo LM, Apaza-Ticona L, Bermejo-Benito P (2012) The *Artemisia* L. genus: a review of bioactive sesquiterpene lactones. In: Atta-ur-Rahman FRS (ed) Studies in natural products chemistry: bioactive natural products, vol 37. Elsevier, Amsterdam, pp 43–65

Abe I (2007) Enzymatic synthesis of cyclic triterpenes. Nat Prod Rep 24:1311–1331

Abe I (2010) Engineered biosynthesis of plant polyketides. Structure-based and precursor-directed approach. In: Piel J (ed) Topics in current chemistry: natural products via enzymatic reactions, vol 297. Springer, Heidenberg, pp 45–66

Abrar M, Anjum FM, Butt MS, Pasha I, Randhawa MA, Saeed F, Waqas K (2013) Aflatoxins: biosynthesis, occurrence, toxicity, and remedies. Crit Rev Food Sci Nutr 53:862–874

Ahuja I, Kissen R, Bones AM (2012) Phytoalexins in defense against pathogens. Trends Plant Sci 17:73–90

Akhila A (2007) Metabolic engineering of biosynthetic pathways leading to isoprenoids: mono- and sesquiterpenes in plastids and cytosol. J Plant Interact 2:195–204

Amthor JS (2003) Efficiency of lignin biosynthesis: a quantitative analysis. Ann Bot 91:673–695

Apers S, Vlietinck A, Pieters L (2004) Lignans and neolignans as lead compounds. Phytochem Rev 2:201–207

Ashihara H, Sano H, Crozier A (2008) Caffeine and related purine alkaloids: biosynthesis, catabolism, function and genetic engineering. Phytochemistry 69:841–856

Ashihara H, Yokota T, Crozier A (2013) Biosynthesis and catabolism of purine alkaloids. In: Giglioli-Guivarc'h N (ed) Advances in botanical research: new light on alkaloid biosynthesis and future prospects, vol 68. Academic, London, pp 111–138

Ashour M, Wink M, Gershenzon J (2010) Biochemistry of terpenoids: monoterpenes, sesquiterpenes and diterpenes. In: Wink M (ed) Annual plant reviews: biochemistry of plant secondary metabolism, vol 40. Wiley-Blackwell, Oxford, pp 258–303

Ayasse M, Stoekl J, Francke W (2011) Chemical ecology and pollinator-driven speciation in sexually deceptive orchids. Phytochemistry 72:1667–1677

Bach TMH, Takagi H (2013) Properties, metabolisms, and applications of L-proline analogues. Appl Microbiol Biotechnol 97:6623–6634

Barry CS (2010) Factors influencing the ripening and quality of fleshy fruits. In: Østergaard L (ed) Annual plant reviews: fruit development and seed dispersal, vol 38. Wiley-Blackwell, Oxford, pp 296–325

Baskar V, Gururani MA, Yu JW, Park SW (2012) Engineering glucosinolates in plants: current knowledge and potential uses. Appl Biochem Biotechnol 168:1694–1717

Bastida J, Lavilla R, Viladomat F (2006) Chemical and biological aspects of *Narcissus* alkaloids. In: Cordell GA (ed) The alkaloids: chemistry and biology, vol 63. Elsevier, San Diego, pp 87–179

Bathori M, Toth N, Hunyadi A, Marki A, Zador E (2008) Phytoecdysteroids and anabolic-androgenic steroids – structure and effects on humans. Curr Med Chem 15:75–91

Bede JC, Tobe SS (2000) Insect juvenile hormones in plants. In: Atta-ur-Rahmen FRS (ed) Studies in natural products chemistry bioactive natural products part C, vol 22. Elsevier, Amsterdam, pp 369–418

Beerhues L, Liu B (2009) Biosynthesis of biphenyls and benzophenones: evolution of benzoic acid-specific type III polyketide synthases in plants. Phytochemistry 70:1719–1727

Bell EA (2003) Nonprotein amino acids of plants: significance in medicine, nutrition, and agriculture. J Agric Food Chem 51:2854–2865

Bence AK, Crooks PA (2003) The mechanism of L-canavanine cytotoxicity: arginyl tRNA synthetase as a novel target for anticancer drug discovery. J Enzym Inhib Med Chem 18:383–394

Bernhardt R (2006) Cytochromes P 450 as versatile biocatalysts. J Biotechnol 124:128–145

Bertin C, Yang X, Weston LA (2003) The role of root exudates and allelochemicals in the rhizosphere. Plant Soil 256:67–83

Blomquist GJ, Figueroa-Teran R, Aw M, Song MM, Gorzalski A, Abbott NL, Chang E, Tittiger C (2010) Pheromone production in bark beetles. Insect Biochem Mol Biol 40:699–712

Bonamore A, Barba M, Botta B, Boffi A, Macone A (2010) Norcoclaurine synthase: mechanism of an enantioselective pictet-spengler catalyzing enzyme. Molecules 15:2070–2207

Boronat A (2010) The methylerythritol 4-phosphate pathway: regulatory role in plastid isoprenoid biosynthesis. In: Rebeiz CA, Benning C, Bohnert HJ, Daniell H, Hoober JK, Lichtenhaler HK, Portis AR, Tripathy B (eds) Advances in photosynthesis and respiration: chloroplast basics and applications, vol 31. Springer, Dordrecht, pp 119–126

Bourgaud F, Hehn A, Larbat R, Doerper S, Gontier E, Kellner S, Matern U (2006) Biosynthesis of coumarins in plants: a major pathway still to be unravelled for cytochrome P_{450} enzymes. Phytochem Rev 5:293–308

Brand S, Hoelscher D, Schierhorn A, Svatos A, Schroeder J, Schneider B (2006) A type III polyketide synthase from Wachendorfia thyrsiflora and its role in diarylheptanoid and phenylphenalenone biosynthesis. Planta 224:413–428

Bringmann G, Irmer A (2008) Acetogenic anthraquinones: biosynthetic convergence and chemical evidence of enzymatic cooperation in nature. Phytochem Rev 7:499–511

Bringmann G, Irmer A, Feineis D, Gulder TAM, Fiedler HP (2009) Convergence in the biosynthesis of acetogenic natural products from plants, fungi, and bacteria. Phytochemistry 70:1776–1786

Brown GD (2010) The biosynthesis of artemisinin (Qinghaosu) and the phytochemistry of Artemisia annua L. (Qinghao). Molecules 15:7603–7698

Bucar F (2013) Phytoestrogens in plants: with special reference to isoflavones. In: Preedy VR (ed) Food and nutritional components in focus: isoflavones, vol 5. RSC Publishing, Cambridge, pp 14–27

Buer CS, Imin N, Djordjevic MA (2010) Flavonoids: new roles for old molecules. J Integr Plant Biol 52:98–111

Bukhari SNA, Jasamai M, Jantan I, Ahmad W (2013) Review of methods and various catalysts used for chalcone synthesis. Mini-Rev Org Chem 10:73–83

Buzzini P, Arapitsas P, Goretti M, Branda E, Turchetti B, Pinelli P, Ieri F, Romani A (2008) Antimicrobial and antiviral activity of hydrolysable tannins. Mini-Rev Org Chem 8:1179–1187

Cai B, Jack AM, Lewis RS, Dewey RE, Bush LP (2013) (*R*)-nicotine biosynthesis, metabolism and translocation in tobacco as determined by nicotine demethylase mutants. Phytochemistry 95:188–196

Cameron RK (2000) Salicylic acid and its role in plant defense responses: what do we really know? Physiol Mol Plant Pathol 56:91–93

Campa C, Rakotomalala JJR, de Kochko A, Hamon S (2008) Chlorogenic acids: diversity in green beans of wild coffee species. Adv Plant Physiol 10:421–437

Cárdenas L, Quinto C (2009) Rhizobium-legume interaction: recent findings in early Nod factor perception. Curr Top Plant Biol 10:17–26

Cazzonelli CI (2011) Carotenoids in nature: insights from plants and beyond. Funct Plant Biol 38:833–847

Chaubet N, Pacquit V, Sivadon P, Grima-Pattenati J (2003) Control of genes involved the phenylpropanoid pathway with emphasis on lignin biosynthesis. Curr Top Plant Biol 4:117–128

Chen Z, Zheng Z, Huang J, Lai Z, Fan B (2009) Biosynthesis of salicylic acid in plants. Plant Signal Behav 4:493–496

Chon SU, Nelson CJ (2010) Allelopathy in compositae plants. A review. Agron Sustain Dev 30:349–358

Chong J, Poutaraud A, Hugueney P (2009) Metabolism and roles of stilbenes in plants. Plant Sci 177:143–155

Croteau RB, Davis EM, Ringer KL, Wildung MR (2005) (−)-Menthol biosynthesis and molecular genetics. Naturwissenschaften 92:562–577

Curini M, Cravotto G, Epifano F, Giannone G (2006) Chemistry and biological activity of natural and synthetic prenyloxycoumarins. Curr Med Chem 13:199–222

Daly JW, Spande TF, Garraffo HM (2005) Alkaloids from amphibian skin: a tabulation of over eight-hundred compounds. J Nat Prod 68:1556–1575

Dao TTH, Linthorst HJM, Verpoorte R (2011) Chalcone synthase and its functions in plant resistance. Phytochem Rev 10:397–412

Dar AA, Arumugam N (2013) Lignans of sesame: purification methods, biological activities and biosynthesis – a review. Bioorg Chem 50:1–10

Dev A, Tapas S, Pratap S, Kumar P (2012) Structure and function of enzymes of shikimate pathway. Curr Bioinform 7:374–391

Dinan L (2001) Phytoecdysteroids: biological aspects. Phytochemistry 57:325–339

Dinkova-Kostova AT (2008) Phytochemicals as protectors against ultraviolet radiation: versatility of effects and mechanisms. Planta Med 74:1548–1559

Dixon RA (2001) Natural products and plant disease resistance. Nature 411:843–847

Dixon RA (2004) Phytoestrogens. Annu Rev Plant Biol 55:225–261

Dixon DP, Skipsey M, Edwards R (2010) Roles for glutathione transferases in plant secondary metabolism. Phytochemistry 71:338–350

Drew DP, Krichau N, Reichwald K, Simonsen HT (2009) Guaianolides in apiaceae: perspectives on pharmacology and biosynthesis. Phytochem Rev 8:581–599

Einhellig FA (2002) The physiology of allelochemical action: clues and views. In: Reigosa MJ, Pedrol N (eds) Allelopathy: from molecules to ecosystems. Science Publishers, Enfield, pp 1–23

Einhellig FA (2004) Mode of allelochemical action of phenolic compounds. In: Macias FA, Galindo JCG, Molinillo JMG, Cutler HG (eds) Allelopathy: chemistry and mode of action of allelochemicals. CRC Press, Boca Raton, pp 217–238

El-Sayed M, Verpoorte R (2007) Catharanthus terpenoid indole alkaloids: biosynthesis and regulation. Phytochem Rev 6:277–305

Erasto P, Viljoen AM (2008) Limonene – a review: biosynthetic, ecological and pharmacological relevance. Nat Prod Commun 3:1193–1202

Facchini PJ (2001) Alkaloid biosynthesis in plants: biochemistry, cell biology, molecular regulation, and metabolic engineering applications. Annu Rev Plant Physiol Plant Mol Biol 52:29–66

Facchini PJ (2006) Regulation of alkaloid biosynthesis in plants. In: Cordell GA (ed) The alkaloids: chemistry and biology, vol 63. Elsevier, San Diego, pp 1–44

Faivre C, Ghedira K, Goetz P, Lejeune R, Staub H (2007) *Humulus lupulus* L. Phytotherapie 5:86–89

Fatope MO, Adoum OA, Takeda Y (2000) C(18) acetylenic fatty acids of *Ximenia americana* with potential pesticidal activity. J Agric Food Chem 48:1872–1874

Ferreira RB, Monteiro S, Freitas R, Santos CN, Chen Z, Batista LM, Duarte J, Borges A, Teixeira AR (2006) Fungal pathogens: the battle for plant infection. Crit Rev Plant Sci 25:505–524

Ferrer JL, Austin MB, Stewart C, Noel JP (2008) Structure and function of enzymes involved in the biosynthesis of phenylpropanoids. Plant Physiol Biochem 46:356–370

Fowler ZL, Koffas MAG (2009) Biosynthesis and biotechnological production of flavanones: current state and perspectives. Appl Microbiol Biotechnol 83:799–808

Fraaije MW, Mattevi A (2008) Cyclization in concert. Nat Chem Biol 4:719–721

Fujii I (2010) Functional analysis of fungal polyketide biosynthesis genes. J Antibiot 63:207–218

Gachon CMM, Langlois-Meurinne M, Saindrenan P (2005) Plant secondary metabolism glycosyltransferases: the emerging functional analysis. Trends Plant Sci 10:542–549

Gaffield W (2000) The veratrum alkaloids: natural tools for studying embryonic development. In: Atta-ur-Rahmen FRS (ed) Studies in natural products chemistry bioactive natural products, vol 23. Elsevier, Amsterdam, pp 563–589

Galliot C, Stuurman J, Kuhlemeier C (2006) The genetic dissection of floral pollination syndromes. Curr Opin Plant Biol 9:78–82

Glas JJ, Schimmel BCJ, Alba JM, Escobar-Bravo R, Schuurink RC, Kant MR (2012) Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. Int J Mol Sci 13:17077–17103

Glenn WS, Runguphan W, O'Connor SE (2013) Recent progress in the metabolic engineering of alkaloids in plant systems. Curr Opin Biotechnol 24:354–365

Gliszczynska A, Brodelius PE (2012) Sesquiterpene coumarins. Phytochem Rev 11:77–96

Gnonlonfin GJB, Sanni A, Brimer L (2012) Review scopoletin – a coumarin phytoalexin with medicinal properties. Crit Rev Plant Sci 31:47–56

Gosch C, Halbwirth H, Stich K (2010) Phloridzin: biosynthesis, distribution and physiological relevance in plants. Phytochemistry 71:838–843

Goswami SK, Das DK (2009) Resveratrol and chemoprevention. Cancer Lett 284:1–6

Graewert T, Groll M, Rohdich F, Bacher A, Eisenreich W (2011) Biochemistry of the non-mevalonate isoprenoid pathway. Cell Mol Life Sci 68:3797–3814

Grayer RJ, Veitch NC (2006) Flavonones and dihydroflavonols. In: Andersen OM, Markham KR (eds) Flavonoids: chemistry, biochemistry and applications. CRC Press, Boca Raton, pp 917–1002

Griffin WJ, Lin GD (2000) Chemotaxonomy and geographical distribution of tropane alkaloids. Phytochemistry 53:623–637

Gronover CS, Wahler D, Pruefer D (2011) Natural rubber biosynthesis and physic-chemical studies on plant derived latex. In: Elnashar M (ed) Biotechnology of biopolymers. InTech Open, Rijeka, pp 75–88

Grunewald J, Marahiel MA (2013) Nonribosomal peptide synthesis. In: Kastin AJ (ed) Handbook of biologically active peptides, 2nd edn. Academic, San Diego, pp 138–149

Grycova L, Dostal J, Marek R (2007) Quaternary protoberberine alkaloids. Phytochemistry 68:150–175

Hagel JM, Facchini PJ (2013) Benzylisoquinoline alkaloid metabolism: a century of discovery and a brave new world. Plant Cell Physiol 54:647–672

Hagel JM, Yeung EC, Facchini PJ (2008) Got milk? The secret life of laticifers. Trends Plant Sci 13:631–639

Hammer SC, Syren PO, Seitz M, Nestl BM, Hauer B (2013) Squalene hopene cyclases: highly promiscuous and evolvable catalysts for stereoselective CC and CX bond formation. Curr Opin Chem Biol 17:293–300

Hammerschmidt R (2009) Systemic acquired resistance. In: Van Loon LC (ed) Advances in botanical research: plant innate immunity, vol 51. Academic, London, pp 173–222

Han YS, Van der Heijden R, Verpoorte R (2001) Biosynthesis of anthraquinones in cell cultures of the Rubiaceae. Plant Cell Tissue Organ Cult 67:201–220

Harborne JB (2007) Secondary metabolites: attracting pollinators. In: Roberts K (ed) Handbook of plant science, vol 2. Wiley, Chichester, pp 1050–1054

Hartmann MA (2004) Sterol metabolism and functions in higher plants. In: Daum G (ed) Topics in current genetics: lipid metabolism and membrane biogenesis, vol 6. Springer, Berlin, pp 183–211

Hartmann T, Ober D (2000) Biosynthesis and metabolism of pyrrolizidine alkaloids in plants and specialized insect herbivores. In: Leeper FJ, Vederas JC (eds) Topics in current chemistry: biosynthesis aromatic polyketides, isoprenoids, alkaloids, vol 209. Springer, Berlin, pp 207–243

Harwood JL (2005) Fatty acid biosynthesis. In: Murphy DJ (ed) Plant lipids: biology, utilisation and manipulation. Blackwell, Oxford, pp 27–66

Hassan S, Mathesius U (2012) The role of flavonoids in root-rhizosphere signaling. J Exp Bot 63:3429–3444

He F, Pan QH, Shi Y, Duan CQ (2008) Biosynthesis and genetic regulation of proanthocyanidins in plants. Molecules 13:2674–2703

He F, Mu L, Yan GL, Liang NN, Pan QH, Wang J, Reeves MJ, Duan CQ (2010) Biosynthesis of anthocyanins

and their regulation in colored grapes. Molecules 15:9057–9091
Hovhannisyan A, Matz M, Gebhardt R (2009) From teratogens to potential therapeutics: natural inhibitors of the Hedgehog signaling network come of age. Planta Med 75:1371–1380
Hunter WN (2007) The non-mevalonate pathway of isoprenoid precursor biosynthesis. J Biol Chem 282:21573–21577
Iglesias A, Meins F Jr (2000) Vacuoles and plant defense. In: Robinson DG, Rogers JC (eds) Annual plant reviews: vacuolar compartments, vol 5. Sheffield Academic Press, Sheffield, pp 112–132
Ionkova I (2010) Podophyllotoxin and related lignans: biotechnological production by in vitro plant cell cultures. In: Arora R (ed) Medicinal plant biotechnology. CABI, Wallingford, pp 138–155
James JT, Dubery IA (2009) Pentacyclic triterpenoids from the medicinal herb, centella asiatica (L.) urban. Molecules 14:3922–3941
Jeandet P, Clement C, Courot E, Cordelier S (2013) Modulation of phytoalexin biosynthesis in engineered plants for disease resistance. Int J Mol Sci 14:14136–14170
Jensen SR, Franzyk H, Wallander E (2002) Chemotaxonomy of the Oleaceae: Iridoids as taxonomic markers. Phytochemistry 60:213–231
Jez JM, Ferrer JL, Bowman ME, Austin MB, Schroder J, Dixon RA, Noel JP (2001) Structure and mechanism of chalcone synthase-like polyketide synthases. J Ind Microbiol Biotechnol 27:393–398
Jones P, Vogt T (2001) Glycosyltransferases in secondary plant metabolism: tranquilizers and stimulant controllers. Planta 213:164–174
Jordan BR (2004) Plant pigments and protection against UV-B radiation. In: Davies K (ed) Annual plant reviews: plant pigments and their manipulation, vol 14. Blackwell, Oxford, pp 275–292
Jorgensen K, Rasmussen AV, Morant M, Nielsen AH, Bjarnholt N, Zagrobelny M, Bak S, Moller BL (2005) Metabolon formation and metabolic channeling in the biosynthesis of plant natural products. Curr Opin Plant Biol 8:280–291
Kato-Noguchi H, Peters RJ (2013) The role of momilactones in rice allelopathy. J Chem Ecol 39:175–185
Kaur B, Chakraborty D (2013) Biotechnological and molecular approaches for vanillin production: a review. Appl Biochem Biotechnol 169:1353–1372
Keeling CI, Bohlmann J (2006) Diterpene resin acids in conifers. Phytochemistry 67:2415–2423
Keeling CI, Bohlmann J (2012) Plant terpenoids. In: Civjan N (ed) Natural products in chemical biology. Wiley, Hoboken, pp 127–142
Koch K, Barthlott W (2006) Plant epicuticular waxes: chemistry, form, self-assembly and function. Nat Prod Commun 1:1067–1072
Koglin A, Walsh CT (2009) Structural insights into nonribosomal peptide enzymatic assembly lines. Nat Prod Rep 26:987–1000
Koleckar V, Kubikova K, Rehakova Z, Kuca K, Jun D, Jahodar L, Opletal L (2008) Condensed and hydrolysable tannins as antioxidants influencing the health. Mini-Rev Med Chem 8:436–447
Kreis W, Mueller-Uri F (2010) Biochemistry of sterols, cardiac glycosides, brassinosteroids, phytoecdysteroids and steroid saponins. In: Wink M (ed) Annual plant reviews: biochemistry of plant secondary metabolism, vol 40. Wiley-Blackwell, Oxford, pp 304–363
Kutchan TM (2005) A role for intra- and intercellular translocation in natural product biosynthesis. Curr Opin Plant Biol 8:292–300
Kutschera U, Wang ZY (2012) Brassinosteroid action in flowering plants: a Darwinian perspective. J Exp Bot 63:3511–3522
Kuzuyama T (2002) Mevalonate and nonmevalonate pathways for the biosynthesis of isoprene units. Biosci Biotechnol Biochem 66:1619–1627
Lange BM, Turner GW (2013) Terpenoid biosynthesis in trichomes-current status and future opportunities. Plant Biotechnol J 11:2–22
Langel D, Ober D, Pelser PB (2011) The evolution of pyrrolizidine alkaloid biosynthesis and diversity in the Senecioneae. Phytochem Rev 10:3–74
Lapcik O (2007) Isoflavonoids in nonleguminous taxa: a rarity or a rule? Phytochemistry 68:2909–2916
Latruffe N, Rifler JP (2013) Bioactive polyphenols from grapes and wine emphasized with resveratrol. Curr Pharm Des 19:6053–6063
Lazniewska J, Macioszek VK, Kononowicz AK (2012) Plant-fungus interface: the role of surface structures in plant resistance and susceptibility to pathogenic fungi. Physiol Mol Plant Pathol 78:24–30
Lichtenthaler HK (2012) Biosynthesis, localization and concentration of carotenoids in plants and algae. In: Eaton-Rye JJ, Tripathy BC, Sharkey TD (eds) Advances in photosynthesis and respiration: photosynthesis, vol 34. Springer, Dordrecht, pp 95–112
Liscombe DK, Facchini PJ (2008) Evolutionary and cellular webs in benzylisoquinoline alkaloid biosynthesis. Curr Opin Biotechnol 19:173–180
Liu YQ, Yang L, Tian X (2007) Podophyllotoxin: current perspectives. Curr Bioact Compd 3:37–66
Liu CJ, Miao YC, Zhang KW (2011) Sequestration and transport of lignin monomeric precursors. Molecules 16:710–727
MacDonald MJ, D'Cunha GB (2007) A modern view of phenylalanine ammonia lyase. Biochem Cell Biol 85:273–282
Macias FA, Molinillo JMG, Varela RM, Galindo JCG (2007) Allelopathy – a natural alternative for weed control. Pest Manag Sci 63:327–348
Maeda H, Dudareva N (2012) The shikimate pathway and aromatic amino acid biosynthesis in plants. Annu Rev Plant Biol 63:73–105
Mahroug S, Burlat V, St-Pierre B (2007) Cellular and subcellular organisation of the monoterpenoid indole alkaloid pathway in *Catharanthus roseus*. Phytochem Rev 6:363–381

Makkar HPS, Sidhuraju P, Becker K (2007) Methods in molecular biology, vol 393, Plant Secondary Metabolites. Humana Press, Totowa

Mandadi KK, Scholthof KBG (2013) Plant immune responses against viruses: how does a virus cause disease? Plant Cell 25:1489–1505

Maor R, Shirasu K (2005) The arms race continues: battle strategies between plants and fungal pathogens. Curr Opin Microbiol 8:399–404

Marsh ENG, Waugh MW (2013) Aldehyde decarbonylases: enigmatic enzymes of hydrocarbon biosynthesis. ACS Catal 3:2515–2521

McKeon TA, Lin JT (2002) Biosynthesis of ricinoleic acid for castor-oil production. In: Kuo TM, Gardner HW (eds) Lipid biotechnology. Marcel Dekker, New York, pp 129–139

Michel T, Halabalaki M, Skaltsounis AL (2013) New concepts, experimental approaches, and dereplication strategies for the discovery of novel phytoestrogens from natural sources. Planta Med 79:514–532

Milborrow BV (2001) The pathway of biosynthesis of abscisic acid in vascular plants: a review of the present state of knowledge of ABA biosynthesis. J Exp Bot 52:1145–1164

Miller RE, Tuck KL (2013) Reports on the distribution of aromatic cyanogenic glycosides in Australian tropical rainforest tree species of the Lauraceae and Sapindaceae. Phytochemistry 92:146–152

Miller K, Neilan B, Sze DMY (2008) Development of taxol and other endophyte produced anti-cancer agents. Recent Pat Anticancer Drug Discov 3:14–19

Mithen R, Bennett R, Marquez J (2010) Glucosinolate biochemical diversity and innovation in the Brassicales. Phytochemistry 71:2074–2086

Mithoefer A (2011) Carnivorous pitcher plants: insights in an old topic. Phytochemistry 72:1678–1682

Mithoefer A, Boland W (2012) Plant defense against herbivores: chemical aspects. Annu Rev Plant Biol 63:431–450

Mueller C (2009) Role of glucosinolates in plant invasiveness. Phytochem Rev 8:227–242

Mueller M, Buchbauer G (2011) Essential oil components as pheromones. A review. Flavour Frag J 26:357–377

Murtaza G, Latif U, Najam-Ul-Haq M, Sajjad A, Karim S, Akhtar M, Hussain I (2013) Resveratrol: an active natural compound in red wines for health. J Food Drug Anal 21:1–12

Musiol EM, Weber T (2012) Discrete acyltransferases involved in polyketide biosynthesis. MedChemComm 3:871–886

Nakata R, Takahashi S, Inoue H (2012) Recent advances in the study on resveratrol. Biol Pharm Bull 35:273–279

Nakayama T (2002) Enzymology of aurone biosynthesis. J Biosci Bioeng 94:487–491

Narula N, Kothe E, Behl RK (2009) Role of root exudates in plant-microbe interactions. J Appl Bot Food Qual 82:122–130

Neilson EH, Goodger JQD, Woodrow IE, Moller BL (2013) Plant chemical defense: at what cost? Trends Plant Sci 18:250–258

Nielsen JS, Møller BL (2000) Cloning and expression of cytochrome P_{450} enzymes catalyzing the conversion of tyrosine to p-hydroxyphenylacetaldoxime in the biosynthesis of cyanogenic glucosides in *Triglochin maritima*. Plant Physiol 122:1311–1321

Novo-Uzal E, Pomar F, Gomez-Ros LV, Espineira JM, Ros-Barcelo A (2012) Evolutionary history of lignins. In: Jouanin L, Lapierre C (eds) Advances in botanical research: lignins biosynthesis, biodegradation and bioengineering, vol 61. Academic, London, pp 311–350

O'Connor SE, McCoy E (2006) Terpene indole alkaloid biosynthesis. In: Romeo JT (ed) Recent advances in phytochemistry: integrative plant biochemistry, vol 40. Elsevier, Amsterdam, pp 1–22

Ober D, Kaltenegger E (2009) Pyrrolizidine alkaloid biosynthesis: evolution of a pathway in plant secondary metabolism. Phytochemistry 70:1687–1695

Ohnishi T, Yokota T, Mizutani M (2009) Insights into the function and evolution of P450s in plant steroid metabolism. Phytochemistry 70:1918–1929

Okada K (2011) The biosynthesis of isoprenoids and the mechanisms regulating it in plants. Biosci Biotechnol Biochem 75:1219–1225

Okuda T, Ito H (2011) Tannins of constant structure in medicinal and food plants - hydrolyzable tannins and polyphenols related to tannins. Molecules 16:2191–2217

Oldfield E, Lin FY (2012) Terpene biosynthesis: modularity rules. Angew Chem Int Ed 51:1124–1137

Oliveira PS, Freitas AVL (2004) Ant-plant-herbivore interactions in the neotropical cerrado savanna. Naturwissenschaften 91:557–570

Oudin A, Courtois M, Rideau M, Clastre M (2007) The iridoid pathway in Catharanthus roseus alkaloid biosynthesis. Phytochem Rev 6:259–276

Ozeki Y, Matsuba Y, Abe Y, Umemoto N, Sasaki N (2011) Pigment biosynthesis I. anthocyanins. In: Ashihara H, Crozier A, Komamine A (eds) Plant metabolism and biotechnology. Wiley, Chichester, pp 321–342

Pacini E, Nepi M, Vesprini JL (2003) Nectar biodiversity: a short review. Plant Syst Evol 238:7–21

Patocka J (2003) Biologically active pentacyclic triterpenes and their current medicine signification. J Appl Biomed 1:7–12

Pattillo CB (2011) Combretastatin derivatives in cancer: recent research reviewed. Drug Future 36:385–390

Pedras MSC, Yaya EE, Glawischnig E (2011) The phytoalexins from cultivated and wild crucifers: chemistry and biology. Nat Prod Rep 28:1381–1405

Peer WA, Murphy AS (2007) Flavonoids and auxin transport: modulators or regulators? Trends Plant Sci 12:556–563

Peiru S, Gramajo H, Menzella HG (2009) Design and synthesis of pathway genes for polyketide biosynthesis. In: Hopwood DA (ed) Methods in enzymology: complex enzymes in microbial natural product biosynthesis, part B, vol 459. Academic, San Diego, pp 319–337

Petersen M, Hans J, Matern U (2010) Biosynthesis of phenylpropanoids and related compounds. In: Wink M

(ed) Annual plant reviews: biochemistry of plant secondary metabolism, vol 40. Wiley-Blackwell, Oxford, pp 182–257

Phillips DR, Rasbery JM, Bartel B, Matsuda SPT (2006) Biosynthetic diversity in plant triterpene cyclization. Curr Opin Plant Biol 9:305–314

Pickard WF (2008) Laticifers and secretory ducts: two other tube systems in plants. New Phytol 177:877–888

Pollastri S, Tattini M (2011) Flavonols: old compounds for old roles. Ann Bot 108:1225–1233

Pourcel L, Bohorquez-Restrepo A, Irani NG, Grotewold E (2012) Anthocyanin biosynthesis, regulation, and transport: new insights from model species. In: Cheynier V, Sarni-Manchado P, Quideau S (eds) Recent advances in polyphenol research, vol 3. Wiley-Blackwell, Oxford, pp 143–160

Rasmann S, Agrawal AA (2009) Plant defense against herbivory: progress in identifying synergism, redundancy, and antagonism between resistance traits. Curr Opin Plant Biol 12:473–478

Razavi SM (2011) Plant coumarins as allelopathic agents. Int J Biol Chem 5:86–90

Reimann E (2007) Synthesis pathways to erythrina alkaloids and erythrina type compounds. In: Herz W, Falk H, Kirby GW (eds) Progress in the chemistry of organic natural products, vol 88. Springer, Wien, pp 1–62

Reina M, González-Coloma A (2007) Structural diversity and defensive properties of diterpenoid alkaloids. Phytochem Rev 6:81–95

Roberts MF, Strack D, Wink M (2010) Biosynthesis of alkaloids and betalains. In: Wink M (ed) Annual plant reviews: biochemistry of plant secondary metabolism, vol 40. Wiley-Blackwell, Oxford, pp 20–91

Rodríguez-Concepción M (2010) Supply of precursors for carotenoid biosynthesis in plants. Arch Biochem Biophys 504:118–122

Ruban AV, Johnson MP (2010) Xanthophylls as modulators of membrane protein function. Arch Biochem Biophys 504:78–85

Ryden AM, Kayser O (2007) Chemistry, biosynthesis and biological activity of artemisinin and related natural peroxides. In: Gupta RR, Khan MTH (eds) Topics in heterocyclic chemistry: bioactive heterocycles III, vol 9. Springer, Berlin, pp 1–31

Salim V, De Luca V (2013) Towards complete elucidation of monoterpene indole alkaloid biosynthesis pathway: *Catharanthus roseus* as a pioneer system. In: Giglioli-Guivarc'h N (ed) Advances in botanical research: new light on alkaloid biosynthesis and future prospects, vol 68. Academic, London, pp 1–37

Samuels L, Kunst L, Jetter R (2008) Sealing plant surfaces: cuticular wax formation by epidermal cells. Annu Rev Plant Physiol Plant Mol Biol 59:683–707

Saporito RA, Donnelly MA, Spande TF, Garraffo HM (2012) A review of chemical ecology in poison frogs. Chemoecology 22:159–168

Sasaki Y, Nagano Y (2004) Plant acetyl-CoA carboxylase: structure, biosynthesis, regulation, and gene manipulation for plant breeding. Biosci Biotechnol Biochem 68:1175–1184

Satake H, Ono E, Murata J (2013) Recent advances in the metabolic engineering of lignan biosynthesis pathways for the production of transgenic plant-based foods and supplements. J Agric Food Chem 61:11721–11729

Sato F, Inui T, Takemura T (2007) Metabolic engineering in isoquinoline alkaloid biosynthesis. Curr Pharm Biotechnol 8:211–218

Schmid KM, Ohlrogge JB (2008) Lipid metabolism in plants. In: Vance DE, Vance JE (eds) Biochemistry of lipids, lipoproteins and membranes, 5th edn. Elsevier, Amsterdam, pp 97–130

Selmar D (2010) Biosynthesis of cyanogenic glycosides, glucosinolates and non-protein amino acids. In: Wink M (ed) Annual plant reviews: biochemistry of plant secondary metabolism, vol 40. Wiley-Blackwell, Oxford, pp 92–181

Seo GW, Cho JY, Moon JH, Park KH (2011) Isolation and identification of cinnamic acid amides as antioxidants from *Allium fistulosum* L. and their free radical scavenging activity. Food Sci Biotechnol 20:555–560

Seybold SJ, Huber DPW, Lee JC, Graves AD, Bohlmann J (2006) Pine monoterpenes and pine bark beetles: a marriage of convenience for defense and chemical communication. Phytochem Rev 5:143–178

Shah J (2003) The salicylic acid loop in plant defense. Curr Opin Plant Biol 6:365–371

Shukla S, Singh SP (2001) Alkaloid profile in relation to different developmental stages of *Papaver somniferum* L. Phyton 41:87–96

Siedenburg G, Jendrossek D (2011) Squalene-hopene cyclases. Appl Environ Microbiol 77:3905–3915

Singh R, Kaur H (2009) Advances in synthetic approaches for the preparation of combretastatin-based anticancer agents. Synthesis 15:2471–2491

Soenderby IE, Geu-Flores F, Halkier BA (2010) Biosynthesis of glucosinolates; gene discovery and beyond. Trends Plant Sci 15:283–290

Spasova M, Ivanova G, Weber H, Ranz A, Lankmayr E, Milkova TS (2007) Amides of substituted cinnamic acids with aliphatic monoamines and their antioxidative potential. Oxid Commun 30:803–813

Stoeckigt J, Barleben L, Panjikar S, Loris EA (2008) 3D-Structure and function of strictosidine synthase – the key enzyme of monoterpenoid indole alkaloid biosynthesis. Plant Physiol Biochem 46: 340–355

Sturaro M, Motto M (2006) Plant cuticular waxes: biosynthesis and functions. Adv Plant Physiol 9:229–251

Subramanian S, Stacey G, Yu O (2007) Distinct, crucial roles of flavonoids during legume nodulation. Trends Plant Sci 12:282–285

Suzuki S, Umezawa T (2007) Biosynthesis of lignans and norlignans. J Wood Sci 53:273–284

Swiezewska E, Danikiewicz W (2005) Polyisoprenoids: structure, biosynthesis and function. Prog Lipid Res 44:235–258

Tanner G (2004) Condensed tannins. In: Davies K (ed) Annual plant reviews: plant pigments and their manipulation, vol 14. Blackwell, Oxford, pp 150–184

Taylor M, Ramsay G (2005) Carotenoid biosynthesis in plant storage organs: recent advances and prospects for improving plant food quality. Physiol Plant 124:143–151

Teixeira da Silva JA, Dobranszki J, Ross S (2013) Phloroglucinol in plant tissue culture. In Vitro Cell Dev Biol Plant 49:1–16

Tian L, Pang Y, Dixon RA (2008) Biosynthesis and genetic engineering of proanthocyanidins and (iso)flavonoids. Phytochem Rev 7:445–465

Tissier A (2012) Glandular trichomes: what comes after expressed sequence tags? Plant J 70:51–68

Tonfack LB, Moummou H, Latche A, Youmbi E, Benichou M, Pech JC, van der Rest B (2011) The plant SDR superfamily: involvement in primary and secondary metabolism. Curr Top Plant Biol 12:41–53

Toyomasu T (2008) Recent advances regarding diterpene cyclase genes in higher plants and fungi. Biosci Biotechnol Biochem 72:1168–1175

Trigo JR (2011) Effects of pyrrolizidine alkaloids through different trophic levels. Phytochem Rev 10:83–98

Tuli HS, Sharma AK, Sandhu SS, Kashyap D (2013) Cordycepin: a bioactive metabolite with therapeutic potential. Life Sci 93:863–869

Tzin V, Galili G (2010) New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. Mol Plant 3:956–972

Usera AR, O'Connor SE (2009) Mechanistic advances in plant natural product enzymes. Curr Opin Chem Biol 13:492–498

Veitch NC, Grayer RJ (2006) Chalcones, dihydrochalcones, and aurones. In: Andersen OM, Markham KR (eds) Flavonoids: chemistry, biochemistry and applications. CRC Press, Boca Raton, pp 1003–1100

Veitch NC, Grayer RJ (2011) Flavonoids and their glycosides, including anthocyanins. Nat Prod Rep 28:1626–1695

Verpoorte R, Contin A, Memelink J (2002) Biotechnology for the production of plant secondary metabolites. Phytochem Rev 1:13–25

Vetter J (2000) Plant cyanogenic glycosides. Toxicon 38:11–36

Vincken JP, Heng L, De Groot A, Gruppen H (2007) Saponins, classification and occurrence in the plant kingdom. Phytochemistry 68:275–297

Vogt T (2010) Phenylpropanoid biosynthesis. Mol Plant 3:2–20

von Lintig J, Vogt K (2004) Vitamin A formation in animals: molecular identification and functional characterization of carotene cleaving enzymes. J Nutr 134:251S–256S

Vranova E, Coman D, Gruissem W (2013) Network analysis of the MVA and MEP pathways for isoprenoid synthesis. Annu Rev Plant Biol 64:665–700

Walling LL (2009) Adaptive defence responses to pathogens and insects. In: Van Loon LC (ed) Advances in botanical research: plant innate immunity, vol 51. Academic, London, pp 551–612

Walton JD (2006) HC-toxin. Phytochemistry 67:1406–1413

Walton NJ, Mayer MJ, Narbad A (2003) Vanillin. Phytochemistry 63:505–515

Walton JD, Hallen-Adams HE, Luo H (2010) Ribosomal biosynthesis of the cyclic peptide toxins of *Amanita* mushrooms. Biopolymers 94:659–664

Wang X (2010) Structural studies and mechanisms of isoflavonoid biosynthesis. In: Thompson MJ (ed) Isoflavones: biosynthesis, occurrence and health effects. Nova Science, Hauppage, pp 239–254

Wang FP, Liang XT (2002) C20-diterpenoid alkaloids. In: Cordell GA (ed) The alkaloids: chemistry and biology, vol 59. Elsevier, San Diego, pp 1–280

Wefer J, Simon K, Lindel T (2013) Cubitane: a rare diterpenoid skeleton. Phytochem Rev 12:95–105

Weston LA, Duke SO (2003) Weed and crop allelopathy. Crit Rev Plant Sci 22:367–389

Weston LA, Mathesius U (2013) Flavonoids: their structure, biosynthesis and role in the rhizosphere, including allelopathy. J Chem Ecol 39:283–297

Wildermuth MC (2006) Variations on a theme: synthesis and modification of plant benzoic acids. Curr Opin Plant Biol 9:288–296

Wink M (2010) Annual plant reviews, vol 40, 2nd edn, Biochemistry of plant secondary metabolism. Wiley-Blackwell, Oxford

Winkel BSJ (2004) Metabolic channeling in plants. Annu Rev Plant Biol 55:85–107

Woronuk G, Demissie Z, Rheault M, Mahmoud S (2011) Biosynthesis and therapeutic properties of *Lavandula* essential oil constituents. Planta Med 77:7–15

Xie DY, Dixon RA (2005) Proanthocyanidin biosynthesis – still more questions than answers? Phytochemistry 66:2127–2144

Yan X, Chen S (2007) Regulation of plant glucosinolate metabolism. Planta 226:1343–1352

Yu F, Utsumi R (2009) Diversity, regulation, and genetic manipulation of plant mono- and sesquiterpenoid biosynthesis. Cell Mol Life Sci 66:3043–3052

Yu B, Sun J, Yang X (2012) Assembly of naturally occurring glycosides, evolved tactics, and glycosylation methods. Acc Chem Res 45:1227–1236

Zhang W, Tang Y (2009) In vitro analysis of type II polyketide synthase. In: Hopwood DA (ed) Methods in enzymology: complex enzymes in microbial natural product biosynthesis, part B, vol 459. Academic, San Diego, pp 367–393

Ziegler J, Facchini PJ, Geissler R, Schmidt J, Ammer C, Kramell R, Voigtlaender S, Gesell A, Pienkny S, Brandt W (2009) Evolution of morphine biosynthesis in opium poppy. Phytochemistry 70:1696–1707

Zulak KG, Bohlmann J (2010) Terpenoid biosynthesis and specialized vascular cells of conifer defense. J Integr Plant Biol 52:86–97

Photosynthesis

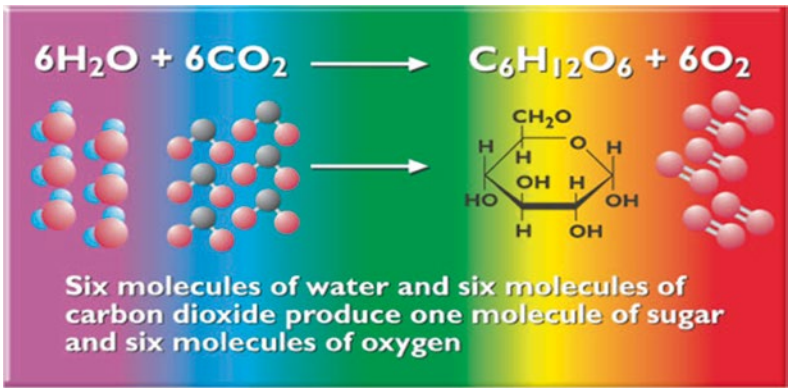
22

B. Sujatha

Abstract

Photosynthesis is the most important biological phenomenon on earth, and it is a multistep process utilizing three substrates (light, water, and carbon dioxide) to yield two primary products (oxygen and reduced carbohydrates) upon which all life in the biosphere is dependent.

The term photosynthesis means literally "synthesis using light." Photosynthetic organisms use solar energy to synthesize carbon compounds that cannot be formed without the input of energy. More specifically, light energy drives the synthesis of carbohydrates from carbon dioxide and water with the generation of oxygen.



Energy stored in these molecules can be used later to power cellular processes in the plant and can serve as the energy source for all forms of life.

B. Sujatha (✉)
Department of Botany, Andhra University,
Visakhapatnam 530 003, Andhra Pradesh, India
e-mail: sujathaau@yahoo.co.in

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_22,

Photosynthesis takes place in three stages:

1. Capturing radiant energy of sunlight by the chloroplast
2. Using the energy to make ATP and NADPH in the light reaction or photochemical reaction
3. Using the ATP and NADPH to power the synthesis of organic molecules from atmospheric carbon dioxide during the dark reaction or biochemical reaction

The most active photosynthetic tissue in higher plants is the mesophyll of leaves. Mesophyll cells have many chloroplasts, which contain the specialized light-absorbing green pigments, the chlorophylls. In photosynthesis, the plant uses solar energy to oxidize water, thereby forming large carbon compounds, primarily sugars. The complex series of reactions that culminate in the reduction of CO_2 include the thylakoid reactions and the carbon-fixation reactions.

The thylakoid reactions of photosynthesis takes place in the specialized internal membranes of the chloroplast called thylakoids. The end products of these thylakoid reactions are the high-energy compounds ATP and NADPH, which are used for the synthesis of sugars in the carbon-fixation reactions. These synthetic processes take place in the stroma of the chloroplasts, the aqueous region that surrounds the thylakoids.

In the chloroplast, light energy is converted into chemical energy by two different functional units called photosystems. The absorbed light energy is used to power the transfer of electrons through a series of compounds that act as electron donors and electron acceptors. The majority of electrons ultimately reduce NADP+ to NADPH and oxidize H_2O to O_2. Light energy is also used to generate a proton motive force across the thylakoid membrane, which is used to synthesis ATP.

Keywords

ATP • Chloroplasts • Photosynthesis • Photosystem I and II • Thylakoid membrane

22.1 Introduction

Life on earth depends on energy derived from the sun. Photosynthesis is the only known process of biological importance that can harvest this energy. Literally, the term photosynthesis means "synthesis using light." Green plants, cyanobacteria, and pigment containing prokaryotes carry out this process. They are called photoautotrophs. Photoautotrophs can use light as energy source and CO_2 as carbon source for the synthesis of carbohydrates. Photoautotrophs contain a photosynthetic apparatus that enables them to absorb light energy and package it as a bond energy in the form of ATP and NADPH. The energy of these compounds is used to drive the reactions that are involved in the fixation of carbon dioxide into carbohydrates. The carbohydrates, in turn, provide cellular source of energy for the synthesis of proteins, lipids, nucleic acids, and other cellular constituents not only in the photoautotrophs that produce them but also in nonphotosynthetic organisms that directly or indirectly consume photosynthetic organisms.

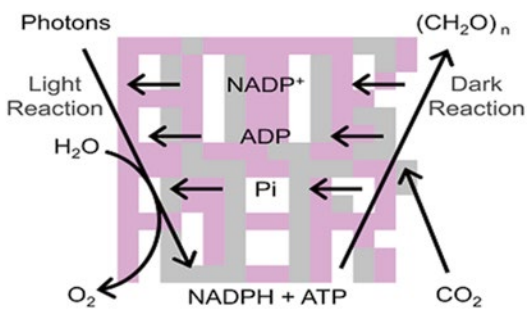
Photosynthesis has been a challenging scientific topic since it leads with the process by which a wide range of organisms from bacteria to higher plants convert solar energy to chemical energy for

producing food, fiber, and fuel out of small molecules of carbon dioxide and water. Deeper and greater understanding of this process holds a promise for a more sustainable earth with enjoyable pollution-free environment with new technologies for food and cleaner energy for a peaceful and progressive society. Thus, there have been global efforts to mimic the photosynthetic process to meet the needs of the future world.

C_4 plants are more productive than C_3 plants when they are grown under their respective optimum conditions. C_4 plants exhibit higher water and nitrogen use efficiencies compared to C_3 plants, which results in an increased dry matter production. Concentrating CO_2 at the site of RuBisCO should allow engineered C_3 plants to reduce stomatal conductance under drought conditions without a dramatic decline in the rate of CO_2 assimilations. This would allow both the use of new areas for crop production required for feeding the growing world population and the reduction of inputs into the system such as fertilizers and thus conserving natural resources. Both factors are much more relevant to today's necessities than the mere increase in biomass production.

Photosynthesis is an important area of research and is a core component of graduate and postgraduate courses in plant biology and the related disciplines of agriculture, horticulture, or biotechnology. Crop production under subtropical conditions should get more importance. Photosynthetic criterion for environmental stress tolerance should be known.

Photosynthesis as a whole is divided into light reactions and dark reactions. In the light reactions, light energy is trapped and converted to chemical energy. This energy is then used to fix CO_2 in the dark reactions.



22.1.1 Historical Background

Establishing the overall chemical equation of photosynthesis required several hundred years and contributions by many scientists. The beginning of understanding of photosynthesis goes back to the 1970s when composition of air by Lavoisier (1774) in France and discovery of O_2 by Joseph Priestley (1771) were made. In 1771, Joseph Priestley observed that a spring of mint growing in air in which a candle had burned out improved the air so that another candle could burn. He has discovered oxygen evolution by plants. A Dutchman, Ingenhousz, documented the essential role of light in photosynthesis in 1779. But neither of them knew the chemical nature of pure or impure air. Further, Lavoisier a French chemist identified in 1785 the "pure" component of air as "oxygen" and "impure" as "carbon dioxide" by the use of a eudiometer followed by gas analysis. Theodore de Saussure (1804) confirmed the equivalence of release of oxygen to consumption of carbon dioxide during photosynthesis. Robert Mayer (1845) reported that the energy used by plants and animals in their metabolism was derived from the energy of the sun and through photosynthesis. It was transformed from radiant to chemical form.

Engelmann (1883) performed an experiment using filamentous alga, *Cladophora*, to determine the wavelengths of light that are most effective for photosynthesis. The efficiency of the visible spectrum was measured on the basis of the evolution of oxygen by them. Localized concentration of aerobic bacteria suggested maximum oxygen evolution in the blue and red regions of the spectrum. Julius Von Sachs (1887) was first to discover that green chloroplasts are the organelles where carbon dioxide is used up and oxygen is released. He also found that starch was the first visible product of photosynthesis.

22.1.2 Chloroplast as Solar Harvesting Enterprise

The chloroplast was first described by a German botanist, Von Mohl (1837). Chloroplast is built

upon system of lamellae – grana lamellae and stroma lamellae. The internal membranes of chloroplast are organized into sacs called thylakoid, and, often, numerous thylakoids are stacked on one another in columns called grana. Interconnecting lamellae between the grana are called stroma lamellae. Surrounding the thylakoid membrane system is a semifluid substance called "stroma." Within the thylakoid membranes, photosynthetic pigments are grouped together in a network called "photosystem" capable of capturing photons of light. The photosystem acts as a large antenna, amplifying the power of individual pigment molecules to gather light. R.Willstaiter and A. Stoll (1912) studied the nature and composition of chloroplast pigments.

Pigments of green plants fall into two categories:

1. Vital (essential) pigments – chl a
2. Accessory pigments – chl b, c, d, e, carotenoids, phycobilins

The thylakoid membranes contain the chloroplast and carotenoid pigments and are the site of the light-dependent energy-conserving reaction of photosynthesis. These thylakoids are lipoproteinaceous bilayer membranes. They contain lipids and proteins. Phospholipids, galactolipids, and sulfolipids are major lipids in thylakoid membranes; these thylakoid membrane lipids contain a high proportion of highly unsaturated fatty acids, the major one being linolenic acid which can comprise as much as 90 % of the total fatty acid content. Because of this, the thylakoid membranes maintain a high degree of fluidity, which is essential for efficient photochemical functioning of the membranes.

The proteins of thylakoid membranes in association with pigments are organized into five major intrinsic and extrinsic pigment-protein complexes. They are designated as follows:

1. PSI pigment-protein complex
2. PSII pigment-protein complex
3. Cytochrome b_6f complex
4. Light-harvesting chlorophyll a/b complex
5. ATP synthase complex

The first three complexes are involved in light-driven electron and proton transport, while the fourth complex acts solely as a light-harvesting antenna and has no photochemical activity. ATP synthase of thylakoids catalyzes the synthesis of ATP from ADP and Pi during photosynthetic electron transport.

The PSII pigment-protein complex, along with its antenna chlorophylls and associated electron transport proteins, is located predominantly in the grana lamella. The PSI pigment-protein complex and its associated antenna pigments, electron transfer proteins, and ATP synthase complex are found almost exclusively in the stroma lamellae (unstacked grana regions) and at the edges of the grana lamellae. The cytochrome b_6f complex that connects the photosystems is evenly distributed.

This spatial separation between photosystem I and II indicates that a strict one-to-one stoichiometry between the two photosystems is not required. The interior space of the thylakoid is known as the lumen. The lumen is the site of water oxidation and, consequently, the source of oxygen evolved in photosynthesis. It is also a place of proton storage during photosynthetic electron transport, and these protons are used to drive ATP synthesis.

Chloroplast also contains starch grains which represent stored photosynthate and lipid droplets, called plastoglobuli. Plastoglobuli appear to function primarily as lipid storage bodies and may contain particularly large amounts of the electron carrier plastoquinone – chloroplasts of aging and senescing leaves may contain an iron-binding protein called phytoferritin.

22.1.3 Photosynthetic Pigments

Photosynthesis is a photobiological phenomenon that requires the participation of a molecule to absorb light energy. A molecule capable of absorbing light is known as pigment. Pigment molecules process the energy and information content of light into a form, which can be used by the organism. The principal pigments found in plants are chlorophylls, carotenoids, and phycobilins.

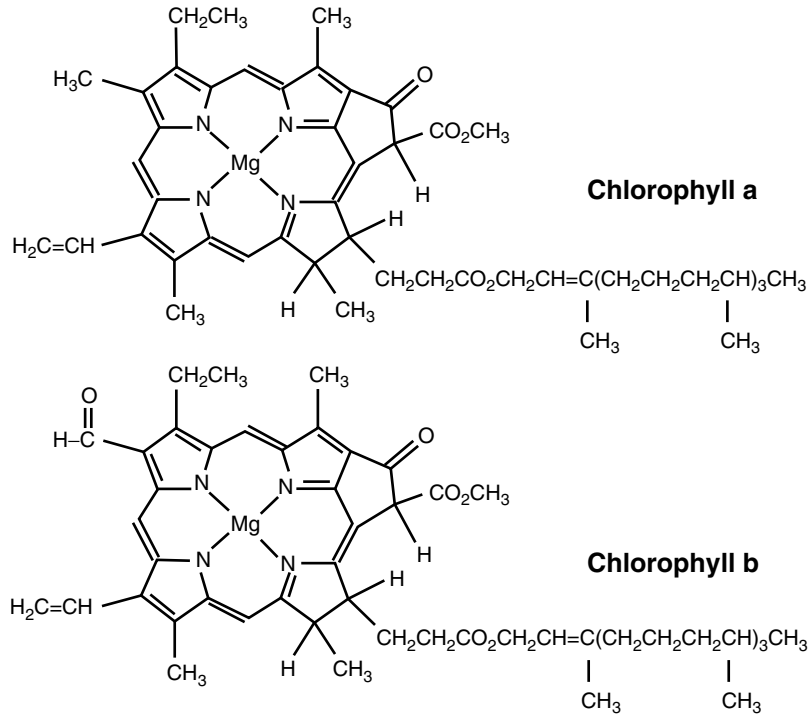


Fig. 22.1 Chemical structure of chlorophyll *a* and *b*

22.1.3.1 Chlorophylls

There are four species of chlorophylls in different types of photosynthetic organisms. They are designated as chlorophylls a, b, c, and d. Chlorophyll a is a principal light-absorbing pigment. It consists of two parts, a porphyrin head and a long hydrophobic hydrocarbon tail also called phytol tail. A porphyrin is a cyclic tetrapyrrole, made up of four nitrogen-containing pyrrole rings arranged in cyclic fashion. Magnesium is present at its center. A long lipid soluble hydrocarbon phytol tail can extend from ring IV of porphyrin head. The chemical structure of chlorophyll a is shown in (Fig. 22.1).

Chlorophyll b differs from chlorophyll a only in having formyl group (CHO) on ring II in place of methyl group. Chlorophyll b is found in virtually all higher plants and green algae. It is absent in cyanobacteria. The principal difference between chlorophyll a and chlorophyll c which is found in the diatoms, dinoflagellates and brown algae, chlorophyll d, found only in the red algae, is similar to chlorophyll a except that (-0-CHO) group replaces the (-CH=CH_2) group on ring II (Fig. 22.1).

Chlorophyll a has absorption maxima in the blue (435 nm) and red (663 nm) region of the visible spectrum. Chlorophyll does not absorb strongly in the green (490–550 nm). The strong absorbance in the blue and red and transmittance in the green are responsible for the chlorophyll to have characteristic green color. In thylakoid membranes, chlorophylls present as water-insoluble pigment-protein complexes.

22.1.3.2 Phycobilins

There are three photosynthetic phycobilins found only in cyanobacteria and red algae. They are phycoerythrin, phycocyanin (phycocyanobilin), and allophycocyanin. These pigments differ from chlorophyll "a" in containing open-chain tetrapyrrole with covalently linked protein molecule (Fig. 22.2). A pigment that contains protein as an integral part of the molecule is known as a chromoprotein. Hence phycobilins are chromoproteins. On the surface of thylakoid membranes, the phycobiliproteins are organized into large macromolecular antenna complexes called phycobilisomes.

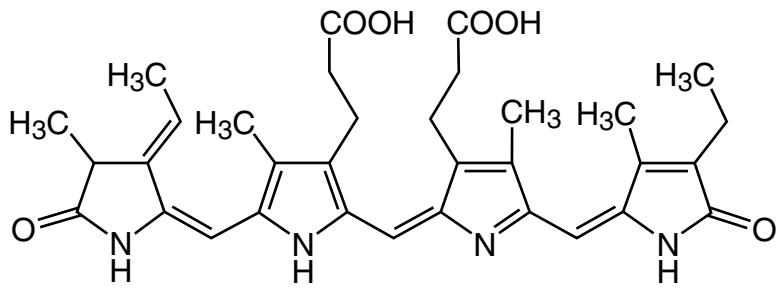


Fig. 22.2 Tetrapyrrole with covalently linked protein molecule

The phycobilins are water-soluble photosynthetic accessory pigments. They impart blue color to the thallus and chlorophyll a imparts green. For this reason, the cyanobacteria are also called blue-green algae. These pigments absorb light energy in the green region where chlorophyll a does not absorb it. Phycobilins absorb light energy between 500 and 600 nm range and transfer that absorbed energy to chlorophyll a for its convention into chemical energy.

22.1.3.3 Carotenoids

These are soluble in lipids, and they are called lipochromes or chromolipids. They have eight isoprene units in their structure and thus are called tetraterpenes. In higher plants, they are present not only in leaves but also in tubes (carrot), roots (*Ipomoea batatas*), and fruits of tomato.

They are of two types. Oxygen derivatives are xanthophylls and carotenes. In carrot roots, α, β, and γ carotenes are present in concentration of 10, 8.7, and 0.1 %, respectively. The molecular formula is C_{40} H_{56}. They are present in green leaves, flowers like dianthus, and fruits like tomato and apricot. The red-colored "lycopene" in tomato (unsaturated simple hydrocarbon chain) has two 15-carbon molecules with double bonds similar to each other. Xanthophylls are also called carotenols. The molecular formula is C_{40} H_{56} O_2. Examples for xanthophylls are lutein, zeaxanthin, physalin, etc.; lutein is present in all green parts, and zeaxanthin and physalins are present in maize leaves.

Carotenoids absorb light energy and transfer it to chlorophyll "a." Further, they protect chlorophylls from photooxidation. So they are called shield pigments. All the pigments, other than chlorophyll "a," are called accessory pigments. Pigment molecules exist as protein complexes.

Photooxidation is a potential problem in all plants. During periods of peak irradiance, plants absorb more energy than they can utilize in the reduction of CO_2. For example, rapidly growing plants may utilize only less than 50 % of absorbed light, while other species, such as evergreen, may utilize as little as 10 %. Any excess absorbed energy must be dissipated. If not, the reduced products of PSI, particularly ferredoxin, may

react with oxygen instead of NADP and produce a toxic form of oxygen known as a superoxide radical (O_2^-). It is highly reactive, and with that potential it will oxidize and destroy not only chlorophyll but also organic molecules in the cell. Formation of such toxic oxygen radical article in chloroplasts is prevented by the carotenoids. Recent studies have established an important link between excess energy dissipation and the presence of the xanthophylls and zeaxanthin. Zeaxanthin is formed from violaxanthin by a process known as xanthophyll cycle. Violaxanthin is a diepoxide. It contains two epoxy groups, one on each ring. Under conditions of excess light, violaxanthin is converted to zeaxanthin through the removal of those two oxygens by a process known as de-epoxidation.

De-epoxidation is induced by light, low pH, and reduced ascorbate. Zeaxanthin contains increased number of carbon-carbon double bonds and can accept a downhill transfer of energy from excited chlorophyll. Under low light, the energy of excited chlorophyll (chl) is preferentially transferred to be used in photosynthesis. As irradiance increases, zeaxanthin is formed from violaxanthin, and an increasing proportion of excitation energy is transferred to zeaxanthin to be dissipated as heat. The xanthophyll cycle thus operates as an effective switch, generating zeaxanthin whenever dissipation of excess energy is required and its formation is stopped under conditions of low light when more of the energy is required for photosynthesis.

22.2 Role of Light

22.2.1 Absorption Spectra

Different pigments in an organism absorb radiant energy. Further, all organic molecules absorb visible light near adjacent wavelength. It is difficult to determine the involvement of a pigment in a photochemical reaction.

In a specific photochemical reaction, the pigment involved can be determined by absorption spectra and action spectra. At first, a plot is drawn indicating the rate of photochemical reaction corresponding to different wavelength using monochromatic light. Photosynthetic rate is maximum in blue (400 nm) and red regions (700 nm) of the spectrum. Later the absorption spectrum of different pigments is compared with the action spectrum of a photochemical reaction to determine the nature of the pigment involved in the process.

The absorption spectra of chlorophylls show near relation with action spectra of photosynthesis. Thus, it is ascertained that the pigments involved in photosynthesis are chlorophylls. However, as "absorption spectra" and action spectra are determined in in vitro system, the organization may be different in in vivo systems. Absorption spectra are generally specific to pigments. So they are called "finger prints" of pigments. Usually, they are used to identify the existence of unknown pigments.

22.2.2 Photophysiology

The absorption of a photon of light by a molecule or atom initiates a photochemical reaction. The molecules absorbing radiant energy in the visible spectrum present in biological systems are called "pigments." Due to absorption of radiant energy by an atom, one of the electrons with less energy in the inner orbit is raised to higher energy level and reaches the outer orbit. This state is called excited state.

Excited pigment molecule, depending on the light energy absorbed and structure of electrons in the pigment, may react in different ways. For example, chlorophyll a absorbs red and blue light with equal efficiency. However, blue light (29.9 J/mole) is more energetic than red light (17.1 J/mole), and the electron is raised to second excited level in blue light and to first excited level in red light. It should be remembered that pairs of electrons are rotating in opposite directions in their specific locations. In both the above excited states, though one of the electrons is raised to higher energy state, the direction of rotation does not change.

Not only chlorophyll, any pigment molecule must reach the ground state after absorption of light energy. It is possible in four methods.

1. Energy in excited electron may be released as heat and reach ground state.
2. However, generally the energy is released partly as heat energy and partly as radiant energy. The light emitted in this condition is with less energy than the light energy absorbed by the electron:
   (i) The quick emission of radiant energy within 10^{-8} to 10^{-9} s is called "fluorescence."
   (ii) Sometimes, excited electron may use some energy and change the direction of rotation and falls back to the lower energy level. It takes some time and this may occur either in first excited state or second excited state. In this state, both the electrons in the electron pair will have same direction of rotation and are in different energy levels. Electron in higher energy level before reaching the ground state should have to change its direction of rotation. Thus, it takes some time period before energy transfers. This emission of light energy by the electron after a delayed period is called phosphorescence.
3. The light energy absorbed by the electron may be transferred to other pigment molecules by "resonance transfer." The light energy absorbed by accessory pigments like carotenoids and chlorophyll b is transferred to chlorophyll a in a similar manner. This is also called "Foster" transfer.
4. The light energy absorbed by an electron may be used to bring out a chemical reaction.

The reaction depends on the concentration of chlorophyll molecules and molecules involved in the reaction and also on structural organization of the light-absorbing molecules. Pigment molecules can transfer energy only when they are very nearer to the molecules participating in the reaction. The definite arrangement of various electron carriers and enzymes and their vicinity to pigment molecules in thylakoid membranes facilitate the conversion of light energy to chemical energy.

When chloroplasts are isolated, they are separated from the photochemical reaction and thus the light energy absorbed by them is emitted as light by fluorescence. Chlorophyll shows red-colored fluorescence, and light energy absorbed by chlorophyll molecule emits a part of energy as heat energy and falls to second state from first excited state. Later, by loss of some more energy as heat, it reaches moderate state called triplet state which is suitable for photosynthesis.

Thus, the pigments are responsible for conversion of light energy to chemical energy by suitable photochemical reactions as described above. ATP and NADPH are synthesized and are together called "assimilatory power." Carotenoids not only transfer light energy absorbed by them to chlorophylls but also protect them from photooxidation.

Green plants release oxygen as by-product during photosynthesis. It is called (oxygenic) photosynthesis. In bacteria, compounds other than water are used to reduce carbon dioxide. As such, oxygen is not evolved during the process. Thus, the photosynthesis in these organisms is called "anoxygenic" photosynthesis.

Van Niel (1931) studied photosynthesis of anoxygenic organisms like *Chromatium vinosum* and indicated that organic acids or sulfur compounds are used for the reduction of carbon dioxide. The reaction can be indicated as below.

$$6CO_2 + 2H_2A \xrightarrow[\text{Bacteria}]{\text{Light}} (CHO)n + 2A + H_2O$$

By comparison of these reactions with the green plant photosynthesis, it is proposed that in oxygen-releasing organisms, water acts as a reductant and photosynthesis is an oxidation reduction reaction. Bacteria like *Chromatium* synthesize complex substances by oxidation of simple inorganic substances, and, thus, they are called "chemophotoautotrophs."

In 1937, Robert Hill expressed that isolated chloroplasts can release oxygen, in presence of light even in absence of external electron acceptors like iron salts. This is called Hill reaction and the external electron acceptors are called "Hill oxidants":

$$4Fe^{3+} + 12H_2O^{18} \rightarrow 4Fe^{2+} + O_2 + 4H^+$$

NADP+ acts as electron acceptor under natural conditions. Ruben and Kamen (1941) proved using water with $^{18}O_2$, a heavy isotope in CO_2 and H_2O, that oxygen released in photosynthesis is derived from water but not from CO_2:

$$6CO_2 + 12H_2O^{18} \rightarrow C_6H_{12}O_6 + 6H_2O + 6O_2^{18}$$

These experiments proved that photosynthesis is a "photochemical reduction" and carbon dioxide is reduced to carbohydrate.

22.2.3 Emerson Effect

While studying the photosynthetic role of accessory pigments in algae, several investigators, working independently, observed a curious phenomenon. They found that light absorbed directly by chlorophyll a was less efficient in photosynthesis than light absorbed by the accessory pigments such as phycocyanin in cyanobacteria and both phycocyanin and phycoerythrin in red algae. There is a conspicuous lack of activity in 675 and 680 nm regions, although thallus spectrum shows definite absorption peak over that range. This superiority of the accessory pigments to chlorophyll a was further worked out by Emerson and Lewis in the 1943. Emerson and Lewis worked on the quantum yield of photosynthesis by using the monochromatic light of different wavelengths. Quantum yield is defined as the number of O_2 molecules released for light quanta absorbed. Emerson found that 8 quanta of light energy would be required for the reduction of one molecule of CO_2 to carbohydrate, that is, one molecule of O_2 is produced.

In a series of experiments, they found that light between 680 and 720 nm is inefficient in exciting photosynthesis, although the absorption due to chlorophyll in this spectral region is still strong. This deficiency in the ability of red light on the per quantum yield of photosynthesis was termed the red drop.

In continuing and extending studies on the action spectra of photosynthesis, Emerson and his coworkers discovered that the efficiency of photosynthesis at wavelengths exceeding 680 nm can be restored by a simultaneous application of a shorter wavelength. The effect of the two superimposed beams of light on the rate of photosynthesis exceeds the sum effect of both beams of light used separately. In other words, illumination of a sample with quanta of orange/red and far-red light wavelengths simultaneously gives a higher rate of O_2 evolution than the sum of the two effects measured separately. This photosynthetic enhancement is referred to as the Emerson enhancement effect.

In the late 1950s and early 1960s, the Emerson effect received a great deal of attention, particularly by Louis Duysens. Chloroplasts contain cytochromes, iron-containing proteins that function as intermediate electron carriers in photosynthesis. Duysens found that when a sample of red alga was illuminated with long wavelength light, the cytochrome became mostly oxidized. If light of a shorter wavelength was also present, the effect was partly reversed. These antagonistic effects can be explained by a mechanism involving two photochemical events: one that tends to oxidize the cytochrome (far-red light) and one that reduces it (green light).

We know now that in the red region of the spectrum, one of the photoreactions, known as photosystem 1 (PSI), absorbs preferentially far-red light of wavelengths greater than 680 nm, while the second, known as photosystem II (PSII), absorbs light of 680 nm well and is driven very poorly by far-red light. The wavelength dependence explains the enhancement effect and the red drop effect.

Another difference between the photosystems is that photosystem 1 produces a strong reductant, capable of reducing NADP+ and a weak oxidant. PSII produces a very strong oxidant, capable of oxidizing water, and a weaker reductant than the one produced by PSI. This reductant reduces the oxidant produces by PSI, which explains the antagonistic effect.

22.3 Important Reaction of Photosynthesis

The light reactions of photosynthesis occur in membranes. In bacteria like those studied by Van Niel, the plasma membrane itself is the photosynthetic membrane.

22.3.1 Organizing Pigments into Photosystems

In plants and algae, by contrast, photosynthesis is carried out by organelles that are the evolutionary descendants of photosynthetic bacteria, chloroplasts – the photosynthetic membranes exist within the chloroplasts. The light reactions take place in four stages:

1. *Primary photoevent*: A photon of light is captured by a pigment. The result of this primary photoevent is the excitation of electrons within the pigment.
2. *Charge separation*: This excitation energy is transferred to a specialized chlorophyll pigment termed as reaction center, which reacts by transferring an energetic electron to an acceptor molecule, thus initiating electron transport.
3. *Electron transport*: The excited electron is shuttled along a series of electron carrier molecules embedded within the photosynthetic membrane. Its arrival at the pump induces the transport of a proton across the membrane. The electron is then passed to an acceptor.
4. *Chemiosmosis*: The protons that accumulate on one side of the membrane now flow back across the membrane through specific protein complexes where chemiosmosis synthesis of ATP takes place, just as it does in aerobic respiration.

22.3.2 Discovery of Photosystems

One way to study how pigments absorb light is to measure the dependence of the output of photosynthesis on the intensity of illumination, that is, how much photosynthesis is produced by how much light. When experiments of this sort are done on plants, they show that the output of photosynthesis increases linearly at low intensities but lesser at higher intensities, finally saturating at high intensity of light (Fig. 22.3). Saturation occurs because all of the light-absorbing capacity of the plant is in use; additional light doesn't increase the output because there is nothing to absorb the added photons.

It is tempting to think that at saturation, all of a plant's pigment molecules are in use. In 1932, plant physiologists Robert Emerson and William Arnold set out to test this hypothesis in an organism where they could measure both the number of chlorophyll molecules and the output of photosynthesis. In their experiment, they measured the oxygen yield of photosynthesis when *Chlorella* (unicellular green algae) were exposed to very brief light flashes lasting only a few microseconds. Assuming the hypothesis of pigment saturation to be correct, they expected to find that as they increased the intensity of the flashes, the yield per flash would increase, until each chlorophyll molecule absorbed a photon, which would then be used in the light reactions, producing a molecule of O_2.

Unexpectedly, this is not what happened. Instead, saturation was achieved much earlier, with only one molecule of O_2 per 2,500 chlorophyll molecules. This led Emerson and Arnold to conclude that light is absorbed not by independent pigment molecules, but rather by clusters of chlorophyll and accessory pigment molecules which have come to be collect photosystems. Light is absorbed by any one of the hundreds of pigment molecules in a photosystem, which transfer their excitation energy to one with a lower energy level than the others. This reaction center of the photosystem acts at an energy sink, trapping the excitation energy. It was the saturation of these reaction centers, not individual molecules, that was observed by Emerson and Arnold.

22.3.3 Architecture of a Photosystem

In chloroplasts and all but the most primitive bacteria, light is captured by such photosystems. Each photosystem is a network of chlorophyll a

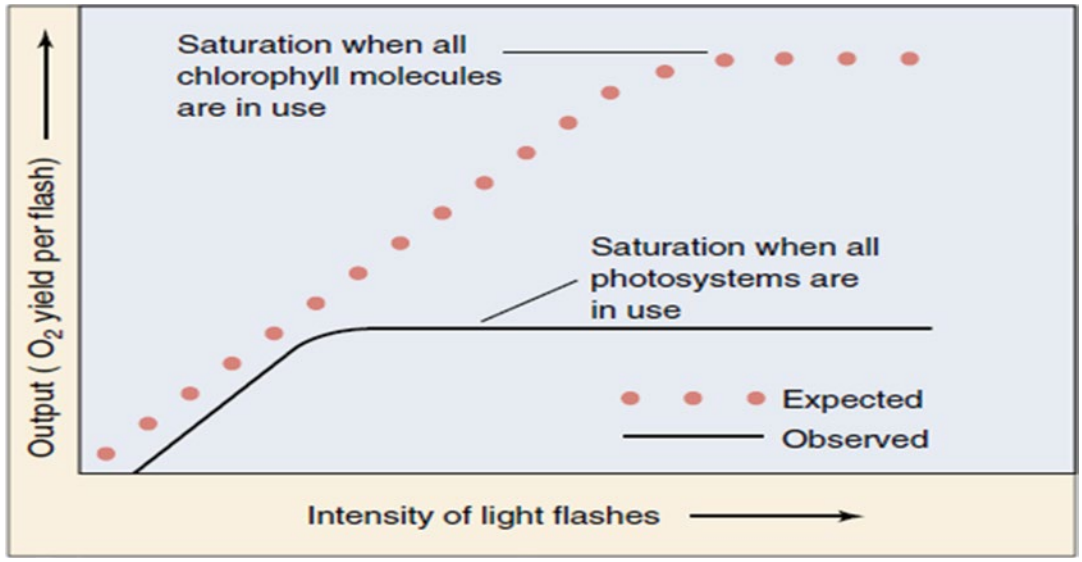


Fig. 22.3 Emerson and Arnold's experiment. When photosynthetic saturation is achieved, further increases in intensity cause no increase in output

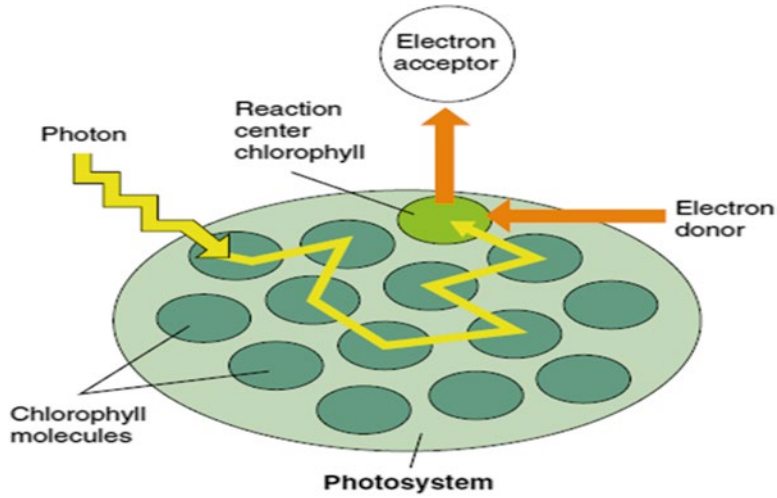


Fig. 22.4 The antenna complex captures photons from sunlight

molecules, accessory pigments, and associated proteins held within a protein matrix on the surface of the photosynthetic membrane. Like a magnifying glass focusing light on a precise point, a photosystem channels the excitation energy gathered by any one of its pigment molecules to a specific molecule, the reaction center chlorophyll. This molecule then passes the energy out of the photosystem so it can be put to work driving the synthesis of ATP and organic molecules. A photosystem thus consists of two closely linked components:

1. An antenna complex of hundreds of pigment molecules that gather photons and captured light energy to the reaction center
2. A reaction center, consisting of one or more chlorophyll a molecules in a matrix of protein that passes the energy out of the photosystem

22.3.4 The Antenna Complex

The antenna complex captures photons from sunlight (Fig. 22.4). In chloroplasts, the antenna

complex is a web of chlorophyll molecules linked together and held tightly on the thylakoid membrane by a matrix of proteins. Varying amounts of carotenoid accessory pigments may also be present. The excitation energy resulting from the absorption of a photon passes from one pigment molecule to an adjacent molecule on its way to the reaction center. After the transfer, the excited electron in each molecule returns to the low-energy level it had before the photon was absorbed. Consequently, it is energy, not the excited electrons themselves, that passes from one pigment molecule to the next. The antenna complex funnels the energy from many electrons to the reaction center.

When light of the proper wavelength strikes any pigment molecule within a photosystem, the light is absorbed by that pigment molecule. The excitation energy is then transferred from one molecule to another within the cluster of pigment molecules until it encounters the reaction center chlorophyll a. When excitation energy reaches the reaction center chlorophyll, electron transfer is initiated.

22.3.5 The Reaction Center

The reaction center is a transmembrane pigment-protein complex. In the reaction center of purple photosynthetic bacteria, which is simpler than in chloroplasts but better understood, a pair of chlorophyll a molecules acts as a trap for photon energy, passing an excited electron to an acceptor precisely positioned as its neighbor. Note that here the excited electrons themselves are transferred, not just the energy as we saw in pigment-pigment transfers. This allows the photon excitation to move away from the chlorophyll and is the key conversion of light to chemical energy. It shows the transfer of energy from the reaction center to the primary electron acceptor. By energizing an electron of the reaction center chlorophyll, light creates a strong electron donor where none existed before. The chlorophyll transfers the energized electron to the primary acceptor, a molecule of quinine reducing and converting it to a strong electron donor. A weak electron donor then donates a low-energy electron to the chlorophyll, restoring it to its original condition. In plant chloroplasts, water serves as the electron donor.

22.4 How Photosystem Convert Light to Chemical Energy

Photosynthetic pigment arrays are thought to have evolved more than three billion years ago in bacteria similar to the sulfur bacteria studied by Van Niel.

22.4.1 Electron Is Joined with a Proton to Make Hydrogen

In these bacteria, the absorption of a photon of light at a peak absorption of 870 nm (near infrared, not visible to the human eye) by the photosystem results in the transmission of an energetic electron along an electron transport chain, eventually combing with a proton to form a hydrogen atom. In the sulfur bacteria, the proton is extracted from hydrogen sulfide, leaving elemental sulfur as a by-product. In bacteria that evolved later, as well as in plants and algae, the proton comes from water, producing oxygen as a by-product.

22.4.2 Electron Is Recycled to Chlorophyll

The ejection of an electron from the bacterial reaction center leaves it short one electron. Before the photosystem of the sulfur bacteria can function again, an electron must be returned. These bacteria channel the electron back to the pigment through an electron transport system. The electron's passage drives a proton pump that promotes the chemiosmotic synthesis of ATP. One molecule of ATP is produced for every three electrons that follow this path. Viewed overall, the path of the electron transfer process leads to ATP formation cyclic photophosphorylation. For more than a billion years, cyclic photo-

phosphorylation was the only form of photosynthetic light reaction that organisms used. However, its major limitation is that it is geared only toward energy production, not toward biosynthesis. Most photosynthetic organisms incorporate atmospheric carbon dioxide into carbohydrates. Because the carbohydrate molecules are more reduced (have more hydrogen atoms) than carbon dioxide, a source of reducing power (that is, hydrogen) must be provided. Cyclic photophosphorylation does not do this. The hydrogen atoms extracted from H_2S are used as a source of protons and are not available to join to carbon. Thus, bacteria that are restricted to this process must scavenge hydrogens from other sources, an inefficient undertaking.

22.5 Why Plants Use Two Photosystems

After the sulfur bacteria appeared, other kinds of bacteria evolved an improved version of the photosystem that overcame the limitation of cyclic photophosphorylation in a neat and simple way: a second, more powerful photosystem using another arrangement of chlorophyll a was combined with the original.

In this second photosystem, called photosystem II, molecules of chlorophyll a are arranged with a different geometry, so that more shorter wavelength and higher energy photons are absorbed than in the ancestral photosystem, which is called photosystem I. As in the ancestral photosystem, energy is transmitted from one pigment molecule to another within the antenna complex of these photosystems until it reaches the reaction center, a particular pigment molecule positioned near a strong membrane-bound electron acceptor. In photosystem II, the absorption peak (that is, the wavelength of light most strongly absorbed) of the pigments is approximately 680 nm; therefore, the reaction center pigment is called P_{680}. The absorption peak of photosystem I pigments in plants is 700 nm, so its reaction center pigment is called P_{700}. Working together, the two photosystems carry out a noncyclic electron transfer (Fig. 22.5).

When the rate of photosynthesis is measured using two light beams of different wavelengths (one red and the other far red), the rate was greater than the sum of the rates using individual beams of red and far-red light. This surprising result, called the enhancement effect, can be explained by a mechanism involving two photosystems acting in series (i.e., one after the other, one of which absorbs preferentially in the red, the other in the far red).

The use of two photosystems solves the problem of obtaining reducing power in a simple and direct way, by harnessing the energy of two photosystems. The scheme shown in Fig. 22.5, called a Z diagram, illustrates the two electron-energizing steps, one catalyzed by each photosystem. The electrons originate from water, which holds its electrons very tightly (redox potential = + 820 mV), and end up in NADPH, which holds its electrons much more loosely (redox potential = 320 mV).

22.5.1 How the Two Photosystems of Plants Work Together

Plants use the two photosystems discussed earlier in series, first one and then the other, to produce both ATP and NADPH. This two-stage process is called noncyclic photophosphorylation, because the path of the electrons is not a circle – the electrons ejected from the photosystems do not return to it, but rather end up in NADPH. The photosystems are replenished instead with electrons obtained by splitting water. Photosystem II acts first. High-energy electrons generated by photosystem II are used to synthesize ATP and then passed to photosystem I to drive the production of NADPH. For every pair of electrons obtained from water, one molecule of NADPH and slightly more than one molecule of ATP are produced.

22.5.2 Photosystem II

The reaction center of photosystem II, called P_{680}, closely resembles the reaction center of purple bacteria. It consists of more than ten transmem-

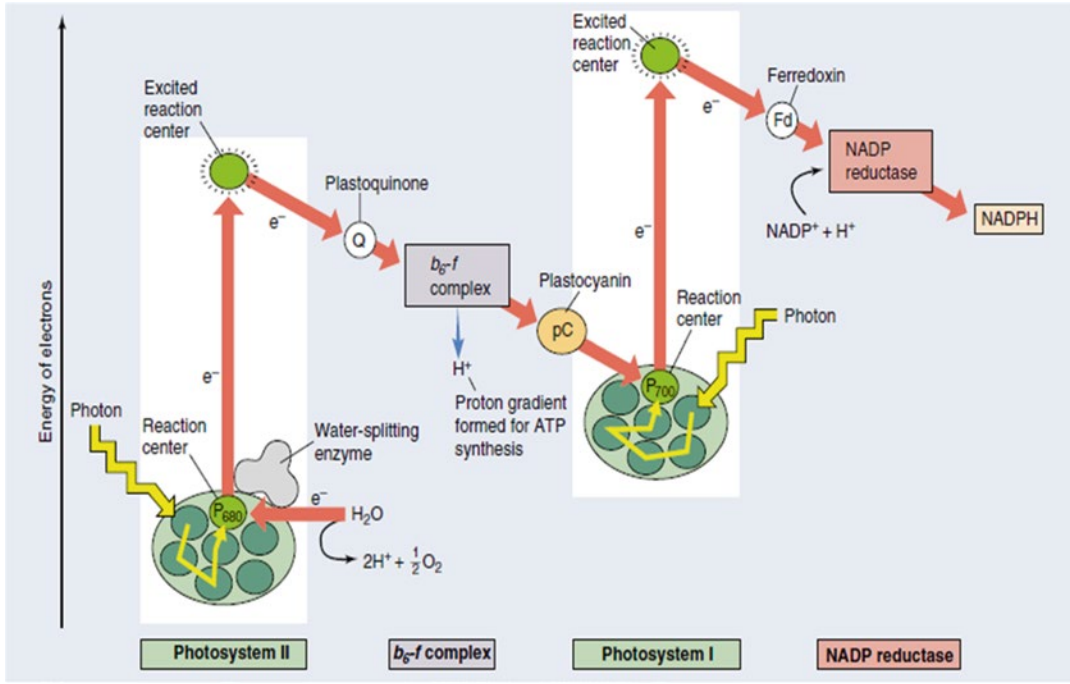


Fig. 22.5 Z diagram of photosystems I and II. Two photosystems work sequentially. First, a photon of light ejects a high-energy electron from photosystem II; the electron is used to pump a proton across the membrane, contributing chemiosmotically to the production of a molecule of ATP. The ejected electron then passes along a chain of cytochromes to photosystem I. When photosystem I absorbs a photon of light, it ejects a high-energy electron used to drive the formation of NADPH (Source: Veit and Govindjee 2000)

brane protein subunits. The light-harvesting antenna complex consists of some 250 molecules of chlorophyll a and accessory pigments bound to several protein chains. In photosystem II, the oxygen atoms of two water molecules bind to a cluster of manganese atoms which are embedded within an enzyme and bound to the reaction center, in a way that is poorly understood. This enzyme splits water, removing electrons one at a time to fill the holes left in the reaction center by departure of light-energized electrons. As soon as four electrons have been removed from the two water molecules, O_2 is released.

22.5.3 Photosystem I

The reaction center of photosystem I, called P_{700} is a transmembrane complex consisting of at least protein subunits. Energy is fed to it by an antenna complex consisting of chlorophyll a and accessory pigment molecules. Photosystem I accepts an electron from plastocyanin into the hole created by the exit of a light-excited energy; almost half remains. Thus, the absorption of a photon of light energy by photosystem I boosts the electron leaving the reaction center to a very high energy level. Unlike photosystem II and the bacterial photosystem, photosystem I does not rely on quinones as electron acceptors. Instead, it passes electrons to an iron-sulfur protein called ferredoxin (Fd).

22.5.4 Making NADPH

Photosystem I passes electrons to ferredoxin on the stromal side of the membrane (outside the thylakoid). The reduced ferredoxin carries a very high potential electron. Two of them, from two molecules of reduced ferredoxin, are then donated to a molecule of NADP+ to form

NADPH. The reaction is catalyzed by the membrane-bound enzyme NADP reductase. Because the reaction occurs on the stromal side of the membrane and involves the uptake of a proton in forming NADPH, it contributes further to the proton gradient established during photosynthetic electron transport.

22.5.5 Making More ATP

The passage of an electron from water to NADPH in the noncyclic photophosphorylation described previously generates one molecule of NADPH and slightly more than one molecule of ATP.

To produce the extra ATP, many plant species are capable of short-circuiting photosystem I, switching photosynthesis into a cyclic photophosphorylation mode, so that the light-excited electron leaving photosystem I is used to make ATP instead of NADPH. The energetic electron is simply passed back to the b_6f complex rather than passing on to NADPH. The b_6f complex pumps out a proton, adding to the proton gradient driving the chemiosmotic synthesis of ATP. The relative proportions of cyclic and noncyclic photophosphorylation in these plants determine the relative amounts of ATP and NADPH available for building organic molecules.

22.6 Carbon Reactions of Photosynthesis

In 1961, Melvin Calvin identified different reactions during dark phase of photosynthesis and formation of carbohydrate using $^{14}CO_2$ (radioactive carbon dioxide) in the unicellular algal member *Chlorella*. Different intermediate compounds are separated and identified using chromatography and autoradiography. The reaction takes place in a cyclic manner and thus it is called "Calvin Cycle." The first stable compound formed during this process is a C_3 acid identified as phosphoglyceric acid.

Hence the cycle is also called C_3 cycle. It is also called reductive pentose phosphate pathway (RPP pathway). Calvin received noble prize for explaining these reactions. The plants with C_3 cycle are called C_3 plants. In the beginning, it was assumed that CO_2 fixation during dark phase of photosynthesis takes place by only one pathway (C_3 pathway).

Later, an alternative pathway called C_4 pathway was identified. In addition, it is noticed that certain plants open their stomata during nighttime and close their stomata during daytime. Such plants have a different type of carbon fixation which takes place during nighttime. It is called dark carbon assimilation or crassulacean acid metabolism as it is first described in crassulacean members like *Bryophyllum*.

Thus, the carbon assimilation in all plants could be grouped into three categories:
1. C_3 pathway
2. C_4 pathway
3. CAM pathway or dark carbon assimilation

22.6.1 C_3 Cycle or Calvin Cycle or RPP Pathway

Different reactions taking place in Calvin cycle can be classified into three phases:
1. Carboxylation
2. Reduction
3. RUBP regeneration

22.6.1.1 Carboxylation

The compound which reacts with carbon dioxide immediately after its entry into the chloroplasts is called "CO_2 acceptor." CO_2 acceptor binds CO_2 to form a first stable compound phosphoglyceric acid. So it was assumed that CO_2 acceptor might be a C_2 compound and investigated for such a compound and failed to identify such compound, than C_5 compound ribulose-1,5-bisphosphate (RUBP) identified in later experiments was considered to combine with CO_2 to form unstable C_6 intermediate which immediately breaks into two molecules of C_3 compound 3-phosphoglyceric acid. The reaction is catalyzed by RUBP carboxylase.

It is identified that RUBP carboxylase acts as oxygenase at high oxygen concentrations. Thus, it is also called RUBP carboxylase/oxygenase (RuBisCO). It is a soluble protein present in very

high concentration in leaves. It constitutes about 40 % of the total soluble protein. Its molecular weight is 560 KDa. The enzyme has eight larger subunits (LSU), each with a molecular weight of 55 KDa, and eight smaller subunits (SSU), each with a molecular weight of 14 KDa. Larger subunits are synthesized by the genetic codons of chloroplasts genome while the smaller subunits are synthesized by the codons of nuclear genes. Membrane proteins called "chaperones" combine LSU and SSU into functional RUBP carboxylase enzyme. Carboxylation results in the formation of C_6 unstable compound which immediately breaks into two molecules of 3-phosphoglyceric acid.

22.6.1.2 Reduction

During this phase, phosphoglycerate (phosphoglyceric acid) is first converted to 1, 3-bisphosphoglycerate utilizing the energy from the ATP synthesized during light phase in the presence of the enzyme 3-phosphoglycerate kinase.

Later, 1, 3-bisphosphoglycerate is converted to glyceraldehyde 3-phosphate in the presence of NADPH formed during light phase. The reaction is catalyzed by the enzyme NADP-glyceraldehyde-3-phosphate dehydrogenase. Only one sixth of the triose formed in the reactions is used for hexose synthesis. The remaining five sixths is used for the regeneration of RUBP.

Thus, the reduction of two molecules of PGA formed during carboxylation, 2ATP and 2 NADPH, are essential. Out of the six carbon atoms in two triose phosphate molecules formed, five carbon atoms are used for the regeneration of RUBP. That means only one carbon atom is used for the synthesis of carbohydrate. Thus, for the synthesis of one molecule of hexose (carbohydrate), six carbon dioxide molecules are used in which the cycle should operate six times.

Only one sixth of triose phosphate formed in reduction reactions is translocated to cytoplasm from chloroplasts and converted to hexose sugars, and sucrose is formed from hexose. Synthesis of starch again takes place in chloroplasts.

22.6.1.3 RUBP Regeneration

Triose phosphate (glyceraldehyde 3-phosphate) formed by reduction reactions is converted to RUBP by several biochemical reactions. Sugar phosphates with 4, 5, 6, and 7 carbon atoms are formed as intermediate.

Ribulose 5-phosphate formed in the last reaction is converted to RUBP in the presence of ATP. Thus, for the conversion of six molecules of ribulose 5-phosphate to RUBP, six ATP molecules are required which are derived from light phase. The CO_2 fixation by Calvin cycle to form one molecule of carbohydrate 3ATP and $2NADPH^+ + 2H^+$ in most molecules is required. Most of the plants grow in temperate and tropical regions to fix CO_2 by Calvin cycle Fig. 22.6.

22.6.1.3.1 Significance

Calvin cycle results in the synthesis of carbohydrate. The various intermediates of the cycle are used in the synthesis of several compounds. Phosphoglyceric acid is used for the synthesis of several organic acids and amino acids. ATP and NADPH formed in light phase are used to reduce nitrate to ammonia.

Calvin cycle acts in an auto catalytic manner. If the concentration of the intermediate is increased, the rate of the cycle is increased, and synthesis of CO_2 acceptor also takes place as a part of the cycle. Five enzymes of Calvin cycle are regulated in the presence of light. These are RuBisCO, NADP – glyceraldehyde-3-phosphate dehydrogenase, fructose-1,6-bisphosphate phosphatase, sedoheptulose-1,7-bisphosphate phosphatase, ribulose-5-phosphate kinase. Thus, the reactions of dark phase (Calvin cycle) cannot take place in the dark.

The regulation of Calvin cycle takes place by two methods:

1. By altering the enzymes chemically through covalent bonds and disulfide bonds reduction and carboxylation of amino groups
2. Conversion of the metabolic products as enzyme homologues by noncovalent changes

Further, to increase the efficiency of Calvin cycle, the enzymes are bound to thylakoid membranes achieving high localization and act as channels for substrates and protect them.

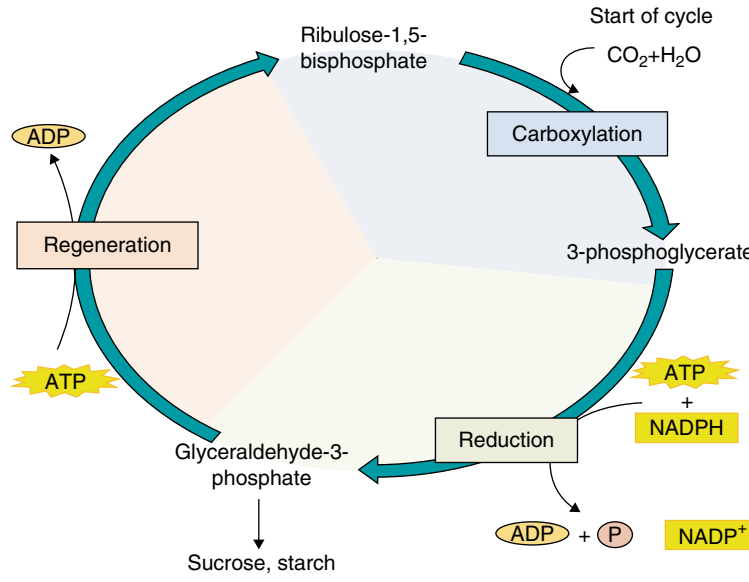


Fig. 22.6 The Calvin cycle proceeds in three stages: (1) Carboxylation, during which CO_2 is covalently linked to a carbon skeleton; (2) Reduction, during which carbohydrate is formed at the expense of the photochemically derived ATP and reducing equivalents in the form of NADPH; and (3) Regeneration, during which the CO_2 acceptor ribulose-1,5-bisphosphate re-forms. (Source: Taiz and Zeiger 2010)

TABLE 8.1
Reactions of the Calvin cycle

Enzyme	Reaction
1. Ribulose-1,5-bisphosphate carboxylase/oxygenase	6 Ribulose-1,5-bisphosphate + 6 CO_2 + 6 H_2O → 12 (3-phosphoglycerate) + 12 H^+
2. 3-Phosphoglycerate kinase	12 (3-Phosphoglycerate) + 12 ATP → 12 (1,3-bisphosphoglycerate) + 12 ADP
3. NADP-glyceraldehyde-3-phosphate dehydrogenase	12 (1,3-Bisphosphoglycerate) + 12 NADPH + 12 H^+ → 12 glyceraldehye-3-phosphate + 12 $NADP^+$ + 12 P_i
4. Triose phosphate isomerase	5 Glyceraldehyde-3-phosphate → 5 dihydroxyacetone-3-phosphate
5. Aldolase	3 Glyceraldehyde-3-phosphate + 3 dihydroxyacetone-3-phosphate → 3 fructose-1,6-bisphosphate
6. Fructose-1,6-bisphosphatase	3 Fructose-1,6-bisphosphate + 3 H_2O → 3 fructose-6-phosphate + 3 P_i
7. Transketolase	2 Fructose-6-phosphate + 2 glyceraldehyde-3-phosphate → 2 erythrose-4-phosphate + 2 xylulose-5-phosphate
8. Aldolase	2 Erythrose-4-phosphate + 2 dihydroxyacetone-3-phosphate → 2 sedoheptulose-1,7-bisphosphate
9. Sedoheptulose-1,7-bisphosphatase	2 Sedoheptulose-1,7-bisphosphate + 2 H_2O → 2 sedoheptulose-7-phosphate + 2 P_i
10. Transketolase	2 Sedoheptulose-7-phosphate + 2 glyceraldehyde-3-phosphate → 2 ribose-5-phosphate + 2 xylulose-5-phosphate
11a. Ribulose-5-phosphate epimerase	4 Xylulose-5-phosphate → 4 ribulose-5-phosphate
11b. Ribose-5-phosphate isomerase	2 Ribose-5-phosphate → 2 ribulose-5-phosphate
12. Ribulose-5-phosphate kinase	6 Ribulose-5-phosphate + 6 ATP → 6 ribulose-1,5-bisphosphate + 6 ADP + 6 H^+
	Net: 6 CO_2 + 11 H_2O + 12 NADPH + 18 ATP → Fructose-6-phosphate + 12 $NADP^+$ + 6 H^+ + 18 ADP + 17 P_i

Note: P_i stands for inorganic phosphate.

22.6.1.4 C_4 Cycle or Hatch-Slack Cycle

Kortchak and Burr in 1965 identified that in some plants growing in tropical regions, the first stable compounds formed in dark reaction are C_4 dicarboxylic acids like oxaloacetic acid or malic acid or aspartic acid. They conducted experiments in sugarcane using radioactively labeled carbon dioxide $^{14}CO_2$ (Fig. 22.7).

Later M.D. Hatch and C.R. Slack in 1966 indicated that C_4 compounds are the first formed stable compounds in photosynthesis of sugarcane leaves and that C_4 pathway operates as an alternative to C_3 pathway. C_4 pathway is reported in 19 genera and 90 species belonging to 11 families of angiosperms. It is identified in millets like jowar, maize, pearl millet, and finger millet of the family Poaceae; in members of Cyperaceae; and in dicots belonging to the families Amaranthaceae, Portulacaceae, and Boraginaceae.

22.6.1.5 Leaf Anatomy in C_4 Plants

C_4 plants, though belonging to diverse families and are taxonomically unrelated, share common features in leaf anatomy. All these plants possess a bundle sheath made of large-sized cells and the bundle sheath resembles flower wreath. It is called "Kranz anatomy" (German Kranz: flower wreath) Fig. 22.8. The chloroplasts of mesophyll and bundle sheath differ in structure and function. Mesophyll chloroplasts are normal in size with grana. Both PSI and PSII are active in them; PEP carboxylase is more active.

Mesophyll chloroplasts are specialized for primary carboxylation, while bundle sheath chloroplasts are specialized for carbohydrate system.

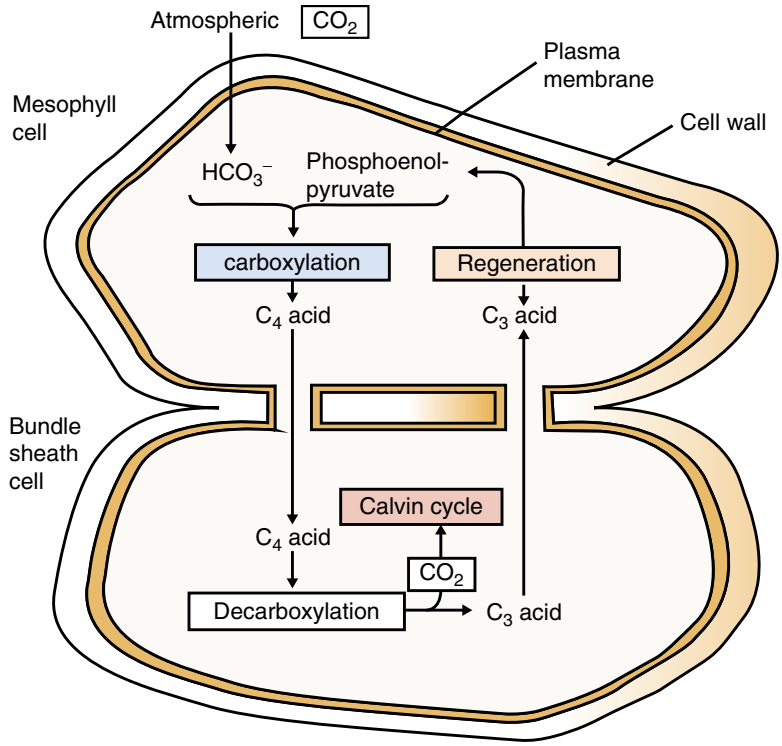


Fig. 22.7 The basic C_4 photosynthetic carbon cycle involves four stages in two different cell types (1) Fixation of CO_2 into a four-carbon acid in a mesophyll cell; (2) Transport of the four-carbon acid from the mesophyll cell to a bundle sheath cell; (3) Decarboxylation of the four-carbon acid, and the generation of a high CO_2 concentration in the bundle sheath cell. The CO_2 released is fixed by RuBisCO and converted to carbohydrate by the Calvin cycle; (4) transport of the residual three carbon acid back to the mesophyll cell, where the original CO_2 acceptor, phosphoenolpyruvate, is regenerated (Source: Taiz and Zeiger 2010)

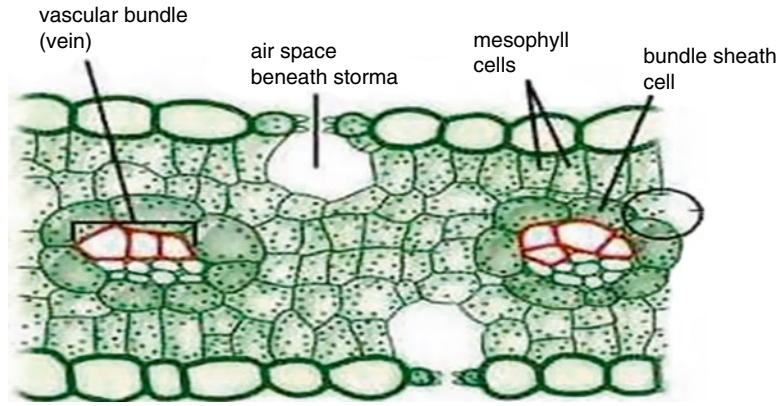


Fig. 22.8 Portion of a cross section of a leaf with C_4 photosynthesis (From authors desk)

This type of dimorphism of chloroplasts with division of labor is exhibited by all C_4 plants.

In C_4 cycle, basically there are four phases:

1. Formation of C_4 acids with the help of PEP carboxylase enzyme in mesophyll
2. Transport of C_4 acids into bundle sheath
3. Decarboxylation of C_4 acids to release CO_2 which is fixed as carbohydrate by C_3 cycle
4. C_3 acid (pyruvate) formed in decarboxylation moves back to mesophyll and helps in PEP regeneration

PEP carboxylase catalyzes primary carboxylation. The enzyme is concentrated in cytoplasm and uses HCO_3 (instead of CO_2) as substrate. Carbonic anhydrase enzyme enables the formation of carbonic acid (H_2Co_3) ions. The reactions of C_4 pathway are explained by Hatch and Slack and so the cycle is also called Hatch-Slack cycle.

CO_2 compensation point is less in C_4 plants (1–5 ppm of CO_2). The concentration of CO_2 at which the rate of respiration (release of CO_2) and the rate of photosynthesis (CO_2 used) are equal is called CO_2 compensation point. In C_3 plants, CO_2 compensation point is more (40–60 ppm CO_2). At compensation point, the net gain of photosynthesis is "zero." In C_4 plants, as this net photosynthetic rate is more, growth is more. But unfortunately, most of the C_4 plants are weed plants. Trials are in progress to incorporate C_4 cycle in C_3 crop plants.

Reactions of the C_4 photosynthetic carbon cycle

Enzyme	Reaction
1. Phosphoenolpyruvate (PEP) carboxylase	Phosphoenolpyruvate + $HCO_3^- \rightarrow$ oxaloacetate + P_i
2. NADP-malate dehydrogenase	Oxaloacetate + NADPH + $H^+ \rightarrow$ malate + $NADP^+$
3. Aspartate aminotransferase	Oxaloacetate + glutamate $\rightarrow$ aspartate + α-ketoglutarate
4. NAD(P) malic enzyme	Malate + $NAD(P)^+ \rightarrow$ pyruvate + CO_2 + NAD(P)H + H^+
5. Phosphoenolpyruvate carboxykinase	Oxaloacetate + ATP $\rightarrow$ phosphoenolpyruvate + CO_2 + ADP
6. Alanine aminotransferase	Pyruvate + glutamate $\leftrightarrow$ alanine + α-ketoglutarate
7. Adenylate kinase	AMP + ATP $\rightarrow$ 2 ADP
8. Pyruvate–orthophosphate dikinase	Pyruvate + P_i + ATP $\rightarrow$ phosphoenolpyruvate + AMP + PP_i
9. Pyrophosphatase	$PP_i + H_2O \rightarrow 2\,P_i$

Note: P_i and PP_i stand for inorganic phosphate and pyrophosphate, respectively.

Energetics of the C_4 photosynthetic carbon cycle		
Phosphoenolpyruvate + H_2O + NADPH + CO_2 (mesophyll)	→	malate + $NADP^+$ + P_i (mesophyll)
Malate + $NADP^+$	→	pyruvate + NADPH + CO_2 (bundle sheath)
Pyruvate + P_i + ATP	→	phosphoenolpyruvate + AMP + PP_i (mesophyll)
PP_i + H_2O	→	2 P_i (mesophyll)
AMP + ATP	→	2ADP
Net: CO_2 (mesophyll) + ATP + 2 H_2O	→	CO_2 (bundle sheath) + 2ADP + 2 P_i
Cost of concentrating CO_2 within the bundle sheath cell = 2 ATP per CO_2		

Note: As shown in reaction 1 of Table 8.3, the H_2O and CO_2 shown in the first line of this table actually react with phosphoenolpyruvate as HCO_3^-.
P_i and PP_i stand for inorganic phosphate and pyrophosphate, respectively.

22.6.1.6 Types of C_4 Plants

Three different types of C_4 plants are recognized. The primary carboxylation is similar in all of them. But depending on the C_4 acid that is transported from mesophyll to bundle sheath, the nature of the enzyme involved in decarboxylation reactions in bundle sheath cells and their intercellular localization differences are recognized.

They are as follows:

1. NADP-ME type: e.g., sugarcane, maize
2. NAD-ME type: e.g., amaranthus
3. PEPCK type: e.g., chloris, panicum

22.6.1.7 NADP-ME Type

These are malate formers. As explained previously, in these plants, first oxaloacetic acid and then malic acid are formed and they move to bundle sheath chloroplasts. There it undergoes oxidative decarboxylation to form carbon dioxide, and pyruvate moves back to mesophyll and is converted to PEP. The enzyme catalyzing decarboxylation is malic enzyme with NADP as coenzyme, and thus, these plants are called NADP-ME type.

22.6.1.8 NAD-ME Type

These are aspartate formers. In these plants, malic acid is converted to aspartic acid and in mitochondria of bundle sheath cells undergoes transamination to produce oxaloacetic acid (OAA). OAA is reduced to malic acid. In the chloroplasts, malic acid releases carbon dioxide, and pyruvate is formed with the help of the malic enzyme with NAD^+ as coenzyme. Pyruvate is converted to alanine by amination. Alanine enters mesophyll and by deamination converted to pyruvic acid. Pyruvic acid is converted to PEP in presence of pyruvate dikinase.

22.6.1.9 PEPCK Type

Phosphoenolpyruvate carboxykinase type. These are also called aspartate formers. However, aspartic acid entering bundle sheath is converted to oxaloacetate and, instead of being reduced as in NAD-ME type, releases CO_2 in chloroplasts in the presence of ATP and PEP carboxykinase enzymes. CO_2 is used in carbohydrate synthesis by Calvin cycle. Pyruvic acid is converted to alanine and enters mesophyll. As the enzyme involved in decarboxylation is PEP carboxykinase, these plants are called PCK type.

22.6.1.9.1 Significance

In C_4 plants, as both the pathways (C_3 and C_4) operate in the same plant, the scope of efficient usage of CO_2 is more. It acts as an adaptation in plants growing in high temperature and drought conditions and enables the plants to grow efficiently in those high temperatures and water scarcity. The studies of C_4 pathway paved the way for the concept of getting high yield by suitable modification in plants. Further, the understanding of C_4 plants may help in selection of plants with efficient photosynthesis.

22.6.1.10 Crassulacean Acid Metabolism (CAM Pathway)

Some plants growing under water scarcity and high temperature close their stomata during day-

time and open their stomata during nighttime. In these plants, CO_2 fixation takes place in nighttime, and thus, this method is called "dark carbon assimilation." This method of carbon assimilation is identified in 20 angiospermic families like Crassulaceae (e.g., *Kalanchoe*, *Sedum*), Cactaceae, Agavaceae, Orchidaceae, Portulacaceae, and Euphorbiaceae. All such plants are called CAM plants and the pathway is called CAM pathway or dark carbon assimilation.

In CAM plants, during daytime the organic acids like malic acid are in low concentration and found in high concentration during nighttime. Further, the concentration of reserve materials like starch is more during daytime and less during nighttime. The leaves and sometimes stems or petioles are succulent. The cells along with other cell organelles have large water-filled vacuoles.

All CAM plants are succulents. But all succulents are not CAM plants. The process of carbon fixation in CAM plants resembles those of C_4 plants. While C_4 plants show Kranz anatomy, formation of C_4 acids, and decarboxylation and refixation of carbon dioxide and exhibit spatial separation of these reactions, CAM plants show temporal separation (separation by time) of formation of C_3 and C_4 acids. Oxaloacetic acid is formed in cytoplasm. During nighttime, by carboxylation, PEP is converted to malic acid and stored in vacuoles. During day time, malic acid from vacuoles enters chloroplasts and releases CO_2 by decarboxylation. CO_2 released is fixed by Calvin cycle. The reactions of CAM pathway are shown as follows (Fig. 22.9).

22.6.1.10.1 Significance

The CAM pathway helps the plants to tolerate drought conditions. C_4 and C_3 plants lose 250–300 g of water, respectively, for every gram of CO_2 obtained, but CAM plants can lose only 50–100 g of water. Further, the reaction takes place in such a way that more CO_2 is available for RuBisCO, and the carbon loss by photorespiration is prevented.

However, the CO_2 stored in dicarboxylic acids limits photosynthesis. Thus, the rate of photosynthesis is less. This pathway acts only as an adaptation to plants to enable them to grow in drought conditions.

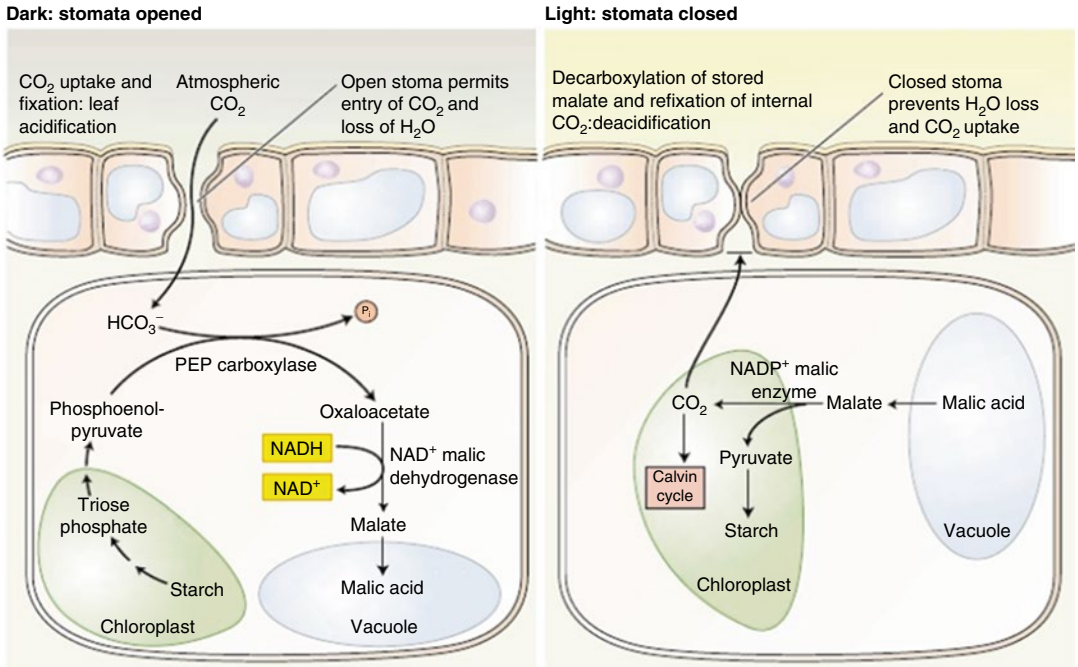


Fig. 22.9 Crassulacean acid metabolism (CAM). Temporal separation of CO_2 uptake from photosynthetic reactions: CO_2 uptake and fixation takes place at night and decarboxylation and refixation of the internally released CO_2 occurs during the day. The adaptive advantage of CAM is the reduction of water loss by transpiration, achieved by the stomatal opening during the night (Source: Buchanan et al. 2000)

Comparison of C_3, C_4, and CAM pathways

S.No.	Character	C_3 plants	C_4 plants	CAM plants
1.	Leaf anatomy	Non-Kranz	Kranz type	Non-Kranz
2.	Enzyme catalyzing	RUBP case	PEP case	PEP case
3.	Primary carboxylation	Ribulose-1,5-bisphosphate carboxylase/oxygenase	Phosphoenolpyruvate	Phosphoenolpyruvate
4.	First stable compound formed during photosynthesis	3 PGA 3-phosphoglycerate (3 carbon compound)	Malic acid or aspartic acid	Malic acid
5.	Photorespiration	Present	Absent	Absent
6.	CO_2 compensation point	>50 µl/l	>50 µl/l	Dark <50 µl/l
				Light >50 µl/l
7.	Carbon assimilation rate mg/ CO2 dm_2/h	15–20	35–35	10–25
8.	Optimum temperature for photosynthesis	15–25 °C	35–35 °C	35–35 °C
9.	Inhibition of photosynthesis by oxygen	30 %	Absent	Absent
10.	Translocation of organic compounds in leaves	Slow	Quick	Slow
11.	Transpiration/photosynthesis ratio	More	Less	Very less
12.	Growth rate in plants	Less	More	Very less

References

Adams WW, Demming-Adams B, Rosenstiel TN, Ebbert V (2001) Dependence of photosynthesis and energy dissipation activity upon growth from light environment during the winter. Photosynth Res 67:51–62

Aran M, Ferrero DS, Pagano E, Wolosink RA (2009) Modulation of typical 2 cys peroxiredoxins by covalent transformations and non-covalent interactions. FEBS J 276:2478–2493

Buchanan BB, Balmer Y (2005) Redox regulations: a broadening horizon. Annu Rev Plant Biol 56:187–220

Buchanan BB, Gruissem W, Jones RL (2000) Biochemistry and molecular biology of plants. The American Society of Plant Physiologists, Rockville

Engelmann TW (1883) *Bacterium photometricum*. Ein Betrag zur vergleichenden Physiologie des Licht-und farbensinnes. Arch Physiol 30:95–124

Gutteridge S, Galensly AA (1995) Rubisco synthesis, assembly, mechanism and regulation. Plant Cell 7:809–819

Hopkins WG, Huner NPA (2009) Introduction to plant physiology, 4th edn. Wiley, London

Levitt J (1969) Introduction of plant physiology. The CV Mosbry Company, Saint Louis

Rabinowitch, Govindjee (1970) Photosynthesis. Eugene Wiley, Borbieur, New York

Raghavendra AS (1998) Photosynthesis a comprehensive treatise. Cambridge University Press, Cambridge

Raghavendra AS, Das VSR (1978) The occurrence of C4-photosynthesis: a supplementary list of C4 plants reported during late 1974-mid –1977. Photosynthetica 12:200–208

Rainer E, Hirsch H-J, Kreuzaler F, Peterhansel C (2002) Overexpression of C_4 cycle enzymes in transgenic C_3 plants: a biotechnological approach to improve C_3-photosynthesis. J Exp Bot 53:591–607

Rochaix JD, Goldschmidt-Clermont M, Merchant S (1998) The molecular biology of chloroplasts and mitochondria in *Chlamydomonas*. Kulwar Academic Publishers, Dordrecht

Ruben S, Kamen MD (1941) Long-lived radioactive carbon: C14. Phys Rev 59:349–354

Salisbury FB, Ross CW (1992) Plant physiology, 4th edn. Wadsworth Publishing Co. Ltd, California

Taiz L, Zeiger E (2010) Plant physiology, 5th edn. Sinauer Associates, Sunderland

Van Niel CB (1931) On the morphology and physiology of the purple and the green bacteria. Arch Mikrobiol 3:1–12

Veit W, Govindjee (2000). Z-scheme diagram of photosynthesis. University of Illinois at Urbana-Champaign

Von Caemmerer S (2000) Biochemical models of leaf photosynthesis. CSIRO, Melbourne

Von Sachs J (1887) Lectures on the physiology plants (trans: Ward HM). pp 309–322. Clarendon Press, Oxford

Zeeman S, Smith SM, Smith AM (2007) The diurnal metabolism of leaf starch. Biochem J 401:13–28

Induced Mutations and Crop Improvement

23

P. Suprasanna, S.J. Mirajkar, and S.G. Bhagwat

Abstract

Genetic variation is the mainstay which plant breeders require to produce new and improved cultivars. The opportunity of obtaining novel traits exists through induction of mutations. Induced mutations have played a significant role in meeting challenges related to world food and nutritional security by way of mutant germplasm enhancement and their utilisation for the development of new mutant varieties. A wide range of genetic variability has been induced by physical and chemical mutagens. In the past several decades, induced mutations have contributed immensely to the development of improved varieties in several crop plants. Cellular and molecular biology tools have led to enhanced efficiency of induction, detection and deployment of mutations. Till date, 3,218 mutant varieties have been released worldwide. More than 60 % of officially released mutant varieties are from Asia with China, India and Japan topping the list. The mutant varieties developed and released in major crops have been cultivated by farmers in large areas and have resulted in increased food production, thus contributing to food security. In this chapter, various aspects of mutation induction, applications and examples of successful use of induced mutants in crop improvement programmes are presented.

Keywords

Mutation breeding • Physical and chemical mutagens • Induced mutants • Irradiation • Crop improvement

P. Suprasanna (✉) • S.G. Bhagwat (Ex-Scientist)
Nuclear Agriculture and Biotechnology Division,
Bhabha Atomic Research Centre,
Trombay, Mumbai, MS 400085, India
e-mail: prasanna@barc.gov.in

S.J. Mirajkar
Department of Agricultural Botany, Dr. Panjabrao
Deshmukh Krishi Vidyapeeth,
Akola, MS 444104, India

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_23,

23.1 Introduction

Plant breeding methods have contributed immensely to the development of genetically improved crop varieties. These methods continue to enrich the crop germplasm base by evolving genetically superior varieties for cultivation. Existing germplasm resources may not be adequate to meet the food needs of an ever-increasing human population, estimated to swell to nine billion by 2050 (Green et al. 2005). Further increase in agricultural productivity, equitably and in an environmentally sustainable manner, in the face of limiting resources, is a challenging task. Although both domestication and modern breeding have led to present-day crops that are far superior in agronomic traits to their wild counterparts, in many cases these also resulted in a narrowed genetic diversity (Tanksley and McCouch 1997; Lee 1998). It is estimated that for most crop species, less than 5 % of the biodiversity known to exist is being utilised in agriculture, particularly in the case of self-pollinated crops (Tanksley and McCouch 1997).

The use of induced mutations has played a key role in the improvement of superior plant varieties (Ahloowalia and Maluszynski 2001; Maluszynski et al. 2004; Jain 2005). A large number of improved mutant varieties have been released for commercial cultivation in different crop species demonstrating economic value of the mutation breeding technology (Kharkwal and Shu 2009; Jain and Suprasanna 2011). In addition to the currently practised methods of genetic improvement, there is a greater need for developing new and innovative research for evolving sustainable agriculture systems. Compared to methods of crossbreeding, mutagenesis enables modification of one or a few characters in an otherwise promising cultivar without significantly altering the remaining (and often unique) genetic background. Mutagenesis techniques have also been integrated with other molecular biology technologies, such as molecular marker techniques and high-throughput mutation screening techniques, thereby becoming more powerful and effective in crop breeding (Shu 2009).

Induced mutations provide a viable option by generation of a novel source of resistance to biotic/abiotic stress factors whereby a new resistant variety can be developed. Since the very early part of the twentieth century, several experimental breakthroughs were made in the area of induced mutagenesis (Fig. 23.1). Mutations occur spontaneously,

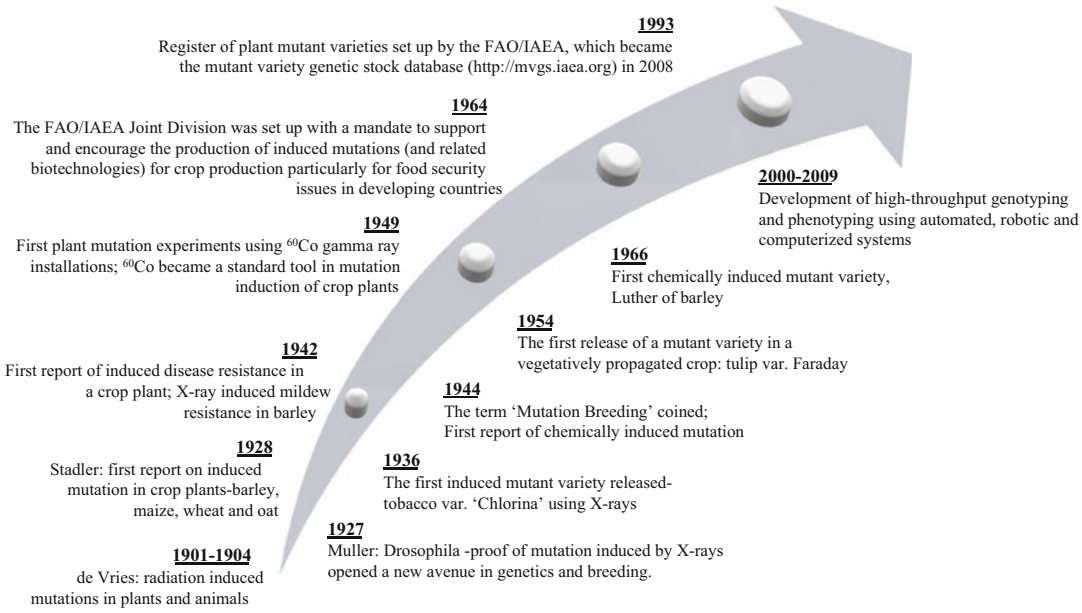


Fig. 23.1 Milestones in the development of induced mutagenesis

however, at a very low frequency. They can happen due to mistakes or errors occurred during DNA replication or repair. It is estimated that a mutation occurs every 10^{-8} base pair per generation in eukaryotic genomes (Drake et al. 1998). In corn (*Zea mays*), mutations occur from 10^{-6} to 5×10^{-4} per base pair per generation (Stadler 1930). Those that we can track easily in the offspring are mutations occurring either in the gametes or cells that give rise to gametes. Mutations in somatic cells cannot be easily tracked nor can these be passed on to future generations; therefore, these are important only in vegetatively propagated species (Suprasanna et al. 2012). Mutations can be induced at higher frequency by exposing cells to mutagens; thus, making use of induced mutations is an important choice in crop improvement (Jain 2000, 2010, 2012).

A mutant variety is a new plant variety that is bred through either:

1. Direct use of a mutant line that is developed through physical and chemical mutagenesis or somaclonal variation
2. Indirect use of a mutant line, which is used as a parental variety in crossbreeding (cross between mutant lines or with a commercial variety)
3. Use of mutant gene allele (trait)
4. Use of wild species' genes translocated into plant genomes through irradiation/mutagen-derived translocations, e.g. genes of wheat wild relative species

23.2 Mutagens: Tools for Inducing Genetic Variability

Several types of mutagenic agents are used extensively to create genetic variation for use in genetics and/or crop improvement. HJ Muller (1928) first discovered that X-rays had mutagenic properties. Since then, ionising radiations and chemical mutagens have found their application and contributed a great deal in induced mutagenesis. Physical and chemical mutagens cause DNA damage. Living cells can respond quickly to DNA damage and, in turn, initiate different mechanisms either by killing the damaged cell or by repairing DNA lesions; the consequences of these processes are directly linked to mutation breeding. Ionising radiations cause single- and double-strand breaks (SSBs and DSBs) and also produce chemically reactive species that interact with the cellular/molecular environment.

23.2.1 Ionising Radiations

Ionising radiations include X-ray, gamma (γ) rays, neutrons and high-energy ion beams. These can cause double-strand DNA breaks. The γ-ray bombardment is less destructive causing point mutations and small deletions, whereas fast neutron bombardment causes translocations, chromosome losses and large deletions. Among the physical mutagens, gamma rays are the most popular among mutation breeders because of the convenience of use and their ability to penetrate deep into a biological matter (Table 23.1). Gamma rays induce nucleotide substitutions and small deletions of 2–16 bp and the mutation frequency is estimated to be one mutation/6.2 Mb (Sato et al. 2006). Fast neutrons are believed to result in kilobase-scale deletions (Li et al. 2001).

Gamma rays obtained from radioactive isotope of cobalt (^{60}Co) are widely used. The isotope has a half-life of 5.3 years and emits radiations of energies 1.33 MeV and 1.17 MeV. Since the dose rate for a given irradiator is fixed, the dose is varied by determining duration for which the sample should be exposed to the source. The material does not have added or induced radioactivity and hence can be handled after treatment without any precautions. In case of neutron irradiation, energy is distributed in irradiated tissues (Ekram et al. 2013). Gamma irradiation is used in two ways in mutation induction experiments: acute or chronic irradiation. In case of acute irradiation, a single dose of radiation is given to plant material using a relatively high dose rate and shorter time exposure in a gamma cell/chamber. Gamma irradiation can also be used for prolonged exposure at a lower dose rate in a gamma facility placed in a glasshouse, in a field or in a growth chamber

Table 23.1 Types and properties of ionising radiations used for plant-induced mutagenesis

	Properties		
Type of radiation	Description	Energy	Penetration in plant tissue
X-rays	Electromagnetic radiation	50–300 keV	A few mm to many cm
Gamma rays	Electromagnetic radiations similar to X-rays	Up to several MeV	Through whole parts
Neutron (fast, slow and thermal)	Uncharged particle, slightly heavier than proton, observable only through interaction with nuclei	From less than 1 eV to several MeV	Many cm
Alpha particles	A helium nucleus, ionising heavily	2–9 MeV	Small fraction of a mm
Beta particles, fast electrons or cathode rays	An electron (− or +) ionising much less densely than alpha particles	Up to several MeV	Up to several cm
Protons or deuterons	Nucleus of hydrogen	Up to several GeV	Up to many cm
Low-energy ion beams	Ionised nucleus of various elements	Dozens of keV	A fraction of mm
High-energy ion beams	Ionised nucleus of various elements	Up to GeV	A fraction of cm

Adopted and modified from Mba et al. (2010)

under controlled environmental conditions so that plants can be irradiated as they grow over extended periods of time.

23.2.2 Ion Beams

Ion beams are usually generated by particle accelerators, e.g. cyclotrons, using ^{20}Ne, ^{14}N, ^{12}C, ^{7}Li, ^{40}Ar or ^{56}Fe as radioisotope sources (Tanaka et al. 2010). These ion beams are responsible for linear energy transfer (LET), and as LET increases, higher biological effects such as lethality, chromosomal aberration, etc., are induced as compared to most commonly used physical mutagens (Watanabe 2001). The LET for gamma rays and X-rays accounts in the range of 0.2–2 keV/μm and hence is called low-LET radiations. In contrast the high-LET radiations from carbon (23 keV/μm) and iron (640 keV/μm) ion beams provide much larger and wider ionisation energy. High-LET ion beam radiations cause more localised, dense ionisation within cells than those of low-LET radiations (Abe et al. 2012). The choice of ion beam depends on the characteristics of the ion with respect to electrical charge and velocity. When a high LET is required, a heavier, highly charged ion having low velocity is selected. Dose (in Gy) is proportional to the LET (in keV/μm) and number of particles. Determining an optimal irradiation dose of ion beams is most important, and an ideal irradiation dose is a dose at which ion beams show the highest mutation rate at any locus of interest (Magori et al. 2010). Hence, irradiation doses should be chosen by testing different doses at a time and screening the irradiated population for desired mutants. Since this requires considerable investment of time and efforts, researchers may consider traits such as survival rate, growth rate, chlorophyll mutation, and so on, which are early indicators for occurrence of mutation (Magori et al. 2010).

Energetic heavy-ion beams are used for generating mutants in higher plants because these induce mutations with high frequency at a relatively low dose (i.e. at which virtually all plants survive) and thereby induce a broad spectrum of phenotypes without affecting other plant characteristics (Tanaka et al. 2010). Advantages of ion beam mutagenesis include low dose with high survival rates, induction of high mutation rates and wide range of variation. Thus, a new

crop variety can be obtained by selecting a mutant with modification to a target trait while retaining the existing valuable ones. However, stepwise trait improvement is expected to be especially effective for plants, those which cannot be crossbred and/or those which have highly duplicated genomes. Hase et al. (2012) demonstrated a novel approach to facilitate directional mutagenesis by altering growth conditions which increased mutation frequency in violet-flowered Petunia. The researchers used a combination of ion beam and high-sucrose pretreatment and obtained higher number of M_2 lines containing flower-colour mutants (3 %) as against using only ion beam (0.5 %). If mutation frequency can be controlled in this way, using a combination of suitable ion type and suitable plant tissue or organ, then the pretreatment may enable occurrence of desirable mutations much easily (Tanaka et al. 2010).

Ion beam is an excellent tool for mutation breeding to improve horticultural and agricultural crops with high efficiency. In rice, four salt-resistant lines were developed through ion beam irradiation, one of which was grown in a saline paddy field with good yield (Hayashi et al. 2007, 2008). Other examples include development of semi-dwarf buckwheat that is shorter and sturdier than normal varieties (Morishita et al. 2003) and development of mutants that enable molecular understanding of the mechanisms of flowering in sterile Eucalyptus and wheat (Shitsukawa et al. 2006). Many varieties of ornamental plants including Carnations, Chrysanthemums and Petunias were also generated using this technology (Okamura et al. 2001; Magori et al. 2010). Yu and Anantulabochai (2011) have described success in obtaining useful mutants in rice and gerbera by using 30–60 keV low-energy ion beam.

23.2.3 Aerospace Mutagenesis

More than 90 % of the space radiation is composed of protons, neutrons, heavy particles, rays and microgravity (Ohnishi et al. 2002). Spaceflight has been demonstrated to affect plant gnome mutations, gene expression patterns, methylation patterns and metabolism products (Porterfield et al. 2000; Paul et al. 2005; Ou et al. 2009). In China, more than 400 varieties belonging to 50 different species have been sent to outer space by 8 recoverable satellites. Profitable genetic variations were produced, and more than 50 new varieties have been commercialised (Liu et al. 2007). Using these tools, genetic diversity and the stability of mutations induced in the space environment were analysed (Lu et al. 2008). Ou et al. (2009) reported spaceflight-induced epigenetic variations through analysing methylation patterns in space-flown plants. To improve upland rice, seed samples of Huhan 3 and Huhan 7 varieties were sent to the outer space, with two recoverable spaceships, for 1 and 5 days and were propagated for seven and five generations, respectively (Yu et al. 2013). The study revealed more lines with high yield, high quality and drought tolerance through aerospace breeding. Although progress is being made, mechanisms of mutation induction and their biological effects still remain to be investigated.

23.2.4 Chemical Mutagens

Chemical mutagens provide high mutation rates and induce mostly point mutations. These include base analogues, acridine dyes, nitrous acid, hydroxylamine, etc. (Table 23.2). Leitão (2012) has detailed various plant chemical mutagens and their action. Alkylating agents, such as ethyl methanesulphonate (EMS), react with guanine or

Table 23.2 Chemical mutagens commonly used in induced mutagenesis (Maluszynski et al. 2009)

Name of the mutagen	Abbreviation	Molecular weight
Ethyleneimine	EI	43.07
Dimethyl sulphate	DMS	126.13
Diethyl sulphate	dES (DES)	154.19
Ethyl methanesulphonate	EMS	124.20
N-ethyl-N-nitrosourea	ENU (ENH)	117.11
Methylnitrosourea	MNU (MNH)	103.08
N-methyl-N[1]- nitro-N-nitrosoguanidine	MNNG	147.09
Sodium azide	NaN3 (Az)	65.01

thymine by adding an ethyl group which causes the DNA replication machinery to recognise the modified base as an adenine or cytosine, respectively. Chemical mutagenesis induces a high frequency of nucleotide substitutions, and a majority of the changes (70–99 %) in EMS-mutated populations are GC to AT base pair transitions (Till et al. 2004, 2007). Sodium azide (Az) and methylnitrosourea (MNU) are also used in combination. Az-MNU mutagenesis can induce a shift in either direction of GC to AT shifts or AT to GC shifts (Till et al. 2007). The mutation frequency induced by Methylnitrosourea (MNU) in *Glycine max* (one mutation/140 kb) was comparable to the frequency induced by EMS (Cooper et al. 2008). In rice, however, higher frequency of mutations was induced by MNU (one mutation/135 kb) than EMS (one mutation/300 kb) (Till et al. 2007; Suzuki et al. 2008; Martín et al. 2009).

The determination of dose for chemical mutagens is often made by varying the concentration and duration of treatment; the solvent used, e.g. dimethyl sulfoxide (DMSO); or the pH of the solution. All these chemical mutagens are strongly carcinogenic and extreme care should be taken while handling and disposal. EMS is an IARC group 2B carcinogen. Working with MNU can be sometimes difficult as it is unstable above 20 °C. EMS solutions can be deactivated in a solution of 4 % (w/v) NaOH and 0.5 % (v/v) thioglycolic acid. Chemical mutagens (EMS, DES, Az) have been applied for treating banana shoot tips to produce variants for tolerance to *Fusarium* wilt (Bhagwat and Duncan 1998). EMS has also been successful in obtaining a wide range of variations in petal colour in Chrysanthemums (Jain 2006) and salt-tolerant lines in sweet potato (Luan et al. 2007).

23.3 Considerations While Performing Induced Mutagenesis

Various aspects need to be taken under consideration before initiating an induced mutagenesis programme. There are three factors important to the success of mutation breeding: the efficiency of mutagenesis, the starting plant material and the mutant screening (Hase et al. 2010). Major stages and routes of induced mutagenesis programme are illustrated in Fig. 23.2.

23.3.1 Choice of Plant Material for Mutagenic Treatment

Mutations are induced by exposing plant propagules to physical and chemical mutagenic agents (Bhagwat 2009; Mba et al. 2010). The plant material such as seeds in the case of seed-propagated crops and plant parts such as stem cuttings, twigs, buds and tubers in vegetatively propagated plants can be exposed to mutagen(s). Purity of the parental material used for mutagenesis is extremely important. The dose rate or intensity, type of mutagen and concentration of mutagen to be used may vary depending upon the type of material chosen. Tissues that are metabolically active or have high water content are more sensitive to radiation damage (Bhagwat 2009). In case of most vegetatively propagated crops (VPCs), *in vitro* cultures are now used as starting material for mutation induction (Suprasanna et al. 2012). Well-dried seeds with good germination ability can be used for irradiation. Since mutation is a chance event, larger experimental population is recommended in early generations (M_1 and M_2). If the trait under consideration is governed by a single gene and if the species is not polyploid, identification of the mutant is easier. However, in case of quantitative (polygenic) traits and in plants with higher ploidy, observing a mutant is possible if the mutagenised population is sufficiently large. If a mutation is likely to occur at a frequency of one in thousand plants, at least 1,000 M_2 generation plants will have to be screened to attain the statistical probability.

These in turn are obtained from the selfed M_1 generation plants and usually twenty seeds from each M_1 plant are sown to raise the M_2 generation population. This would mean, to obtain 1,000 M_2 plants, at least 50 M_1 plants have to survive and set adequate number of seeds. To have these, 100 plants would be required as the starting number in the M_1 generation (if LD_{50} dose is employed). Thus, if 100 seeds are irradiated at LD_{50} dose,

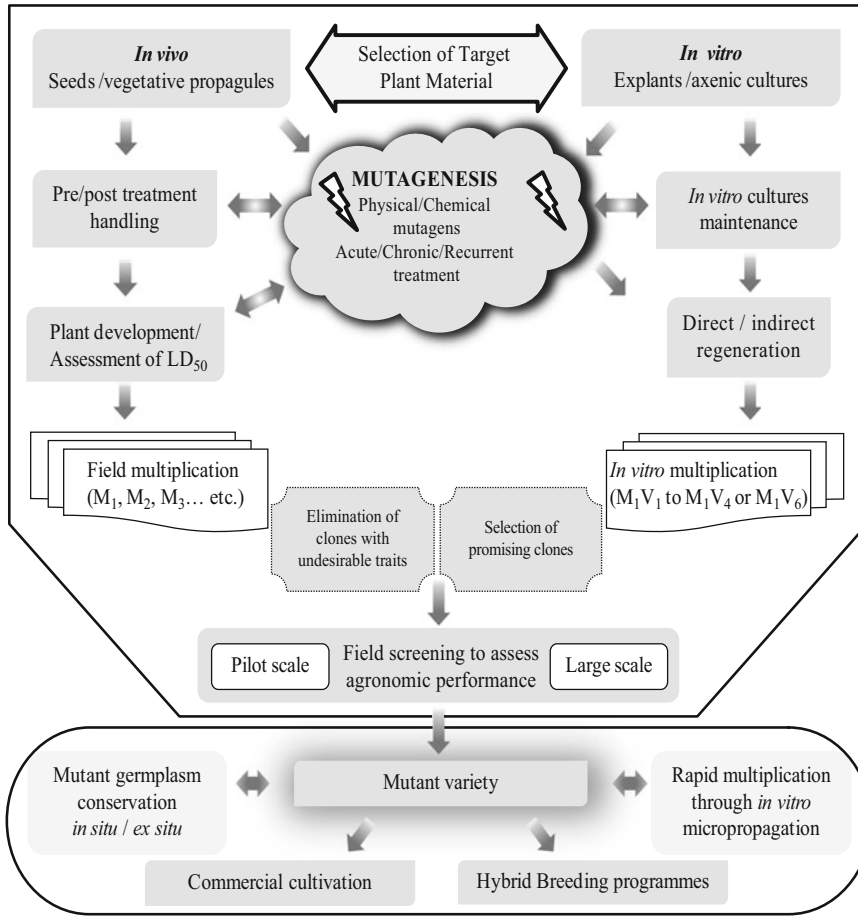


Fig. 23.2 Stages in the development of a mutant crop variety using induced mutagenesis

there is a subsequent possibility of obtaining one mutant of that particular type. To ensure that the mutant occurs, it is recommended that a higher number of seeds are irradiated. Also, if several mutants of a particular phenotype are identified, it presents an opportunity to select and carry forward the one with the potential for the best economic returns.

Although mutations can be induced in all types of plants, to obtain desired mutant in prescribed time, effort and facilities, it is important to consider suitability of the plant species for mutation breeding. Self-pollinating, seasonal plants are more suitable as they can be grown in large numbers in smaller field area and generation time is shorter. Thus, mutants can be identified and confirmed in shorter time periods. Cross-pollinated plants, large-sized trees or plants with long generation time are not as suitable; however, they can be used for mutation breeding. Similarly, plants with higher levels of ploidy may continue to segregate before the mutant phenotype is identified and stabilised. Mutagenesis in a cross-pollinated plant species is more complicated as most mutations remain recessive and fail to express since they cannot reach homozygosity and subsequently may get eliminated. The alternative is to self-pollinate the mutagenised population; however, self pollination may result in loss of vigour.

One of the bottlenecks of plant mutation breeding is the occurrence of chimeras following the mutagenic treatment of multicellular tissues. In vegetatively propagated crop plants (VPPs), a mutated cell gives rise to a shoot or a branch

which needs to be separated and propagated to attain a uniform and non-chimeric mutant plant (Geier 2012). An *in vitro* technique was utilised for isolating new ornamental varieties through retrieval of chimeric tissues derived by induced mutagenesis in Chrysanthemums (Datta and Chakrabarty 2009). This technique also has practical application for mutation in other ornamental plants. The combined method of irradiation and *in vitro* culture yielded a mutation rate eight times higher than the conventional chronic irradiation of cutting which produced non-chimeric mutants in Chrysanthemum (Nagatomi and Degi 2009).

23.3.2 Dose of Mutagen

To obtain a mutant, the dose of the mutagen should be sufficiently high to increase the probability of inducing a mutation; however, it should not be so high as to cause damage to the cells/tissues resulting in lethality. Radiation dose is expressed in rads (radiation-absorbed dose) which is equivalent to absorption of 100 ergs/g. The unit kilorad (kR which is 1,000 rads) which was in use earlier is replaced by gray (Gy) which is currently used. The two can be interconverted as 1 kR is equivalent to 10 Gy. A concept of LD_{50} (lethal dose 50 %) is used to refer the optimum dose to be used in the experiment. By definition LD_{50} is the dose which causes 50 % lethality in the organism used for irradiation in defined time. It varies with the plant species, the type and status of the material and the stage at which lethality is measured. Generally, irradiated populations are generated by using an LD_{50} dose treatment and with a dose lower than LD_{50}. Since induction of mutation is a chance event, and recovery of a mutation is dependent upon chance of the survival of that individual plant, this strategy improves the probability of obtaining a desirable mutant. In a case where LD_{50} dose is already reported, it can be used as a guideline; otherwise, it can be determined by exposing different subsamples of the target plant material (seeds) to a range of doses (low to high) and monitoring survival of the plants in field (up to flowering or maturity). In plants which are sensitive to radiation, doses lower than LD_{50} are also used to reduce the mutation load (Shu et al. 2012). Therefore, it is preferred to work out radiosensitivity test between LD_{25} or LD_{30} and LD_{50} to obtain mutation for desired and optimum traits (Choudhury 1983; Maluszynski et al. 2003).

Chemical mutagen dose is determined in account of the properties of the mutagen (half-life, penetrability, solubility, toxicity or reactivity); type and condition of the treated material before, during and after treatment; interaction with target tissue and culture medium; pH of the medium; and posttreatment handling of the material (van Harten 1998). Pre-soaking and prolonged treatment at lower concentrations in combination with the right temperature are practised where the exterior of the target material (viz., hard seed coat in conifers and legumes) obstructs tissue penetrability of the mutagen. The optimum dose of physical and chemical mutagens for seed treatment of major cereal crop plants is given in Table 23.3. The dose may also depend on the genome size of the plant species under study and often negatively reciprocate to the genome size (Table 23.4). To maximise recovery of mutants, it is possible to adjust the dose of the mutagen, for example, the germinating seeds of tetraploid cotton (*Gossypium hirsutum*) were exposed to three to five times the LD_{50} rate of EMS (3 % v/v) to ensure recovery of mutants (Auld et al. 1998). In contrast, EMS treatment of germinating sugar beet seeds required only 0.5 % (v/v) EMS to elicit a sufficiently mutagenic response (Hohmann et al. 2005). Over 3,200 M_2 families were derived from an early bolting line treated with EMS, of these only 9 families exhibited the desired non-bolting trait and eventually gave rise to 5 lines with the non-bolting phenotype (Hohmann et al. 2005).

The combined treatment of physical and chemical mutagens is of apparent interest to a mutation breeder with an intention of enhancing mutation spectrum and frequency, thereby maximising efforts to obtain positive results. In practice, seeds are exposed to physical mutagen first, followed by a treatment of chemical mutagen in solution (van Harten 1998). The other

Table 23.3 Optimum dose rate of physical and chemical mutagens for seed treatment of cereals

	Crop species		
Mutagen	Rice	Wheat	Barley
Physical mutagens			
Fast neutrons	3–8 Gy	2–6 Gy	2–5 Gy
Thermal neutrons	–	1×10^{11} to 8×10^{12} N/cm^2	4×10^{7} to 6.5×10^{7} N/cm^2
X-rays	95–250 Gy	150–250 Gy	60–200 Gy
Gamma rays	100–350 Gy	50–350 Gy	150–400 Gy
Chemical mutagens			
EMS	(0.2–0.5 %)×(8–20 h)	(0.01–0.04 %)×(10–30 h)	(0.02–2.5 %)×(8–20 h)
NaN_3	(0.5–2 mM)×(3–5 h)	(0.5–2.0 mM)×5 h	(0.5–1.5 mM)×5 h
DMS	(0.01–0.05 %)×(4–6 h)	(0.005–0.04 %)×5 h	(1.0–1.5 %)×(8–12 h)
EI	(0.01–0.03)×(3–6 h)	(0.04–0.09 %)×(3–5 h)	(0.03–0.06 %)×(8–12 h)
MNH (MNU)	(0.7–1.5 mM)×(3–5 h)	(0.75–1.5 mM)×5 h	(0.5–1.0 mM)×5 h
ENH (ENU)	(1.7–2.5 mM)×(3–5 h)	–	(1.0–2.5 mM)×5 h
DES	–	(0.4–1.0 %)×5 h	–
Ethylene oxide	–	–	(0.02–0.04 %)×(15–20 h)

Maluszynski et al. (2009)

Table 23.4 Comparison of optimal irradiation doses (LD_{50}) and genome size of the plant materials (Magori et al. 2010)

	Radiation (in Gy)		
Plant material	18.3 MeV/u C[a]	12.5 MeV/u He[a]	Low-LET radiations[b]
(a) Dry seeds (genome size)			
Arabidopsis (130 Mb)	300	1,100	1,200 (electrons)
Rice (430 Mb)	40–50	200	350
Tomato (950 Mb)	70	240	–
Barley (4.8 Gb)	10–20	–	–
Wheat (16 Gb)	25	–	–
(b) Tissue cultures			
Chrysanthemum var. Taihei	15	10–20	~60–80
Chrysanthemum var. Jimba	3	2–3	~10
Carnation	15	40	60

[a]Carbon and helium ions

[b]Gamma rays

way of recurrent mutagen treatment signifies recovery of hundreds of mutants in a very short time span. Preliminary studies on recurrent X-ray irradiation in tetraploid and hexaploid wheat species for six consecutive seed generations showed a sharp decrease in mutation frequency for chlorophyll mutations after the first cycle of irradiation as the ploidy increased, but after the second cycle, the number of mutants detected was greatly increased in the tetraploid species (Kao and Caldecott 1966). Such recurrent irradiation X-ray treatment has resulted in the development of eight new cultivars of chrysanthemum starting from a single parent cultivar 'Horim' in a span of just 3 years (Broertjes et al. 1980; Micke et al. 1990). Recurrent gamma irradiation of chrysanthemum and rose resulted in enhanced genetic variability with increased percentage of mutations and spectrum of mutations (Datta 2009). In chrysanthemum, two new flower-colour mutants were recovered through the recurrent irradiation (Datta 1991). This also

evidenced utility of recurrent mutagen treatment in deriving greater genetic variability than a single mutagen treatment in vegetatively propagated ornamental plants (Datta 2009).

23.3.3 Furthering Generations of the Mutants Developed

It is important to have a precisely defined objective in a mutation breeding programme. A clearly specified objective, such as resistance to a disease, helps identify one or more plants with the mutant phenotype, thus narrowing the number of plants/lines to be carried forward to subsequent generations. An ideal situation is where a high-yielding and otherwise acceptable variety has one defect such as susceptibility to a specific disease. Examples of other traits that can be modified are change in plant height, days to flowering/maturity, non-shattering pods, etc. Simultaneous improvement in more than one trait is possible if provision to handle sufficiently large populations is available. Also, the use of more than one variety as a parent for mutagenesis is possible in situations where there is no constraint on resources such as land, irrigation and trained manpower. M_1 generation population should be grown in a constraint-free environment to ensure maximum survival and good seed set.

Mutagen-treated seeds are sown in field to obtain their first generation (M_1). Although a mutation event takes place in a cell in the germ part of the seed, it is in a heterozygous condition. Most mutations are recessive in nature and are not expressed in the first generation. M_1 generation may show variation in the growth of individual plants due to physiological effects, with the plants showing lethality at various stages of growth and development. The significance of M_1 population is that it is the source for M_2 generation, and ensuring maximum survival of M_1 generation plants is beneficial. Therefore, M_1 plants are numbered and harvested individually. At least twenty seeds from each M_1 progeny are used for raising the next (M_2) generation, in a plant-to-row fashion. The remaining seeds are saved to further recover mutants when required. The M_2 plants are often space planted to allow full expression of each individual plant. After meiosis in the M_1 plant, some seeds are formed by fusion of male and female gametes which are carriers of the same mutation and thus are homozygous for the mutation. These seeds give rise to an individual in which the mutant phenotype can be observed. The M_2 generation contains mutants of different kinds. The ones which can be detected visually are seedling colour, leaf shape and size, stem height, branching habit, flowering time, flower and fruit shape, size or colour, etc. There are however many types of mutations which may be in homozygous condition but cannot be observed by unaided eye.

23.3.4 Methods for Screening Mutants

Appropriate screening method is an important prerequisite for success in a mutation breeding programme. Since a large number of individual plants have to be screened in the M_2 generation, a rapid and economical method is necessary. It is easier to screen the populations for mutations in plant height, flowering date or pigmentation as these can be visually determined. For proper comparison the original parent should be planted in sufficient numbers. The parent variety rows may be introduced after certain number of rows of the M_2 population. In case of selection of a disease-resistant mutant, it is necessary that the M_2 population is grown at a hot spot location where the disease is present all the time. If conditions are not suitable for the disease to occur or spread on its own, spray of inoculum may be carried out. Rows of susceptible parent variety not only allow proper comparison but also help in the spreading of the disease in the entire M_2 population. Depending upon the nature of the trait of interest, screening methodology can be employed. For instance, to isolate mutants for biotic or abiotic stress resistance/tolerance, a relevant stress factor has to be employed during screening after the mutagen treatment in M_2 or later generation.

Disease symptoms may be observed as per the rating scale for any particular disease. All plants showing absence of disease or resistance reaction should be tagged. If the mutation involves a single locus, it may segregate in a monogenic ratio and out of the 20 plants in the row more than one mutant may be observed. The M_1 plant corresponding to the M_2 row is marked, and the remaining seeds of the specific M_1 plant may be sown to recover more mutants.

All plants with resistant reaction are putative mutants. The plants are allowed to self-pollinate. Seeds from individual plants are collected and each individual plant harvest is given an identification number. In the subsequent season, these have to be sown as plant-to-row progeny (M_3 generation). A putative mutant may confirm the presence of resistance mutation by showing a row which is true breeding for resistance. Often, mutation events are complex, and segregation may not throw up any mutations in the M_2 generation. It is possible that in the subsequent generations, such as M_3 or M_4, the mutation may appear. The mutant with resistance is grown for few generations in the location where disease occurs while practicing single-plant selection till a completely homozygous progeny is obtained. The resistant mutant may or may not have the ability to outyield the parent variety in the absence of the disease; however, it must show significant yield advantage in the presence of the disease. Agronomic performance of the mutant can be improved by using the mutant in a backcrossing or crossbreeding programme.

The mass screening for abiotic stress tolerance relies on quick, sensitive, efficient and preferably nondestructive methods. Conventional means to measure effects of stress may lead to false-positive findings. Screening for abiotic stress tolerance can be undertaken by assessing chlorophyll fluorescence, net photosynthetic rate, transpiration rate, stomatal conductance, water-use efficiency, free proline content, etc. (de Ronde and Spreeth 2007; Boureima et al. 2012; Cha-um et al. 2013, Mantri et al. 2014). Alongside to these physiological parameters, biochemical and molecular marker screening may also be followed.

23.4 *In Vitro* Mutagenesis and Mutant Selection

In vitro mutagenesis refers to mutations induced by treating explants or *in vitro* cultures (protoplasts, cells, tissues and organs) with a mutagen, followed by mutant screening/selection and characterisation. *In vitro* mutagenesis can be applied to both seed-propagated crops and vegetatively propagated crops. It is particularly advantageous in the latter case where a large number of uniformly growing *in vitro* cultures can be subjected to mutagenic treatment. Cultured cells, organs and tissues have a developmental pattern, therefore those can be synchronised and separation of chimeras can be done more efficiently (Suprasanna et al. 2012). For seed crops, the use of haploid culture may provide additional benefits. The whole process of *in vitro* mutagenesis involves a series of important steps: selection of proper target material (explants or cultures); selection of a mutagen and determination of a proper dose, posttreatment handling and subcultures; and regeneration of plants for mutant selection. Mutagenic treatments can be applied to explants or *in vitro* cultures (e.g. multiple shoot cultures, callus, cell suspensions, protoplasts, microspores, etc.). A variety of explants are available, e.g. apical meristems, axillary buds, roots and tubers. Whether explants or *in vitro* cultures are used for mutagenic treatment, all mutagenised cultures need to undergo a series of subcultures to dissociate chimeras and produce plants for selection of desired mutants. The number of subcultures needed depend on the type of species, the material mutagenised and the way of plant regeneration. It is not advisable to screen for mutations in the first vegetative generation after mutagenesis (M_1V_1) as mutations may remain masked or remain undetectable by chimeras in this generation. If useful mutants are found early, these should be monitored for stability in subsequent generations, up to M_1V_4 or M_1V_6 generations. Using recurrent irradiation *in vitro*, increased *in vitro* shoot multiplication and morphological variations were observed in shoot cultures of banana (Mishra et al. 2007). In an

attempt of carbon-ion beam irradiation of *in vitro* plantlets of banana (cv. Williams and Cavendish Enano) and their field evaluation resulted in six plants of cultivar Williams and two plants of cultivar Cavendish Enano being resistant to black sigatoka (Reyes-Borja et al. 2007).

The tissue structures from which plants originate are either multicellular or of single-cell origins. Normally chimeras are major problems in plants regenerated after mutagen treatment of multicellular structures such as shoot tips or axillary buds. However, chimeras can be easily dissociated in *in vitro* culture by repetitive subculturing, normally involving about four generations (M_1V_4). Compared to *in vivo* bud mutagenesis, this translates into a lower frequency of chimeric plants and a higher probability of mutant phenotype expression. It also facilitates rapid completion of propagation cycles (subculturing) aimed at separating the mutated from non-mutated sectors. *In vitro* selection and cloning can, therefore, capitalise on early evaluation and large-scale multiplication systems. *In vitro* mutagenesis with pingyangmycin (PYM) as the mutagen in peanut and *in vitro* selection for salinity tolerance led to isolation of 23 salt-tolerant M_3 progenies (Zhao et al. 2013). *In vitro* mutagenesis in combination with cellular selection was employed for obtaining salt tolerance in sugarcane (Patade et al. 2006; Nikam et al. 2014). Embryogenic calli were irradiated with different doses of gamma rays, subcultured and exposed to inhibitory level of NaCl. Salt-selected plants grown to maturity exhibited better agronomic performance under control and saline conditions. The mutant clones were characterised at the biochemical level for proline, glycine betaine, Na+ and K+ content and at the field level for agronomic characters and yield (Nikam et al. 2014).

It is apparent that *in vitro* mutagenesis is particularly pertinent to VPCs, since sexually propagated crops can be mutagenised using seed materials and resultant mutant lines can be developed and selected in subsequent generations, which is not possible in VPCs. In the case of seed crops, backcross to the original line can exclude unwanted mutant genes, but in vegetatively propagated crops, this procedure cannot be applied. Table 23.5 presents examples of successful *in vitro* mutagenesis in some vegetatively propagated crop plants. During *in vitro* culture, it is possible to exercise selection of mutants for agronomical useful and genetically determined traits. For selection, one can use a culture medium containing a certain amount of herbicide, salt or aluminium or by exposure of cultures to physical stress such as cold or heat (Table 23.6). Such a stress situation kills cells not equipped with required tolerance or resistance and allows tolerant/resistant surviving cells to grow. These can be picked up, multiplied through subcultures and regenerated into plants.

In comparison to methodologies involving treatment of *in vivo* material, *in vitro* cultured explants provide a wider choice of controlled selection following mutagenic treatment. Screening performed *in vitro* allows for handling of large populations, avoiding the problem of working with a low number of individuals as is often in the case of *in vivo* plant material. Mutagenised cell suspensions, microspores and protoplast cultures have the greatest potential for *in vitro* selection owing to their uniformity. *In vitro* selection of mutated cells involves exposure to herbicides, salts, toxins, culture filtrates and low or high heavy metals or temperatures. The trait of interest is selectable at the cellular level and is expressed in regenerated plants. Not all traits can be selected at the cellular level, for example, yield, seed colour or plant height. Disease resistance, abiotic stress tolerance (particularly to salt and drought), enriched nutritional quality and herbicide tolerance are some of the traits that have been successfully selected in crop plants including VPCs. *In vitro* selected variants should always undergo specific *ex vitro* testing to confirm the existence of improved selected traits and to exclude the possible emergence of other undesired traits such as instability and nonuniformity.

Table 23.5 Reports of *in vitro* mutagenesis in vegetatively propagated crops (Suprasanna et al. 2012)

Crop species	Treated material	Mutagen and dose (LD_{50} or applied dose)	Plant regeneration route	Selected mutants/lines	Reference
Chrysanthemum morifolium	Rooted cuttings	γ-rays (25 Gy)	Direct shoot organogenesis	Yellow flower mutants (from white and red flower varieties)	Ahloowalia et al. (2004)
Banana (*Musa* spp.)	Shoot tips	Carbon-ion beam (0.5–128 Gy)	Direct regeneration	Disease-resistant lines	Reyes-Borja et al. (2007)
Banana (*Musa* spp.)	Shoot tips	γ-rays (60 Gy)	Direct regeneration	Mutant Novaria; earliness	FAO-IAEA mutant variety database
Banana var. Lakatan, Latundan	Shoot tips	γ-rays (40 Gy)	Direct regeneration	Height reduction, larger fruit size	Roux (2004)
	Shoot tips	γ-rays (25 Gy)	Direct regeneration	Mutant variety Klue Hom Thong KU1	
Pineapple var. Queen (*Ananas comosus* (L.) Merr.)	Crowns	γ-rays	Axillary bud regeneration	Lines with reduced spines	FNCA
Begonia rex	*In vitro* cultured leaflets	γ-rays (30–40 Gy)	Adventitious bud regeneration	Leaf colour and shape mutants	Buiatti (1990)
Weigela		γ-rays (30 Gy)	Bud neoformation *in vitro*	Leaf and flower-colour mutant varieties 'Courtadur'	Duron and Decourtye 1986
Potato	Callus cultures	γ-rays (30–50 Gy)	Adventitious bud regeneration	Tuber colour mutants	Ancora and Sonnino (1987)
Sugarcane	Buds/callus cultures	γ-rays (20–25 Gy)	Organogenesis/embryogenesis	Mutants for agronomic traits	Patade and Suprasanna (2008)
Cassava	Somatic embryos	γ-rays (50 Gy)	Embryogenesis	Morphological mutants; mutants with storage root yield, altered cyanogen	Roy et al. (2004)
Sweet potato	Embryogenic suspensions	γ-rays (80 Gy)	Embryogenesis	Mutants for salt tolerance	He et al. (2009)
Pear	*In vitro* shoots	γ-rays (3.5 Gy)	Microcuttings from shoots	Mutants for russeting, fruit shape and size	Predieri and Zimmerman (2001)
				Small tree size wide branch angle and short internodes	Predieri et al. (1997)

Table 23.6 Agronomic traits that can be selectable in cultured plant cells

Trait	Selection agents
Disease resistance	Pathotoxin/crude toxin/culture filtrate (CF)
Herbicide tolerance	Herbicide
Salt tolerance	High salt (NaCl) concentration or sea water
Metal tolerance	High concentration of toxic metals
Cold tolerance	Cold temperature
Drought	Polyethylene glycol or other high osmoticum
Enhanced amino acid accumulation	Amino acid analogues or high concentrations of amino acids
Flooding tolerance	Anaerobic conditions

23.5 High-Throughput Mutation Detection and Screening Techniques

23.5.1 DNA Molecular Markers

DNA marker techniques can also be used widely in research on plant mutation breeding and genetics for increasing both efficiency and efficacy of the mutation techniques (see box below). They can be used for tracing the pedigree of induced mutants and tagging important mutations. Consequently, closely linked markers of mutant traits can be used for marker-assisted selection (MAS), pyramiding and cloning of mutant genes. Simple sequence repeats (SSRs) are codominant and highly polymorphic. SSRs are not confined to noncoding regions of the genome, and many are found in coding sequences of genes, allowing the development of EST (expressed sequence tag)-SSR markers (Varshney et al. 2005). Multiple regions in the genome can be targeted simultaneously with random decamer primers without the need of knowledge of genomic makeup of the organism. The RAPD technique is fairly simple and does not require restriction digestion, labelled probes, hybridisation or prior knowledge of sequence and use of hazardous detection chemicals. A downside to RAPD markers is that they are dominant and reproducibility is not high enough.

Box: Types of DNA Markers Used for Analysing Genetic Variation

Type of marker	Markers used
Genomic DNA restriction site-based markers	Restriction fragment length polymorphism (RFLP)
PCR-based markers	Random amplified polymorphic DNA (RAPD)
	Sequence-characterised amplified region (SCAR)
	Simple sequence repeats (SSR)
	EST (expressed sequence tag)-SSR
	Inter-simple sequence repeats (ISSR)
	Single-nucleotide polymorphism (SNP)
	Sequence-related amplified polymorphism (SRAP)
	Allele-specific associated primers (ASAP)
	Variable number tandem repeats (VNTR)
	Sequence-tagged sites (STS)
Combination of restriction and PCR	Amplified-fragment length polymorphism (AFLP)
	Cleaved amplified polymorphic sequence (CAPS)
Array-based molecular markers	Diversity array technology (DArT)
	Microarray-based marker BeadArray

AFLP typically allows the analysis of dozens of DNA markers simultaneously. Combinations of primers with alternate supplementary bases allow many different subsets of DNA marker combinations, or genomic representations, to be analysed, a feature that makes this technique very versatile. SNPs (single-nucleotide polymorphisms) are increasingly used as DNA markers because the techniques are becoming more widespread, cost-effective and automated and the genome sequence information of major crops are becoming increasingly available. With SNPs, as opposed to other types of DNA markers, marker density can be measured on a kilobase scale, instead of a megabase scale. Over 37,000 SNPs were identified in a comparison of shotgun sequences between *Arabidopsis* accessions: Columbia and *Landsberg erecta* (Jander et al. 2002). Because of their high density in the genome, SNPs are extremely well suited for high-resolution gene mapping, marker-assisted selection and studies assessing the level of diversity throughout the genome. SNP frequency is such that more than one SNP can be detected within a DNA fragment or across a region of interest, and the variation detected can be converted into haplotypes if required. In addition to these, a number of other markers, i.e. CAPS, STS, SCAR, ISSR, SRAP, VNTR, DArT, etc., have been developed and are being utilised in mutation breeding research.

23.5.2 TILLING (*Targeting Induced Local Lesion IN Genomes*)

A novel, reverse genetics approach that combines advantages of point mutations provided by chemical mutagenesis, with advantages of PCR-based mutant screening, has been introduced and is known as TILLING (McCallum et al. 2000). EMS is considered useful as high frequency of single-nucleotide mutations is induced and distributed randomly throughout the genome (Greene et al. 2003; Till et al. 2003; Slade et al. 2005). TILLING allows EMS-induced G:C to A:T transition point mutation detection and enables recovery of a range of alleles including knockouts and missense changes. The mutation densities estimated for various mutagenised populations range between 1/23.3 kilobases and 1/6,190 kilobases (Sato et al. 2006; Dong et al. 2009). Typically, TILLING protocol includes PCR amplification of a target DNA fragment of interest from pooled DNAs of multiple individuals of mutagenised population. In sample pools, heteroduplexes with a mismatched base pair are formed between wild-type and mutated DNA fragments by denaturing and re-annealing PCR products. Heteroduplexes are cleaved by an endonuclease enzyme able to recognise the mismatch position. Cleaved products are then resolved using denaturing polyacrylamide gel or capillary electrophoresis. When a positive signal is identified, individual DNA samples of a pool are separately analysed to identify an individual mutant plant; the induced mutations are eventually confirmed by sequencing. Although the mutations are induced randomly across the plant genome, they are detected only in the gene of interest. This allows the researcher to keep only those plants with mutations in the desired region of the DNA. Thus, TILLING involves generation of a mutant population, selection of target genes, molecular screening and recovery of mutants.

Ideal targets for TILLING are species with a diploid and gene-rich genome where phenotypic alterations induced by mutagenesis are easy to identify because point mutations often occur in a functional region and they are not masked by gene redundancy. To date, mutagenised populations have been developed exclusively for seed-propagated crops using either seed or pollen mutagenesis strategies (McCallum et al. 2000; Till et al. 2004). Although difficulties may arise due to polypoidy nature of the genome as in the case of cultivated wheat and the extensive segmental genome duplications as in case of *Arabidopsis*. Despite these limitations, excellent TILLING results have been reported in both *Arabidopsis* (Till et al. 2003) and wheat (Slade et al. 2005). TILLING has so far been adapted to over 20 species, and many groups host websites

describing projects and progress (Tadele et al. 2010). Jankowicz-Cieslak et al. (2011) have reviewed the progress in the development and adaptation of TILLING for different plant species to harness the power of induced mutations to target and recover lesions in specific genes.

With the advancement in sequencing methodologies, various high-/ultra-high-throughput platforms are now available. TILLING by sequencing approach could discriminate the heteroduplexes from homoduplex DNA targets of rice and wheat in multidimensionally pooled samples (Tsai et al. 2011). This method together with comparative computational approaches favoured to analyse rare mutations with high sensitivity and specificity. A modified TILLING was demonstrated to screen 2,400 mutant individuals (out of 6,912) obtained from EMS-treated mature seed-derived calli of rice for two senescence-related genes. The study revealed 7 sense change mutations out of 15 point mutations (Serrat et al. 2014).

23.5.3 HRM (*H*igh-*R*esolution *M*elting) Genotyping

The HRM is an efficient, accurate and cost-effective PCR-based genotyping assay used to discover SNPs and IN/DELs which enable genotyping of multiple samples in a single run. HRM is typically used for high-throughput genotypic screening of multiple TILLING mutant libraries of complex crop genomes. HRM has been successfully used to identify mutants and point mutations in *Brassica* (Lochlainn et al. 2011), wheat (Botticella et al. 2011), peach (Chen and Wilde 2011), olive (Muleo et al. 2009), etc.

23.5.4 KeyPoint™ Technology

This high-throughput technique runs on massive parallel sequencing of target genes amplified from mutant or natural populations. It combines multidimensional pooling of large numbers of individual DNA samples and the use of sample identification tags or sample barcodes with next-generation sequencing (NGS) technology. This has been successfully demonstrated identifying two mutants in the tomato eIF4E gene based on screening of more than 3,000 M_2 families in a single sequencing run (Rigola et al. 2009).

23.5.5 EMAIL (*E*ndonucleolytic *M*utation *A*nalysis by *I*nternal *L*abelling)

EMAIL is a mismatch scanning assay involving capillary electrophoresis and internal amplicon labelling by PCR incorporation of fluorescently labelled deoxynucleotides. This technique demonstrated its strength by reclassifying a rice line as being heterozygous for starch genes, which was previously assigned homozygous by other sequencing studies (Cross et al. 2008).

23.6 Mutation Breeding in Crop Plants: Some Constraints

Breeding mutant traits is fairly simple and achievable in self-fertilising plants. Cross-fertilising plant species, however, pose some difficulties as these exhibit inbreeding depression; also, the number of selfing generations can reduce plant vigour. This could worsen the problems in effectively identifying mutations. Dominant mutations can be identified, but these occur very rarely. Induction of mutations in vegetatively propagated plant species is compounded by chimerism. All cells exposed to the mutagen will not incur mutations, but those that do incur mutations will give rise to cells exhibiting the mutation. For this reason it is important to treat parts of the plant that will give rise to either seed or vegetative propagules. Identification of a mutant in a large population is difficult in several vegetatively propagated plants; however, once a mutant is identified, the mutation can be fixed in the cloned progeny. Crop species where *in vitro* techniques exist and can be used for mutating plant material allow for regeneration of large numbers of plantlets. This system is highly amenable to both vegetatively and seed-propagated species.

In crops that do not produce seeds, such as edible bananas or seedless grapes, induction of mutation may be the only acceptable way of

increasing variability for developing new cultivars (Jain et al. 2009). This also applies to many root and tuber crops (van Harten 1998; Predieri 2001) and the development of novel colours and variations in ornamental plant species propagated vegetatively (Datta 2009). Crossbreeding in perennial crop species, which in practice are vegetatively propagated such as many fruit crops, is also subject to constraints of time, growing space and clonal identity, and mutation induction can be a valuable breeding strategy (Predieri 2001; Suprasanna et al. 2012).

23.7 Global Status and Impact of Mutant Crop Varieties

Increase in the crop yields attributed to altered plant architecture to suit modern agronomic practices is also a result of induced or incorporated mutant genes into the old cultivars (Pathirana 2011). A plethora of mutant crop varieties have been developed and are greatly contributing to the sustainable global food production. The International Atomic Energy Agency (FAO-IAEA), Vienna, Austria, hosts the mutant variety database (MVD) which provides comprehensive information on induced mutations suitable for breeding programmes and genetic analysis. MVD collects information on crop mutant varieties, mutagen used and characters improved. Since 1960, several mutant varieties have been officially released in 60 countries. The top six countries are China, India, the former USSR, the Netherlands, Japan and the USA (Fig. 23.3). Rice stands first by having the largest number (700 mutants) of mutant varieties, followed by barley, wheat, maize, durum wheat, oat, millet, sorghum and rye. As per FAO-IAEA mutant variety database, out of the total number of mutants registered, about 1,825 (accounting for 57 %) possess better agronomic and botanical traits; 577 (18 %) show increased yield and yield-contributing traits; about 321 (10 %) have better quality and nutritional content; about 200 (6 %) have superior biotic stress resistance; and 125 (4 %) are attrib-

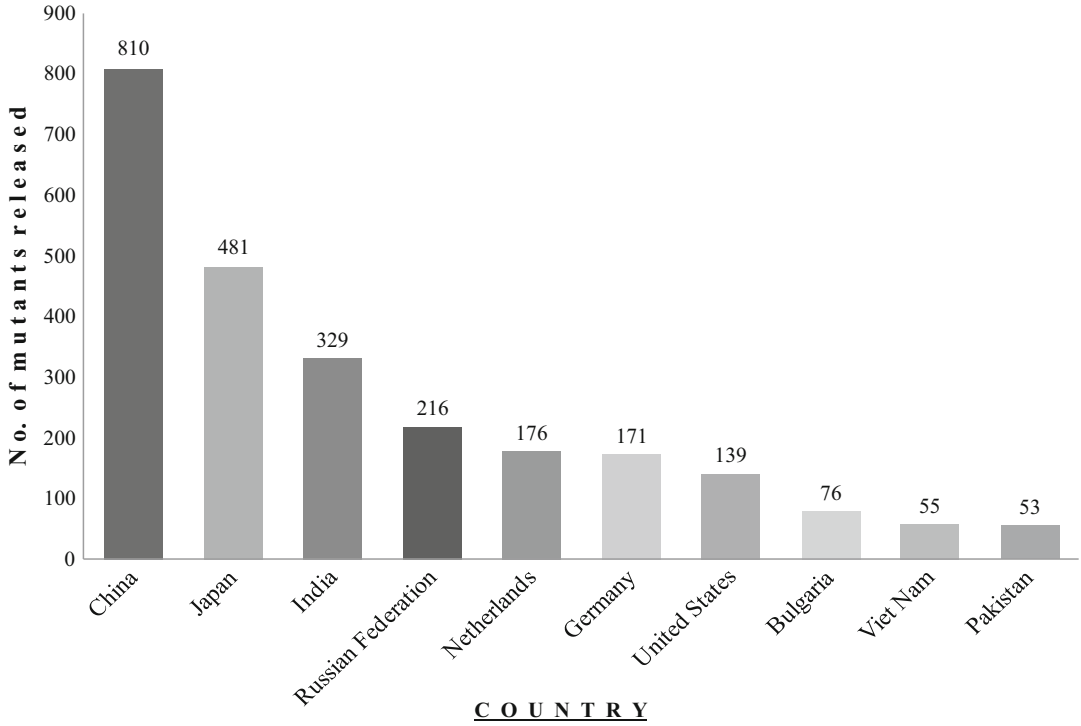


Fig. 23.3 Global status of officially released mutant varieties in top ten countries (Based on FAO-IAEA mutant variety database 2014)

uted with abiotic stress tolerance (http://mvgs.iaea.org). There are several examples of disease-resistant mutants developed either through direct selection after mutation induction or through crossbreeding with other mutants (Pathirana 2011). Several mutants attributing moderate-to-high levels of tolerance to abiotic stress factors have been developed and are serving either directly or indirectly for crop improvement. Many of these mutants having tolerance to salinity, drought, alkalinity or low temperature were developed using different mutagenic agents, mainly physical mutagens. It is interesting to see that more than 50 % of the mutants are developed by using gamma rays (Fig. 23.4). The FAO-IAEA mutant variety database (MVD) has listed around 160 mutant varieties (directly and indirectly bred varieties) that are resistant/tolerant to abiotic stresses. Of these, 110 mutant varieties are identified as drought-tolerant followed by salinity-tolerant (36) or low-temperature-tolerant (15) mutant varieties. Most of the mutants for low temperature or salinity are in rice, while majority of the drought-tolerant ones are in wheat (Suprasanna et al. 2014). Further, it is evident that mutagenesis in seed-propagated crops (SPCs) is more practised or successful, as about 77 % of mutants belong to SPCs and the remaining 23 % are mutants of vegetatively propagated crops (VPCs).

Rapid and free access to such mutants is essential to determine their value and potential for use in locations other than where they were produced. In addition, mutants of crops are becoming very valuable in functional genomics and proteomics. Hence, it is essential to collect, conserve and facilitate distribution of mutants of important crops ensuring unrestricted access to plant breeders and geneticists around the world. Moreover, collections of such mutants help maintain germplasm diversity. According to FAO-IAEA mutant variety database (MVD), before the 1960s, there were only 176 mutant varieties released, which grew rapidly to 777 and 1,806 by 1979 and 1989, respectively, with the all-time highest gain of 1,029 during the 1980s. As of now 3,218 mutant varieties are registered in the FAO-IAEA database (IAEA 2014; http://mvgs.iaea.org). Among all the registered mutant varieties, rice mutant varieties share nearly 25 % (815 varieties) and of these around 36 % are developed alone by China. In India, several mutant cultivars of crops, belonging to 56 plant species, were approved and/or released (Fig. 23.5). The largest number of mutant cultivars has been produced in ornamentals, followed by cereals, legumes and oilseeds.

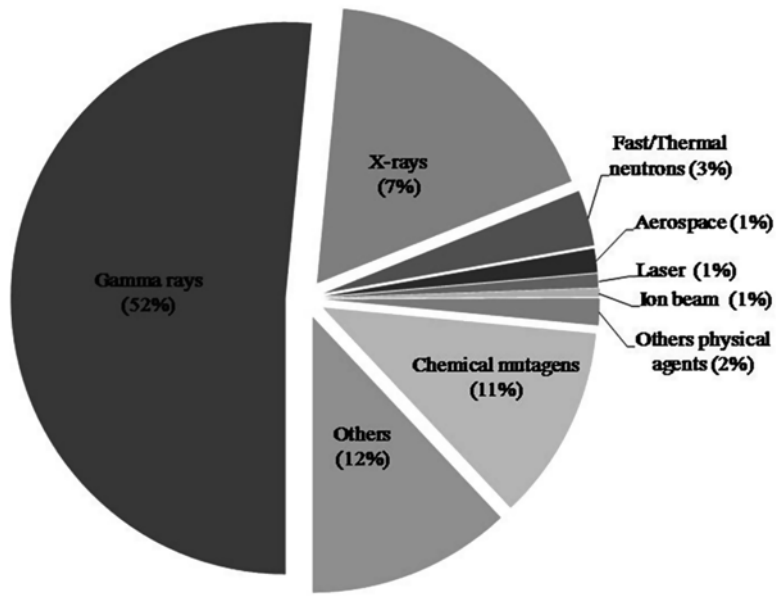


Fig. 23.4 Distribution of types of mutagen used for induced mutagenesis (Based on FAO-IAEA mutant variety database 2014)

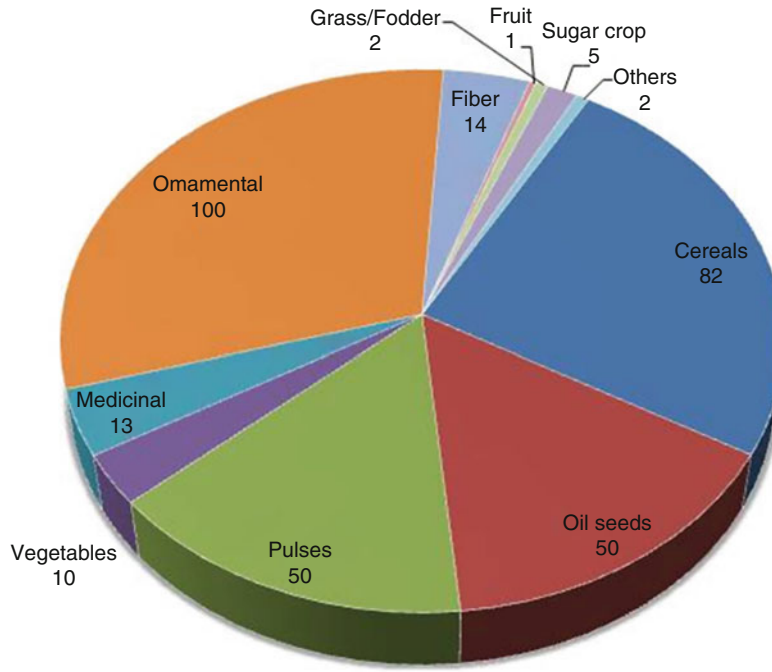


Fig. 23.5 Development of mutant varieties – Indian scenario

Many of these induced mutants were released directly as new varieties, while others were used indirectly as parents to derive new varieties. Mutation induction with gamma-ray radiation was the most frequently used method to develop direct mutant varieties (89 %). Mutation techniques have played a significant role in increasing rice production in the Asia-Pacific Region. By 2000, 434 mutant varieties of rice were released with improved characters such as semi-dwarf stature, early maturity, improved grain yield, disease and cold tolerance and improved grain quality (Maluszynski et al. 2000). During 2000–2014, a total of 62 mutant varieties of rice attributing abiotic stress tolerance in addition to other agronomic characters were released.

Mutation breeding of cereals, legumes, oil crops, ornamentals and fruit trees has produced significant benefits. For example, a mutated durum wheat variety 'Creso' and varieties derived from it make up over half the wheat used for pasta in Italy (Ahloowalia et al. 2004). Rice varieties derived from mutation breeding are grown extensively in Asia and Australia, generating billions of US dollars annually (Mba 2013). The mutant barley varieties 'Golden Promise' and 'Diamant' and varieties derived from them have been important to the brewing industry in Europe (Ahloowalia et al. 2004).

There is immense scope to enhance mineral nutrient (biofortification) and essential amino acid contents of human food as well as animal feed along with altered protein and fatty acid profiles, physicochemical properties of starch, enhance phytonutrients in fruits and reduced anti-nutritional factors in staple food grains. Induced mutations could play an important role in inducing mutations for enhancing nutritional quality in crop plants. Of the 3,000 mutant varieties developed globally, 776 are identified for their superior nutritional quality (Jain and Suprasanna 2011).

Several mutant genes have been successfully introduced into commercial crop varieties that significantly enhance the nutritional value of crops like maize, barley, soybean and sunflower. In maize, quality protein maize (QPM) varieties are grown on hundreds of hectares of lands. They have almost twice the amount of lysine and tryptophan compared to normal varieties and 30 % less leucine as compared to parental lines. Inclusion of these in diet has shown a dramatic effect on human and animal nutrition, growth and

performance. In cassava, three mutants have been isolated showing different sizes of starch grains. They have high economic potential for the industrial use of starch and influence on cooking quality. Small starch grain size seems to be highly suitable for bioethanol production. In sweet sorghum, a mutant variety Yuantian No.1 has been developed in China, which has 20 % more total carbohydrates as compared to the parental lines, well suited for food, feed and bioenergy (three in one). Five rice giant embryo mutants, characterised by enlarged embryo than that of wild type, were found to have an increase in the contents of protein; vitamin B1; vitamin B2; vitamin E; essential amino acids such as arginine, aspartic acid, glutamic acid, lysine and methionine; and mineral elements such as calcium, iron, potassium, phosphorus and zinc (Zhang et al. 2007). In Vietnam, more than 50 % of its soybean and 15 % of rice cultivated area are occupied by mutant varieties, contributing significantly to increased productivity (Vinh et al. 2009). In a global initiative taken by FAO-IAEA to eradicate deadly wheat stem rust caused by the fungus Ug99, a remarkable success has been made with the development of 13 putative resistant mutant lines. A field evaluation for resistance in Kenya has led to the release of the world's first Ug99-resistant wheat mutant variety in February 2014 and was named 'Eldo Ngano 1'. Lately, a second advanced mutant line was also tested for varietal status and a promising advanced mutant line ready for official testing and release in 2015 (Forster 2014).

23.8 Integrating Genomics into Mutation Breeding

Mutational genomics is becoming a valuable tool to the understanding of the molecular basis of the plant stress response based on information gathered from mutants of *Arabidopsis* and other model plant studies (Papdi et al. 2010). High-throughput genomics platforms such as cDNA-AFLP, single-strand conformational polymorphism (SSCP), serial analysis of gene expression (SAGE), microarray, differential display, TILLING, high-resolution melt (HRM) analysis, etc., allow rapid and in-depth global analysis of mutational events. T-DNA insertional mutagenesis has been successfully utilised to create saturation mutants of rice and *Arabidopsis* covering nearly all (>90 %) the genes and was employed to characterise stress-responsive genes (Panjabi-Sabharwal et al. 2010).

A random insertional mutagenesis approach using a transposon or T-DNA as a mutagen offers a viable method for obtaining insertion mutants for genes of interest (Parinov and Sundaresan 2000). Routine targeted mutagenesis opens up a new dimension in plant biology and should help to generate mutants in previously difficult-to-access genes, as well as simultaneously mutate multiple loci and generate large deletions (Mao et al. 2013). Both these strategies allow the mutagenesis of large numbers of genes. Loss and/or gain of function mutants have been used to identify components of stress reception, signal transduction and transcription factors involved in the stress responses (Cushman and Bohnert 2000).

Considerable progress has been made in induced mutation breeding of horticultural crops resulting in the development of cultivars with traits of commercial importance, such as extended shelf life (tomato, melon), improved starch quality (potato), viral disease resistance (peppers, tomato), etc. With the availability of high-throughput genome sequencing tools and candidate gene sequence data, numbers of novel alleles have been identified (Wilde et al. 2012).

Developments in genomics such as the use of high-throughput sequencing have enabled relatively inexpensive and fast genome sequencing of plants. TILLING, zinc-finger nuclease-mediated mutagenesis and use of meganucleases have allowed generation of targeted mutations in crop plants, to delineate gene function besides improving cultivars. These new and more specific methods are very promising.

Zinc-finger nucleases (ZFN) and meganucleases (MN) present a more targeted approach to an induced mutation. ZFNs can be tailored to bind to specific recognition sites associated with the desired sequence. Once dimerised, the target DNA is cleaved and a donor sequence introduced (Bibikova et al. 2003). The donor sequence typi-

cally exhibits desired mutations or it can be used to introduce new transgenes altogether into the target genome. Meganucleases have a similarly specific mode of action, and a great deal of research is going into both of these promising techniques for targeted mutagenesis as well as plant transformation.

23.9 Conclusion

Mutations can be induced by physical or chemical mutagens. This is an ideal approach to broaden genetic variation for application in functional genomics and plant breeding. So far, 3,218 mutant varieties have been released worldwide. Optimum utilisation of mutant lines worldwide can be made by free exchange of a mutant material and its deployment in regions where a farmers' need is paramount. With the aid of well-defined objectives in national research programmes, availability of efficient mutagenesis protocols and high-throughput, efficient phenotype screening methods, mutagenesis can be made more useful in the improvement of crop plants. Mutant identification/selection at the genotypic level, using new, high-throughput technologies, has changed the way mutations are now used in genetics and breeding. The possibility of directed mutation by using mutagens and certain chemical pretreatments should help in achieving the goal. Induced mutations are poised to play a more significant role in the times to come.

References

Abe T, Ryuto H, Fukunishi N (2012) Ion beam radiation mutagenesis. In: Shu QY, Forster BP, Nakagawa H (eds) Plant mutation breeding and biotechnology. CABI, Oxford, pp 99–106

Ahloowalia B, Maluszynski M (2001) Induced mutations-a new paradigm in plant breeding. Euphytica 118:167–173

Ahloowalia BS, Maluszynski M, Nichterlein K (2004) Global impact of mutation-derived varieties. Euphytica 135:187–204

Ancora G, Sonnino A (1987) In vitro induction of mutation in potato. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 3. Springer, Berlin, pp 408–424

Auld D, Ethridge M, Dever J, Dotray P (1998) Chemical mutagenesis as a tool in cotton improvement. Proceedings of the Beltwide Cotton Conference of USA, Memphis, pp 550–552

Bhagwat SG (2009) Fundamentals of mutation breeding. Proceedings of the IAEA/RCA regional training course on mutation breeding approaches to improving disease resistance. BARC, Mumbai, pp 10–15

Bhagwat B, Duncan EJ (1998) Mutation breeding of banana cv. Highgate (*Musa acuminate* AAA Group) for tolerance to *Fusarium oxysporum* f. sp. cubense using chemical mutagens. Sci Hortic 73:11–22

Bibikova M, Beumer K, Trautman JK, Carroll D (2003) Enhancing gene targeting with designed zinc finger nucleases. Science 300:764–765

Botticella E, Sestili F, Hernandez-Lopez A, Phillips A, Lafiandra D (2011) High resolution melting analysis for the detection of EMS induced mutations in wheat SbeIIa genes. BMC Plant Biol 11:156

Boureima S, Oukarroum A, Diouf M, Cisse N, Damme PV (2012) Screening for drought tolerance in mutant germplasm of sesame (*Sesamum indicum*) probing by chlorophyll a fluorescence. Environ Exp Bot 81:37–43

Broertjes C, Koene P, van Veen JWH (1980) A mutant of a mutant of a…: irradiation of progressive radiation induced mutants in a mutation breeding programmes with *Chrysanthemum morifolium* Ram. Euphytica 29:525–530

Buiatti M (1990) The use of cell and tissue cultures for mutation breeding. In: Parey P (ed) Science for plant breeding. Eucarpia, Gottingen, pp 179–201

Cha-um S, Chantawong S, Siriwatana C, Ashraf M, Kirdmanee C (2013) Field screening of sugarcane (*Saccharum* spp.) mutant and commercial genotypes for salt tolerance. Not Bot Horti Agrobo 41(1):286–293

Chen Y, Wilde HD (2011) Mutation scanning of peach floral genes. BMC Plant Biol 11:96

Choudhury RK (1983) A note on the effect of gamma irradiation in wheat. Wheat Info Sci 57:21–23

Cooper JL, Till BJ, Laport RG, Darlow MC, Kleffner JM, Jamai A, El-Mellouki T, Liu S, Ritchie R, Nielsen N, Bilyeu KD, Meksem K, Comai L, Henikoff S (2008) TILLING to detect induced mutations in soybean. BMC Plant Biol 8:9

Cross MJ, Waters DL, Lee LS, Henry RJ (2008) Endonucleolytic mutation analysis by internal labeling (EMAIL). Electrophoresis 29(6):1291–1301

Cushman JC, Bohnert HJ (2000) Genomic approaches to plant stress tolerance. Curr Opin Plant Biol 3:117–124

Datta SK (1991) Evaluation of recurrent irradiation on vegetatively propagated ornamental: *Chrysanthemum*. J Nucl Agric Biol 20(2):81–86

Datta (2009) Role of classical mutagenesis for development of new ornamental varieties. In: Shu QY (ed) Induced plant mutations in the genomics era. Food and

Agriculture Organization of the United Nations, Rome, pp 300–302
Datta SK, Chakrabarty D (2009) Management of chimera and *in vitro* mutagenesis for development of new flower color/shape and chlorophyll variegated mutants in Chrysanthemum. In: Shu QY (ed) Induced plant mutations in the genomics era. Food and Agriculture Organization of the United Nations, Rome, pp 303–305
de Ronde JA, Spreeth MH (2007) Development and evaluation of drought resistant mutant germ-plasm of *Vigna unguiculata*. Water SA 33(3):381–386
de Vries H (1909) The mutation theory. Open Court Pub Co, Chicago
Dong C, Dalton-Morgan J, Vincent K, Sharp P (2009) A modified TILLING method for wheat breeding. Plant Genome 2:39–47
Drake JW, Charlesworth B, Charlesworth D, Crow JF (1998) Rates of spontaneous mutation. Genetics 148:1667–1686
Duron M, Decourtye L (1986) Effets biologiques des rayons gamma appliqués a des plantes de *Weigela* cv. Bristol Ruby cultivees *in vitro*. In: Proceedings of international symposium on nuclear techniques and *in vitro* culture for plant improvement, IAEA, Vienna, pp 103–111
Ekram AH, Hanan A, Asma AAH (2013) Oxidative damage and mutagenic potency of fast neutron and UV-b radiation in pollen mother cells and seed yield of *Vicia faba* L. BioMed Res Int. doi:10.1155/2013/824656, Article ID 824656
Forster BP (2014) The development of "Eldo Ngano I": the world's first Ug99 resistant mutant wheat variety. IAEA Bull 55:18–19
Geier T (2012) Chimeras: properties and dissociation in vegetatively propagated plants. In: Shu QY, Forster BP, Nakagawa H (eds) Plant mutation breeding and biotechnology. CABI Publishing, Wallingford, pp 191–201
Green RE, Cornell SJ, Scharlemann JP, Balmford A (2005) Farming and the fate of wild nature. Science 307:550–555
Greene EA, Codomo CA, Taylor NE, Henikoff JG, Till BJ, Reynolds SH, Enns LC, Burtner C, Johnson JE, Odden AR (2003) Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in *Arabidopsis*. Genetics 164:731–740
Hase Y, Okamura M, Takeshita D, Narumi I, Tanaka A (2010) Efficient induction of flower-color mutants by ion beam irradiation in petunia seedlings treated with high sucrose concentration. Plant Biotechnol 27:99–103
Hase Y, Akita Y, Kitamura S, Narumi I, Tanaka A (2012) Development of an efficient mutagenesis technique using ion beams: toward more controlled mutation breeding. Plant Biotechnol 29:193–200
Hayashi Y, Takehisa H, Kazama Y, Ichida H, Ryuto H, Fukunishi N, Abe T (2007) Effects of ion beam irradiation on mutation induction in rice. Cyclotrons and their applications, Eighteenth International Conference, pp 237–239
Hayashi Y, Takehisa H, Kazama Y et al (2008) Characterization of salt-tolerant mutants of rice induced by heavy-ion irradiation. RIKEN Accel Prog Rep 41:234–238
He SZ, Han YF, Wang YP, Zhai H, Liu QC (2009) In vitro selection and identification of sweet potato (*Ipomoea batatas* (L.) Lam.) plants tolerant to NaCl. Plant Cell Tissue Organ Cult 96:69–74
Hohmann U, Jacobs G, Jung C (2005) An EMS mutagenesis protocol for sugar beet and isolation of non bolting mutants. Plant Breed 124:317–321
Jain SM (2000) Mechanisms of spontaneous and induced mutations in plants. In: Proceedings of the 11th International Congress of Radiation Research. Dublin, 18–23 July 1999, pp 255–258
Jain SM (2005) Major mutation-assisted plant breeding programmes supported by FAO/IAEA. Plant Cell Tissue Organ Cult 82:113–121
Jain SM (2006) Mutation-assisted breeding in ornamental plant improvement. Acta Horticult 714:85–98
Jain SM (2010) Mutagenesis in crop improvement under the climate change. Rom Biotechnol Lett 15(2):88–106
Jain SM (2012) *In vitro* mutagenesis for improving date palm (*Phoenix dactylifera* L.). Emirates J Food Agric 24(5):386–399
Jain SM, Suprasanna P (2011) Induced mutations for enhancing nutrition and food production. Gene Conserve 40:201–215
Jain SM, Reddy AVR, Venugopal V, Sinha RK, Banerjee S (2009) Application of radiation induced mutations in the improvement of vegetatively propagated crops. Bhabha Atomic Research Centre, India
Jander G, Norris S, Rounsley S, Bush D, Levi I, Last R (2002) Arabidopsis map-based cloning in the post-genome era. Plant Physiol 129:440–450
Jankowicz-Cieslak J, Huynh OA, Bado S, Matijevic M, Till BJ (2011) Reverse-genetics by TILLING expands through the plant kingdom. Emir J Food Agric 23(4):290–300
FAO/IAEA Mutant Variety Database (2014) http://mvgs.iaea.org
Kao FT, Caldecott RS (1966) Genetic effects of recurrent irradiation in diploid and polyploid *Triticum* species. Genetics 54(3):845–858
Kharkwal MC, Shu QY (2009) The role of induced mutations in world food security. In: Induced plant mutations in the genomics era. Proceedings of the International Joint FAO/IAEA Symp IAEA, Vienna, pp 33–38
Lee M (1998) Genome projects and gene pools: new germplasm for plant breeding? Proc Natl Acad Sci U S A 95:2001–2004
Leitão JM (2012) Chemical mutagenesis. In: Shu QY, Forster BP, Nakagawa H (eds) Plant mutation breeding and biotechnology. CABI Publishing, Wallingford, pp 135–158

Li X, Song Y, Century K (2001) Fast neutron deletion mutagenesis-based reverse genetics system for plants. Plant J 27:235–242

Liu LX, Guo H, Zhao L, Gu J, Zhao S (2007) Achievements in the past twenty years and perspective outlook of crop space breeding in China. J Nucl Agric Sci 21(6):589–592

Lochlainn S, Amoah S, Graham NS, Alamer K, Rios JJ, Kurup S, Stoute A, Hammond JP, Østergaard L, King GJ, White PJ, Broadley MR (2011) High Resolution Melt (HRM) analysis is an efficient tool to genotype EMS mutants in complex crop genomes. Plant Methods 7:43

Lu WH, Wang XZ, Zheng Q, Guan SH, Xin P, Sun YQ (2008) Diversity and stability study on rice mutants induced in space environment. Genomics Proteome Bioinforma 6:51–60

Luan YS, Zhang J, Gao XR, An LJ (2007) Mutation induced by ethyl methane sulphonate (EMS), in vitro screening for salt tolerance and plant regeneration of sweet potato (*Ipomoea batatas* L.). Plant Cell Tissue Organ Cult 88:77–81

Magori S, Tanaka A, Kawaguchi M (2010) Physically induced mutation: ion beam mutagenesis. In: Meksem K, Kahl K (eds) The handbook of plant mutation screening: mining of natural and induced. WILEY-VCH Verlag GmbH & Co KGaA, Weinheim, pp 3–16

Maluszynski M, Nichterlein K, van Zanten L, Ahloowalia BS (2000) Officially released mutant varieties – the FAO/IAEA Database. Mutat Breed Rev 12:1–84

Maluszynski M, Szarejko I, Maluszynski J (2003) Mutation techniques. In: Thomas B, Murphy DJ, Murray BG (eds) Encyclopedia of applied plant sciences. Elsevier Academic Press, San Diego, pp 186–201

Maluszynski M, Szarejko I, Maluszynska J (2004) Mutation techniques. Encycl Appl Plant Sci 1–3:186–201

Maluszynski M, Szarejko I, Bhatia CR, Nichterlein K, Lagoda PJL (2009) Methodologies for generating variability. Part 4: mutation techniques. In: Ceccarelli S, Guimarães EP, Weltzien E (eds) Plant breeding and farmers participation. Food and Agriculture Organization of the United Nations, Rome, pp 159–194

Mantri N, Patade V, Pang E (2014) Recent advances in rapid and sensitive screening for abiotic stress tolerance. In: Ahmad P, Wani MR, Azooz MM, Tran LSP (eds) Improvement of crops in the era of climate changes. Springer Science+Business Media, New York, pp 37–47

Mao Y, Zhang H, Xu N, Zhang B, Gao F, Zhu JK (2013) Application of the CRISPR-Cas system for efficient genome engineering in plants. Mol Plant 9:2008–2011

Martín B, Ramiro M, Martínez-Zapater JM, Alonso-Blanco C (2009) A high-density collection of EMS-induced mutations for TILLING in *Landsberg erecta* genetic background of Arabidopsis. BMC Plant Biol 9:147

Mba C (2013) Induced mutations unleash the potentials of plant genetic resources for food and agriculture. Agronomy 3:200–231

Mba C, Afza R, Bado S, Jain SM (2010) Induced mutagenesis in plants using physical and chemical agents. In: Davey MR, Anthony P (eds) Plant cell culture: essential methods. John Wiley & Sons, New York, pp 111–130

McCallum CM, Comai L, Greene EA, Henikoff S (2000) Targeted screening for induced mutations. Nat Biotechnol 18:455–457

Micke A, Dinini B, Maluszynski M (1990) Induced mutations for crop improvement. Mut Breed Rev 7:1–41

Mishra PJ, Ganapathi TR, Suprasanna P, Bapat VA (2007) Effect of single and recurrent gamma irradiation on *in vitro* shoot cultures of banana. Int J Fruit Science 7(1):47–57

Morishita T, Yamaguchi H, Degi K et al (2003) Dose response and mutation induction by ion beam irradiation in buckwheat. Nucl Inst Methods Phys Res B 206:565–569

Muleo R, Colao MC, Miano D, Cirilli M, Intrieri MC, Baldoni L, Rugini E (2009) Mutation scanning and genotyping by high-resolution DNA melting analysis in olive germplasm. Genome 52(3):252–260

Muller HJ (1927) Artificial transmutation of the gene. Science 66:84–87

Muller H (1928) The production of mutations by X-rays. Proc Natl Acad Sci U S A 14:714–726

Nagatomi S, Degi K (2009) Mutation breeding of Chrysanthemum by gamma field irradiation and *in vitro* culture. In: Shu QY (ed) Induced plant mutations in the genomics era. Food and Agriculture Organization of the United Nations, Rome, pp 258–261

Nikam AA, Devarumath RM, Shitole MG, Ghole VS, Tawar PN, Suprasanna P (2014) Gamma radiation, *in vitro* selection for salt (NaCl) tolerance, and characterization of mutants in sugarcane (*Saccharum officinarum* L.). In Vitro Cell Dev Biol Plant. doi:10.1007/s11627-014-9630-4

Ohnishi T, Ohnishi K, Takahashi A, Taniguchi Y, Sato M, Nakano T, Nagaoka S (2002) Detection of DNA damage induced by space radiation in Mir and space shuttle. J Rad Res 43:S133–S136

Okamura M, Ohtsuka M, Yasuno N, Hirosawa T, Tanaka A, Shikazono N, Hase Y, Tanase M (2001) Mutation generation in carnation plants regenerated from *in vitro* leaf cultures irradiated with ion beams. JAERI Rev 39:52–54

Ou XF, Long LK, Zhang YH, Xue YQ, Liu JC, Lin XY, Liu B (2009) Spaceflight induces both transient and heritable alterations in DNA methylation and gene expression in rice (*Oryza sativa* L.). Mut Res 662:44–53

Panjabi-Sabharwal V, Karan R, Khan T, Pareek A (2010) Abiotic stress adaptation in plants. In: Pareek A, Sopory SK, Bohnert HJ, Govindjee (eds) Physiological, molecular and genomic foundation. Springer, Dordrecht, pp 177–198

Papdi C, Leung J, Joseph MP, Salamo IP, Szabados L (2010) Genetic screens to identify plant stress genes. In: Sunkar R (ed) Plant stress tolerance: methods in molecular biology. Springer Science+Business Media LLC, Berlin, pp 121–139, 639

Parinov S, Sundaresan V (2000) Functional genomics in Arabidopsis: large-scale insertional mutagenesis complements the genome sequencing project. Curr Opin Biotechnol 11(2):157–161

Patade VY, Suprasanna P (2008) Radiation induced in vitro mutagenesis for sugarcane improvement. Sugar Tech 10(1):14–19

Patade VY, Suprasanna P, Bapat VA, Kulkarni UG (2006) Selection for abiotic (salinity and drought) stress tolerance and molecular characterization of tolerant lines in sugarcane. BARC Newsl 27:244–257

Pathirana R (2011) Plant mutation breeding in agriculture. In: CAB Reviews: Persp Agric Vet Sci Nutri and Natur Resour 6(32):1–20

Paul AL, Popp MP, Gurley WB, Guy C, Norwood KL, Ferl RJ (2005) *Arabidopsis* gene expression patterns are altered during spaceflight. Adv Space Res 36:1175–1181

Porterfield DM, Barta DJ, Ming DW, Morrow RC, Musgrave ME (2000) Astroculture (TM) root metabolism and cytochemical analysis. Adv Space Res 26:315–318

Predieri S (2001) Mutation induction and tissue culture in improving fruits. Plant Cell Tissue Organ Cult 64(2–3):185–210

Predieri S, Zimmerman RH (2001) Pear mutagenesis: *in vitro* treatment with gamma-rays and field selection for productivity and fruit traits. Euphytica 117(3):217–227

Predieri S, Magli M, Zimmerman RH (1997) Pear mutagenesis: *in vitro* treatment with gamma-rays and field selection for vegetative form traits. Euphytica 93(2):227–237

Reyes-Borja WO, Sotomayor I, Garzón I, Vera D, Cedeño M, Castillo B, Tanaka A, Hase Y, Sekozawa Y, Sugaya S, Gemma H (2007) Alteration of resistance to black Sigatoka (*Mycosphaerella fijiensis* Morelet) in banana by *in vitro* irradiation using carbon ion-beam. Plant Biotechnol 24:349–353

Rigola D, van Oeveren J, Janssen A, Bonne A, Schneiders H, van der Poel HJA, van Orsouw NJ, Hogers RCJ, de Both MTJ, van Eijk MJT (2009) High-throughput detection of induced mutations and natural variation using KeyPoint™ technology. PLoS One 4(3):e4761

Roux NS (2004) Mutation induction in *Musa* – review In: Jain SM, Swennen R (eds) Banana improvement: cellular, molecular biology and induced mutations. Science Publishers, Enfield, New Hampshire, USA, pp 23–32

Roy J, Yeoh HH, Loh CS (2004) Induced mutations in cassava using somatic embryos and the identification of mutant plants with alters starch yield and composition. Plant Cell Rep 23:91–98

Sato Y, Shirasawa K, Takahashi Y, Nishimura M, Nishio T (2006) Mutant selection from progeny of gamma-ray-irradiated rice by DNA heteroduplex cleavage using *Brassica* petiole extract. Breed Sci 56:179–183

Serrat X, Esteban R, Guibourt N, Moysset L, Nogués S, Lalanne E (2014) EMS mutagenesis in mature seed-derived rice calli as a new method for rapidly obtaining TILLING mutant populations. Plant Methods 10:5

Shitsukawa N, Takagishi A, Ikari C, Takumi S, Murai K (2006) *WFL*, a wheat *FLORICAULA/LEAFY* ortholog, is associated with spikelet formation as lateral branch of the inflorescence meristem. Genes Genet Syst 81:13–20

Shu QY (2009) A summary of the international symposium on induced mutations in plants. In: Shu QY (ed) Induced plant mutations in the genomics era. FAO, UN, Rome, pp 15–18

Shu QY, Forster BP, Nakagawa H (2012) Principles and applications of plant mutation breeding. In: Shu QY, Forster BP, Nakagawa H (eds) Plant mutation breeding and biotechnology. CABI Publishing, Wallingford, pp 301–324

Slade AJ, Fuerstenberg SI, Loeffler D, Steine MN, Facciotti D (2005) A reverse genetic, non transgenic approach to wheat crop improvement by TILLING. Nat Biotechnol 23:75–81

Stadler L (1930) The frequency of mutation of specific genes in maize. Anat Rec 47:381

Suprasanna P, Jain SM, Ochatt SJ, Kulkarni VM, Predieri S (2012) Applications of *in vitro* techniques in mutation breeding of vegetatively propagated crops. In: Shu QY, Forster BP, Nakagawa H (eds) Plant mutation breeding and biotechnology. CABI Publishing, Wallingford, pp 371–385

Suprasanna P, Mirajkar SJ, Patade VY, Jain SM (2014) Induced mutagenesis for improving plant abiotic stress tolerance. In: Tomlekova NB, Kozgar MI, Wani MR (eds) Mutagenesis: exploring genetic diversity of crops. Wageningen Academic Publishers, The Netherlands, pp 345–374

Suzuki T, Eiguchi M, Kumamaru T, Satoh H, Matsusaka H, Moriguchi K, Nagato Y, Kurata N (2008) MNU-induced mutant pools and high performance TILLING enable finding of any gene mutation in rice. Mol Genet Genomics 279:213–223

Tadele Z, Chikelu MBA, Till BJ (2010) TILLING for mutations in model plants and crops. In: Jain SM, Brar DS (eds) Molecular techniques in crop improvement. Springer Science+Business Media BV, Netherlands, pp 307–332

Tanaka A, Shikazono N, Hase Y (2010) Studies on biological effects of ion beams on lethality, molecular nature of mutation, mutation rate, and spectrum of mutation phenotype for mutation breeding in higher plants. J Rad Res 51(3):223–233

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Till BJ, Reynolds SH, Greene EA, Codomo CA, Enns LC, Johnson JE, Burtner C, Odden AR, Young K, Taylor NE, Henikoff JG, ComaiL HS (2003) Large-scale discovery of induced point mutations with high throughput TILLING. Genome Res 13:524–530

Till BJ, Reynolds SH, Weil C, Springer N, Burtner N, Young K, Bowers E, Codomo CA, Enns LC, Odden AR et al (2004) Discovery of induced point mutations in maize genes by TILLING. BMC Plant Biol 4:1–8

Till BJ, Cooper J, Tai TH, Colowit P, Greene EA, Henikoff S, Comai L (2007) Discovery of chemically induced mutations in rice by TILLING. BMC Plant Biol 7:1–12

Tsai H, Howell T, Nitcher R, Missirian V, Watson B, Ngo KJ, Lieberman M, Fass J, Uauy C, Tran RK, Khan AA, Filkov V, Tai TH, Dubcovsky J, Comai L (2011) Discovery of rare mutations in populations: TILLING by sequencing. Plant Physiol 156(3):1257–1268

van Harten AM (1998) Mutation breeding: theory and practical applications. Cambridge University Press, Cambridge, pp 137–158

Varshney RK, Graner A, Sorrells ME (2005) Genic microsatellite markers in plants: features and applications. Trends Biotechnol 23(1):48–55

Vinh MQ, Thinh DK, Bang DT, At DH, Ham LH (2009) Current status and research directions of induced mutation application to seed crops improvement in Vietnam. In: Shu QY (ed) Induced plant mutations in the genomics era. FAO, UN, Rome, pp 341–345

Watanabe H (2001) Significance and expectations of ion beam breeding. In: Gamma Field Symposia, No. 40. Institute of Breeding, NIAS, Ibaraki, pp 15–19

Wilde HD, Chen Y, Jiang P, Bhattacharya A (2012) Targeted mutation breeding of horticultural plants. Emir J Food Agric 24(1):31–41

Yu LD, Anantabochai S (2011) Low energy ion beam research at Chiang Mai University. Trans Mat Res Soc Japan 36(1):123–127

Yu S, Luo H, Li J, Yu X (2013) Molecular variation and application from aerospace mutagenesis in upland rice Huhan 3 and Huhan 7. Rice Sci 20(4):249–258

Zhang L, Shu XL, Wang XY, Lu HJ, Shu QY, Wu DX (2007) Characterization of indica-type giant embryo mutant rice enriched with nutritional components. Cereal Res Commun 35:1459–1468

Zhao MX, Sun HY, Ji RR, Hu XH, Sui JM, Qiao LX, Chen J, Wang JS (2013) *In vitro* mutagenesis and directed screening for salt-tolerant mutants in peanut. Euphytica 193:89–99

Polyploidy in Crop Improvement and Evolution

24

Dinesh Narayan Bharadwaj

Abstract

Breeding of polyploid crops has been in progress since the domestication of crop plants, while genetic gains can be obtained through selection, evaluation and recombination, the successful selection of crop improvement may depend on understanding and unravelling the complexities of genetic variation that underlies the phenotype. The genomic sequence analysis has vastly enhanced our knowledge of plant genomes, leading to an understanding of the behaviour of polyploid plant genomes. A better understanding of polyploidy holds a great promise for crop improvement by better association between genotype and phenotype and bridging gaps for the genetic transmission of desired agronomic traits between crop species and their wild relatives.

For a long period of time, polyploidy in plants has been considered to be an important phenomenon because of genome buffering, increased allelic diversity, fixing heterozygosity and the opportunity for novel phenotypic variations because of duplicated genes which acquire new function (Stebbins, Variation and evolution in plants. Columbia University Press, Columbia, 1950). Polyploidisation followed by fractionation and duplicate gene diversification provides the opportunity to reconsider the importance of polyploidy for crop improvement.

Keywords

Domestication • Evolution • Heterozygosity • Genotype • Phenotype and genome

24.1 Introduction

Prokaryotes consist of a haploid (single) set of chromosomes, but normal eukaryotic (diploid = $2n$) cells/organisms consist of two homologous sets

D.N. Bharadwaj (✉)
Department of Genetics and Plant Breeding,
C.S. Azad University of Agriculture & Technology,
Kanpur 208 001, Uttar Pradesh, India
e-mail: bharadwajdncsau@gmail.com

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_24,

Table 24.1 Major crops, their chromosome number and ploidy level

Sl. no.	Crops	Chromosome no.	Ploidy level
1	Rice	$2x=24$	Diploid
2	Wheat	$6x=42$	Hexaploid
3	Maize	$2x=20$	Diploid
4	Barley	$2x=14$	Diploid
5	Cotton	$2x=56$	Tetraploid
6	Potato	$4x=48$	Tetraploid
7	Soybean	$2x=40$	Diploid
8	Tomato	$2x=24$	Diploid
9	Banana	$3x=33$	Triploid
10	Sugarcane	$8x=80$	Octaploid
12	Watermelon	$2x=22$	Diploid
13	Cassava	$2x=36$	Diploid

of chromosomes, where each set of haploid or monoploid set of chromosome is inherited from its parents. Generally eukaryotic diploid cells produce haploid gametes by meiotic cell division, which after fertilisation again produce diploid zygote that later on by its mitotic division produces a diploid organism. Polyploid cells contain more than two sets of genome/chromosomes that are most commonly found in plants (Table 24.1), rarely in animals except in amphibians and some tissues of human muscles. Polyploidy is heritable in nature. Polyploid cells are produced due to mutation that causes numerical change in genome/chromosomes. On the basis of number of chromosomes, polyploidy can be divided into euploidy and aneuploidy.

24.2 Euploidy

Euploids are polyploids with exact multiples of the complete set of chromosomes specific to a species. Euploids can be of the following types according to the number of genome/chromosome sets in the nucleus:

Triploid (three sets; $3x$): examples are ginger, apple, banana, citrus and seedless watermelons

Tetraploid (four sets; $4x$): examples are apple, durum or macaroni wheat, potato, cabbage, tobacco, peanut, kinnow, *Pelargonium* and cotton (*Gossypium hirsutum* L.)

Pentaploid (five sets; $5x$): examples are Kenai birch (*Betula papyrifera* var. *kenaica*)

Hexaploid (six sets; $6x$): examples are chrysanthemum, triticale, oat, kiwifruit and bread wheat (*Triticum aestivum* L.)

Octaploid (eight sets; $8x$): examples are strawberry, dahlia, sugar cane and oca (*Oxalis tuberosa*)

Decaploid (ten sets; $10x$): an example is certain strawberries

Dodecaploid (12 sets; $12x$): examples are *Celosia argentea* and amphibians

Some crops are found in a variety of ploidies such as: tulips and lilies are commonly found as both diploid and triploid; day lilies (*Hemerocallis* cultivars) are available as either diploid or tetraploid; apples and kinnows can be diploid, triploid or tetraploid. These euploids can be of the following types:

24.2.1 Autopolyploidy

Autopolyploids are polyploids with multiple chromosome sets derived from a single species. They arise from a spontaneous mutation in genome by its doubling such as potato and, in some other cases, by fusion of $2n$ gametes (unreduced gametes) and produce autotriploids such as bananas and apples which typically display polysomic inheritance and are therefore often sterile and propagated clonally. Autopolyploids (autoploids) consist of multiple copies of the basic (×) genome/chromosomes (Acquaah 2007; Chen 2010). Autopolyploids occur in nature due to the union of unreduced gametes and can also be induced artificially (Chen 2010). Natural autopolyploids include tetraploid crops such as alfalfa, peanut, potato coffee and triploid bananas. Spontaneously they develop through the process of chromosome doubling. Chromosome doubling in autopolyploids has varying effects based on the species, generally in ornamentals (tulip and hyacinth) and forage grasses (rye grasses) with increased vigour which yielded superior varieties due to this advantage; breeders have started the induction of chromosome doubling *in vitro* to produce

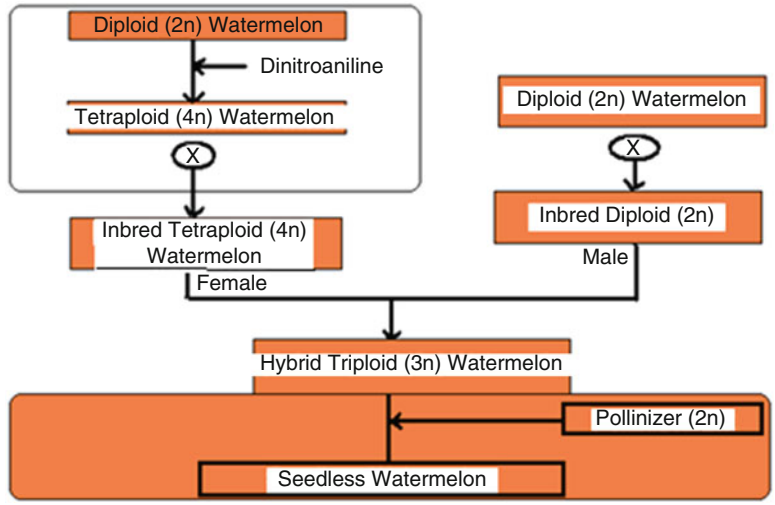


Fig. 24.1 Production of triploid watermelon (Source: Wehner 2008)

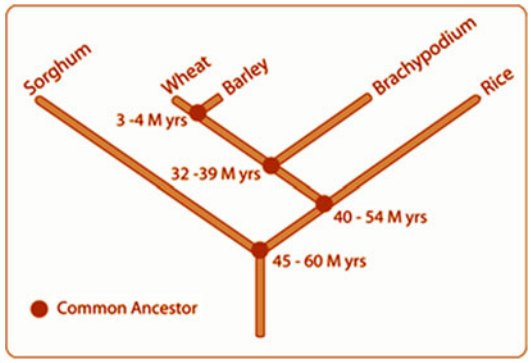


Fig. 24.2 Evolutionary relationships between some major cereal grasses (Source: Grass Phylogeny Working Group 2001)

superior crops such as autotetraploid seedless watermelon (Fig. 24.1).

24.2.2 Allopolyploidy

Allopolyploids are polyploids whose chromosomes are derived from different species. Precisely it is the result of multiplying the chromosome number in an F1 hybrid, for example, *Triticale* which have six chromosome sets (allohexaploid) where four sets are from wheat (*Triticum turgidum*) and two from rye (*Secale cereale*) (Fig. 24.2). Amphidiploids are a type of allopolyploids (tetraploid, containing the diploid chromosome sets of both parents), for example, brassicas, which contain relationships among the three common diploids (*B. oleracea*, *B. rapa* and *B. nigra*) and three allotetraploids (*B. napus*, *B. juncea* and *B. carinata*) derived from hybridisation among the diploids (Fig. 24.3). Allopolyploids or alloploids are a combination of genomes of different species (Acquaah 2007) due to hybridisation of two or more genomes followed by chromosome doubling or by the fusion of unreduced gametes between species (Acquaah 2007; Chen 2010; Jones et al. 2008; Ramsey and Schemske 1998). This is the key process of speciation for angiosperms and ferns in nature (Chen 2010). Economically important natural alloploid crops are wheat, oat, upland cotton, strawberry, blueberry and mustard. To differentiate between the sources of the genomes in any alloploid, the genomes are designated by a different letter as given below (Fig. 24.3) in the *Brassica* triangle by Nagaharu (Bellostas et al. 2007; Nelson et al. 2009). When segments of the chromosomes of the combining genomes differ, they are called as segmental alloploids. These chromosomes are similar but not homologous and are called homologous chromosomes. Such chromosomes indicate ancestral homology (Acquaah 2007). Induced alloploidy is not common; it has been used in the genus *Cucumis* to elucidate the molecular mechanisms involved in diploidisation, a tendency of polyploids to act as diploids (Chen et al. 2007). An allotetraploid was induced

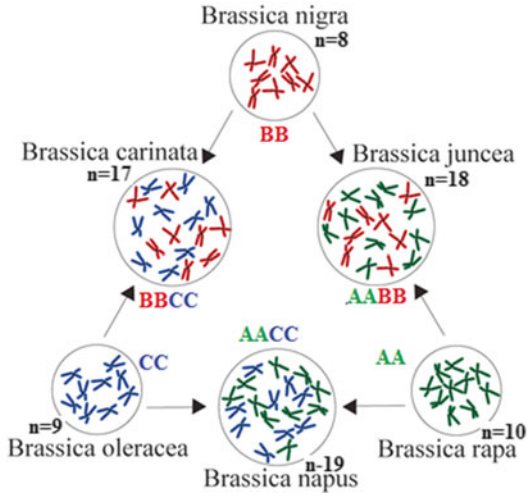


Fig. 24.3 U triangle showing the origin of cultivated mustard (Source: Nagaharu 1935)

by hybridisation between *Cucumis sativus* and *Cucumis hystrix*, followed by chromosome doubling.

Allopolyploidy shows uncertainty because it possesses both a diversifying force and a genetic bottleneck (Stebbins 1971). However, the genetic bottleneck problem may be solved by the fact that population-level genetic studies of polyploid plants and animals indicate that polyploidy is not a rare event leading to produce unique and uniform genotypes. Rather, the multiple independent formations of polyploid species from heterozygous diploid progenitors may provide a significant source of genetic variation (Soltis and Soltis 2000, 1993, 1999). Polyploids attracted considerable attention because of their unique cytogenetics, distinctive phenotypes and hybrid vigour that are useful in agriculture (Randolph 1941; Levin 2002; Ramsey and Schemske 2002). Hybrid vigour (heterosis) is an important phenomenon of agriculture where two genetically distinct offspring inbred varieties produce higher yields than either one of their parents. In contrast to hybrid vigour, some allopolyploids show decreased vigour compared to their diploid progenitors for some traits. Naturally occurring *Arabidopsis suecica* does not show seed lethality, but new allopolyploids can be unstable, indicating that trait changed over the generation of natural selection due to 'genomic shock' (union of different genomes). This 'genomic shock' may be caused by the first steps in the diploidisation process as a result of changes in gene expression (McClintock 1984).

24.2.2.1 Natural Alloploids

Some important natural allopolyploid crops are wheat, cotton, tobacco, mustard, oat, etc. These allopolyploid crops originated naturally by interspecific crossing followed by chromosome doubling; some natural allopolyploid crops are given bellow:

1. *Bread Wheat (Triticum aestivum L.):* Evolutionary history of wheat showed that diploid species of wheat contributing three genomes (A, B and C). *Triticum aestivum* (formerly *Triticum spelta*) is a hexaploid wheat artificially synthesised by McFadden and Sears (1946). They crossed an emmer wheat, *Triticum turgidum* (formerly *Triticum dicoccum*; tetraploid: $2n=28$), with goat grass, *Triticum tauschii* (formerly *Aegilops squarrosa*; diploid; $2n=14$), and doubled the chromosome number in the F1 hybrids. This artificially synthesised hexaploid wheat was found to be similar to the primitive wheat *Triticum aestivum*. This synthesised hexaploid wheat was crossed with the naturally occurring *Triticum aestivum*; the F1 hybrid was fertile; this suggested that hexaploid wheat must have originated in nature due to hybridisation between tetraploid wheat and goat grass, followed by chromosome doubling in subsequent generation (Fig. 24.4).
2. *Tobacco (Nicotiana tabacum):* The genus *Nicotiana* (family Solanaceae) comprises 76 chromosomes and naturally occurring species that are subdivided into 13 sections (Knapp et al. 2004). *Nicotiana tabacum* ($n=24$) is a classic amphidiploid arisen from the hybridisation between *Nicotiana sylvestris* and *Nicotiana tomentosa*; both these species are diploid with $n=12$. There is a very strong evidence of *Nicotiana sylvestris* as the maternal parent and the donor of 'S' genome (Bland et al. 1985; Olmstead and Palmer 1991; Aoki and Ito 2000; Yukawa et al. 2006). It is also evidenced that the 'T' genome was contributed by *Tomentosae* (viz. *Nicotiana*

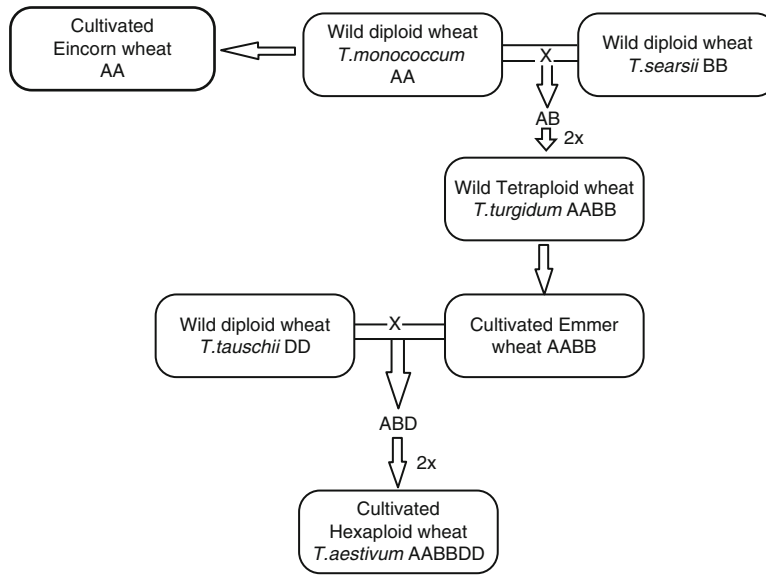


Fig. 24.4 Evolution of wheat (Source: Feldman et al. 1995)

tomentosiformis, *Nicotiana otophora* or an introgressive hybrid between the two) (Kenton et al. 1993; Riechers and Timko 1999; Kitamura et al. 2001; Ren and Timko 2001).

3. *Cotton:* The diploid species of the genus *Gossypium* possess $n=13$ and fall into seven different 'genome types', designated from A to G based on chromosome pairing relationships (Beasley 1942); five tetraploid ($n=2x=26$) species are recognised, exhibiting disomic chromosome pairing (Kimber 1961). Chromosome pairing in interspecific crosses between diploid and tetraploid cottons suggests that tetraploids contain two distinct genomes, which resemble the extant A genome of *G. herbaceum* ($n=13$) and D genome of *G. raimondii* ($n=13$), respectively. The A and D genome species diverged about 6–11 million years ago from a common ancestor (Wendeil 1989). It is assumed that polyploidisation of A X D genome has occurred in the New World, about 1.1–1.9 million years ago. The transoceanic migration of the maternal A genome which is indigenous to the Old World (Fryxelp 1979). This polyploidisation was followed by radiation and divergence, with distinct AD genome ($n=26$) species now indigenous to Central America (*G. hirsutum*), South America (*G. barbadense*, *G. mustelinum*), the Hawaiian Islands (*G. tomentosum*) and the Galapagos Islands (*G. danuinii*) (Fryxelp 1979). The tetraploid (*Gossypium hirsutum*, $2n=52$) is believed to be an amphidiploid between the larger chromosome of *Gossypium africanum* and smaller chromosome of *Gossypium raimondii* with $2n=26$ (Hutchinson et al. 1947).
4. *Oat (Avena sativa):* Oat is a self-pollinated allohexaploid species with a basic chromosome number of $n=3x=21$, which consists of three basic genomes A, C and D (Rajathy and Thomas 1974) derived from a cross between *A. barbara* (tetraploid, $n=14$) and *A. strigosa* (diploid, $n=7$).
5. *Amphidiploid Brassica Species:* The origin of amphidiploid *Brassica* species is based on the U triangle proposed by Nagaharu (1935) (Fig. 24.3) where *Brassica juncea* (2=18) is an amphidiploid from an interspecific cross between *Brassica nigra* ($n=8$) and *Brassica campestris* ($n=10$), whereas *Brassica napus* ($n=19$) is an amphidiploid from an interspecific cross between *Brassica oleracea* ($n=9$) and *Brassica campestris* (Osborn et al. 2003a) and *Brassica carinata* ($n=17$) is a result of an interspecific cross between *Brassica nigra* ($n=8$) and *Brassica oleracea* ($n=9$).

24.2.2.2 Artificial Allopolyploids

Artificial allopolyploids have been synthesised in some crops with two main objectives, viz. (1) either to study the origin of naturally available

alloploids or (2) to explore the possibilities of creating new species. The following are some artificial alloploids:

1. *Raphanobrassica*: This is a classical example of artificially created alloploidy, developed between radish (*Raphanus sativus*, $n=9$) and cabbage (*Brassica oleracea*, $n=9$) by Russian geneticist Karpechenko (1927) with the object of developing a fertile hybrid between these two species with roots of radish and leaves of cabbage. An unfortunate fertile amphidiploid ($4n=36$) was developed by spontaneous chromosome doubling which had roots of cabbage and leaves of radish (Fig. 24.5).
2. *Triticale*: Triticale is a new synthesised crop species from a cross between hexaploid wheat (*Triticum aestivum*) and rye (*Secale cereale*, $n=9$). Some triticales were developed from the cross between tetraploid wheat (*Triticum turgidum*) and rye and some other from the cross between hexaploid wheat (*Triticum aestivum*) and rye. The F_1 was sterile and made fertile by the doubling of chromosomes with colchicines treatment. Triticales produced by using tetraploid and hexaploid wheat are hexaploid and octaploid, respectively. Now triticales are being commonly grown in Canada, Mexico, Hungary and some other countries (Fig. 24.6).

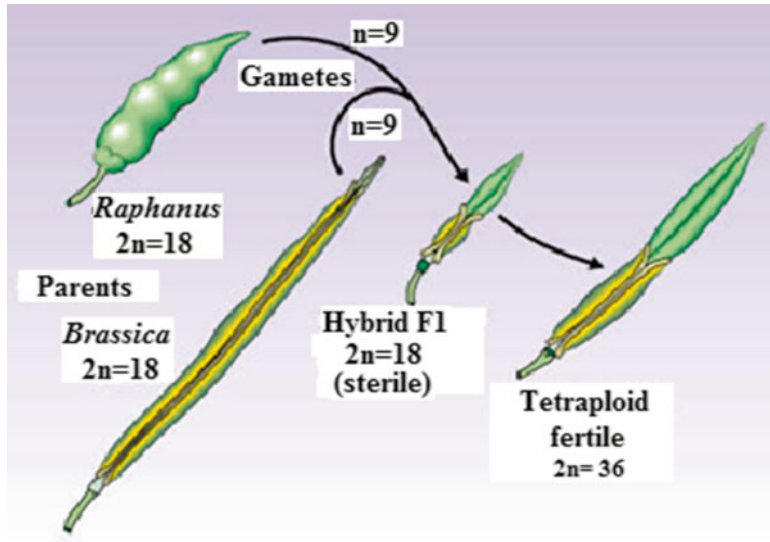


Fig. 24.5 Development of *Raphanobrassica* (Source: Karpechenko 1928/1989)

Fig. 24.6 Evolution of triticale from wheat and rye. Seeds of wheat (*left*), triticale (*centre*) and rye (*right*) (Source: A. Stephen Wilson 1875)

24.2.3 Palaeopolyploidy

It refers to an ancient polyploid that later became a diploid again due to sequence divergence between duplicated chromosomes as in case of human genome. They generally have large basic chromosome numbers. Ancient genome duplications probably occurred in the evolutionary history of all life, evolutionary lineages can be difficult to detect because of subsequent diploidisation and polyploid starts to behave cytogenetically as a diploid because mutations and gene translations gradually make one copy of each chromosome unlike the other copy and become inactive pseudogenes. Examples are baker's yeast (*Saccharomyces cerevisiae*), mustard weed/thale cress (*Arabidopsis thaliana*) and rice (*Oryza sativa*), and some flowering angiosperms have palaeopolyploidy in their ancestry.

24.2.4 Neopolyploidy

Neopolyploids are newly formed auto- and allopolyploids that have individuals with a series of ploidy levels within the species. The ploidy series may consist of individuals with even or odd multiples of the basic chromosome number (*Chrysanthemum*, $x=9$, series $2x$, $4x$, $6x$, $8x$ and $10x$) or odd multiples of the basic chromosome number (*Crepis occidentalis*, $x=11$, series $2x$, $3x$, $4x$, $5x$, $7x$ and $8x$ forms).

24.3 Aneuploidy

Aneuploids are polyploids that contain either an addition or deletion of one or more specific chromosome(s) to the total number of chromosomes that usually make up the ploidy of a species (Acquaah 2007; Ramsey and Schemske 1998). Aneuploids are formed due to the formation of univalents and multivalents during meiosis of euploids (Acquaah 2007). Autotetraploid maize are aneuploids (Comai 2005), and univalents cannot divide equally among daughter cells during meiosis anaphase I; therefore, some cells inherit more genetic material than others (Ramsey and Schemske 1998). Similarly, multivalents such as homologous chromosomes may fail to separate during meiosis, leading to the unequal migration of chromosomes to opposite poles; this phenomenon is called non-disjunction (Acquaah 2007). These meiotic aberrances result in plants with reduced vigour. These represent a succession of allopolyploid series based on different basic chromosome numbers. Dibasic polyploids are the sum of two different diploid numbers (e.g. *Brassica oleracea* ($2n=18$) and *Brassica campestris* ($2n=20$) and their tetraploid derivative *B. napus* ($2n=4x=38$)). Polyploids often tolerate the loss of one or more chromosome pairs which at times may give rise to modified polyploid series, what Darlington called a 'polyploid drop' (e.g. a modified series found in *Hesperis* where different species have gametic numbers of $n=7$, 12, 13 and 14). Aneuploids are classified according to the number of chromosomes as shown in Table 24.2.

Table 24.2 Classification of aneuploids according to the number of chromosomes

Term	Chromosome number
Monosomy	$2n-1$
Double monosomy	$2n-1-1$
Nullisomy	$2n-2$
Trisomy	$2n+1$
Tetrasomy	$2n+2$
Pentasomy	$2n+3$

24.4 Types of Polyploidy

On the basis of nature of mutation, polyploidy can be divided into following types:

24.4.1 Spontaneous Polyploidy

Mutations arise spontaneously at low frequency, owing to the chemical instability of purine and pyrimidine bases and due to errors which occur during DNA replication. Natural exposure of an organism to certain environmental factors, such as ultraviolet light and chemical carcinogens (e.g. aflatoxin B1), can cause mutations. A common cause of spontaneous point mutations is the deamination of cytosine to uracil in the DNA

double helix. Subsequent replication leads to a mutant daughter cell in which a T–A base pair replaces the wild- type C–G base pair. Another cause of spontaneous mutations is copying during DNA replication. Although replication generally is carried out with high fidelity, errors rarely occur due to spontaneous mutations on the molecular level; these may be caused by:

- *Tautomerism* – A base is changed by the repositioning of a hydrogen atom, altering the hydrogen bonding pattern of that base, resulting in incorrect base pairing during replication.
- *Depurination* – Loss of a purine base (A or G) to form an apurinic site (AP site).
- *Deamination* – In general hydrolysis changes a normal base to an atypical base containing a keto group in place of the original amine group. Examples include C→U and A→HX (hypoxanthine), which can be corrected by DNA repair mechanisms, and 5MeC (5-methylcytosine) →T, which is likely to be less detected as a mutation because thymine is a normal DNA base.
- *Slipped strand mispairing* – Denaturation of the new strand from the template during replication, followed by renaturation in a different spot (slipping). This can lead to insertions or deletions.

24.4.2 Induced Polyploidy

The polyploids that are produced artificially in the laboratory at molecular level with efforts to change the DNA sequence which is passed from the parent to offspring. As a result of induced mutation, produced crops have more desirable traits such as the strong ability to resist diseases and insects. In order to increase the frequency of mutations in organisms, researchers treat them with high doses of chemical mutagens to expose them with ionising radiation. Mutations arising in response to such treatments are referred to as *induced* mutations. Generally, chemical mutagens induce point mutations, whereas ionising radiation gives rise to large chromosomal abnormalities. Ethyl methanesulfonate (EMS), a commonly used mutagen, alkylates guanine in DNA, forming O^6-ethylguanine. During subsequent DNA replication, O^6-ethylguanine directs the incorporation of deoxythymidylate, not deoxycytidylate, resulting in the formation of mutant cells in which a G–C base pair is replaced with an A–T base pair.

Induced mutation: The following mutagens are used to create induced mutations on the molecular level of the plant concerned:

1. *Chemicals*
   (a) Hydroxylamine NH_2OH.
   (b) Base analogues (e.g. BrdU).
   (c) Alkylating agents (e.g. *N*-ethyl-*N*-*nitrosourea*). These agents can mutate both replicating and non-replicating DNA. Each of these chemical mutagens has certain effects that can lead to transitions, transversions or deletions to DNA sequence.
   (d) Agents that form DNA adducts (e.g. ochratoxin A metabolites).
   (e) DNA intercalating agents (e.g. ethidium bromide).
   (f) DNA crosslinkers.
   (g) Oxidative damage.
   (h) Nitrous acid converts amine groups on A and C to diazo groups, altering their hydrogen bonding patterns that leads to incorrect base pairing during replication.
2. *Radiation:* Ultraviolet radiation (nonionising radiation). Two nucleotide bases in DNA – cytosine and thymine – are most vulnerable to radiation that can change their properties. UV light can induce adjacent pyrimidine bases in a DNA strand to become covalent.

24.5 Factors Promoting Polyploidy

Listed below are some factors that promote polyploidy:

1. *Polyspermy* is observed in many plants, but its contribution as a mechanism for polyploid formation is rather rare except in some orchids.

2. *Endo-reduplication* is a form of nuclear polyploidisation resulting in multiple uniform copies of chromosomes. It is observed in the endosperm and the cotyledons of developing seeds, leaves and stems of bolting plants. In animals it is observed in certain tissues of liver cells and megakaryocytes (precursor of thrombocytes).
3. *Other miscellaneous factors promoting polyploidy:* Besides the above, several other factors can promote polyploidy, i.e. mode of reproduction, pollination, fertilisation, growth habit, size of chromosomes, etc. Polyploidy is mostly favoured in perennial plants possessing various vegetative means of propagation (e.g. *Fragaria, Rubus, Artemisia, Potamogeton*, etc.) and is also frequent in natural interspecific hybridisations. Cross-fertilisation and allogamy are also favouring polyploidy, while autogamy was supposed to restrict polyploidy. Various ecological factors such as wet soils and meadows are also favouring polyploidy. A reciprocal relationship has been established between cell size, chromosome size and chromosome numbers in polyploids.

24.6 Mechanism of Polyploidy

Polyploids arise due to a rare mitotic or meiotic catastrophe, such as non-disjunction that causes the formation of gametes which have a complete set of duplicate chromosomes where diploid gametes are frequently formed. When this diploid gamete fuses with a haploid gamete, a triploid zygote is formed, but these triploids are generally unstable and mostly are sterile. If a diploid gamete fuses with another diploid gamete, this will produce a fertile and stable tetraploid zygote. In this way, many polyploids (tetraploids, hexaploids, etc.) are formed in nature. Different species exhibit different levels of tolerance for polyploidy. Flowering plants show polyploids at a relatively high frequency, i.e. 1/100,000 individuals, indicating that plants have a high tolerance for polyploidy in comparison to higher vertebrates (do not tolerate polyploidy).

24.7 Origin of Polyploidy

There are various modes for the origin of polyploids but mainly arise due to somatic doubling during mitosis, nonreduction in meiosis leading to the production of unreduced gametes, polyspermy (fertilisation of the egg by two male nuclei) and endo-replication (replication of the DNA but without cytokinesis). Chromosome doubling can occur either in the zygote to produce a completely polyploid individual or in some apical meristems to give polyploid chimaeras. This doubling may occur in vegetative tissues (as in root nodules of some leguminous plants). Spontaneous somatic chromosome doubling is a rare event (*Primula kewensis*) which arose by somatic doubling in certain flowering branches of a diploid hybrid. The phenomenon of chromosome doubling in the zygotes is generally occurring due to high temperatures. The zygotic chromosome doubling is spontaneous appearance of tetraploids as in *Oenothera lamarckiana* and amphidiploid hybrids of tobacco (*Nicotiana tabacum*) that are results of zygotic chromosome doubling.

The second major cause of polyploid formation involves gametic nonreduction or meiotic nuclear restitution during micro-sporogenesis and mega-sporogenesis which results in unreduced 2*n* gametes. Nonreduction could be due to meiotic non-disjunction (failure of chromosome separation and reduction in chromosome number), failure of cell wall formation or formation of gametes by mitosis instead of meiosis. The classic example has occurred in *Raphanobrassica* that originated by a one-step process of fusion of two unreduced gametes. Another route may involve nonreduction occurring in one of the germ lines (pollens or eggs) only. A tetraploid individual can then result from a two-step process (triploid bridge mechanism) from the fusion of an unreduced 2*n* gamete with a reduced 1*n* gamete to give a 3*n* zygote, followed by the subsequent fusion of a 3*n* gamete with a normal 1*n* gamete in the next generation to give rise to tetraploid individuals as in the case of

Galeopsis tetrahit. This is a more common process in polyploid formation from unreduced gametes (produces sterile triploids). The production of unreduced gametes is also a function of the environment and genotype (*Gilia* sp.) and maize wherein the gene *elongate* on chromosome 3 was found to increase the proportion of diploid egg production.

24.7.1 Chromosome Evolution

In higher plants polyploidy is a prominent feature of chromosome evolution; it is common in many species and genera that are characterised by diploid and polyploid races. Polyploidy is an evolutionary process, not an event. Polyploid may involve somatic chromosome doubling or sexual functioning of cytologically unreduced gametes. Spontaneous chromosome doubling, either in the zygote to produce a polyploid plant or in an apical meristem to produce a chimaera, is a rare event. The common mode of synthesis of polyploidy is through the formation of cytologically unreduced gametes as given above it is commonly a two-step process – a diploid ($2n$) female gamete is fertilised by a haploid (n) male gamete to produce a triploid ($3x$) unreduced female gamete that can be fertilised by haploid (n) gametes of the diploid parents, and this results into a tetraploid ($4x$) offspring. Fertilisation of a rare diploid ($2n$) female gamete by an equally rare diploid ($2n$) male gamete may directly produce a tetraploid ($4x$) and is extremely rare but possible. Fertility in polyploids is restored through cytological diploidisation of the genomes or through gametophytic apomixis. Reversible tetraploidy is a part of polyploid evolution.

24.8 Polyploidy in Crop Improvement

Considering the high importance of polyploidy in plant evolution, the induced polyploidy was first discovered in the 1930s, developed in several major crops but mostly found inferior to their diploid progenitors. Somatic doubling does not introduce any new genetic material, but rather produces additional copies of existing chromosomes. This extra DNA must be replicated with each cell division. Enlarged cell size is often associated with polyploids, which can result in anatomical imbalances. Other deleterious effects can include abnormal bearing, brittle wood and watery fruits (Sanford 1983). High-level polyploids, i.e. more than octaploids, are generally stunted and malformed because of extreme genetic redundancy and somatic instability that leads to chimaeral tissue. Despite several drawbacks of induced autopolyploids, these plants may be valuable in several breeding programmes to enhance the degree of heterozygosity and further selection of desirable traits. In most cases it is apparent that inducing autopolyploidy can improve substantial heterozygosity in plants (Sanford 1983). Polyploidy can result in a wide range of effects on plants that relate to gene silencing, gene interactions, gene dose effects and regulation of specific traits and processes.

24.9 Polyploidy and Creation of Novel Variations

Polyploids can create variation through mechanisms of gene flow with diploids and multiple origins of polyploids (population genetics) and through mechanisms that generate 'de novo variation' such as chromosomal rearrangements and epigenetic phenomena (Mittelstein Scheid et al. 1996). Polyploidy has long been considered as an important example of instant or sympatric speciation, since polyploid species are mostly reproductively isolated from their diploid progenitors (Stebbins 1950, 1971; Levin 1983). The members of the same species are commonly known as related population individuals that can interbreed and produce fertile offspring. For example, the horse and the donkey are considered separate species; their hybrid offspring mules are viable but infertile. In plants, hybridisation of different species is quite common and many of them produce allopolyploids resulting from interspecies hybrids. These allopolyploids pose a challenge to phylogenetic species concepts, which define species on strict monophyletic criteria. Multiple origins of polyploid species have been reported in ferns,

mosses and many angiosperms (Soltis and Soltis 2000).

Now it has become apparent that polyploid genomes are not always a simple sum of their constituent genomes, but is a product of dynamic genetic and epigenetic changes that occur due to polyploid formation. Epigenetic changes involve alterations of gene expression without a change in DNA sequence and are particularly intriguing because they play essential roles in plant development and plant defence against viruses and transposons. In nascent polyploids, epigenetic phenomena observed which include nuclear dominance and changes in DNA methylation and chromatin structure, triggering silencing or activation of genes and creating novel phenotypes.

An advance study in polyploidy has indicated that the degree of genetic and epigenetic changes in recent natural and synthetic allopolyploids varies across taxa as in the case of *Arabidopsis*, *Brassica* (Song et al. 1995), *Triticum* (Ozkan et al. 2001; Kashkush et al. 2002) and *Nicotiana*; this demonstrated rapid genomic and epigenetic changes. In contrast, synthetic *Gossypium* polyploids show few changes in overall genome sequences, which display differential expression of genes in different tissue types (Liu et al. 2001; Adams et al. 2003). These recent studies suggested that genetic and epigenetic changes contribute to the potentially dynamic nature of polyploids (Soltis and Soltis 1995) and reveal a link between these epigenetic changes and the evolutionarily success of polyploid speciation.

24.10 Advantages and Disadvantages of Polyploidy

Various advantages and disadvantages of being polyploids are discussed below.

24.10.1 Advantages of Polyploidy

The high incidence of polyploidy in plants and lower vertebrates (fish, frogs, etc.) showed advantages to being polyploids; in plants they demonstrated hybrid vigour (superior heterosis), where polyploid offspring of two diploid progenitors are more vigorous and healthier than either of the diploid parents. This superiority can be explained as enforced pairing of homologous chromosomes within an allotetraploid which prevents recombination between the genomes of the original progenitors, effectively maintaining heterozygosity throughout generations that prevents the accumulation of recessive mutations in the genomes of subsequent generations, thus maintaining hybrid vigour. Another important factor is gene redundancy because polyploid offspring have two or more copies of any particular gene, so the offspring are protected from the deleterious effects of recessive mutations. In a haploid cell, it can be imagined that any allele that is recessive for a deleterious mutation will not be masked by the presence of a normal dominant allele. Conversely, a diploid gamete permits the masking of the deleterious allele's effect by the presence of dominant normal allele, thus protecting the pollen or egg sac from developmental dysfunction. This dominant protective effect of polyploidy might be important in smaller, isolated populations that are forced to inbreed. Another advantage of gene redundancy is the ability to diversify gene function over the time, therefore, unwanted extra copies of genes in an organism might end up, leading to new opportunities in evolutionary selection (Adams and Wendel 2005a, b). Polyploidy can affect sexuality which provides selective advantages by disrupting certain self-incompatibility systems, thereby allowing self-fertilisation. In allopolyploids, this may be another way of favouring the onset of asexual reproduction in static environments (Comai 2000).

24.10.2 Disadvantages of Polyploidy

Besides several advantages of being a polyploid organism, there are a huge number of disadvantages which proportionally enhance changes in the ratio of the genome and the volume of the cell in the cell's nucleus. The doubling of a cell's genome is expected to double the volume of the nucleus, but it can increase up to 1.6-fold only in the surface area of the nuclear envelope (Melaragno et al. 1993) which disrupts the balance

of factors to mediate interactions between the chromosomes and nuclear components. This disrupts the peripheral position of telomeric and centromeric heterochromatin because there is less relative surface area on the nuclear envelope to accommodate cell components (Fransz et al. 2002). Polyploidy can also disrupt the normal completion of mitosis and meiosis due to the increase of spindle irregularities, which leads to the irregular segregation of chromatids and production of aneuploid cells. Autopolyploids have the potential to form multiple arrangements of homologous chromosomes at meiotic metaphase I, which can result in abnormal segregation patterns, such as 3:1 or 2:1 plus one laggard (that does not attach properly to the spindle apparatus and thus segregate randomly in daughter cells). These abnormal segregation patterns form unbalanced aneuploid gametes. Chromosome pairing at meiosis I is more constrained in allopolyploids than in autopolyploids because of unbalanced gametes.

Another disadvantage of polyploidy is potential changes in gene expression; any increase in the copy number of chromosomes would affect gene expression. Epigenetic instability can pose another challenge for polyploids which refers to changes in phenotype and gene expression that are not caused by changes in DNA sequence. According to the genomic shock hypothesis, disturbances in the genome, such as polyploidisation, may lead to widespread changes in epigenetic regulation. In some cases, transgene silencing occurred more frequently in tetraploids (*Arabidopsis thaliana*) than in diploids (*A. thaliana*), thus suggesting an effect of ploidy on chromosome remodelling. Structural genomic changes, such as DNA methylation, and expression changes due to the transition of chromosomes in alloploidy results into regulatory changes as in wheat (Shaked et al. 2001), and in *Arabidopsis thaliana* about 2–2.5 % changes were estimated during the transition to allopolyploidy.

Aneuploidy might also be a factor in epigenetic remodelling in neoallopolyploids, either by altering the dosage of factors that are encoded by chromosomes that have greater or fewer than the expected number of copies, leading to changes in imprinted loci, or by exposing unpaired chromatin regions to epigenetic remodelling mechanisms. Therefore, some of the epigenetic instability that is observed in allopolyploids might result from aneuploidy.

24.11 Evolutionary Potential of Polyploid Organisms

The epigenetic changes in polyploids are observed to be deleterious because of the disruptive effects on regulatory patterns of genes on selection which show increased diversity and plasticity for rapid adaptation of polyploids. Polyploidy is believed to play an important role in the rapid adaptation of some allopolyploid arctic flora, possibly due to their genomes that confer hybrid vigour and buffer against the effects of inbreeding. However, epigenetic fertility barriers between species often need to be overcome in successful allopolyploids. Unbalanced gametic polyploids arise due to their chromosomes which could not pair, and zygotes would perish because their zygotic chromosomes might split longitudinally, each making a pair (Winge 1917); this allowed the zygotes to develop the hybrid carrying 2*x* parental number of chromosomes. The cross between *Raphanus* and *Brassica* (*Raphanobrassica*) showed that meiotic failure resulted in unreduced gametes (Karpechenko 1927).

24.11.1 Genome Evolution in Polyploids

Polyploidy has been a constant natural force in plant evolution and speciation. Modern plant genomes are evidence of multiple rounds of polyploidisation and often followed by gene silencing or elimination of duplicated genes which has extensive effects on gene expression. Most polyploids undergo widespread and rapid genomic alterations that arise with the onset of polyploidy; the survivorship of duplicated genes is differential and is prone to retention. Interdisciplinary

approaches to combine phylogenetic and molecular genetics perspectives have improved the understanding of genetic interactions through polyploidy in plants. Genes duplicated by polyploidy may retain their original or similar function and undergo diversification in protein regulation, or one copy may become silenced through mutation or epigenetic process. Duplicated genes may also interact through inter-locus recombination, gene conversion and evolution. The intergenomic chromosomal exchange and non-Mendelian genomic evolution in nascent polyploids are the cause of intergenomic invasion and cytonuclear stabilisation. The role of transposable elements in structural and regulatory gene evolution; processes and significance of epigenetic silencing are underlying within controls of chromosome pairing. The mechanisms and functional significance of rapid genome changes i.e. cytonuclear accommodation and coordination of regulatory factors are contributed by two or more divergent progenitor genomes. The application of molecular genetic approaches to polyploid genome evolution holds promise for producing novel genotypes and facilitates evolution and adaptation. Survivorship of duplicated genes is differential across gene classes, with some duplicate genes being more prone to retention. Recent evidence shows that genes that are retained in duplicate typically diversify in function or undergo sub-functionalisation. Polyploidy has extensive effects on gene expression, with gene silencing accompanying polyploid formation and continuing over evolutionary time.

24.11.2 Speciation and Polyploidy

Early changes in the ploidy level of organisms played an important role in diversification and represent an ongoing phenomenon. The prevalence of polyploid ancestry indicates that it is a common and successful evolutionary transition and has a significant effect on patterns and rates of diversification. The incidence of polyploidy in ferns and flowering plants indicates transitions between odd and even base chromosome numbers which indicates that ploidy changes may represent from 2 % to 4 % of speciation in flowering plants and 7 % in ferns. Speciation via polyploidy is likely to be one of the more predominant modes of sympatric speciation in plants, potentially effects on gene regulation and developmental processes that can create immediate shifts in morphology, breeding system and ecological tolerances.

Polyploidy has caused many rapid speciation events in sympatry because offspring produced through tetraploid × diploid matings often result in triploid sterile progeny (Barluenga et al. 2006). However, all polyploids are not isolated reproductively from their parents, and gene flow may still occur through triploid hybrid × diploid matings that produce tetraploids or matings between meiotically unreduced gametes from diploids and gametes from tetraploids. Reproduction of successful polyploid species is sometimes asexual by parthenogenesis or apomixis for unknown reasons. Instant speciation in plants can almost change the ploidy of their chromosomes reproduced via asexual reproduction; they spread vegetatively but lack sexual reproduction.

1. *Sympatric speciation* is the process through which new species evolve from a single ancestral species while existing in the same geographic region. If these organisms are closely related, such a distribution may be the result of sympatric speciation. Sympatric speciation is one of the traditional geographic categories for the phenomenon of speciation (Fitzpatrick et al. 2008).
2. *Allopatric speciation* is the evolution of geographically isolated populations into distinct species. Here, divergence is facilitated by the absence of gene flow, which tends to keep populations genetically similar.
3. *Parapatric speciation* is the evolution of geographically adjacent populations into distinct species. Here, divergence occurs despite limited interbreeding where the two diverging groups come into contact.

In sympatric speciation, there is no geographic constraint to interbreeding. These categories are special cases of a continuum from zero (sympatric) to complete (allopatric) spatial segregation of diverging groups. In multicellular eukaryotic

organisms, sympatric speciation is thought to be an uncommon but plausible process by which genetic divergence (reproductive isolation) of various populations from a single parent species and inhabiting the same geographic region leads to the creation of new species (Bolnick and Fitzpatrick 2007).

24.12 Polyploidy in Evolution

Polyploidy is an interesting phenomenon that leads to an important pathway for evolution and speciation in plants. The first polyploid was discovered over a century ago; the genetic and evolutionary implications of polyploidy are still being elucidated (Bennett 2004). The gradual evolution abruptly created new species from isolated populations through natural polyploidy; thus, polyploidy has provided a valuable tool to conventional plant breeders in breeding programmes. Once a tetraploid arises in a population, it can generally hybridise with other tetraploids; however, these tetraploids are reproductively isolated from their parental species so tetraploids that cross with diploids of the parental species will produce a sterile triploid, which provides a reproductive barrier between the polyploids and the parental species. About 47–70 % of flowering plants are of polyploids in origin (Ramsey and Schemske 1998) as in case of rosaceous plants (*Malus*, *Pyrus*, *Chaenomeles*, etc.) that are supposed to originate from ancient allopolyploids with basic chromosomes $n=17$, whereas other subfamilies of rosaceous plants have $n=8$ or 9 (Rowley 1993). In many genera, different species have different ploidy levels in multiples of a base number, representing a series of polyploids.

Polyploids comprise a number of factors with adaptive and evolutionary advantages that are significantly more heterozygous than their diploid parents. This degree of heterozygosity may be a main factor in the growth, performance and adaptability of a polyploid. Allopolyploids have a greater degree of heterozygosity that can contribute to heterosis or hybrid vigour where chromosomes preferably pair with similar homologous chromosomes during meiosis, ensuring both parental species' genomes to function. The addition of multiple copies of homozygous chromosomes in autopolyploids does little to enhance genetic superiority and can actually reduce vigour and fertility by creating a more inbred situation.

However, allopolyploids have some adaptive benefits that exhibit enzyme multiplicity (Soltis and Soltis 1993) because of the fusion of two distinct genomes; these can potentially produce all the enzymes produced by each parent as well as new hybrid enzymes, and this results in greater biochemical flexibility (Roose and Gottlieb 1976). Other changes in gene expression, altered regulatory interactions and rapid genetic and epigenetic changes could further contribute to increased variation and new phenotypes (Osborn et al. 2003b).

24.13 Utilisation of Polyploids

The origin of a polyploid can often determine its fertility and indicates how it can best be utilised in a plant improvement programme. If a tetraploid arises from spontaneous doubling in a shoot or from the union of unreduced gametes from two closely related or same species of diploid individuals, it will have four similar (homologous) sets of each chromosome. Despite different origins, both of these polyploids behave similar reproductively and are often referred to as autotetraploids. Autopolyploids may or may not be fertile. In diploids, meiosis involves the pairing of homologous chromosomes, which segregate to form two separate gametes, each with one set of chromosomes. Infertility can arise in autopolyploids due to the presence of more than two homologous chromosomes. The presence of multiple homologous chromosomes often results in false pairing between multiple chromosomes, unpaired chromosomes and gametes with unbalanced chromosome numbers known as aneuploids. The offspring that result from sexual reproduction of unreduced gametes of different

species are commonly referred to as allopolyploids (amphidiploids or disomic polyploids). These plants also have four genomes, the two from one parent (nonhomologous) and the other two from another parent, which generally do not pair during meiosis. Due to this composition, allopolyploids are typically fertile. During meiosis, each chromosome can pair with its homologous partner, and meiosis continues, resulting in fertile germ cells.

24.14 Mechanism of Plant Evolution

In contrast to the gradual evolutionary process where new species evolve from isolated populations, new species of plants can also arise abruptly through polyploidy. The most common mechanism for abrupt speciation is through the formation of natural polyploids. Once a tetraploid species arises in a population, it can generally hybridise with other tetraploids. However, these tetraploids are reproductively isolated from their parental species. Tetraploids that cross with diploids of the parental species will result into sterile triploids. This phenomenon provides a 'reproductive barrier' between the polyploids and the parental species – a driving force for speciation. Polyploids that arise at a low frequency are generally self-fertile and apomictic that helps to compensate for their minority disadvantage and would provide opportunities in stressful environments. It has often been observed that disproportionate numbers of polyploids are found in cold and dry regions. Inbreeding is less deleterious in allopolyploids, because of their extreme heterozygosity.

24.15 Polyploidy and Plant Improvement

Considering the extreme importance of polyploidy in plant evolution, polyploids have been developed in many crops, but except ferns these plants are mostly inferior to their diploid progenitors. Somatic doubling does not introduce any new genetic recombination; instead adding additional copies of existing chromosomes, this extra DNA replicated with each cell division; thus, polyploids are characterised by enlarged cell size that is associated with anatomical imbalances and other deleterious effects that include unpredictable bearing, brittle wood and watery fruit (Sanford 1983). High-level polyploids, i.e. the above octaploids, can be stunted and malformed, because of extreme genetic redundancy and somatic instability that leads to chimaeral tissue. The development of allopolyploids may be used to study gene silencing, gene interactions, gene dose effects and regulation of specific traits and processes. The following are the main goals of polyploidy in crop improvement:

- Gene buffering: slower response to selection but more adaptive potential.
- Dosage effect: additive effect of the alleles increases the number of phenotypes.
- Increased allele diversity and heterozygosis: more possible allele combinations and opportunities for breeding.
- Novel phenotypic variation: genome interactions and changes in gene expression in new synthesised allopolyploids.

24.15.1 Opportunities of Polyploidy

Polyploidy provides the following opportunities to plants.

24.15.1.1 Overcoming Barriers of Hybridisation

Due to differences in ploidy levels of prospective parents, desirable crosses are difficult to obtain in several cases; these interploid barriers mainly arise due to endosperm imbalance and the seeds can develop normally with a ratio of 2:1 (maternal and paternal) in the genomic makeup of the endosperm for two diploid parents. The seeds that do not meet this requirement are generally aborted because the above ratio is not exact (Sanford 1983). These interploid barriers of hybridisation may be overcome by manipulating the matching ploidy levels prior to hybridisation.

24.15.1.2 Developments of Sterile Cultivars

The introduction of invasive species may face a threat to some ecosystems. The development of sterile forms of several nursery crops is an ideal approach to overcome this problem to grow them for landscaping and eliminating the possibility to reproduce sexually and become invasive. Several methods are employed to develop sterile plants by producing polyploidy. In most cases, these plants function normally with the exception of sexual reproduction; autotetraploidy (doubling of the chromosomes) may develop sterility due to multiple homologous chromosomes and complications during meiosis, but in some species autotetraploidy can produce fertile seeds. These tetraploids then hybridised with diploids can create sterile triploids that results into unequal segregation of the chromosomes (aneuploids in triploid apples) where seedlings rarely survive. The development of triploids is complicated due to the presence of an interploid block which prevents the normal development of its triploid embryo. In such cases, the embryo culture technique can be employed to overcome this problem and produce sterile triploid plants. Alternatively, triploid plants can be produced by the regeneration of plants from seed endosperm. The embryo of most angiospermic seeds is diploid, but the nutritive tissue endosperm is 3*n* (one male and two female gametophytes), resulting in a triploid tissue. This tissue can be excised from developing seeds and cultured *in vitro* to regenerate embryos and plantlets as in the case of kiwifruit, loquat, acacia, citrus, rice, etc.

24.15.1.3 Restoration of Fertility in Wide Hybrids

The hybrids of distant species (genera) are usually sterile, due to the failure of chromosomes to correctly synapse during meiosis. The doubling of the chromosomes of a wide hybrid can create fertile allopolyploid.

24.15.1.4 Enhancing Pest Resistance and Stress Tolerance

The increase in the chromosome number by polyploidy of related gene dose can mostly enhance the gene expression and production of some secondary metabolites that results in enhancing defence or pest resistance mechanisms in plants. For example, autotetraploid rye grass had better disease resistance and more structural carbohydrates than diploids (van Bogaert 1975), because of change in relationship between gene dose, gene silencing and expression of secondary metabolites. This polyploid approach may be helpful to create allopolyploids between plants with diverse endogenous defence chemicals and secondary metabolites as compared to parental species. The allopolyploids often produce all the enzymes and metabolites of both parents, those effectively enhancing the pest-resistant characteristics with the more horizontal form of pest resistance. This approach may also enhance tolerance to certain environmental stresses.

24.15.1.5 Enhancement in Vigour

Polyploidy has significant enlargement of cells and most often have undesirable effects, but sometimes it can be beneficial. Fruit from tetraploid apples can be bigger by twice as the diploid fruit, but they are mostly watery and distorted, but triploids have to bear larger fruit with good quality and are grown for commercial production. This approach is also used for enlargement of ornamental flowers with thicker flower petals, and flowers are longer lasting in polyploid plants (Kehr 1996) over diploids.

24.15.1.6 Production of Double Haploids

Doubled haploid (DH) plants are potentially useful in the development of inbred lines to save time and can be achieved in a single generation. Double haploids also express deleterious recessive alleles; otherwise, they are masked by dominance effects in a genome containing more than one copy of allele on each chromosome. Various techniques have been employed to create double haploids; often, androgenesis (anthers and microspores) is used in '*in vitro*' culture to develop pollen. Several plant species and cultivars of recalcitrant seeds (including triticale) are poor to generate DH. Genotypes and culture medium interaction is generally responsible for varying

success rates (Johansson et al. 2000). Chromosome elimination technique is another method of producing DHs and involves hybridisation of wheat with maize (*Zea mays* L.), followed by auxin treatment and rescue technique which are used to develop haploid embryos before they abort.

24.15.1.7 Increased Allelic Diversity and Heterozygosity

An increase in allelic copy number with ploidy level leads to create novel phenotypes via dosage effects. The allelic diversity also increases in allopolyploidy by recombining two or more divergent genomes in a nucleus. This intergenomic heterozygosity will apply to the entire genome such as the intergenomic heterozygosity of *B. napus* (Osborn et al. 2003b) where low seed yield is associated with a loss of intergenomic heterozygosity. The diploids *G. arboretum* and *G. herbaceum* and tetraploids *G. barbadense* and *G. hirsutum* (AD genome) have been domesticated for their epidermal seed trichomes (cotton fibre), while the D genome diploids of Central and South America produce short fuzz on their seed (Applequist et al. 2001; Wendel and Cronn 2003). The tetraploid cotton produces long, fine and strong fibre than their diploid cultivars. Jiang et al. (1998) investigated several QTL that are located on the D genome and suggested that D genome loci are responsible for the synthesis of fibre subsequent to polyploid formation. In bread wheat, rye translocations have been used to introgress novel phenotypic variation, including abiotic stress resistance (Singh et al. 1998) and green bug resistance (Sebesta and Wood 1978).

24.15.1.8 Polyploidisation and Genetic Bottlenecks

The allelic diversity within a polyploid genome is greater than diploid genomes; often there exist higher levels of natural variation than diploid species because the process of polyploidisation entails a genetic bottleneck. Several polyploids originate repeatedly from identical or similar progenitors. Accordingly, much effort has been done for gene pool enrichment through trait introgression and interploidal hybridisation as in polyploid crops (cotton, wheat, canola, potato, etc.). In autopolyploids, the transfer of genetic material between diploid and tetraploid levels is somewhat simplified by a single genome, a common cytoplasm, diploid gametes and a recognition of endosperm balance (Bushell et al. 2003; Carputo et al. 2003).

24.15.1.9 Genomic Consequences of Polyploidy

The successful fusion of two divergent genomes, doubling of one genome, requires a series of genetic and genomic adjustments that governs proper centromere recognition, chromosome pairing and balanced assortment of chromosomes during meiosis division. Genome doubling may be complicated due to 'genomic shock' (McClintock 1984). These include a diverse suite of genetic and epigenetic mechanisms that influence gene expression and function as well as genomic organisation.

24.16 Conclusion

Polyploidy has been a major force of plant evolution (Winge 1917; Karpechenko 1927; Stebbins 1950, 1971) as in the case of ferns and flowering plants. Several crop plants are evidently polyploids, i.e. alfalfa, cotton, wheat, potato, etc., while some others are vestiges of ancient polyploidy (palaeopolyploids), i.e. cabbage, maize, soybean, etc. Polyploids have unique cytogenetics with their reproductive isolation to their progenitors (Blackeslee 1921; Jorgensen 1928) and differ in ecological, morphological, physiological and cytological characteristics. Most polyploids exhibit distinct phenotypic traits and hybrid vigour that played a useful role in agriculture (Levin 2002; Ramsey and Schemske 2002) which provided an important source of genetic variation (Soltis and Soltis 2000). Thus, polyploidy is the most important mechanism of adaptation and speciation in plants (Clausen et al. 1945; Stebbins 1950; Levin 2002). Polyploidy is used in breeding programmes to develop new crops by interspecific gene recombination and to create the 'origin of new crops'. Now polyploidy is

a fascinating field of study to unravel the evolution of crop plants and utilise their variability in the plant breeding.

References

Acquaah G (2007) Principles of plant genetics and breeding. Wiley-Blackwell, Malden

Adams KL, Wendel JF (2005a) Polyploidy and genome evolution in plants: genome studies and molecular genetics. Curr Opin Plant Biol 8:135–141

Adams KL, Wendel JF (2005b) Novel patterns of gene expression in polyploid plants. Trends Genet 21:539–543

Adams KL, Cronn R, Percifield R, Wendel JF (2003) Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. Proc Natl Acad Sci U S A 100:4649–4654

Aoki S, Ito M (2000) Molecular phylogeny of *Nicotiana* (solanaceae) based on the nucleotide sequence of the matK gene. Plant Biol 2:316–324

Applequist WL, Cronn R, Wendel JF (2001) Comparative development of fiber in wild and cultivated cotton. Evol Dev 3:3–17

Barluenga M, Stölting KN, Salzburger W, Muschick M, Meyer A (2006) Sympatric speciation in Nicaraguan Crater Lake cichlid fish. Nature 439:719–723

Beasley YO (1942) Meiotic chromosome behavior in species hybrids, haploids, and induced polyploids of Gossrplzlm. Genetics 27(1):25–54

Bellostas N, Sorensen JC, Sorensen H (2007) Profiling glucosinolates in vegetative and reproductive tissues of four Brassica species of the U triangle for their biofumigation potential. J Sci Food Agric 87:1586–1594

Bennett MD (2004) Perspectives on polyploidy in plants – ancient and neo. Biol J Linn Soc 82(4):411–423

Blackeslee (1921) Types of mutation and their possible significance in evolution. Am Nat 55:254–267

Bland MM, Matzinger DF, Levings CS III (1985) Comparison of the mitochondrial genome of *Nicotiana tabacum* with its progenitor species. Theor Appl Genet 69:535–541

Bolnick DI, Fitzpatrick BM (2007) Sympatric speciation: models and empirical evidence. Ann Rev Ecol Evol Syst 38:459–487

Bushell C, Spielman M, Scott RJ (2003) The basis of natural and artificial postzygotic hybridization barriers in *Arabidopsis* species. Plant Cell 15:1430–1442

Carputo D, Frusciante L, Peloquin SJ (2003) The role of 2n gametes and endosperm balance number in the origin and evolution of polyploids in the tuber-bearing Solanums. Genetics 163:287–294

Chen Z (2010) Molecular mechanisms of polyploidy and hybrid vigour. Trends Plant Sci 15:57–71

Chen L, Lou Q, Zhuang Y, Chen J, Zhang X, Wolukau JN (2007) Cytological diploidization and rapid genome changes of the newly synthesized allotetraploids *Cucumis*× hytivus. Planta 225:603–614

Clausen J, Keck DD, Hiesey WM (1945) Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autopolyploidy, with examples from the Madiinae. Carnegie Institute of Washington, Washington, DC

Comai L (2000) Genetic and epigenetic interactions in allopolyploid plants. Plant Mol Biol 43:387–399

Comai L (2005) The advantages and disadvantages of being polyploid. Nat Rev Genet 6:836–846

Feldman M, Lupton FGH, Miller TE (1995) Wheats. Triticum spp. (Gramineae- Triticinae). In: Smartt J, Simmonds NW (eds) Evolution of crop plants. Longman Scientific & Technical Press, London, pp 184–192

Fitzpatrick BM, FordyceJ A, Gavrilets S (2008) What, if anything, is sympatric speciation? J Evol Biol 21:1452–1459

Fransz P, De Jong JH, Lysak M, Castiglione MR, Schubert I (2002) Interphase chromosomes in *Arabidopsis* are organized as well defined chromocenters from which euchromatin loops emanate. Proc Natl Acad Sci 99:14584–14589

Fryxelp A (1979) The natural history of the cotton tribe. Texas A&M University Press, College Station

Grass Phylogeny Working Group (2001) Phylogeny and classification of Poaceae. Ann Mo Bot Gard 88(3):431–457

Hutchinson JB, Silow RA, Stephens SG (1947) The evolution of Gossypium and the differentiation of the cultivated cottons. Oxford University Press, London

Jiang C, Wright RJ, El-Zik KM, Paterson AH (1998) Polyploid formation created unique avenues for response to selection in *Gossypium* (cotton). Proc Natl Acad Sci U S A 95:4419–4424

Johansson N, Tuvesson C, Ljungberg A, Karlsson KE, Suijs LW, Josset JP (2000) Large-scale production of wheat and triticale double haploids through the use of a single-anther culture method. Plant Breed 119(6):455–459

Jones JR, Ranney TG, Eaker TA (2008) A novel method for inducing polyploidy in Rhodo-dendron seedlings. J Am Rhododendron Soc 62:130–135

Jorgensen (1928) The experimental formation of heteroploid plants in the genus *Solanum*. J Genet 19:133–211

Karpechenko GD (1927) Polyploid hybrids of *Raphanus sativus* X *Brassica oleracea* L. Bull Appl Bot 17:305–408

Karpechenko GD (1928/1989) Polyploid hybrids of *Raphanus sativus* L. X *Brassica oleracea* L. In: Janick J (ed) Classic papers in horticultural science. The Blackburn Press, New Jersey, pp 442–525

Kashkush K, Feldman M, Levy AA (2002) Gene loss, silencing and activation in a newly synthesized wheat allotetraploid. Genetics 160:1651–1659

Kehr AE (1996) Woody plant polyploidy. Am Nurserym 183(3):38–47

Kenton A, Parokonny AS, Gleba YY, Bennett MD (1993) Characterization of the *Nicotiana tabacum* L. genome by nuclear cytogenetics. Mol Gen Genet 240:159–169

Kimber G (1961) Basis of the diploid-like meiotic behavior of polyploid cotton. Nature 191:98–99
Kitamura S, Inoue M, Shikazono N, Tanaka A (2001) Relationships among *Nicotiana* species revealed by the 5S rDNA spacer sequence and fluorescence in situ hybridization. Theor Appl Genet 103:678–686
Knapp S, Chase MW, Clarkson JJ (2004) Nomenclatural changes and a new section classification in *Nicotiana* (Solanaceae). Taxon 53:73–82
Levin DA (1983) Polyploidy and novelty in flowering plants. Am Nat 122(1):1–24
Levin DA (2002) The role of chromosomal change in plant evolution. Oxford University Press, New York
Liu B, Brubaker CL, Mergeai G, Cronn RC, Wendel JF (2001) Polyploid formation in cotton is not accompanied by rapid genomic changes. Genome 44: 321–330
McClintock B (1984) The significance of responses of the genome to challenge. Science 226:792–801
McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J Hered 37:81–89, 107–116
Melaragno JE, Mehrotra B, Coleman AW (1993) Relationship between endopolyploidy and cell size in epidermal tissue of *Arabidopsis*. Plant Cell 5:1661–1668
Mittelstein Scheid O, Jakovleva L, Afsar K, Maluszynska J, Paszkowski J (1996) A change in ploidy can modify epigenetic silencing. Proc Natl Acad Sci U S A 93:7114–7119
Nagaharu U (1935) Genome analysis in Brassica with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn J Bot 7:389–452
Nelson MN, Mason AS, Castello MC, Thomson L, Yan G, Cowling WA (2009) Microspore culture preferentially selects unreduced (2n) gametes from an interspecific hybrid of *Brassica napus* L.×*Brassica carinata* Braun. TAG Theor Appl Genet 119:497–505
Olmstead RG, Palmer JD (1991) Chloroplast DNA and systematics of the Solanaceae. In: Hawkes JG, Lester RN, Nee M, Estrada N (eds) Solanaceae III: taxonomy, chemistry, evolution. Royal Botanic Gardens, Kew, pp 161–168
Osborn TC, Butrulle DV, Sharpe AG, Pickering KJ, Parkin IAP, Parke JS, Lydiate DJ (2003a) Detection and effects of a homoeologous reciprocal transposition in *Brassica napus*. Genetics 165:1569–1577
Osborn TC, Pires JC, Birchler JA, Auger DL, Chen ZJ, Lee HS, Comai L, Madlung A, Doerge RW, Colot V, Martienssen RA (2003b) Understanding mechanisms of novel gene expression in polyploids. Trends Genet 19(3):141–147
Ozkan H, Levy AA, Feldman M (2001) Allopolyploidy induced rapid genome evolution in the wheat (*Aegilops-Triticum*) group. Plant Cell 13:1735–1747
Rajathy T, Thomas H (1974) Cytogenetics of oats, 2nd edn. The Genetics Society of Canada, Ottawa, p 90
Ramsey J, Schemske DW (1998) Pathways, mechanisms and rates of polyploidy formation in flowering plants. Ann Rev Ecol Syst 29:467–501
Ramsey J, Schemske DW (2002) Neopolyploidy in flowering plants. Ann Rev Ecol Syst 33:589–639
Randolph LF (1941) An evaluation of induced polyploidy as a method of breeding crop plants. Am Nat 75:347–363
Ren N, Timko MP (2001) AFLP analysis of genetic polymorphism and evolutionary relationships among cultivated and wild *Nicotiana* species. Genome 44:559–571
Riechers DE, Timko MP (1999) Structure and expression of the gene family encoding putrescine N-methyltransferase in *Nicotiana tabacum*: new clues to the evolutionary origin of cultivated tobacco. Plant Mol Biol 41:387–401
Roose ML, Gottlieb LD (1976) Genetic and biochemical consequences of polyploidy in *Tragopogon*. Evolution 30:818–830
Rowley GD (1993) Rosaceae: the rose family. In: Heywood VH (ed) Flowering plants of the world. Batsford Pub, London, pp 141–144
Sanford JC (1983) Ploidy manipulations. In: Moore JN, Janick J (eds) Methods in fruit breeding. Purdue University Press, West Lafayette, pp 100–123
Sebesta EE, Wood EA (1978) Transfer of green bug resistance from rye to wheat with x-rays. In: Agronomy abstracts, pp 61–62
Shaked H, Kashkush K, Ozkan H, Feldman M, Levy AA (2001) Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. Plant Cell 13:1749–1759
Singh RP, Huerta-Espino J, Rajaram S, Crossa J (1998) Agronomic effects from chromosome translocations 7DL.7AL and 1BL.1RS in spring wheat. Crop Sci 38:27–33
Soltis DE, Soltis PS (1993) Molecular data and the dynamic nature of polyploidy. Crit Rev Plant Sci 12:243–273
Soltis DE, Soltis PS (1995) The dynamic nature of polyploid genomes. Proc Natl Acad Sci 92(18):8089–8091
Soltis DE, Soltis PS (1999) Polyploidy: recurrent formation and genome evolution. Tree 14(9):348–352
Soltis PS, Soltis DE (2000) The role of genetic and genomic attributes in the success of polyploids. Proc Natl Acad Sci U S A 97(13):7051–7057
Song K, Lu P, Tang K, Osborn TC (1995) Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. Proc Natl Acad Sci 2(17):7719–7723
Stebbins GL (1950) Variation and evolution in plants. Columbia University Press, Columbia
Stebbins GL (1971) Chromosomal evolution of higher plants. Edward Arnold Ltd, London
Van Bogaert G (1975) A comparison between colchicine induced tetraploid and diploid cultivars of Lolium species. In: Neusch B (ed) Ploidy in fodder crops. Eucarpia Report, Zurich
Wehner TC (2008) Watermelon (pp 381–418). In: Prohens J, Nuez F (eds) Handbook of plant breeding; vegetables I: asteraceae, brassicaceae, chenopodiaceae, and cucurbitaceae. Springer Science+Business LLC, New York, 426 p. 17

Wendeil F (1989) New world cottons contain old world cytoplasm. Proc Natl Acad Sci U S A 86: 4132–4136

Wendel JF, Cronn RC (2003) Polyploidy and the evolutionary history of cotton. Adv Agron 78: 139–186

Wilson AS (1875) On wheat and rice hybrids. Trans Proc Bot Soc 12:286–288

Winge O (1917) The chromosomes, their number and general importance. CR Trav Lab Carlsberg 13:131–275

Yukawa M, Tsudzuki T, Sugiura M (2006) The chloroplast genome of *Nicotiana sylvestris* and *Nicotiana tomentosiformis*: complete sequencing confirms that the *Nicotiana sylvestris* progenitor is the maternal genome donor of *Nicotiana tabacum*. Mol Genet Genom 275:367–373

Male Sterility Systems in Major Field Crops and Their Potential Role in Crop Improvement

25

K.B. Saxena and Anupama J. Hingane

Abstract

Male sterility is a phenomenon where the male reproductive parts of the plants do not develop normally and fail to participate in sexual reproduction. The male sterility is of different kinds and can arise through a number of biological abnormalities. Among these, cytoplasmic nuclear male sterility has been extensively used by plant breeders to achieve breakthrough in the productivity of various field and horticultural crops through the development of hybrid cultivars. The impact of this technology is visible in crops like maize, rice, sorghum, pearl millet, etc., and this has helped in encountering the challenges of global food security. Among high-protein legumes, the world's first hybrid was released recently with record 3–4 t/ha of grain yield. This chapter briefly discusses the types of male sterility systems available in different crop species and their potential uses. Besides this, various methods of creating different male sterility systems are also described.

Keywords

Male sterility • Fertility restoration • Microsporogenesis • Genetic male sterility • Cytoplasmic nuclear male sterility • Mitochondria

25.1 Introduction

Male sterility is a unique gift of nature to mankind. The contribution of this system in combating global hunger through its use in developing high-yielding hybrids in various food crops has been immense. Male sterility is a situation where the male reproductive parts of a plant are either absent, aborted, or nonfunctional, and hence they fail to participate in the process of

K.B. Saxena (✉)
Pigeonpea Breeding, Grain Legumes, ICRISAT, 2, ICRISAT Colony (Phase I), Bowenpally, Secunderabad 502324, Telangana, India
e-mail: kbsaxena1949@gmail.com

A.J. Hingane
Pigeonpea Breeding, Grain Legumes, ICRISAT, Building No-305, Room No 124, ICRISAT Campus, Patancheru 502324, India

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_25,

natural sexual reproduction. This situation can arise due to any developmental defect at any stage of microsporogenesis or release of pollen grains. Kolreuter (1763) was the first to record the existence of plants in nature with impaired anthers in some natural populations. Since then numerous reports of such abnormal events have been published in a number of crop species. Darwin (1877) recognized the importance of this phenomenon and hypothesized that the loss of reproducing ability of plant helps evolutionary processes in enhancing adaptation through gene transfer from various related and unrelated individuals through cross-pollination.

With respect to utilization of male sterility in crop breeding, it is essential that the individuals with altered male fertility keep their female fertility intact. The fertilization of such plants with the pollen grains from other plants that may be transferred with the help of external agencies such as wind, insects, or human beings produces viable seeds. Historically, the male-sterile mutants had appeared naturally in the populations of cultivars and germplasm, but at that time their economic value was not recognized, and hence these were lost over a period of few generations. However, with the evolution of the concept of heterosis by Shull (1908) and subsequently by others, the potential benefits of male sterility in enhancing productivity of crops were realized. In this context it should be mentioned that Stephens (1937) for the first time utilized male sterility in hybrid seed production in sorghum. At the same time Jones and Emsweller (1937) also demonstrated its use in hybrid seed production of onion. The male-sterile trait may arise in nature through mutations, or it can be bred through induced mutagenesis or hybridization and selection. Considering the economic importance of this unique natural phenomenon, vast scientific information has been generated on its genetics, physiology, and genomics. This information has been very elegantly compiled by Kaul (1988) in his monograph *Male Sterility in Higher Plants*. In the present chapter, the author has not made any hard attempt to compile available literature on various aspects of male sterility, and rather issues of general importance have been briefly explained with focus on its origin and utilization in 12 major field crops involving cereals, legumes, and oilseeds.

25.2 Fundamentals of Different Male Sterility Systems

As mentioned earlier the male sterility is an abnormality that is observed occasionally in higher plants, where the male reproductive system of an individual fails to participate in producing its progenies. Such plant defects can arise due to a number of reasons such as inability of anther tissues to grow and differentiate normally, failure of normal microsporogenesis, failure to release the mature pollen grains, and/or inability of mature pollen grains to germinate on the stigmatic surface. These abnormalities, however, do not impair the female reproductive system, and if such plants are pollinated through manual or natural means, they produce fertile seeds. Since male sterility is a manifestation of abnormal growth and development, the action of genes controlling male sterility may also be variable and inconsistent across the crops and genotypes. Based on the type of malfunctioning of the androecium, the male sterility systems have been classified as structural (absence or deformity of anthers), sporogenous (defective microsporogenesis), and functional (failure of mature pollen to germinate). In addition, on the basis of genetic control mechanisms, it has also been classified as genetic, cytoplasmic, and cytoplasmic nuclear (or genetic) male sterility.

25.2.1 Genetic Male Sterility

This is the most common form of male sterility found in a number of plant species in both monocots as well as dicots (Kaul 1988). In this system the male sterility is controlled by nuclear genetic factors, and it is independent of cytoplasmic influences. In most cases its expression is controlled by one or two pairs of recessive alleles, which segregate independently. However, a few exceptions are also found where the male sterility is

reported to be controlled by one or two dominant genes. Also in certain cases, the male sterility is linked to some easily identifiable morphological traits such as pigmentation of the stem, translucent anthers, sparse podding and delayed flowering etc. (Kaul 1988; Verulkar and Singh 1997).

The mutant male-sterile plants may arise spontaneously carrying homozygous alleles (*msms*), and these will be lost if not maintained as heterozygotes (*Msms*). For this to happen, the male-sterile mutants need to be pollinated with fertile homozygous (*MsMs*) or heterozygote (*Msms*) counterparts. In cases where male sterility is controlled by dominant alleles, its maintenance through reproductive means is very difficult.

25.2.2 Cytoplasmic Male Sterility

This type of male sterility is governed by cytoplasm which contains defective mitochondrial DNA. This happens due to deleterious interactions of mitochondrial genes with those present in the nucleus. This type of cytoplasm is designated as "sterile" (S), and it can originate spontaneously or through wide hybridization. Such plants do not produce fertile pollen grains because its nucleus also contains a pair of recessive non-restoring (*msms*) alleles. The cytoplasmic male sterility is maintained by the genotypes which carry "fertile" (F) or "normal" (N) cytoplasm (Fig. 25.1) and non-restoring recessive nuclear alleles. According to Kaul (1988), about 150 plant species have been reported to carry this type of male sterility.

In the cytoplasmic male sterility system, the diversification of hybrid parents may be difficult due to incorporation of recessive non-restorer nuclear alleles. At genotypic level the male hybrid parent should resemble its maintainer but with diverse nuclear genome that is capable of producing heterotic hybrid progenies. This type of male sterility cannot be used for field crops due to absence of fertility-restoring genes and difficulties in producing large quantities of hybrid seed. Alternatively, this system has been used in horticultural crops where fruits are consumed or the seeds are noncommercial entity.

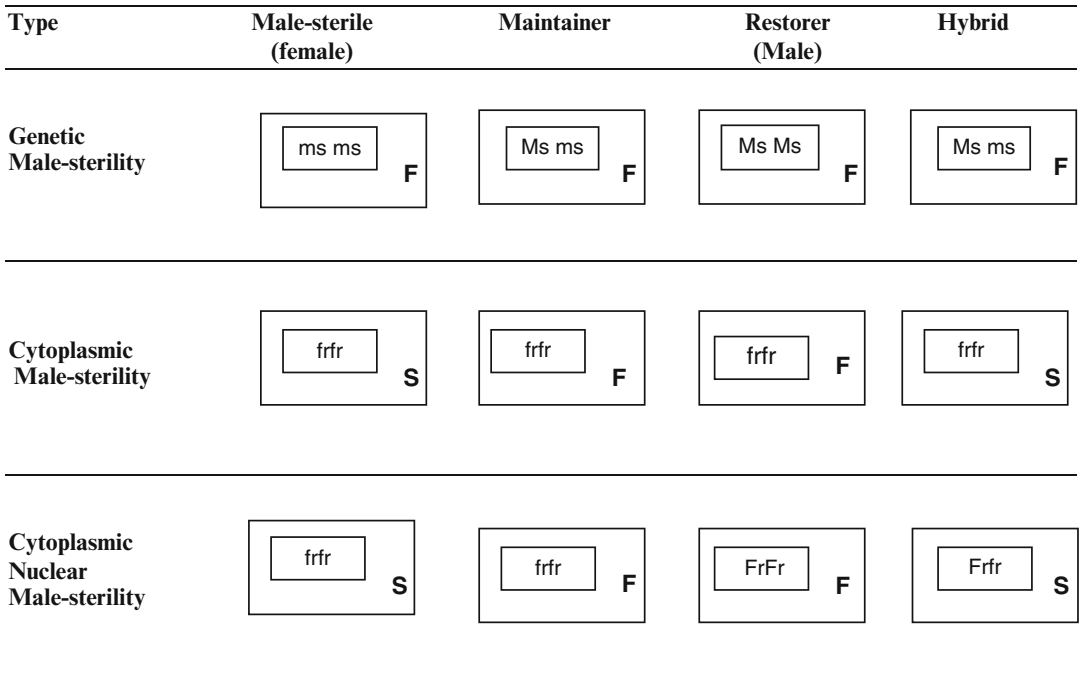


Fig. 25.1 Generalized hereditary constitution of the nucleus and cytoplasm of the three male sterility systems

25.2.3 Cytoplasmic Nuclear Male Sterility

Like that of cytoplasmic male sterility, in cytoplasmic nuclear male sterility also, the manifestation of male sterility is a consequence of interaction between cytoplasmic and nuclear genomes. The difference between the two types is in their fertility restoration mechanisms. In the former male fertility is controlled by its "N" cytoplasm of the maintainer lines, while in the latter type dominant fertility-restoring genes are located in the nucleus of restorer line (Fig. 25.1). Hence, it is termed as cytoplasmic nuclear/genetic male sterility. Further depending on the type of fertility-restoring gene, the expression of male fertility/sterility could be total or partial. Sometimes such expressions are also affected by prevailing environmental conditions such as photoperiod, temperature, or both. This form of male sterility has been used most extensively in hybrid breeding programs in a number of field crops. The complete hybrid system involves three distinct genotypes:

- "A" line is the male-sterile female line with "S" cytoplasm and recessive fertility nuclear alleles (*frfr*).
- "B" line is a maintainer of the female line, and it has fertile "F" cytoplasm and recessive nuclear alleles (*frfr*). When this line is crossed with "A" line, the entire progeny is male sterile.
- Third parent is designated as "R" line and it contains dominant fertility-restoring gene (*FrFr*). This line has the ability to restore the male fertility of the hybrid plants produced by crossing with "A" line.

Molecular studies on this male sterility system revealed that the expression of male sterility is associated with mitochondrial genome rearrangements that results in the production of toxic proteins and reduction in respiration. In fact various theories have been proposed, but still the molecular basis of this male sterility is not well understood in most crops. Recent studies have shown that the male sterility is associated with chimeric mitochondrial ORFs (open reading frames). Wang et al. (2006) demonstrated that in rice the ORF encodes a cytotoxin peptide which determines the expression of male sterility. Iwabuchi et al. (1993) showed that an abnormal copy of a mitochondrial gene produced aberrant mRNA transcripts containing an additional ORF. Hanson and Bentolila (2004) reported that male sterility may also be associated with alterations in promoter regions and portions of coding regions of mitochondrial ATP synthase. This may impair the activity of ATP synthase. The genomic studies on mitochondrial genome of pigeon pea A_4 cytoplasm recognized 13 ORFs which can trigger male sterility (Tuteja et al. 2013). Further, Sinha et al. (unpublished) reported involvement of 10 bp deletion in *nad7a* gene which was responsible for producing male-sterile plants in pigeon pea.

25.2.4 Environment-Sensitive Male Sterility

This is a unique male sterility system where the expression of male sterility and fertility of the plants is controlled by environmental factors. Under this system the male sterility gene expresses only under specific environment such as low or high temperature, short or long photoperiod, variable light intensity, different soilborne stresses, or their specific combinations (Kaul 1988). This situation can arise both in genetic as well as cytoplasmic nuclear male sterility systems. According to Levings et al. (1980), the reversion of sterility is influenced by cytoplasmic rather than nuclear genetic factors, and loss of such factors is correlated with reversion of male sterility to male fertility. Escote et al. (1985) and Small et al. (1988) showed that no DNA loss was associated with the reversion of male sterility. The conversion of male sterility to fertility and its reversal is a complex genetic phenomenon, and more research is required at genomics and physiological levels to understand it better.

The environment-sensitive male-sterile line was first reported by Shi (1981) in rice, and later Yuan (1987) proposed its use in hybrid breeding program. Since it eliminates the requirement of maintainer "B" line, this hybrid system is

popularly called as "two-parent hybrid" breeding. At present this male sterility system is being used in China commercially, and in 1994 the hybrids based on this system of male sterility covered over 30,000 ha areas with yields as high as 8–9 t/ha (Lu et al. 1994).

25.2.5 Fertility Restoration of Male-Sterile Germplasm

Perfect male fertility restoration of cytoplasmic nuclear male sterility-based hybrids is an integral part of any hybrid breeding technology. Once an "R" line is crossed with "A" line, the dominant *Fr* nuclear gene of "R" line overcomes the ill effects of defective mitochondrial genome. The *Fr* gene produces certain proteins which repair the damage and make the plant male fertile. In most crops one or two dominant fertility restorer genes have been reported to control the pollen fertility (Kaul 1988). Saxena et al. (2011) reported that in pigeon pea two dominant genes were responsible for fertility restoration of A_4 cytoplasm. However, the hybrids with a single dominant gene were also fertile, but they produced less quantity of pollen and also showed a lot of instability with respect to fertility restoration in diverse environments. Similarly in maize also, four fertility-restoring genes were reported, and of these, two were major genes while the remaining two only resulted in partial restoration of male sterility (Wise et al. 1999). Further, it was also reported that one of the major fertility-restoring genes reduced sterility-causing protein by 80 % (Kennel et al. 1987). The fertility restoration has also been associated with genes encoding pentatricopeptide repeat proteins (Hanson and Bentolila 2004).

25.3 Origin of Male Sterility Systems

25.3.1 Selection from Natural Variation

Nature has provided unlimited variability and in the past it has yielded a number of economic traits in different crops. There are numerous examples of it, and among these, various male sterility systems are unique, and these have benefitted millions through the cultivation of high-yielding hybrids. Over the period different types of male sterility systems have emerged, and in the future also we can expect some unexpected genetic variation which can be tapped by plant breeders.

25.3.1.1 Genetic Male Sterility

It arises due to mutation of the male fertility nuclear gene (*MsMs*) to its recessive form to produce heterozygote individuals. Self-pollination of such plants reveals male-sterile segregants. Under natural conditions in the self-pollinated crops, the male-sterile mutants are generally lost, but in cross-pollinated or partially cross-pollinated crops, such mutants are preserved by natural hybridization. According to Kaul (1988), genetic male sterility arising due to spontaneous variation has been reported in over 175 plant species.

25.3.1.2 Cytoplasmic Male Sterility

The frequency of this form of male sterility in nature is relatively less, because it requires natural mutation in mitochondrial genome to make its cytoplasm male sterile.

25.3.1.3 Cytoplasmic Nuclear Male Sterility

The natural occurrence of this form of male sterility system is also low since it requires simultaneously double occurrence of mutation; one in the mitochondria and the other in the nucleus. According to Kaul (1988), so far only 46 plant species are credited to have produced this form of male sterility under natural conditions.

25.3.2 Integration of Cultivated Genome into Alien Cytoplasm

This technology has been successfully used to develop cytoplasmic nuclear male sterility systems in cereals, oilseeds, legumes, and various other groups of crops. It is based on the concept

of bringing cytoplasmic and nuclear genomes of diverse origins within a single genotype. This is achieved by crossing a wild relative of a crop as female parent with a cultivated line as male parent. This combination integrates the cultivated nucleus into the cytoplasm of wild species and brings together the two diverse entities in a new genotype.

25.3.2.1 Intergeneric Hybridization

In this approach the wild relatives from different genera are crossed as female parent with cultivated types. Sometimes due to large diversity between the two parents, the crosses may not be successful, and it may require rescuing the young hybrid embryo. The resultant hybrid plants may be both male and female sterile due to severe abnormalities occurring during meiosis. Also, there may be problems with plant type itself with abnormal growth of vegetative and reproductive parts. It has also been observed at ICRISAT that in pigeon pea such hybrid plants failed to produce seeds. In many cases the male-sterile plants found through this approach failed to survive in the absence of any maintainer.

25.3.2.2 Interspecific Hybridization

This is the most common and successful approach used in breeding cytoplasmic nuclear male-sterile genotypes in various species of cereals, legumes, and other field crops. It also involves hybridizing the wild relatives as female parent. In this group of materials, the success from crossing is generally high because of relatively closer relationship between the two species. The process used in developing the male sterility in pigeon pea has been outlined in Fig. 25.2. Initially, due to strong linkage drag, there will be a lot of unwanted traits in the hybrid and in segregating generations, and breeders must be careful in selecting individual genotypes for backcrossing. The selection of an appropriate maintainer is also important, and it should be done with care while maintaining its genetic purity. In some cases the fertility restorers can be identified from cultivated germplasm, but in case it is not available, then the breeders need to select fertile segregants originating from the same cross.

25.3.3 Selection from Recombinant Populations

Large breeding populations derived from various intervarietal crosses sometimes reveal new genetic variation, and it may arise due to rare recombination of recessive alleles or transgressive segregation. In crops like pearl millet, soybean, and cotton, cytoplasmic nuclear male sterility has been derived from recombinant populations in the past (Kaul 1988). The frequency of such useful recombination is, however, very low.

25.3.4 Induced Mutations

Various known chemical and physical mutagenic agents have been used by breeders in different crops to create new variability in important economic traits. Scientists have also succeeded in developing male sterility systems in a number of crops. According to Kaul (1988), over 35 plant species have been tried successfully to breed male sterility systems. Among the mutagenic agents tried, gamma rays were the most effective. This, however, cannot be generalized due to differential crop x mutagen x application rate interactions. In some crops the success rate of EB has also been found to be very high. In most cases these mutagens have yielded genetic male sterility systems. In soybean and pearl millet, cytoplasmic nuclear male-sterile lines have also been developed through mutagenesis (Burton and Hanna 1976; Kaul 1988).

25.3.5 Chemical Hybridizing Agents

Some of the chemicals are known to have gametocide properties; these as a group are called as "chemical hybridizing agents" (CHA). Moore (1950) and Naylor and Davis (1950) were the first to induce male sterility by spraying a chemical called MH (maleic hydrazide) in maize. Soon other chemicals (alpha naphthalene acetic acid and beta indole acetic acid) were reported to have induced female flowers in cucumber

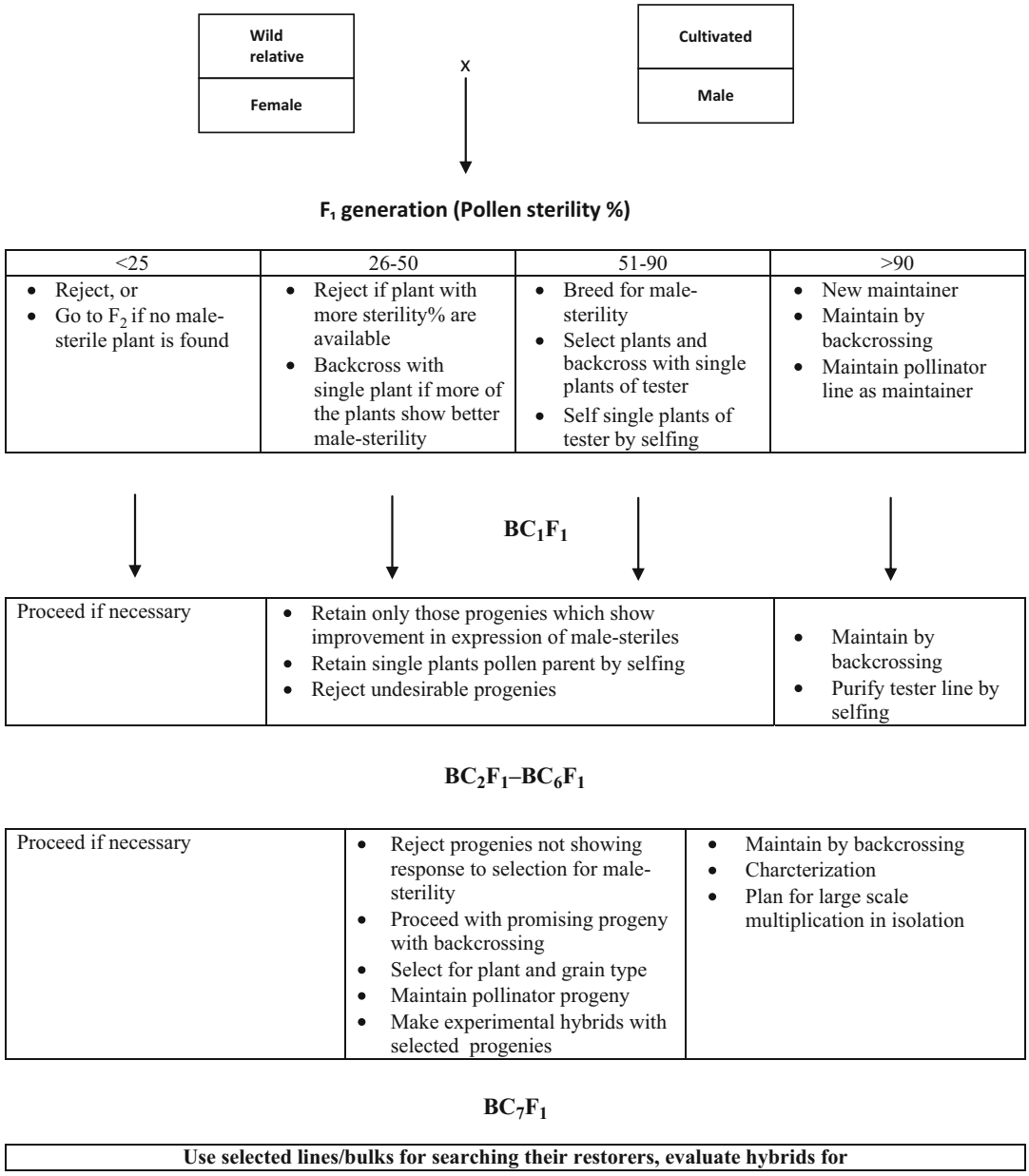


Fig. 25.2 Flow diagram followed in breeding alloplasmic CGMS line in pigeon pea

(Laibach and Kriben 1951). Besides this, some chemicals were also found accidently which altered the reproductive parts in crops such as rice. Subsequently, a large number of chemicals were screened for their potential use in the development of hybrids in different crops. According to Colhoun and Steer (1982), the ideal chemical hybridizing agents must be very specific and should not affect other parts of the plants and at the same time should not be transmitted to the progeny in any form. Besides this, these should be environment friendly and economical in use. Good CHAs should be specific in action with respect to crop, time of application, and doses.

In this system of hybrid production, the fertile (normal) crop is sprayed with CHA as per

the recommendations, and it results in the production of male-sterile female-fertile flowers in large proportions. Such induced male-sterile flowers can be pollinated with selected male parents to produce heterotic hybrids. The major advantage of this system is that there is no need of any maintainer line and it also allows the production and testing of a large number of experimental hybrids without many resources. The heterotic hybrid combinations can be selected in less time for promotion. It is not advisable to use this system of hybrid breeding in the field crops which produce more nonsynchronous tillers for relatively more time or have a perennial growth habit. In such cases the effect of chemical in producing male-sterile flowers will not be uniform, and there is a danger of late-emerging tillers or branches producing fertile or partial fertile flowers. This will adversely affect hybrid seed quality and the expected hybrid vigor may not be realized. Tu and Banga (1998) reviewed this subject and reported that chemicals like "dalapon," a known herbicide, can cause male sterility in cotton, pearl millet, wheat, linseed, sesame, capsicum, and some other crops. "Ethrel" is effective in barley, mustard, oat, pearl millet, rice, and wheat. Similarly, "gibberellic acid" has been found effective in inducing male sterility in crops like rice, maize, barley, oats, sunflower, and onion. "Maleic hydrazide" is another CHA which has been found effective on capsicum, cotton, oats, sorghum, and onion.

25.3.6 Genetically Engineered Male-Sterile Plants

Recent advances in DNA recombination technology have made it possible to synthesize male-sterile lines and their restorers. Mariani et al. (1990) were the first to develop such a genotype. This was achieved by transferring tobacco and rapeseed plants with a chimeric dominant gene from *Bacillus amyloliquefaciens*. This gene disrupts the normal process of pollen formation and causes male sterility. Besides this, some other technologies such as induction of modified glucanase gene (Worrall et al. 1992) and hormone engineering (Schmulling et al. 1988) have been explored in the past. Considering the scope of this paper, the details of these technologies are not discussed here. The cytoplasmic nuclear male sterility can also be produced through asexual recombination. The asexual method of somatic hybridization and transformation offers a positive alternative. Their use, however, has not found favor with plant breeders in any commercial hybrid crop.

25.3.7 Summary of Recorded Male Sterility Systems in Different Crops

In an attempt to enhance productivity, plant breeders have tried to exploit the well-known phenomenon of hybrid vigor in various field crops. In order to achieve this goal, it was essential that the ways for an effective seed production were developed, and hence, attempts were made to breed stable male sterility systems using different approaches. As a first step screening of germplasm was undertaken and natural mutants were selected. In addition, attempts were also made to breed for a reliable male sterility system using different mutagens, wide crosses, and chemical hybridizing agents. The review of literature on the 12 crops (Table 25.1) showed spontaneous occurrence of genetic male sterility in all the crops. A similar observation was also recorded in identifying environment-sensitive genetic or cytoplasmic nuclear male sterility. The use of different chemical or physical mutagens yielded genetic male sterility. The exceptions were sorghum and pearl millet where cytoplasmic nuclear male sterility systems were developed through mutagenesis. In some cereals, certain intervarietal crosses also yielded the male-sterile lines, while in rice and wheat, chemical hybridizing agents were successfully used (Table 25.1) to create temporary nonheritable male sterility for the purpose of hybrid seed production.

Table 25.1 Brief summary about the origin of different male sterility systems in some important field crops

Crop	Spontaneous	Spontaneous	Spontaneous	Mutagen	Mutagen	Intervariety	Interspecific	Intergeneric	CHA
	GMS	CMS	ESMS	GMS	CMS	CMS	CMS	CMS	
Rice	*	*	*	*		*		*	*
Maize	*			*		*	*	*	*
Wheat	*		*	*		*	*	*	*
Sorghum	*	*	*	*	*	*	*		*
Pearl millet	*	*	*	*	*				*
Cotton	*	*	*				*	*	*
Mustard	*	*	*	*			*	*	*
Sunflower	*	*	*			*	*	*	*
Pigeon pea	*		*	*			*		
Fava bean	*	*	*	*					
Soybean	*		*	*					
Safflower	*								

Source: Kaul (1988), Tu and Banga (1998), and various other papers
*Denotes presence of particular male sterility system in the crop

25.3.8 Microsporogenesis and Male Sterility

The development of seedling into an adult plant is characterized by a number of morphological changes. The plants are genetically programmed to enter into reproductive phase to pass on its genetic information to the progeny. This in many cases is controlled by photoperiod and/or temperature. The differentiation of meristem cells to pollen mother cells and finally pollen production is the result of many complicated biochemical events that are controlled by a network of genes. Any abnormality in a gene or two disturbs the natural process of microsporogenesis and that leads to male sterility. Such events could be spontaneous or induced. Since male sterility is of economic importance, a lot of research is being undertaken in a number of laboratories to unlock the mystery of this complex phenomenon.

The process of microsporogenesis can be impaired at premeiotic, meiotic, or postmeiotic stages to produce structural, sporogenous, or functional male sterility. It is a well-known fact that the most internal layer of meiotic cell wall, called tapetum, plays an important role in the development of pollen grains. The tapetum cells are hyperactive and constantly feed the meiotic cells with vital nutrients. Once the normal functioning of tapetum is disrupted, the food supply chain breaks and it triggers abnormalities. The anther and pollen morphology of such plants are generally determined by the time of breakdown of microsporogenesis.

Kaul (1988) while reviewing the subject concluded that in genetic male sterility the male-sterile gene acts at either early or late stage of meiosis, and only a few cases of premeiotic abortion of microsporogenesis are reported. In a large number of cases, the male-sterile gene has been reported to act toward the end of meiosis when tetrads are ready to be released. In certain cases the pollen mother cells develop normally, but they start degenerating even before meiosis starts; this may happen due to protein starvation of pollen mother cells. According to Vasal (1967), any abnormality in nutrient supply generally leads to aberrant outputs such as fusion of cells or degeneration of tapetum, and ultimately leading to abnormal development of pollen mother cells. Worrall et al. (1992) found that in case of male sterility, callose is synthesized due to the presence of high concentrations of cellular calcium. Katti et al. (1994) reported that a gradual reduction in concentration of sugars and proteins in the tetrads was responsible for disorientation of cytoplasm leading to malnutrition and poor tetrad growth. In contrast to many reports, Frankel and Galum (1997) suggested that early impairment of microsporogenesis was also associated with sterility of female gametes. Saxena et al. (1983) reported that in the male-sterile plants degeneration of pollen mother cells occurred at young tetrad stage, and it was accompanied with vacuolation and subsequent rupturing of nuclear membrane. In an interesting experiment, Saxena and Kumar (2001) demonstrated that if two male sterility genes *ms1* and *ms2* were incorporated into a single genotype, then the *ms2* gene, which acts first during early prophase, dominated the proceedings of microsporogenesis, and all the plants had phenotype of *ms2*, and none of the individual showed any sign of the presence of *ms1* gene, which acts at late tetrad stage. In the segregating generation (F_2), both the genotypes were present. These observations showed that the action sites of the two male sterility genes were different and independent of each other. Kuranouchi et al. (2000) demonstrated that male reproductive machinery is more sensitive than the female counterparts, and it results in high frequency of male sterility cases in nature as compared to female sterility.

In the last two decades, a lot of investigations have been carried out in the areas of physiology, genomics, and embryology to understand the phenomenon of male sterility, but the basic question, such as how the mitochondrial genomic abnormalities control the events of microsporogenesis in the anthers, still remains unanswered. However, some of the important observations recorded in the recent publications are:

- Some anther-specific substances might interact with *urf 13* proteins to cause sterile phenotypes. These proteins have also been

found to be detrimental to cell viability (Flavell 1974).
- The *urf 13* gene may be overexpressed in tapetal cells (Levings 1993).
- The tapetal cells exhibited characteristic features of prolonged cell death (Balk and Leaver 2001).
- Cytotoxic gene products were associated with male sterility (Nakai et al. 1995).
- Mitochondrial open reading frames played an important role in the expression of male sterility (Wang et al. 2006).
- The proposed "genomic conflict theory" explains the results of interaction of cytoplasmic determinant that prevent pollen production and nuclear restorers that restore fertility (Cosmides and Tooby 1981).
- The *urf 13* gene caused male sterility by programmed cell death (Wu and Cheung 2000).
- Polyhydroxybutyrate causes abnormal development of the epidermis and endothecium with a broken tapetal layer (Poirier et al. 1992).
- PhaA (a-ketothiolase) gene caused 100 % male sterility, and light illumination reverted the male-sterile lines to male fertility (Ruiz and Daniell 2005).

25.4 Utilization of Male Sterility in Crop Breeding

25.4.1 Population Breeding

Most breeding programs suffer from limited genetic variability, and it may arise due to nonavailability of diverse germplasm or lack of recombination to allow breeders to select new genotypes. Since recombination breeding is primarily based on human resources, both in partially or completely cross-pollinated species, breeders have attempted to accumulate favorable alleles from diverse sources in one population. Besides serving as gene pool for deriving useful variability from time to time, these heterogeneous populations can also be released for cultivation, especially for stress environments. The exercise of breeding populations is cumbersome as it requires large-scale hybridization and selection of genotypes with required genetic constitution. To facilitate random mating and enhance gene frequency of favorable alleles, several breeding populations using genetic male sterility were developed in the past. This also helped in maintaining genetic diversity within and across the populations. This, when achieved, would yield populations with high yield, wide adaptation, and greater stability against various biotic and abiotic stresses (Doggett 1972; Eberhardt 1972). Utilization of genetic male sterility in reciprocal recurrent selection allows breeders to exploit additive, additive x additive, and epistatic genetic variations for crop improvement (Comstock and Robinson 1952). Lukhele and Obilana (1980) and Obilana and El-Rouby (1980) reported about 40 % gain in productivity after three cycles of recurrent mass selection. The genetic male sterility-based populations can also be bred to create vast gene pools for specific traits to encounter different production constraints for specific ecosystem with locally preferred market traits. Besides releasing elite populations for cultivation, some breeders have successfully derived high-yielding pure line cultivars from such genetic pools (Murthy and Rao 1997). In population breeding schemes, breeders should always ensure that the male sterility gene is not lost while advancing the generations. This will restrict recombination among genotypes in the population.

25.4.2 Hybrid Breeding

Enhancement of productivity has been an important goal for most breeding programs. Although the hybrid breeding was known as a potential way of yield increases, but the constraint of large-scale hybrid seed production prevented the commercialization of this technology in many crops. In maize the hybrid technology grew with the aid of detasseling (removing the male part from female rows) and wind-supported cross-pollination. In dioecious plants where male and female reproductive parts are in the same flower, the exploitation of hybrid vigor remained a challenge.

Stephens (1937) was the first to demonstrate the use of genetic male sterility in producing quality hybrid seed in sorghum. In the genetic male sterility system, where the male-sterile genotype is multiplied only by crossing heterozygotes with male-sterile plants, and in the resultant population, the male-fertile and male-sterile plants will be equal proportion. This means that it will be a difficult task to produce quality seed of female line and its hybrids. The development of cytoplasmic nuclear male sterility system has changed the seed production scenario, and its application can be seen in a large number of crops (Singhal 2013). Thus the problems faced by seed producers in using genetic male sterility were overcome, and hybrid seed production became easy. With the advent of this technology, the hybrid seed tonnage of all kinds of crops has increased by many folds, and now commercial hybrid breeding has become a well-established industry across the world.

25.5 Diversification of Male-Sterile Lines

25.5.1 Genetic Male Sterility

The genetic diversity of genetic male-sterile lines is accomplished by following a standard backcrossing procedure, and since the male sterility is controlled by recessive alleles, each cycle of backcross should be followed by selfing. This will expose the male-sterile segregants, and these should be used for pollinations for the next cycle of backcross. Theoretically, six backcrosses are recommended to transfer the male-sterile trait in the desired genetic background, and it will consume about 12 crop seasons.

25.5.2 Nuclear Diversity of Cytoplasmic Nuclear Male-Sterile Lines

Genetic diversity with respect to various traits of economic importance is the key in any breeding program. It has generally been observed that most hybrid breeding programs are based on one or two cytoplasm, and to develop hybrids for different production niches, it is essential that sufficient nuclear diversity is available among the male-sterile lines. This can be achieved by selecting a fair number of unrelated testers, selected on the basis of their phenotypic, genotypic, and geographic diversity. These testers should be crossed with selected "A" lines with good agronomy traits and high general combining ability in a line x tester design. In F_1 generation all the hybrid plants of each cross should be tested for the expression of male fertility and sterility. The fertile combinations should be transferred to hybrid breeding program for the assessment of their productivity and other traits, while the hybrids showing male sterility should be examined very carefully. The testers of such hybrid combinations should be maintained, keeping their genetic purity intact. After reconfirming their pollen sterility, the hybrids and their testers should be selected for backcrossing (Fig. 25.2). In each generation the breeder needs to double-check the male sterility of the plants before going for the next cycle of backcrossing. If need arises some selection pressure can also be imposed for one or two important agronomy traits. This may enhance the chances of getting a good "A" line. If required, then some promising "B" lines can also be converted to male sterility by simple backcrossing, using "A" line as female parent and "B" line as recurrent parent.

25.5.3 Cytoplasmic Diversity of "A" Lines

For a sustainable hybrid breeding program based on cytoplasmic nuclear male sterility system, it is essential that a fair amount of cytoplasmic diversity is also maintained. This will protect the breeding program from any potential threat of single cytoplasmic susceptibility against certain specific biotic or abiotic stresses.

Before launching such an expensive diversification breeding program, it is necessary that the candidate cytoplasm should be tested for their genetic diversity. This can be done in two ways,

the first being the traditional way of making experimental hybrids with a few male-sterile lines of a specific cytoplasm and a set of common testers. These hybrids are evaluated for their genetic variation with respect to some important traits and prepare an inventory of diverse traits. This method, however, does not give accurate information about the real genetic diversity among different sources of cytoplasm due to various interactions with known and unknown environmental factors. The other method is based on genomics technologies. An RFLP analysis of mitochondrial DNA based on specific male sterility enzyme probe combinations can be adopted, and this will provide information on the similarity or dissimilarity of the cytoplasm sources. The differences in the RFLP patterns will suggest mitochondrial genomic differences. It seems that from practical breeding points of view, both the traditional restoration patterns and molecular studies would provide more or less conclusive evidences on the diversity of the available cytoplasm sources. In rice, Virmani and Shinjyo (1988) listed several CMS sources; however, Brar et al. (1998) reported that 95 % of the hybrid rice in China represents only a single WA cytoplasm. It is because this cytoplasm has high frequency of fertility restorers and it provides an opportunity to breed high-yielding hybrid combinations. In pigeon pea also, eight cytoplasmic male sterility sources have been reported (Saxena et al. 2010; Saxena 2013), but only one (A_4) cytoplasm derived from a wild relative of pigeon pea is being used for commercial hybrid breeding program. In summary, diversification of cytoplasm is an essential activity of a dynamic breeding program, and plant breeders should take a serious view of it. A breeding program to introduce a new cytoplasmic male sterility that was adopted in pigeon pea at ICRISAT is outlined in Fig. 25.3.

25.6 Seed Production of Male-Sterile Lines

For any technology to become popular among users, it is important that quality of the product is maintained, and it should be economically viable. In seed business also, these two factors are of prime importance. Since the hybrid seed is a product of two parents, it is essential that the seed of the parental lines is of highest quality. To achieve this, care should be taken at every step of seed production, its storage, and its distribution. The hybrids involving different type of male sterility would require specialized approach.

25.6.1 Genetic Male Sterility

Since genetic male sterility is controlled by a pair of recessive genes, it needs to be maintained in heterozygote form. For the seed production of male-sterile line, the heterozygote (*Msms*) seeds are planted in isolation, and these segregate in the Mendelian fashion and produce about 50 % fertile and 50 % sterile plants. In this population these two types of the plants should be tagged with different identity. At maturity the seed should be harvested only from the male-sterile plants, which get pollinated by heterozygote fertile segregants.

The production of hybrid seed with genetic male sterility lines is very tricky and requires greater attention and resources. The seed of male and female parents is planted in isolation. As mentioned earlier the female rows will segregate for fertility/sterility. It is very important that each and every plant is examined, and the fertile plants should to be removed from the field. To maintain the quality of the hybrid seed, it is essential that the fertile segregants should be removed before they start shedding pollen. This will permit crossing of the male-sterile plants with designated male parent only. In case the male sterility gene is linked to any morphological trait, then rouging becomes very easy. In the field crops, there are only a few cases, such as safflower, where male sterility is linked to dwarf phenotype (Vijender Singh, personal communication).

25.6.2 Cytoplasmic Nuclear Male Sterility

In both cytoplasmic and cytoplasmic nuclear male sterility systems, the male-sterile ("A"

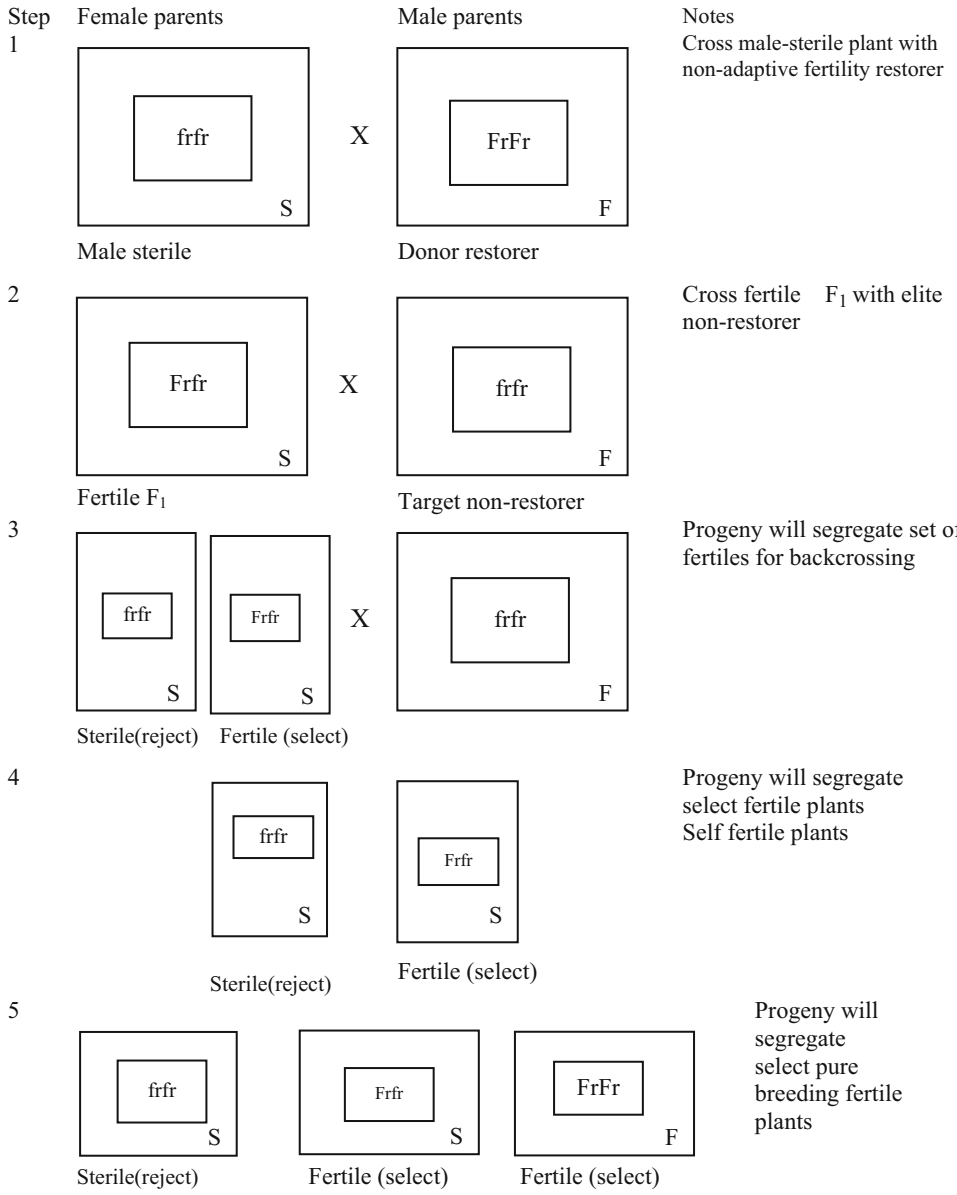


Fig. 25.3 Conversion of elite B-line or other non-restorers into fertility restorers with CGMS system

line) genotypes with sterile cytoplasm and recessive nuclear fertility genes (Fig. 25.1) are crossed with lines having fertile cytoplasm and recessive nuclear fertility genes ("B" line). The seed of "A" and "B" lines is planted in isolation in rows in certain proportions, determined by the availability of pollen and pollinators. Since all the plants in the female rows are expected to be male sterile, examination of each plant for male sterility is not required and crossed seeds are harvested from "A" line only. The production of hybrid seed in the cytoplasmic nuclear system is easy, and "A" and restorer line "R" are planted in isolation in rows, and the seeds set on the "A" lines through cross-pollination are harvested.

25.6.3 Environment-Sensitive Male Sterility

The seed production system involving environment-sensitive line is interesting, and selection of production sites is the most critical. In this system two different sites with distinct and stable temperature requirements during crop growth are essential. These sites should also satisfy the requirement of the length of photoperiod. Site # 1 should have the temperatures under which the male sterility of the line will be maintained. In this site all the plants will be male sterile and these are used for hybrid seed production. For this, the male and female lines are grown in specific ratio, and the cross-pollinated seed is harvested from the male-sterile rows.

For the maintenance of male-sterile line, its seed is planted in site # 2. This site should have the temperatures under which the male fertility is induced and all the plants should be male fertile. Thus, the seed produced from this site will be the self-seed. The next season can be used for the second cycle of seed production.

25.7 General Discussion

Providing quality seed to masses has been an important goal for most national and international organizations dealing with global food security issues. A gradual reduction in arable land and ground water has created challenges for meeting food needs in many countries. With little or no chance of horizontal expansion, scientists from both public and private sectors are aiming for vertical production growth. This can only be achieved through introduction of new technologies. Among these, the most economic and stable technology is hybrid breeding.

The pivoting role of hybrids in enhancing global food availability is well established. The concept of hybrid breeding was developed by Shull (1908) in maize and involved crossing of two diverse pure breeding lines. For large-scale seed production of high-yielding combinations, detasseling (manual removal of male parts) of female parents was adopted. Subsequently, this activity was replaced by introducing cytoplasmic nuclear male-sterile lines. Since 1930, there has been a sixfold increase in the productivity of maize in the USA by the turn of the century (Fig. 25.4; Trayer 1991). This has been possible due to remarkable progress made in breeding high-yielding hybrids and the role of male sterility in easing the seed production technology that allowed access of hybrid seed to most crop growers. Rice is another staple food crop where a tremendous progress has been made in their productivity. This endeavor began in China in 1976, and since then over 55 % rice area has come under hybrid crop production, and it

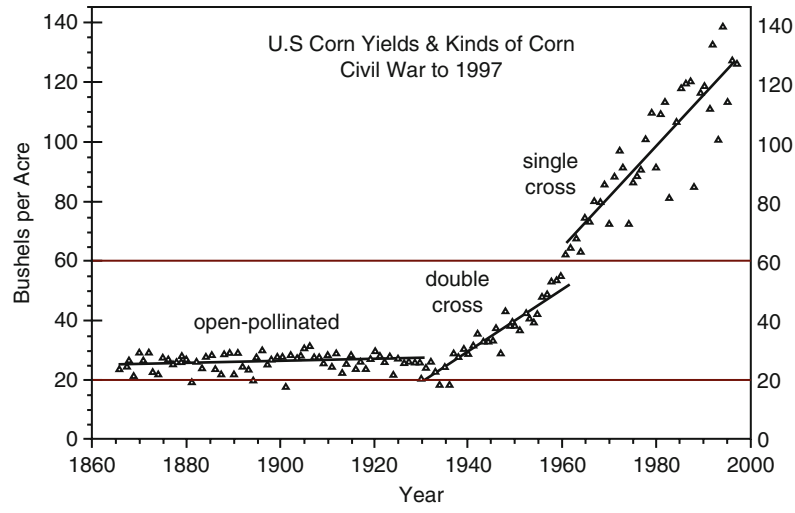


Fig. 25.4 Trends of maize production in America (Source: Trayer 1991)

contributes to 65 % of total production of the country. The adoption of hybrid technology in China has also increased the crop productivity from 2 to over 6 t/ha. This has released over three million ha of rice lands to other crops (Brar et al. 1998). Almost similar achievements have been recorded in cereals like sorghum and pearl millet in India. The other crops which have directly benefitted from hybrid technology are cotton, sunflower, safflower, caster, and a number of vegetable and fruit crops.

Among legumes, although heterotic, male sterility systems have been reported but these could not be utilized for enhancing production and productivity. The exception, however, is pigeon pea where breeders have exploited hybrid vigor commercially very recently. The productivity of pigeon pea had been stagnant at about 700–800 kg/ha for the past six decades, and breeders did not succeed in spite of using different breeding approaches. The breakthrough in the productivity was achieved recently in 2010, when the first high-yielding hybrid was released in India (Saxena et al. 2013). This hybrid is based on cytoplasmic nuclear male sterility and partial natural outcrossing. It has demonstrated 30–40 % yield advantage over 3 years of testing in farmers' fields. Also, under high-input conditions and good management yields, up to 4,000–5,000 kg/ha have been recorded by farmers (Kumar RV, personal communication).

The examples of rice, wheat, and pigeon pea have shown that with concerted research efforts it is also possible to breed hybrids in the non-cross-pollinated crops. This fact demonstrates that heterosis can be exploited in both self- as well as cross-pollinated crops, provided their seed production technology is good enough for adoption by both large- and small-scale seed producers. It can be facilitated by the use of suitable male sterility system. The genetic and cytoplasmic diversifications of male-sterile lines with high combining ability are the key for the future, and it can break low-yield barrier in other food crops also. The better understanding of the phenomenon of heterosis at genetic, physiological, agronomic, and molecular levels can pave the way for obtaining record high yields. Besides seed yield, the hybrid technology can also take care of delicate issues like nutrition, drought, stability, and response to inputs. In this context, maize, rice, pearl millet, and sorghum are good examples where breeding of new hybrids involves a balance between productivity and nutrition. In essence, the success of hybrid technology in continuously enhancing yield levels, as has been demonstrated in maize in the USA, will require robust breeding programs to develop elite hybrid parents with sufficient nuclear and cytoplasmic diversity and accurate tools to predict the productivity of potential hybrid combinations for specific and wide adaptation.

Acknowledgments The author would like to acknowledge the technical support of Mr. R. V. Kumar, Ms. Dipali Thakre, and Mr. Uttam Chand in the preparation of this manuscript.

References

Balk J, Leaver C (2001) The PET-1 CMS mitochondrial mutation in sunflower is associated with pre mature programmed cell death and cytochrome release. Plant Cell 13:1803–1818

Brar DS, Zhu YG, Ahamad MI, Jachuck PJ, Virmani SS (1998) Diversifying the CMS system to improve the sustainability of hybrid rice technology. In: Hybrid rice technology. 3rd International Symposium on Hybrid Rice 14–16 Nov 1996. Hyderabad

Burton GW, Hanna WW (1976) Ethidium bromide – induced cytoplasmic male sterility in pearl millet. Crop Sci 16:731–732

Colhoun CW, Steer MW (1982) Gametocide induction of male sterility: a review and observations on the site of action in the anther. Rev Cytol Biol Veg 5:283–302

Comstock RE, Robinson HF (1952) Genetic parameters, their estimation and significance. In: Proceedings of the 6th international grasslands congress, Simi Valley, pp 284–291

Cosmides L, Tooby J (1981) Cytoplasmic inheritance and inter-genomic conflict. J Theor Biol 89:83–129

Darwin C (1877) Different forms of flowers on plants of the same species. Murray, London

Doggett H (1972) The importance of sorghum in East Africa. In: Rao NGP, House LR (eds) Sorghum in seventies. Oxford & IBH Publ, New Delhi, pp 47–59

Eberhardt SA (1972) Techniques and methods for more efficient population improvement in sorghum. In: Rao NGP, House LR (eds) Sorghum in seventies. Oxford & IBH Publ, New Delhi, pp 195–213

Escote L, Gabay-Laughnan S, Laughnan J (1985) Cytoplasmic reversion to fertility in CMS-S maize

need not involve loss of linear mitochondrial plasmids. Plasmid 14:264–267
Flavell R (1974) A model for mechanism of cytoplasmic male sterility in plants, with special reference to maize. Plant Sci 3:259–283
Frankel R, Galum E (1977) Pollination mechanisms, reproduction and plant breeding. Springer-Verlag, Berlin, p 215
Hanson MR, Bentolila S (2004) Interaction of mitochondrial and nuclear genes that affect male gametophyte development. Plant Cell 16:5154–5169
Iwabuchi M, Kyozuka J, Shimamoto K (1993) Processing followed by complete editing of altered mitochondrial *atp6* RNA restores fertility of cytoplasmic male sterile rice. EMBO J 12:1437–1446
Jones HA, Emsweller SL (1937) A male sterile onion. Proc Am Soc Horticult Sci 34:583–585
Kaul MLH (1988) Male sterility in higher plants. Springer, Berlin
Katti RY, Gaddanavar HS, Naik S, Agadi SN, Rr H (1994) Persistence of callose and tapetum in the microsporogenesis of genetic male sterile line (*Cajanus cajan* L. Mills) with well formed endothecium. Cytologia 59:65–72
Kennel J, Wiss R, Pring D (1987) Influence of nuclear background on transcription of a maize mitochondrial region associated with Texas male sterility of cytoplasm. Mol Gen Genet 210:399–406
Kolreuter DJG (1763) Vorlaufige Nachricht von einigen das Geschlecht der Pflanzenbetreffenden Versuchenund Beobachtungen Fortsetzung. 1. Ostwalds Klassiker der Exakten Wissenschaften Nr 41. Engelmann, Leipzig
Kuranouchi T, Kawaguchi K, Tanaka M (2000) Male sterility in sugar beet induced by cooling treatment and its application to cross pollination for breeding. Breed Sci 50:283–289
Laibach F, Kriben FJ (1951) Der Einfluss von Wuchstoff auf das Geschlecht der Blutenbeieinermonoezischen Pflanze (*Cucumis sativus* L.). Beitr Biol Pflanzenbau 28:64–67
Levings C (1993) Thoughts on cytoplasmic male sterility in CMS-T maize. Plant Cell 5:1285–1290
Levings C, Kim B, Pring D, Conde M, Mans R, Laughnan J, Gabay Laughnan S (1980) Cytoplasmic reversion of CMS-S in maize: association with a tRNA positional event. Science 209:1021–1023
Lu XG, Zhang G, Maruyama K, Virmani SS (1994) Current status of two line method of hybrid rice breeding. In: Virmani SS (ed) Hybrid rice breeding: new developments and future prospects. Intl Rice Res Inst, Manila, pp 37–50
Lukhele PB, Obilana AB (1980) Genetic variability in a long season random mating grain sorghum population. Z Pflanzensuchtung 92:40–51
Mariani C, Beukelear M, Truethner J, Leemans J, Goldberg R (1990) Induction of male sterility in plants by chimeric ribonuclease gene. Nature 347:737–741
Moore JF (1950) Several effects of maleic hydrate on plants. Science 112:474–479
Murthy UR, Rao NGP (1997) Sorghum. In: Bahl PN, Salimath PM, Mandal AK (eds) Genetics, cytogenetics, and breeding of crop plants, vol 2, Cereal and commercial crops. Oxford & IBH Publ, New Delhi, pp 198–239
Nakai S, Noda D, Kondo M, Terachi T (1995) High-level expression of a mitochondrial orf 552 gene from the male sterile sunflower is lethal to *E. coli*. Breed Sci 45:233–236
Naylor AW, Davis EA (1950) Maleic hydrazide as a plant growth inhibitor. Bot Gaz 112:112–116
Obilana AB, El-Rouby MM (1980) Recurrent mass selection for yield in two random mating populations of sorghum (*Sorghum bicolor*). Maydica 25:127–133
Poirier Y, Dennis D, Klomparens K, Somerville C (1992) Polyhydroxybutyrate, a biodegradable thermoplastic, produced in transgenic plants. Science 256:520–523
Ruiz O, Daniell H (2005) Engineering cytoplasmic male sterility viz., chloroplast genome by expression of ketothiolase. Plant Physiol 138:1232–1246
Saxena KB (2013) A novel source of CMS in pigeonpea derived from *Cajanus reticulatus*. Indian J Genet 73:259–263
Saxena KB, Kumar RV (2001) Genetics of a new male sterility locus in pigeonpea (*Cajanus cajan* L. Millsp). J Hered 92:437–439
Saxena KB, Wallis ES, Byth DE (1983) A new gene for male sterility in pigeon pea. Heredity 51:419
Saxena KB, Sultana R, Mallikarjuna N, Saxena RK, Kumar RV, Sawargaonkar SL, Varshney RK (2010) Male sterility systems in pigeonpea and their role in enhancing yield. Plant Breed 129:125–134
Saxena KB, Sultana R, Saxena RK, Kumar RV, Sandhu JS, Rathore A, Kavikishore PB, Varshney RK (2011) Genetics of fertility restoration in A4-based diverse maturity hybrids of pigeonpea (*Cajanus cajan* L. Millsp.). Crop Sci 51:574–578
Saxena KB, Kumar RV et al (2013) ICPH 2671- The world's first commercial food legume hybrid. Plant Breed 132:479–485
Schmulling C, Schell J, Spena A (1988) Single gene from *Agrobacterium rhizogenes* influence plant development. EMBO J 7:2621–2629
Shi MS (1981) Preliminary report of later japonica natural 2-line and applications. Hubei Agric Sci 7:1–3
Shull GF (1908) The composition of a field of maize. Rep Am Breeders Assoc 4:296–301
Singhal NC (2013) Hybrid seed production in field crops-principle and practices. Oxford-IBH Publication, New Delhi
Sinha P, Saxena KB, Saxena RK, Singh VK, Suryanarayana V, Katta MAVS, Khan AW, Varshney R. (unpublished). *Nad7*: a candidate gene responsible for cytoplasmic male sterility in pigeonpea (*Cajanus cajan*)
Small I, Earle L, Escote L, Gabay-Laughanan S, Laughnan J, Leaver C (1988) A comparison of cytoplasmic revertants to fertility from different CMS-S maize sources. Theor Appl Genet 76:609–618
Stephens JC (1937) Male sterility in sorghum: its possible utilization in production of hybrid seed. J Am Soc Agron 29:690–696

Trayer AF (1991) Breeding corn for the export market. Proceedings of the 46th Annual Corn and Sorghum Research Conference. Seed Trade Assoc 46:165–176

Tu ZP, Banga SK (1998) Chemical hybridizing agents. In: Banga SS, Banga SK (eds) Hybrid cultivar development. Narosa Publishing House, New Delhi, pp 160–180

Tuteja R, Saxena RK, Davila J, Shah T, Chen W, Xiao Y, Fan G, Saxena KB, Alverson A, Spillane C, Town C (2013) Cytoplasmic male sterility associated chimeric open reading frames identified by mitochondrial genome sequencing of four *Cajanus* genotypes. DNA Research 20(5):485–495. ISSN 1756-1663

Vasal IK (1967) Physiology and cytology of anther development. Bot Rev 42:327–373

Verulkar SB, Singh DP (1997) Inheritance of spontaneous male-sterility in pigeonpea. Theor Appl Genet 94:1102–1103

Virmani SS, Shinjyo C (1988) Current status of analysis and symbols for male sterility cytoplasm and fertility restoring genes. Rice Genet Newsl 5:9–15

Wang Z, Yetal Z (2006) Cytoplasmic male sterility of rice with Boro II cytoplasm is caused by cytotoxic peptide and is restored by two related PPR motif genes via district modes of mRNA silencing. Plant Cell 18:676–687

Wise RP, Gobelman KW, Pei D, Dell CL, Sehnabla PS (1999) Mitochondrial transcript processing and restoration of male fertility in T- cytoplasm maize. J Hered 90:350–385

Worrall D, Hird DL, Hegde R, Paul W, Draper J, Scot TR (1992) Premature dissolution of microsporocyte callose wall causes male sterility in transgenic tobacco. Plant Cell 4:759–771

Wu H, Cheung A (2000) Programmed cell death in plant reproduction. Plant Mol Biol 44:267–281

Yuan LP (1987) Strategy concepts of hybrid rice breeding. Hybrid Rice 1:1–4

Apomixis in Crop Improvement

26

Tavva S.S. Mohan Dev, Y. Venkateswara Rao,
B. Venkateswara Rao, and M.V. Subba Rao

Abstract

Apomixis is a method of asexual reproduction in plants with three main variants, viz., apospory, diplospory, and adventitious embryony. Genetic understanding of apomixis has been handicapped for a long time due to lack of techniques for a rapid and accurate identification of apomictic and normal plants. Subsequent development of techniques for isolation of embryo sacs, use of flow cytometry, and availability of molecular markers facilitated an early identification of apomictic genotypes. Apomixis is now considered to be a consequence of deregulation of the genes involved in sexual reproduction. Though the inheritance of apomixis appears to follow Mendelian principles, every conceivable complication for genetic analysis such as epistatic gene interactions, components that are expressed sporophytically and gametophytically, expression modifiers, polyploidy, segregation distortion, and suppressed recombination is now thought to have accumulated in apomicts. Biotechnological work carried out on some plant systems—*Pennisetum*, *Brachiaria*, and *Paspalum*—where apomixis has been subjected to detailed molecular genetic analysis is summarized here because of the importance of concepts and experimental strategies involved. The three features of apomixis, viz., (1) ease of multiplying and maintaining elite hybrid genotypes, (2) ease of producing high-quality pure seed without isolation requirements, and (3) possibility for selection of a diversity of more closely adapted genotypes, are expected to provide means for indefinite fixation of hybrid vigor and lower the cost of hybrid seed production. Though significant advancement has taken place in our

T.S.S. Mohan Dev • Y. Venkateswara Rao
B. Venkateswara Rao • M.V. Subba Rao (✉)
Botany Department, Andhra University,
Visakhapatnam 530003, Andhra Pradesh, India
e-mail: mnpati_srao@yahoo.com

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_26,

understanding and handling of apomixis, no gene has yet been isolated that could convincingly be labeled as "apomixis gene." Nevertheless, attempts made so far have led to the optimism that apomixis can be available to the breeder in a not too distant future.

Keywords
Plant reproduction • Apomixis • Apospory • Diplospory • Adventitious embryony • Genetic basis of apomixis

26.1 Introduction

Present day agriculture relies more on uniformity both in quantity and quality, of the produce, be it the seed or fruit. Apomixis is a natural way of achieving this goal by fixing the genotype of a superior variety, including hybrid cultivars. Especially in the case of hybrids, this phenomenon prevents loss of vigor due to genotypic segregation in advancing generations, thus enabling a significant reduction in the cost of hybrid seed production. Therefore, breeders to date have focused on introgressing the trait of apomixis from distant wild relatives into agricultural crops of economical importance through traditional breeding. However, like most of the classical breeding methods, this is a slow process, and the choice of the type of apomictic mechanism is limited by what is available in the apomictic relatives. These problems could be overcome if molecular knowledge of the gene(s) involved in initiating and controlling apomixis is available, because they could then be transferred to the crop of interest by genetic engineering methods (Koltunow et al. 1995).

Apomixis (*apo* = detached/separate; *mixis* = union/combination) is a method of asexual reproduction found in plants. The discovery of apomixis in higher plants is attributed to the observation by Smith in 1941 that a solitary female plant of *Alchornea ilicifolia* from Australia continued to form seeds when planted at Kew Gardens in England (Lone and Lone 2013). Winkler introduced in 1908 the term apomixis to mean "asexual multiplication process without the fusion of nucleus and cell." In simpler terms, apomixis is defined as "asexual (agamic) reproduction by seeds" (Nogler 1984a). It does not involve either the formation of normal female gametes or their fertilization (syngamy) but involves the formation of seeds; hence, this phenomenon is also referred to as agamospermy. Consequently, an apomictic seed can give rise to a plant that could show nearly hundred percent genetic resemblance to its mother plant. Apomictic reproduction thus enables a plant to maintain its genetic identity through any number of generations, a feature of profound agricultural importance (Bashaw 1980). This possibility has been the point of interest to geneticists and breeders ever since the phenomenon was understood. Identification of the gene(s) governing apomictic reproduction would increase the chances of their cloning and transferring this valuable genetic trait into any desired system.

26.2 Types of Apomixis

A detailed account of the origin of apomixis was dealt with by several authors (Lakshmanan and Ambegaokar 1984; Nogler 1984a; Asker and Jerling 1992; Quarin 1992; Savidan 2000). The definition of apomixis has varied over time. Winkler's usage of the term seems to have included all modes of asexual reproduction under apomixis (Nogler 1984a). Crane (2001) suggested that up to 45 different types of apomixis are theoretically possible, as unreduced embryo sacs can be produced in nine different ways while

endosperm and embryo can develop following at least five different patterns. However, the now widely accepted definition is that of Nogler (1984a) which defines apomixis as agamospermy, i.e., asexual reproduction through seeds. This definition restricts apomixis to asexual reproduction or cloning through seeds, thereby excluding all types of vegetative propagation.

Apomixis is recognized into three main types according to the origin and development of the maternal embryos. Two groups entail the gametophytic apomixis of Stebbins (1950) including *apospory* in which the megagametophyte originates from a somatic, usually nucellar cell and *diplospory* in which the megaspore originates from the reproductive cell itself with the latter failing to successfully complete meiosis; thus, in both these types, the embryo arises from the egg cell of an unreduced embryo sac. Apomeiosis is a common term covering both apospory and diplospory. The third type is *adventitious embryony* in which the embryo develops directly from a somatic cell instead of from an unreduced megaspore. Adventitious embryony is divided into two subtypes, viz., nucellar and integumentary embryony, based on the origin of the embryos. Polyembryony is a characteristic of adventitious embryony, and fertilization of the central cell of the embryo sac is often necessary for the formation of endosperm and viable seed.

In members of the tribe Paniceae of the family Poaceae where the phenomenon of apomixis has been extensively studied, majority show apospory (Brown and Emery 1958). The system of "sporophytic agamospermy" or "adventitious embryony" is the most widespread form of agamospermy (Carman 1997). An interesting example is the Indian fruit tree *Commiphora wightii* ("guggul") which not only produces sexual and apomictic embryos but can also develop the endosperm autonomously (Gupta et al. 1996). Sporophytic agamosperms are mostly diploid and sexually fertile. Frequently, they are polyembryonous and both sexual and apomictic embryos often coexist. Many are pseudogamous and the endosperm requires primary endosperm nucleus's (PEN) fertilization as in *Garcinia*. It seems that pseudogamy, autonomous embryony, and successful embryony in the absence of an endosperm can all occur together (Richards 1990; Van Baarlen et al. 1999).

26.3 Occurrence

The phenomenon of apomixis appears to be of considerable widespread occurrence having been reported in about 400 species distributed over 40 angiosperm families (Carman 1997). Of the plants known to show gametophytic apomixis, 75 % belong to three families, the Asteraceae, Rosaceae, and Poaceae, which collectively constitute 10 % of flowering plants. Adventitious embryony or sporophytic apomixis is taxonomically scattered with representatives in Orchidaceae, Celastraceae, and Rutaceae and mainly found in tropical and subtropical woody plants with multiseeded fruits (Lone and Lone 2013).

However, apomixis is of common occurrence in perennial tropical forage grasses showing polyploidy and hybrid origin. For example, in *Tripsacum* agamic complex ($x = 18$), the diploids reproduce sexually, whereas the polyploids reproduce through diplospory of gametophytic apomixis followed by parthenogenesis. Diplospory in some species/subspecies of *Tripsacum* is facultative and primarily of the *Antennaria* type where failure of meiosis in megaspore mother cells leads to their direct development into mature unreduced female gametophytes through three to four mitotic divisions (Leblanc et al. 1995a). An interesting phenomenon associated with the failure of meiosis in these species is that there is no callose deposition in the megasporocyte's cell wall while the normal megasporocytes that undergo meiosis and their derivatives show callose deposition. This feature has been successfully applied for the identification of apomictic and non-apomictic embryo sacs.

26.4 Identification of Apomicts

Genetic understanding of apomixis is handicapped due to the nonavailability of techniques for a rapid and accurate identification of apomic-

tic and normal plants. Development of techniques for isolation of embryo sacs and for the analysis of callose deposition to identify the meiotic (normal) and non-meiotic products of megasporogenesis rekindled interest in the genetic analysis of apomixis. This latter technique involves staining of meiocytes with sucrose-aniline blue followed by clearing with methyl benzoate-dibutyl phthalate solution (Crane and Carman 1987; Peel 1993). When these cleared ovules were observed under an epifluorescence microscope, all the enlarged megasporocytes of the diplosporous type show the absence of callose, whereas the normal meiocytes show fluorescence. However, all these procedures are destructive (i.e., involve ovule analysis) and time-consuming and cannot be carried out before flowering, making them difficult to apply when large numbers of progenies have to be screened and/or when low female fertility occurs (Leblanc et al. 1995b). Availability of molecular markers associated with the apomictic character helped to overcome these limitations and facilitated an early screening of the apomictic genotypes (Leblanc and Mazzucato 2014).

26.5 Origin and Evolution of Apomixis

Many flowering plants can choose between no less than three fundamentally different modes of reproduction: (1) outcrossing sex, (2) selfing sex, and (3) asexuality. These influence population structure and evolutionary potential in profoundly different ways. Perennial plants commonly use a combination of all three modes to fine-tune their reproductive strategy to changing ecological circumstances (Richards 1997). Successful individuals may be favored by "symmetrical" selection. Despite its theoretical advantages, apomixis usually coexists with sexuality suggesting "hidden" disadvantages. Agamospermy is relatively uncommon but gains from the attributes of the seed. It forces agamospermy genes, which discourage recombination, to form coadapted linkage groups so that they become targets for disadvantageous recessive mutant accumulation. Consequently, agamospermy genes cannot succeed in diploids and may be highly heterotic. Agamospermous endosperm may suffer from genomic imbalance so that nutritious ovules which can support embryos without endosperm may be preadapted for agamospermy. When fertilization of primary endosperm nucleus ("pseudogamy") continues as a requirement for many aposporous agamosperms, selfing sex becomes preadaptive, and archesporial sex remains an option. Apomictic populations can be quite variable although apomictic families are much less variable than sexuals. Only in some diplosporous species does sex disappear completely, and in such species release of some variability may persist through somatic recombination. The genomes of obligate asexuals act as giant linkage groups as they lack recombination and might form targets for disadvantageous recessive mutant accumulation (Richards 2003).

Nogler (1984a) stated that at least the basic components of apomixis could have originated by mutation. Quoting Petrov (1976), Nogler further endorsed the possibility that most of the latter elements or components of apomixis "lie within the reproductive potentialities of sexual plants." This may be interpreted by suggesting that probably no major genetic change is required in the machinery that controls the regulation of both megasporogenesis and megagametogenesis in sexual plants. Apomixis has also been viewed to be a consequence of the deregulation of genes involved in sexual reproduction (Ozias-Akins and van Dijk 2007).

26.5.1 Apomixis and Biodiversity

Logically, it appears that transfer or introduction of apomixis and its use in crops will have an influence, possibly negative, on biodiversity as it promotes uniformity. However, there are evidences to the contrary. Using comparative isozyme analysis in 2× sexual and 4× apomictic pools in *Panicum maximum*, Assienan and Noirot (1995) demonstrated that apomixis in the wild does not lead to a reduction in diversity and on the contrary, rare alleles, strongly counter-selected at the

diploid (sexual) level, have been maintained by apomixis. Similar conclusion was arrived at by Schmelzer and Renno (1997) while working with the two pools of species—*Pennisetum polystachion* and *Pennisetum subangustum*. Reviewing current knowledge on diversity in wild apomicts and models of evolution, Berthaud (1999) emphasized that obligate apomixis should definitely be considered as exceptional and that the rate of "residual" sexuality found in facultative apomicts will be transmitted and expressed in the new apomictic crops so that farmers in such areas will maintain their ability to select new genetic combinations if favorable, though such new combinations appear less frequently than today while landraces will maintain their evolutionary capacity.

26.6 Genetic Basis

Apomixis involves several interrelated events such as termination of meiosis, formation of aposporous embryo sacs, and their parthenogenetic development. The character has to be transferred only through the male gametes. Probably because of these limitations and complex nature of the character, the quantum of genetic information available on apomixis is rather limited with studies enabling definite conclusions being still scanty. However, surprisingly, these complex and coordinately regulated cascades of events appear to be controlled by only one or a few genes (Nogler 1984b; Asker and Jerling 1992; Savidan 2000). Nevertheless, a significant deviation from Mendelian inheritance was also reported in some cases such as *Paspalum simplex* X *Paspalum procurrens* (Pupilli et al. 2004), *Pennisetum glaucum* X apomictic *Pennisetum squamulatum* (Roche et al. 2001b), *Tripsacum*-maize hybrids (Grimanelli et al. 1998), *Hieracium* (Catanach et al. 2006), *Ranunculus* (Nogler 1984b), *Taraxacum* (Vijverberg et al. 2004), and *Erigeron* (Noyes and Rieseberg 2000). Such deviation was attributed to segregation distortion toward sexual progeny. In several instances, the possibility of pleiotropic effects of a single gene or close linkage of several genes remains open. The work of Ozias-Akins et al. (1993) supports the limited evidence that only one or a few tightly linked genes may be required for the genetic transmission of apomixis in *Pennisetum*.

In *Ranunculus auricomus*, Nogler (1984b) suggests that the allele controlling aposporous embryo sac development (A−) is dominant but has lethal recessive effects in the gametes. Apomixis can therefore be inherited only by diploid gametes heterozygous for A^+ (i.e., A^+ A^-), while haploid gametes carrying "A^-" would not be viable. Recessive lethal effects in the pollen or in the egg cell were also observed in some other apomictic taxa, mainly in Asteraceae and Poaceae (Ozias-Akins and van Dijk 2007). Genetic regulation of diplospory in *Taraxacum* (Mogie 1988) suggested that the control of female meiosis resides on a single chromosome and probably at a single locus.

However, just because agamospermy can be inherited as a single entity, it does not follow that this character is controlled by a single, localized, clonable DNA sequence (Richards 2003). Rather, it seems that "apomixis may be controlled by large sectors of DNA in which recombination is suppressed" (Bicknell et al. 2000), although detailed studies in several genera have suggested that recombination within this "apomixis supergene" does in fact occur regularly. As expressed by Richards (2003), such model of a complex locus which proposes that all the genetic elements that control agamospermy may come together in linkage to recessive lethals has several major implications such as the strong association of agamospermy with polyploidy and also with hybridity and heterozygosity. The existence of a complex locus also might raise the possibility for the involvement of gene silencing as part of the apomixis mechanism. Silencing or repression of genes required for normal sexual reproduction could occur through several mechanisms, either transcriptional or posttranscriptional. If the apospory locus were a silencing locus or could act in trans to repress other genes, the dominance of the trait in many species, its incomplete penetrance in facultative apomicts, and its potential for suppression by other loci could be explained (Richards 2003).

Tripsacum is diplosporous rather than aposporous and forms 8-nucleate embryo sacs from megaspore mother cells that either do not enter meiosis or exit meiosis through first-division restitution. The long arm of *Tripsacum* chromosome Tr16, on which a nucleolus organizing region is located, was shown to be required for apomictic reproduction in maize-*Tripsacum* hybrids, but apomixis was only observed when the hybrids had at least eight other *Tripsacum* chromosomes (Kindiger et al. 1996). Leblanc et al. (1995b) identified three restriction fragment length polmorphic (RFLP) markers linked to diplospory in maize-*Tripsacum* hybrids that were also found to be linked in the maize genetic map supporting the single-locus hypothesis. A mutagenesis population has been built up by crossing apomictic maize-*Tripsacum* hybrid derivatives with $2n = 10$ maize (M) + 18 *Tripsacum* (T) chromosomes onto a maize stock enriched in *mutator* elements. The resulting apomictic $2n + n$ off types, which represent approximately 20 % of the progeny, offered the first opportunity to "dissect" the apomeiotic control beyond the conventional Mendelian genetics (Leblanc et al. 1998). Mapping by Blakey et al. (2001) suggests that multiple chromosomal regions may be required for the full expression of apomixis.

Apomixis in *Erigeron* (Asteraceae) is controlled by two unlinked dominant loci, one for diplospory (D) and the other (F) for both parthenogenesis and autonomous endosperm development (Noyes and Rieseberg 2000). The fact that the $2n = 19$ genotype did not express parthenogenesis, despite carrying the linked marker, suggested initially that parthenogenesis was silenced in the absence of diplospory—in other words, parthenogenesis would be contingent upon diplospory (Noyes 2006). Eleven amplified fragment length polmorphic (AFLP) markers cosegregated strictly with diplospory, and four markers cosegregated significantly with parthenogenesis. This block of diplospory-linked markers was by far the largest in the genome, suggesting a locally low recombination rate in the diplospory linkage group (DLG). By contrast, no lack of recombination was observed in the parthenogenesis linkage group (PLG). Neither the DLG nor the PLG was transferred via haploid pollen grains to diploid offspring explaining the absence of diploid apomicts. However, the cause of non-transmission was different between these two chromosomal regions. The DLG showed univalent inheritance and apparently did not pair with its homologues during pollen meiosis, remaining as a univalent and thus randomly segregating in the diploid pollen. By contrast, the PLG showed polysomic inheritance in the diploid pollen grains, suggesting that its absence in the diploid F_1 was best explained by recessive lethal gametophytic selection against the parthenogenetic linkage group (Noyes et al. 2007).

Apomixis in cassava is controlled by more than one recessive gene, which acts in an additive form. Aneuploidy is associated with apomixis in cassava and can provide the double dosages necessary for recessive gene action (Freitas and Nassar 2013).

Based on the relationship between gametophytic apomixis and polyploidy, Roche et al. (2001a) and Koltunow and Grossniklaus (2003) suggested that apomixis may be under epigenetic control rather than under genetic and that this might be related to polyploidy. Comai (2005) provided empirical data to substantiate the occurrence of both genetic and epigenetic changes shortly after polyploidization. From a review of Mendelian genetics of apomixis, Ozias-Akins and van Dijk (2007) suggested that such heritable epigenetic changes are based not on DNA sequence changes but rather on covalent modifications of nucleotides such as methylation, chromatin remodeling, RNA interference, or more elusive dosage effects. Whether genetic or epigenetic mechanisms control apomixis, there is abundant evidence that specific chromosomal regions transmit the trait in natural apomicts and that frequently observed polysomic inheritance, pointing toward autopolyploidy but abundant heterozygosity, argues against a recent origin of apomixis by hybridization. Though the inheritance of apomixis appears to follow Mendelian genetics, every conceivable complication for genetic analysis such as epistatic gene interactions, components that are expressed sporophytically and gametophytically, expression modifiers,

Table 26.1 A brief overview of the genetic basis of different types of apomixis

Species	Type of apomixis	No. of loci	Genotype
Brachiaria brizantha	A, P	1	Aaaa
Cenchrus ciliaris	A, P	1	Aaaa; +
Erigeron annuus	Diplospory, mitotic, autonomous endosperm	2	D/dd; + Fff
Hieracium caespitosum	A, P	2	Aaaa, Pppp; +
Panicum maximum	A	1	Aaaa; +
Paspalum notatum	A, P	1	Aaaa; +
Paspalum simplex	A, P	1	Aaaa +
Pennisetum squamulatum	A, P	1	Aaaa; +
Poa pratensis	A	2	Aaaa, Pppp
Ranunculus auricomus	A, P	1	*Aaaa*
Taraxacum officinale	Diplospory, meiotic, autonomous endosperm	3	Ddd, Ppp; +
Tripsacum dactyloides	Diplospory, mitotic, P	1?	Dddd; +

Modified after Ozias-Akins and van Dijk (2007)
A apospory, *D* diplosporous, *P* pseudogamous endosperm; + suppression of recombination in apomixis specific chromosomal region

polyploidy, segregation distortion, and suppressed recombination seems to have accumulated in apomicts (Table 26.1) (Ozias-Akins and van Dijk 2007).

It is now widely acknowledged that the first step toward developing new apomicts is to focus on the genetic control of apomixis as the basis of our molecular strategy (Savidan 2000), as apomixis appears to be more complex than what could be explained through Mendelian analysis and might result from more than a single mutation at one locus. Through transcriptome analysis, Polegri et al. (2010) reported that out of nearly 200 genes differentially expressed between apomictic and sexual lines of *P. simplex*, only 10 % were genetically linked to apomixis. This means that the transferring of the apomixis locus from an apomictic "donor" to a sexual "receiver" implies the reprogramming of the regulation of several genes that act downstream of the apomixis-linked factors. Accordingly, the whole process should be divided into individual components, and the mutations affecting each of those should be searched for and identified separately. The focus now is to analyze the genetic basis and molecular mechanisms controlling megasporogenesis, megagametogenesis, and seed development in sexual species including the identification of mutants affecting various aspects of apomictic reproduction.

26.7 Biotechnological Studies

Molecular markers have become very efficient and powerful tools in plant breeding because they facilitate genetic dissection of complex traits and an early selection of desired genotypes. Work carried out on three plant systems where apomixis was subjected to detailed molecular genetic analysis is presented in the following pages because of the importance of concepts and experimental strategies involved.

26.7.1 *Pennisetum*

In the genus *Pennisetum*, several wild species are known to be apomictic (Hanna 1986). Transfer of the gene(s) responsible for apomictic behavior from the undomesticated species to the cultivated pearl millet, *P. glaucum* (L.) R. Br. ($2n=14$), involved a novel approach of using a bridge species. Introgression of the apomictic character was achieved from a wild hexaploid species *P. squamulatum* ($2n=6x=54$) to induced tetraploid pearl millet ($2n=4x=28$) using *P. purpureum* ($2n=4x=28$) (Dujardin and Hanna 1984). This transfer was effected through four successive backcrosses between the double cross trispecific hybrid and the tetraploid pearl millet followed by continuous selection for apomictic mode of reproduction. The chromosomes of the wild species became eliminated by third backcross, and finally, a 29-chromosome containing plant showing apomixis was developed. From a total of 2,268 cDNA fragments displayed between 200

and 600 bp, 8 were identified and cloned. Based on northern analysis, one cDNA was detected in only sexual ovaries, two cDNAs in only apomictic ovaries, and one cDNA was present in both types of ovaries, while one fragment was detected in ovaries, stems, and leaves.

Ozias-Akins et al. (1993, 1998) and Lubbers et al. (1994) reported some markers (Table 26.2) which strictly cosegregated with aposporous embryo sac development clearly defining a contiguous apospory-specific genomic region in which no genetic recombination could be detected. Lack of or suppression of recombination was attributed to either coincidental association with the chromosomal context of the apomixis locus or a consequence of evolutionary process that is essential for preservation of gene function.

A differential display procedure for a comparative study of gene expression in unpollinated ovaries containing either apomictic or sexual female gametophytes was applied by Vielle-Calzada et al. (1996) in another species of this genus, *Pennisetum ciliare* (syn. *Cenchrus ciliaris* L., buffel grass). Such comparison of gene expression during sexual and apomictic development was suggested to represent a new model system and a strategy for investigating female reproductive development in the angiosperms. Akiyama et al. (2004) reported that one retrotransposon family is particularly abundant in two chromosome blocks within the apospory-specific genomic region (ASGR) of *P. squamulatum*. The same retrotransposon family was later found to be abundant on all chromosomes of *C. ciliaris*, particularly in centromeric and pericentromeric regions. In both species, there is a region of low-copy DNA (or at least low in abundance of this particular retroelement) that is sandwiched between regions of highly repetitive DNA. This highly repetitive DNA bordering the ASGRs was suggested to be responsible for the heterochromatic nature of these regions.

Table 26.2 DNA sequences closely associated with the region conferring apomictic character in *Pennisetum*

OPC-04: 5′CCGCATCTAC 3′[a]
OPE-11: 5′GAGTCTCAGG 3′[a]
OPE-14: 5′TGCGGCTGAG 3′[a]
OPF-05: 5′CCGAATTCCC3′[a]
UGT 197 – FP: 5′ CTGCAGACCTCCAAACAG3′; UGT 197 – RP: 5′ CTGCAGCATGTGAACCAT3′
Pca-2: 3′-TCAGAGCGCC-5′
Pca-3: 3′-CTGTTGCTAC-t5′

[a]After Ozias-Akins et al. (1993); others after Lubbers et al. (1994)

26.7.2 *Brachiaria*

The mode of reproduction in the forage grass *Brachiaria* is closely related to its ploidy level. Diploid genotypes are sexual, but polyploid ones reproduce through the formation of aposporous embryo sacs followed by parthenogenetic development of the egg cell and pseudogamous development of the endosperm (Brown and Emery 1958). Apomictic type of reproduction is inherited as a dominant trait under the control of a single gene (do Valle and Glienke 1993; do Valle et al. 1994; Miles and Escandon 1997). Using an F1 population derived from a cross between apomictic *Brachiaria brizantha* and sexual *Brachiaria ruziziensis* and taking advantage of the genomic synteny observed among grass genomes (Moore et al. 1995), the *Brachiaria* genome was systematically scanned using 61 cDNA and maize genomic clones along with some RFLP and RAPD markers (Pessino et al. 1997). Two RFLPs and one RAPD marker were found to be significantly linked to the apomictic trait. One of the RAPDs (OPC4), which was also shown to be associated with apospory in *Pennisetum*, was the only one linked to apospory in *Brachiaria* suggesting a common genomic location for the gene controlling the trait in both the genera. One of the probes belonging to the linkage group associated with the character in *Brachiaria* (csu134) was also found to map to chromosome 5 of maize carrying the apomixis control (Savidan et al. 1995). In a further step, Pessino et al. (1998) used 25 RFLP and 46 AFLP markers to generate a complete map of the region. Two markers, PAM52-5 and PAM49-13, were located, respectively, at 1.2 cM and 5.7 cM at either side of the target locus. The map shows

close synteny to regions of maize chromosome 5 and rice chromosome 2.

26.7.3 *Paspalum*

The genus *Paspalum* shows a variety of reproductive systems such as allogamy (due to self-incompatibility), autogamy (including cleistogamy), and apomixis. In general, diploid species are sexual, whereas most polyploids are apomictic (Quarin 1992). The main type of apomixis in *Paspalum* is apospory. One or more nucellar cells give rise to unreduced embryo sacs by two or three mitotic divisions. The unreduced egg cell gives rise to a functional embryo by parthenogenesis, whereas fertilization of the central cell is necessary for the correct development of the endosperm (Cáceres et al. 2001). Pupilli et al. (2001, 2004) showed that the apomixis controlling locus (ACL) in *P. simplex* is defined by a single dominant allele. This region showed strong repression of recombination and a significant synteny of markers with the telomeric region of the long arm of chromosome 12 of rice. Sequence analysis of this region indicated the presence of a "sea" of repetitive and transposable elements and noncoding DNA. Using fluorescence in situ hybridization (FISH), Calderini et al. (2006) localized the ACL in a non-pericentromere and non-heterochromatic chromosome area which suggested that the genes that are contained in this locus could be transcriptionally active.

Comparative molecular genetic analysis of the ACL in these species was initiated by Pupilli et al. (2004) who showed that a restricted *Paspalum* genomic region homologous to a specific chromosome area of rice was conservatively linked to apomixis in several *Paspalum* species even though some small-scale rearrangements took place among the species analyzed. That the markers linked to apomixis in some *Paspalum* species were not so in other closely related species is probably due to deletions and/or insertions of genes related to transposable elements as was observed by Calderini et al. (2006) while comparing the intron/exon structure of apomixis-linked genes in *Paspalum* with that of their rice homologues. Such mutations could interrupt locally the gene colinearity and linkage with apomixis. Hojsgaard et al. (2011) showed that the apomixis locus in *P. procurrens* underwent a deletion event that spans 2.4 cM in rice corresponding to around 490 kb between the markers C1069 and R4038 when compared with the same region of *P. simplex* and *P. malacophyllum*. The structural changes of the ACL among the three species studied to date are depicted by small rearrangements, and *P. simplex* seems to represent the ancestral situation because the marker synteny with the homoeologous genomic region of rice was much more significant in this species compared with others.

The use of single-copy gene sequence of model species as anchor and the exploitation of synteny make it possible to look for and identify specific genetic features in non-model species. Comparative mapping approach showed that, of all the markers present over the rice regions related to asexuality, only a few are conservatively linked to apomictic reproduction across four *Paspalum* species. This region is further narrowed down in *P. notatum*, a distantly related species in which only two markers (C996A and C1069) located at a distance of 5.8 cM on the rice map (corresponding to a physical distance of around 1,062 kb) showed apomixis-linked fragments. The genomic region of *Paspalum* flanked by these two markers likely includes all the genetic determinants of apomictic reproduction in this species. An example of a possible candidate gene located in this area is the clone C996A in rice which is an expressed sequence tag (EST) of the gene LOC_Os12g42180 that is the homologue of the *Arabidopsis* gene *HUELLENLOS* (*HLL*) encoding for a ribosomal protein essential for normal ovule development (Skinner et al. 2001).

On the basis of their predicted molecular function, other genes (such as "ankyrin-1," "auxin responsive," and "asynaptic mutant 1" that are putatively involved in processes related to cell division and differentiation) located in this area could also have a role in the process of apomixis in *Paspalum*. Furthermore, an AP2-containing homologue of embryogenesis-related gene of *Arabidopsis* BABY BOOM was also found in the apospory locus of *Pennisetum* (Conner et al. 2008).

The comparative mapping approach to identify the genes responsible for apomictic reproduction in *Paspalum* appears to be conceptually similar to the deletion mapping approach of Catanach et al. (2006) in *Hieracium*. Spontaneous deletions occurring during speciation have screened out all those genes that are dispensable for apomictic reproduction. The genus *Paspalum* is well suited for this purpose because it includes many agamic complexes differentially related to each other and each formed by apomictic polyploid species with crossable sexual counterparts (Quarin 1992). The small-scale rearrangements detected at the ACL of *P. procurrens* with respect to *P. simplex* contributed to narrow down the size of the ACL that is conservatively linked to apomixis in several *Paspalum* species. This makes it possible for a core of markers linked to apomixis between closely related and distantly related species within the *Paspalum* genus. Such interspecific recombination helps to overcome the intraspecific block of recombination that severely hampers the isolation of apomixis genes in these species by genetic means, restricting the genomic window to mine the genetic determinants of apomixis (Hojsgaard et al. 2011).

Although apomictic reproduction is genetically controlled, most of the wild species are not amenable for applying the tools of molecular biology commonly used in other model biological systems such as *Arabidopsis* or *Petunia*. Genes that may be involved in embryo initiation (including those for somatic embryogenesis in culture) have been characterized in *Arabidopsis*. Several *Arabidopsis* mutants have also been identified that rather unexpectedly promote endosperm development in the absence of either PEN or egg cell fertilization, or embryony (Spillane et al. 2001). Remarkable progress has been recently made in *Arabidopsis* to reproduce some features of apomictic reproduction, i.e., apomeiosis (Ravi et al. 2008; Olmedo-Monfi et al. 2010) and parthenogenesis (Ravi and Chan 2010) by mutagenesis, but no full apomictic *Arabidopsis* mutants have been produced to date. Furthermore, it is still unclear whether these phenotypes are related to apomixis noticed in wild species (Garcia-Aguilar et al. 2010). Recently, an artificial apomictic *Arabidopsis* has been produced by combining the aforementioned apomeiotic and parthenogenetic mutants by crossing (Marimuthu et al. 2011). However, since this plant still relies on crossing to express full maternal inheritance, it cannot be defined as a genuine apomictic genotype as it stands.

Successful application of apomixis in major crops such as corn and rice will be dependent on the possibility to introduce this trait into these genomes and control its expression so that high yields of genetically pure crops could be obtained. In spite of all the knowledge available on apomixis, information on critical aspects, such as the nature of the character's molecular trigger and the specific differentiation pathways involved in its occurrence, is still lacking. The observation made nearly a decade ago by Richards (2003) that no agamospermy gene has yet been analyzed which "is sufficiently localized and independent, or free of a genetic load such as linkage with lethal genes and accumulation of mutations to function successfully in an isolated clonal state in a foreign genome that lacks dominant heterologues," is still valid. An ideal donor species of apomixis gene should not have agamospermy controlled by multiple-factor systems or linked but recombinable factors and should have a regular male meiosis. A successful map-based gene cloning depends on the ability to position the trait phenotype with respect to molecular markers based on recombination distance. In the absence of genetic recombination, physical mapping becomes a tedious but essential process for ordering molecular markers associated with the trait. Deletion or insertional mutagenesis approach might allow positioning of the apomixis gene(s) with respect to the linked molecular markers.

26.8 Future Prospects

Though significant advancement in our understanding and handling of apomixis has been made, no gene has yet been isolated that could be convincingly labeled as an "apomixis gene." Nevertheless, all the attempts made so far have

increased the level of optimism that apomixis will be brought into the hands of the breeders in a not too distant future. To quote Grossniklaus et al. (1998), there appears to be "a bright future for apomixis." It should also be kept in view, as suggested by Van Dijk and Van Damme (2000), that if apomixis genes were successfully cloned and released into the environment, they should succeed at the expense of sexual genes and might constitute a serious environmental hazard. However unlikely, this possibility would need to be taken care of before a GM apomixis gene is released (Richards 2003).

The three features of apomixis, viz., (1) ease of multiplying and maintaining elite hybrid genotypes, (2) ease of producing high-quality pure seed without isolation requirements, and (3) possibility for selection of a diversity of more closely adapted genotypes, are expected to provide means of indefinite fixation of hybrid vigor, lower cost of hybrid seed production, and help marginal farmers who usually save seed from their last harvest for the next cycle of cultivation. As predicted by Hanna (1995), the greatest impact of apomixis would likely be in the developing countries.

References

Akiyama Y, Conner JA, Goel S, Morishige DT, Mullet JE (2004) High-resolution physical mapping in *Pennisetum squamulatum* reveals extensive chromosomal heteromorphism of the genomic region associated with apomixis. Plant Physiol 134:1733–1741

Asker SE, Jerling L (1992) Apomixis in plants. CRC Press, London

Assienan B, Noirot M (1995) Isozyme polymorphism and organization of the agamic complex of the maximae (*Panicum maximum* Jacq, *P. infestum* Anders, and *P. trichocladum* K. Schum) in Tanzania. Theor Appl Genet 91:672–680

Bashaw EC (1980) Apomixis and its implication in crop improvement. In: Fehr WR, Hadley HH (eds) Hybridization of crop plants. American Society of Agronomy, Madison, pp 45–63

Berthaud J (1999) Apomixis and the management of diversity. In: Savidan Y, Carman JG, Dresselhaus T (eds) Advances in apomixis research. CIMMYT &IRD, Mexico, pp 8–23

Bicknell RA, Borst NK, Koltunow AM (2000) Monogenic inheritance of apomixis in two *Hieracium* species with distinct developmental mechanisms. Heredity 84:228–237

Blakey CA, Goldman SL, Dewald CL (2001) Apomixis in Tripsacum: comparative mapping of a multigene phenomenon. Genome 44:222–230

Brown WV, Emery HP (1958) Apomixis in the Gramineae: Panicoideae. Am J Bot 45:253–263

Cáceres ME, Matzk F, Busti A, Pupilli F, Arcioni S (2001) Apomixis and sexuality in *Paspalum simplex*: characterization of the mode of reproduction in segregating progenies by different methods. Sex Plant Reprod 14:201–206

Calderini O, Chang BS, de Jong H, Busti A, Paolocci F, Arcioni S, de Vries SC, Abma-Henkens MHC, Klein Lankhorst RM, Donnison IS, Pupilli F (2006) Molecular cytogenetics and DNA sequence analysis of an apomixis-linked BAC in *Paspalum simplex* reveal a non pericentromere location and partial microcolinearity with rice. Theor Appl Genet 112:1179–1191

Carman JG (1997) Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory and polyembryony among their relatives. Biol J Linn Soc 61:51–94

Catanach AS, Erasmuson SK, Podivinsky E, Jordan BR, Bicknell R (2006) Deletion mapping of genetic regions associated with apomixis in *Hieracium*. Proc Natl Acad Sci U S A 103:18650–18655

Comai L (2005) The advantages and disadvantages of being polyploid. Nat Rev Genet 6:836–846

Conner JA, Goel S, Gunawan G, Cordonnier-Pratt MM, Johnson VE, Liang C, Wang H, Pratt LH, Mullet JE, DeBarry J, Yang L, Bennetzen JL, Klein PE, Ozias-Akins P (2008) Sequence analysis of bacterial artificial chromosome clones from the apospory-specific genomic region of *Pennisetum* and *Cenchrus*. Plant Physiol 147:1396–1411

Crane CF (2001) Classification of apomictic mechanisms. In: Savidan Y, Carman J, Dresselhaus T (eds) The flowering of apomixis: from mechanisms to genetic engineering. CIMMYT, IRD, Eur. Comm. DG VI (FAIR), Mexico, pp 24–43

Crane CF, Carman JG (1987) Mechanisms of apomixis in *Elymus rectisetus* from Eastern Australia and New Zealand. Am J Bot 74:477–496

do Valle CB, Glienke C (1993) Towards defining the inheritance of apomixis in *Brachiaria*. Apomixis Newsl 6:24–25

do Valle CB, Glienke C, Leguizamon GOC (1994) Inheritance of apomixis in *Brachiaria*, a tropical forage grass. Apomixis Newsl 7:42–43

Dujardin M, Hanna W (1984) Cytogenetics of double cross hybrids between *Pennisetum americanum – P, purpureum* amphiploids and *P. americanum X Pennisetum squamulatum* interspecific hybrids. Theor Appl Genet 69:97–100

Freitas DYH, Nassar NMA (2013) Apomixis in cassava: advances and challenges. Genet Mol Res 12(2): 988–994

Garcia-Aguilar M, Michaud C, Leblanc O, Grimanelli D (2010) Inactivation of a DNA methylation pathway in maize reproductive organs results in apomixis-like phenotypes. Plant Cell 22:3249–3267

Grimanelli D, Leblanc O, Espinosa E, Perotti E, González de León D, Savidan Y (1998) Non-Mendelian transmission of apomixis in maize-*Tripsacum* hybrids caused by a transmission ratio distortion. Heredity 80:40–47

Grossniklaus U, Koltunow AM, van Lookeren M (1998) A bright future for apomixis. Trends Plant Sci 3:415–416

Gupta P, Shivanna KR, Mohan Ram HY (1996) Apomixis and polyembryony in the guggul plant, Commiphora wightii. Ann Bot 78:67–72

Hanna WW (1986) Utilization of wild relatives of pearl millet. In: Proceedings of the International Pearl Millet Workshop. ICRISAT, Patancheru, pp 33–42

Hanna WW (1995) Use of apomixis in cultivar development. Adv Agron 54:333–350

Hojsgaard DH, Martínez EJ, Acuña CA, Quarin CL, Pupilli F (2011) A molecular map of the apomixis-control locus in *Paspalum procurrens* and its comparative analysis with other species of *Paspalum*. Theor Appl Genet 123:959–971

Kindiger B, Sokolov V, Dewald C (1996) A comparison of apomictic reproduction in eastern gamagrass (*Tripsacum dactyloides* (L.) L.) and maize-*Tripsacum* hybrids. Genetica 97:103–110

Koltunow AM, Grossniklaus U (2003) Apomixis: a developmental perspective. Annu Rev Plant Biol 54:547–574

Koltunow AM, Bicknell RA, Chaudhury AM (1995) Apomixis: molecular strategies for the generation of genetically identical seeds without fertilization. Plant Physiol 108:1343–1352

Lakshmanan KK, Ambegaokar KK (1984) Polyembryony. In: Johri BM (ed) Embryology of angiosperms. Springer, Berlin, pp 445–474

Leblanc O, Mazzucato A (2014) Screening procedures to identify and quantify apomixis. In: Savidan Y, Carman JG, Dresselhaus T (eds) The flowering of apomixis: from mechanisms to genetic engineering. CIMMYT, IRD, European Commission DG VI (FAIR), Mexico, pp 121–136

Leblanc O, Peel MD, Carman JG, Savidan Y (1995a) Megasporogenesis and megagametogenesis in several *Tripsacum* species (Poaceae). Am J Bot 82:57–63

Leblanc O, Grimanelli D, Gonzfilez-de-Leon D, Savidan Y (1995b) Detection of the apomictic mode of reproduction in maize-*Tripsacum* hybrids using maize RFLP markers. Theor Appl Genet 90:1198–1203

Leblanc O, Grimanelli D, Perotti E, Serratos A, Pointe C, Pressoir G, Savidan Y (1998) Transferring apomixis through wide crosses: a five year report. Proceedings of the XVth international congress on sexual plant reproduction. Wageningen, pp 16–21

Lone FA, Lone S (2013) Apomixis in flowering plants – an overview. Int J Adv Res 1:130–136

Lubbers EL, Arthur L, Hanna WW, Ozias-Akins P (1994) Molecular markers shared by diverse apomictic *Pennisetum* species. Theor Appl Genet 89(636):642

Marimuthu MPA, Jolivet S, Ravi M, Pereira L, Davda JN, Cromer L, Wang L, Nogué F, Chan SWL, Siddiqi I, Mercier R (2011) Synthetic clonal reproduction through seeds. Science 331:876

Miles JW, Escandon ML (1997) Further evidence on the inheritance of reproductive mode in *Brachiaria*. Can J Plant Sci 77:105–107

Mogie M (1988) A model for the evolution and control of generative apomixis. Biol J Linn Soc 35:127–154

Moore G, Devos KM, Wang Z, Gale MD (1995) Grasses line up and form a circle. Curr Biol 7:737–739

Nogler GA (1984a) Gametophytic apomixis. In: Johri BM (ed) Embryology of angiosperms. Springer, Berlin

Nogler GA (1984b) Genetics of apospory in apomictic *Ranunculus auricomus*. V. Conclusions. Bot Helv 92:123–411

Noyes RD (2006) Apomixis via recombination of genome regions for apomeiosis (diplospory) and parthenogenesis in *Erigeron* (daisy fleabane, Asteraceae). Sex Plant Reprod 19:7–18

Noyes RD, Rieseberg LH (2000) Two independent loci control agamospermy (apomixis) in the triploid flowering plant *Erigeron annuus*. Genetics 155:379–390

Noyes RD, Baker R, Mai B (2007) Mendelian segregation for two-factor apomixis in *Erigeron annuus* (Asteraceae). Heredity 98:92–98

Olmedo-Monfi V, Durán-Figueroa N, Arteaga-Vázquez M, Demesa-Arévalo E, Autran D, Grimanelli D, Slotkin RK, Martienssen RA, Vielle-Calzada J-P (2010) Control of female gamete formation by a small RNA pathway in *Arabidopsis*. Nature 464:628–632

Ozias-Akins, Van Dijk PJ (2007) Mendelian genetics of apomixis in plants. Ann Rev Genet 41:509–537

Ozias-Akins P, Lubbers EL, Hanna WW, MeNay JW (1993) Transmission of the apomictic mode of reproduction in *Pennisetum*: co-inheritance of the trait and molecular markers. Theor Appl Genet 85:632–638

Ozias-Akins P, Roche D, Hanna WW (1998) Tight clustering and hemizygosity of apomixis-linked molecular markers in *Pennisetum squamulatum* implies genetic control of apospory by a divergent locus that may have no allelic form in sexual genotypes. Proc Natl Acad Sci U S A 95:5127–5132

Peel MD (1993) Meiocyte callose in aposporic and diplosporic grasses and in hybrids between bread wheat and *Elymus rectisetus.* MS thesis, Utah State University

Pessino SC, Ortiz JPA, Leblanc O, do Valle CB, Evans C, Hayward MD (1997) Identification of a maize linkage group related to apomixis in *Brachiaria*. Theor Appl Genet 94:439–444

Pessino SC, Evans C, Ortiz JPA, Armstead I, do VaUe CB, Hayward MD (1998) A genetic map of the apospory-region in *Brachiaria* hybrids: identification *of* two markers closely associated with the trait. Hereditas 128:153–158

Petrov DF (1976) Genetically regulated apomixis as a method of fixing heterosis and its significance in breeding. In: Khokhlov SS (ed) Apomixis and breeding. Amerind, New Delhi, pp 18–28

Polegri L, Calderini O, Arcioni S, Pupilli F (2010) Specific expression of apomixis-linked alleles revealed by comparative transcriptomic analysis of sexual and apomictic *Paspalum simplex* Morong flowers. J Exp Bot 61:1869–1883

Pupilli F, Lambobarda P, Cáceres ME, Quarin CL, Arcioni S (2001) The chromosome segment related to apomixis in *Paspalum simplex* is homoeologous to the telomeric region of the long arm of rice chromosome 12. Mol Breed 8:53–61

Pupilli F, Martínez EJ, Busti A, Calderini O, Quarin CL, Arcioni S (2004) Comparative mapping reveals partial conservation of synteny at the apomixis locus in *Paspalum* spp. Mol Genet Genomics 270:539–548

Quarin CL (1992) The nature of apomixis and its origin in Panicoid grasses. Apomixis Newsl 5:8–15

Ravi M, Chan SW (2010) Haploid plants produced by centromere mediated genome elimination. Nature 464:615–618

Ravi M, Marimuthu MP, Siddiqi I (2008) Gamete formation without meiosis in *Arabidopsis*. Nature 451:1121–1124

Richards AJ (1990) The implications of reproductive versatility for the structure of grass populations. In: Chapman GP (ed) Reproductive versatility in the grasses. Cambridge University Press, Cambridge UK, pp 131–153

Richards AJ (1997) Why is gametophytic apomixis almost restricted to polyploids? The gametophyte-expressed model. Apomixis Newsl 9:3–4

Richards AJ (2003) Apomixis in flowering plants. Phil Trans R Soc Lond B 358:1085–1093

Roche D, Hanna WW, Ozias-Akins P (2001a) Gametophytic apomixis, polyploidy, and supernumerary chromatin. Sex Plant Reprod 13:343–349

Roche D, Chen Z, Hanna WW, Ozias-Akins P (2001b) Non-Mendelian transmission of an apospory-specific genomic region in a reciprocal cross between sexual pearl millet (*Pennisetum glaucum*) and an apomictic F1 (*P. glaucum* X *P. squamulatum*). Sex Plant Reprod 13:217–223

Savidan Y (2000) Apomixis: genetics and breeding. Plant Breed Rev 18:13–86

Savidan Y, Grimanelli D, Leblanc O (1995) Transferring apomixis from T*ripsacum* to maize: progress and challenges. In: Taba S (ed) Maize genetic resources. Maize program special report. CIMMYT, Mexico, pp 86–92

Schmelzer GH, Renno JF (1997) Genetic variation in the agamic species complex of *Pennisetum* section *Brevivalvula* (Poaceae) from West Africa: ploidy levels and isozyme polymorphism. Euphytica 96:23–29

Skinner DJ, Baker SC, Meister RJ, Broadhvest J, Schneitz K, Gasser CS (2001) The Arabidopsis *HUELLENLOS* gene, which is essential for normal ovule development encodes a mitochondrial ribosomal protein. Plant Cell 13:2719–2730

Spillane C, Steimer A, Grossnicklaus U (2001) Apomixis in agriculture: the quest for clonal seeds. Sex Plant Reprod 14:179–187

Stebbins GL (1950) Variation and evolution in plants. Columbia University Press, New York

Van Baarlen P, Verduijn M, Van Dijk PJ (1999) What can we learn from natural apomicts? Trends Plant Sci 4:43–44

Van Dijk P, Van Damme J (2000) Apomixis technology and the paradox of sex. Trends Plant Sci 5:81–84

Vielle-Calzada JP, Nuccio ML, Budiman MA, Thomas TL, Burson BL, Hussey MA, Wing RA (1996) Comparative gene expression in sexual and apomictic ovaries of *Pennisetum ciliare* (L.) Link. Plant Mol Biol 32:1085–1092

Vijverberg K, Van der Hulst RGM, Lindhout P, Van Dijk PJ (2004) A genetic linkage map of the diplosporous chromosomal region in *Taraxacum officinale* (common dandelion; *Asteraceae*). Theor Appl Genet 108:725–732

Plant Volatile Chemicals and Insect Responses

27

Pathipati Usha Rani

Abstract

Production and emission of volatile organic chemicals (VOCs) is a general phenomenon in most of the plant communities. Insects respond to plant chemicals in a variety of interesting ways which has tremendous potential in pest management programmes. In a normal state, plants release a spectrum of species-specific VOCs through their leaf, stem, flower, and even root surfaces, and they become host location cues to insects leading to their colonisation on the plant, whereas the plants damaged by insect feeding emit qualitatively and quantitatively different volatiles that become host/prey location signals to the wandering insect natural enemies causing the reduction of the pest population. In addition to this, plant volatiles synergise or deter the insect sex pheromonal activities. Insects possess excellent chemosensory system for detection of volatile chemicals. The advent of electrophysiology, scanning, and transmission electron microscopic techniques made insect sensory physiology/morphology an admirable tool to unravel the mechanisms underlying the insect responses to plant volatile compounds.

Keywords

Plant volatiles • Plant defence • Insect natural enemies • Host location • Sensory receptors

27.1 Introduction

Insects are the most dominating, interesting, and curious forms of life exceeding in numbers as well as species. Among these more than 50 % exploit plants for their feeding, oviposition, and even as habitat. Almost all plants are constantly challenged with facing trade-off between

P. Usha Rani (✉)
Biology and Biotechnology Division, CSIR, Indian Institute of Chemical Technology,
Tarnaka, Hyderabad 500 007, Telangana, India
e-mail: usharani65@yahoo.com; purani@iict.res.in

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_27,

growth, development, and reproduction and, on the other hand, defence against biotic and abiotic stresses. Plants have evolved diverse mechanisms to tackle both the challenges. Of late, the constitutive and induced responses to attack, including systemic emissions of herbivore-induced volatiles, gained utmost interest in the area of insect-plant interactions.

Plants are rich banks of various chemicals, and they comprise multitude of quantitatively and qualitatively different chemicals which even in normal stage without any stimulation also release them into their surrounding atmosphere. These chemicals chiefly volatiles in nature play important role in insect behaviour and plant-insect interactions and have wider importance in plant biology (Holopainen 2004). Release of volatile organic compounds can help plants acclimatise to abiotic stress, contributing to thermotolerance (Sharkey and Yeh 2001). The plant-emitted odours surround them and provide access to different herbivore insects as they act as important clues to select a plant for egg laying or for feeding. These odours are comprised of complex mixtures of chemicals and volatiles produced by different plant biosynthetic pathways. The composition of the blend is species specific as it varies qualitatively and quantitatively according to plant and herbivore species.

In a word, insect's survival and reproduction depends on plant volatile perception and responding to them in appropriate manner. Predators need to know the type of plants eaten by their prey, and this is achieved only by recognising the chemicals emanating from host plants of their prey.

Plants produce secondary metabolites in nature as a defence mechanism against pathogenic and insect attack. They constitute a large source and an amazing diversity of low molecular weight compounds, and about 100,000 have already been isolated from plant sources (Jeong and Hee Park 2006), 50,000 of these have been elucidated (De Luca and St Pierre 2000), whereas 4,000 new compounds are being discovered every year. There is great variation in plant volatile emission between the taxa and sometimes even between the types, or even intraspecific differences occur in the chemical components, concentrations, or total amounts. Some volatiles are characteristic of certain plants, such as sulphides in onions, *Allium* spp., and menthol in *Mentha* spp. (Croteau et al. 2005). The most common plant volatile and insect interaction known till recent is the example of floral scent volatiles evolved to attract insect pollinators such as bees and a few kinds of butterflies. Plant-emitted chemicals are imperative for insects to communicate with plant populations and to solve many challenges they face. In fact, plant species diversity has been linked to the diversity and abundance of herbivores and natural enemies (Haddad et al. 2001). Feeding by insect larvae also enhance the number and quantity of secondary metabolites. Elicitors from insect saliva have been shown to be responsible for modifying the cell metabolism. These modifications are designed to enhance the productivity of useful metabolites in the plant tissues. The emitted volatiles indicate the plant identity (through species-specific volatiles) and even status (infested by other pests or pathogens). The insects, their natural enemies, and even neighbouring plants respond to these volatiles emitted into plant's surrounding atmosphere. Plant constitutive volatiles emitted through their vegetative parts may attract the insect larvae for feeding purpose and gravid females for oviposition, but volatiles from feeding damaged plants may act as repellents to both (Fig. 27.1). Plant volatiles may indicate plant stress status (Dudareva et al. 2006) and also provide information, allowing discrimination between hosts and non-hosts to the approaching insects.

There is a significant progress in understanding of the intriguing complexity of active defences of plants and their effects on insects at different trophic levels. Individual plants are often attacked by multiple herbivores and disease species during their lifetime. The bouquet of volatiles released from insect or pathogen-damaged plants come from at least three biosynthetic pathways. Octadecanoid pathway produces green leaf volatiles and cis-jasmine, while the tryptophan pathways produces indole and methyl salicylate

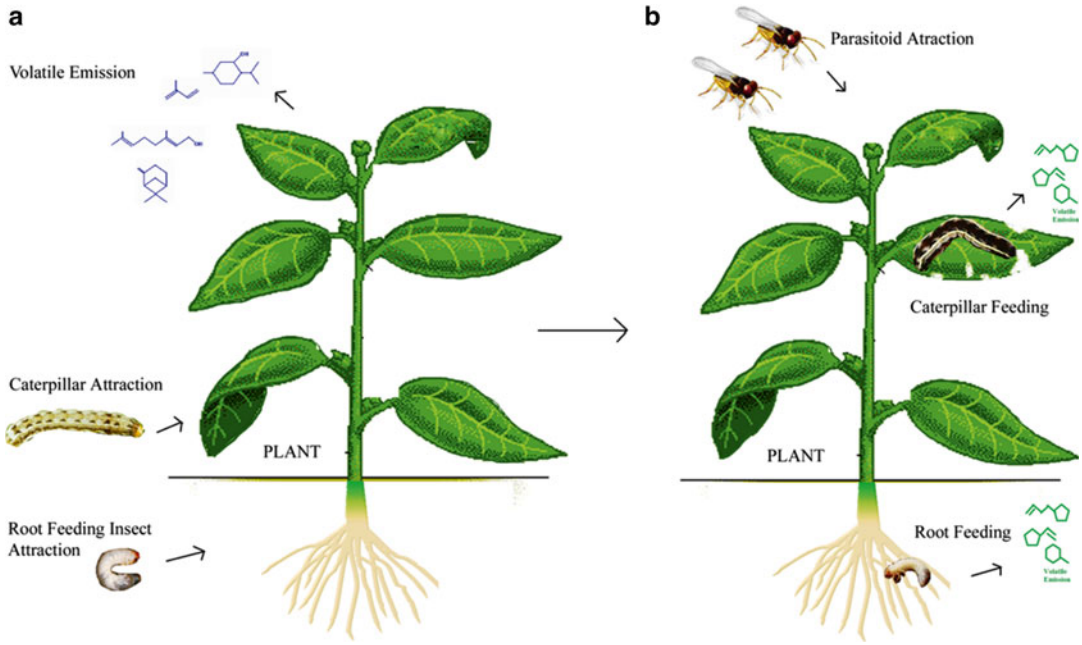


Fig. 27.1 Plant volatile chemical attraction to insects and their natural enemies. (**a**) The chemicals from normal (healthy plants) attracting pests. (**b**) Pest-infested plant chemicals attracting pest's natural enemies (parasitoids and predators)

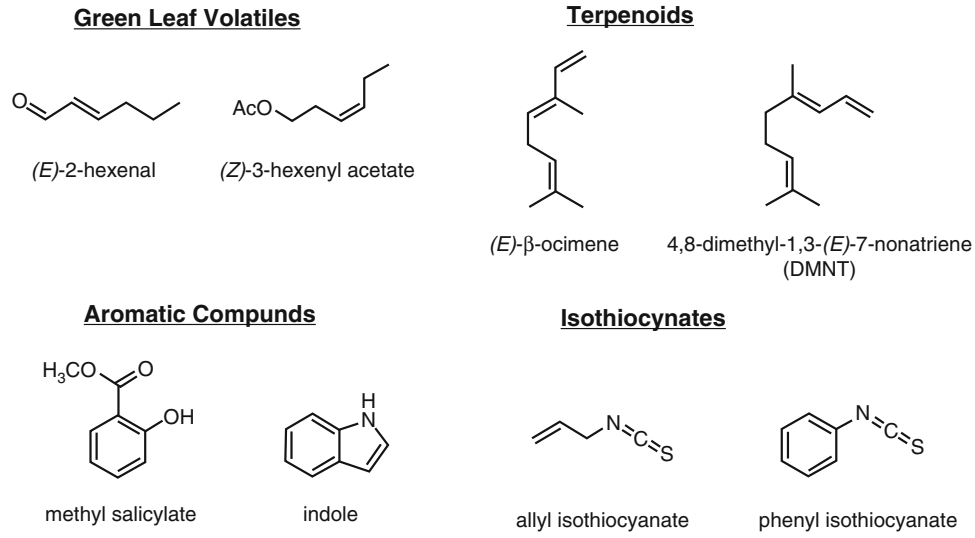


Fig. 27.2 Some common plant volatiles produced in different biosynthetic pathways

and finally the isoprenoid-derived pathways produce terpenes. Many enzymes are involved in plant volatile biosynthesis and even one gene coding one enzyme also can produce several compounds in particular ratio (Schnee et al. 2006) (Fig. 27.2).

27.2 Behavioural Effects of Volatiles on Herbivore Insects

Chemicals from plants play an important role in plant-insect interactions. Oviposition often precedes insect herbivory and is a particularly important event for both insect and plant fitness. Feeding or oviposition occurs often after the herbivore has found and accepted the plant as a suitable host (Miller and Strickler 1984). Insects that feed on plants use host plant volatiles to locate their host plant. Unfortunately an insect has to choose from a vast number of volatiles of varying compositions, and ratios of emissions depend on the plant type, genus or species, and also the time of release, etc., which make the insect job difficult. As a result, the study of insect-plant interactions became complex and needed to be investigated through a multidisciplinary approach, and also these studies are important for understanding ecosystems.

Bruce and Pickett (2011) contributed an excellent review on how phytophagous insects use blends of volatile compounds to distinguish between suitable and unsuitable host plants and the complex-chemical and physiological interactions between insects and their host plants. Host plant location is vital to a phytophagous insect for its nutritional as well as oviposition requirements. Hence, the recognition of proper host plant is done by using either species-specific compounds or specific ratios of ubiquitous compounds.

Plants in normal condition emit several volatile chemicals through their leaf surfaces which are highly beneficial to the prospective pest as these chemicals play a major role in their attraction towards the source, thus aiding greatly in host location. A study on oviposition preferences of the gravid female moths of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) towards the volatile chemicals from the crop plants marigold, maize, sunflower, and pigeon pea in a cotton (non-Bt) ecosystem revealed that young pods of pigeon pea generated attraction to the moths and the young pods have more chemicals emitted through their surfaces.

McCallum et al. (2011) investigated the influence of increased production of volatile monoterpene (S) linalool in transgenic *Nicotiana tabacum* on the attraction of a herbivorous insect, *H. armigera*, and the direct fitness costs of plant volatile production on insect growth and survival of offspring. *H. armigera* laid fewer eggs on transgenic plants compared with non-transformed controls, indicating that increased linalool emissions have a deterrent effect on *H. armigera* oviposition. (S) Linalool, whether in volatile or sequestered form, does not appear to have a direct effect on offspring fitness in this moth (McCallum et al. 2011). Reisenman et al. (2010) using artificial odour blends of linalool has suggested that the R (for *Rectus*, Latin for right) and S (for *Sinister*, Latin for left) isomers of linalool may be perceived differently by the moth, *Manduca sexta*, with both isomers being attractive to nectar feeding moths, but only the R isomer deterring the ovipositing moths. Bidart-Bouzat and Kliebenstein (2011) showed that plant responses were not influenced by the degree of specialisation of insect herbivores but are more strongly shaped by insect taxa (whether it is an aphid or a lepidopteran) likely due to their different feeding modes. Often mode of feeding of insects is also important as it is associated with the loss of biomass, and each type of feeding lead to different ranges of tissue damage. Lepidopteran larvae consume more amount of plant tissue, while sucking pests like aphids and bugs cause little plant tissue damage. Herbivore insects not only remove leaf tissue as such and sometimes even parasitise the vascular transport system of plants, some tunnel through the stems and roots, while a few bore and mine.

Different cultivars of same plant may emit different volatiles and may even differ in attraction to insects. The differences in quality or quantity of volatiles formed among the plant species vary significantly, and this is may be due to natural genetic variation between individuals. Degen et al. (2004) witnessed a great variation in the total amount of volatiles emitted by 31 maize inbred lines. A number of studies reported similar results showing that genetic variation accounts for large variability in volatile blends

released by plants (Takabayashi et al. 1991; Hare 2007). The aphid *Rhopalosiphum padi* responded differently to the four cultivars of barley to which they are exposed, and it is observed that certain combinations of plants became significantly less acceptable (Ninkovic et al. 2002). Plants of the same genotype grown in different environmental conditions also can vary in their emissions so also in different times of the day (Agelopoulos et al. 2000).

The behavioural bioassays with the larvae of *Antheraea assamensis*, Helfer (Lepidoptera: Saturniidae) and the feeding preferences of this insect towards the four host plants, namely, *Persea bombycina* King ex. Hook (Laurales: Lauraceae), *Litsea polhantha* Jussieu, *L. salicifolia* Roxburgh ex. Nees, and *L. citrata* Blume, showed the involvement of several chemicals in host plant finding as well as host plant feeding (Neog et al. 2011). Tests with different plant chemicals indicated a mixture of caryophyllene, decyl aldehyde, and dodecyl aldehyde attracting them towards the host leaves and stimulate feeding. The flavonoids, myricetin and 7, 2', 4' trimethoxy dihydroxy flavone with sterol compound β-sitosterol also accelerated the feeding behaviour by *A. assamensis* larvae. The terpenoids in plants are well-known attractants for host as well as parasitic insects. Herbivores respond to the changes in terpenoid compositions, for example, in response to transgenically upregulated concentrations of linalool and nerolidol (Aharoni et al. 2003) or (*E*)-β-farnesene (Beale et al. 2006) in Arabidopsis. Since even a slight modification qualitative or a quantitative affect the herbivore insect response, thus the modification of crop volatile emissions contribute to increase anti-herbivore effects.

Most of the reports on herbivore-induced volatiles are available on leaf-chewing lepidopteran insects. However, several Coleopterans also respond to these volatiles, and the majority of the published reports are on forest pests. Similar to any other plant-feeding insects, herbivore beetles too react to plant-emitted chemicals. Beetles are attracted to unhealthy trees and shrubs, as weakening trees produce certain volatile chemicals that attract bark beetles and others borers. The Colorado potato beetle was attracted to the specific blend of C6 alcohols and aldehydes (GLVs) in potato, and attraction was lost if any of the components was increased in concentration (Visser and Ave 1978). Traps baited with (−)-α-pinene and ethanol, the major constituents of the pine trees, lured the large wood-boring pine beetles, Cerambycidae: *Acanthocinus nodosus*, *A. obsoletus*, *Arhopalus rusticus nubilus*, *Asemum striatum*, *Monochamus titillator*, *Prionus pocularis*, *Xylotrechus integer* and *X. sagittatus sagittatus*, *Buprestis lineata* (Buprestidae), *Alaus myops* (Elateridae) and *Hylobius pales*. Traps baited with ethanol and (−)-α-pinene are used to detect and monitor common large wood-boring beetles at different entry points between the countries to prevent the spread of the pests (Miller 2006).

In an open track Y olfactometer, both sexes of predatory beetle, *Trogossita japonica* (Coleoptera: Trogosstidae), showed positive responses to volatiles of the host tree *Pinus densiflora*, indicating that secondary attraction is probably important in host selection by this species. The predatory beetles were attracted to the mixtures of terpenoids, −(+)-α-pinene, −(−)-α-pinene, (+)-β-pinene, (−)-β-pinene and the (+)-α-pinene plus ethanol mixtures and a non-host chemical, 1-octene-3-ol. Among these the (+) alpha pinene and the pinene-ethanol mixture was highly attractive (Nakamuta and Usha Rani, unpublished data).

Another interesting report concerning the beetle pests is the occurrence of certain plant-based chemicals in the beetle pests feeding on *Citrus unshiu* plants. Sesquiterpene hydrocarbons (SHC), including β-elemene, β-caryophyllene, α-humulene, α-farnesene and several unidentified compounds, were found in the elytra of the white-spotted longicorn beetle *Anoplophora malasiaca* (Thomson) (Yasui et al. 2008). They detected significant quantities of these chemicals from the air around both mechanically wounded and beetle-infested branches in solid-phase microextraction (SPME) and gas chromatography (GC) analyses. They hypothesised that the citrus SHCs is adsorbed in, retained on and released from the wax layer of the beetle elytra.

The beetle feeding on the tree branches result with the release of these compounds which may provide the indirect signals about the presence of beetles to others in the field as well as act the communication system of *A. malasiaca*.

Different kinds of results were also evident with respect of chemicals effect on their fellow insects belonging to the same species. The feeding-induced volatiles attract the conspecifics and increase their incidence on the plant. In a series of behavioural bioassays, Sun et al. (2010) proved that volatile compounds from plants infested by tea weevil, *Myllocerinus aurolineatus* (Voss) (Coleoptera: Curculionidae), were attractive to conspecific weevils and the weevil infestations dramatically increased the emission of volatiles (Z)-3-hexenal, (Z)-3-hexenol, (E)-beta-ocimene, linalool, phenylethyl alcohol, etc. Role of homoterpenes in attracting parasitoids and predators of herbivores is a known phenomenon. A beetle pest *Meligethes aeneus* F. (Coleoptera: Nitidulidae) is a pollen-feeding insect on oilseed rape, *Brassica napus* L. Both male and female *M. aeneus* were attracted to higher concentrations of VOCs, particularly to the three common volatiles (β-caryophyllene, (*E*)-β-farnesene and (*Z*)-β-ocimene) emitted by these plants 72 h after floral bud injury and oviposition by their conspecifics but avoided much higher doses (Piesik et al. 2013). The western tarnished plant bug, *Lygus hesperus* Knight, causes severe damage to Alfalfa and cotton. It was found that fifth instar *L. hesperus* responded to odours associated with conspecifics and alfalfa, *Medicago sativa* L, whereas female insects only responded preferentially to vegetative and flowering alfalfa where the conspecifics were fed for 1–3 days (Blackmer et al. 2004). Analysis of headspace volatiles revealed differences in the chemical constituents of the bug-damaged and normal vegetative and flowering alfalfa plants.

It is also interesting to find how flower-feeding insects respond to the plant-emitted volatiles containing large number of chemicals. Often folivory or florivory may also alter flower chemistry by increasing toxin concentrations in nectar and flower tissues (Adler et al. 2006). Also plants in the vegetative stage may emit volatile chemicals that are emitted during the flowering stage. In an excellent and elaborate study on the effects of herbivore-induced plant volatiles and their interaction with flower-visiting insect, Lucas-Barbosa et al. (2011) highlighted the need to integrate the study of plant defence and pollination since it is a requisite for the advancement of plant biology. They stressed the need to examine the volatile emission of the entire plants including the flowering stage and the systemic nature of herbivore-induced plant responses and the behavioural responses of antagonistic and mutualistic insect (Lucas-Barbosa et al. 2011). Since flowering plants differ in many physiological and biochemical aspects from vegetative plants, the induced plant responses also might vary. Seven major classes of compounds normally occur in flower volatiles, aliphatics, benzenoids/phenylpropanoids, C5-branched compounds, terpenoids, nitrogen-containing compounds, sulphur-containing compounds and a class of various cyclic compounds (Knudsen et al. 2006). The volatile blend of brassicaceous and solanaceous plants consist of all seven major classes constitutively; however, volatile composition differs significantly among closely related species (Raguso et al. 2003). It appears that both pollinators and herbivores exploit the same chemicals (Wiemer et al. 2009). A homoterpene, 4, 8-di methyl-1.3.7-nonatriene was induced by folivory in brassicaceous plants (Abel et al. 2009) and a solanaceous plant, *Solanum lycopersicum*, and this monoterpene which is known to be emitted by various flowers (Kaiser 1993) is also involved in parasitoid and predator attraction (Mumm and Dicke 2010; Turlings et al. 1990).

Not all plant extracts are attractants or participate in the host location. Some chemicals in their volatile form even repel the pests and thus preventing certain insects reaching that plant. Glinwood et al. (2003) showed that a direct chemical interaction between weeds and crop plants can affect insects that use the plants as hosts. They found that the plants exposed to root exudates from the couch grass, *Elytrigia repens*, became less acceptable to bird cherry-oat aphid *Rhopalosiphum padi*, a serious pest of cereals. The interactions between certain weeds and

Fig. 27.3 A predatory ladybird beetle, *Coccinella septempunctata*, feeding on aphids

barley also affect aphid natural enemies such as *Coccinella septempunctata*. It was found that odour of barley previously exposed to volatiles from *C. arvense* was more attractive to *C. punctata* than odour of unexposed plants (Ninkovic and Pettersson 2003) (Fig. 27.3). Plant chemicals in their volatile form serve as airborne semiochemicals, promoting or deterring insect-plant interactions. Plant volatiles might attract the herbivore insects even from a distance in normal state. These volatiles from the same plant after herbivore feeding can deter the approaching insect pest of even the same species. Healthy and pest uninfested castor bean plants in their normal state attract one of its major pest, *Spodoptera litura*, in field conditions as well as in laboratory behavioural bioassays in Y olfactometers. However, the *S. litura* feeding for few hrs on the plant deter new *S. litura* larvae that attempt to come near the plant. Thus, *S. litura* larvae are avoiding the crowded plants to reduce the competition. We also found that stress frequency, intensity, duration and timing will affect the direction of response to *S. litura* herbivory. Advances in the plant chemical research, namely, the isolations, characterisation and identifications by modern analytical techniques focus on the use of plant kairomones to lure, trap and kill the gravid female moths. The chemicals from the extracts of four plants, *Pimpinella anisum* L. (Apiales: Apiaceae), *Galium longifolium* (Sibth. and SM.) (Gentianales: Rubiaceae), *Retama raetam* Forssk (Genisteae: Fabaceae) and *Ballota undulata* Bentham (Lamiales: Lamiaceae), had a repellent effect and thus prevented the sweet potato whitefly, *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae), from reaching the source (Ateyyat et al. 2009). Fatouros et al. (2012) studied the effects of volatiles on gravid females of specialist cabbage butterfly (*Pieris brassicae*). These butterflies were repelled by volatiles from plants induced by cabbage white butterfly eggs. Two of its parasitic wasps *Trichogramma brassicae* and *Cotesia glomerata* displayed an attraction towards these chemicals from the wild crucifer *Brassica nigra* plant. However, volatiles from plants induced by eggs of the generalist moth *Mamestra brassicae* did not evoke any positive or negative response in these insects. Analysis of the plant's volatile metabolomic profile by gas chromatography-mass spectrometry and the structure of the plant-egg interface by scanning electron microscopy revealed that the plant responds differently to egg deposition by the two lepidopteran species. These observations indicate that insect oviposition can induce specific plant responses that affect the other trophic levels. After a successful mating, a gravid female's prime task is to find a suitable host plant where she can lay her precious eggs, so that, her off springs can grow with minimum obstacles. Biosynthesis and release of mating signals as well as production of eggs may be influenced or even determined by plant chemicals (Hilker and Meiners 2011). In the words of Stadler (2002), an eminent researcher in the insect-plant relation field, 'odour of plants, the plant surface and the plant's interior guide egg-laying herbivorous insects to their host plants and influence the choice of oviposition sites'. The plant-produced toxins may protect the eggs from egg predators and parasitoids (Blum and Hilker 2002). The concentration of leaf volatile and atmospheric gases present in the leaf boundary layer surrounding the eggs may specifically affect embryonic development.

Volatile phytochemicals can serve as airborne semiochemicals, promoting or deterring interactions between plants and insect herbivores. For example, wheat seedlings without herbivore

damage attract aphids, whereas odours released from wheat seedlings with a high density of aphids repel other aphids (Quiroz et al. 1997). For swallowtail butterflies, volatiles from host plants enhance the effect of contact stimulants, increasing landing rates and oviposition relative to non-host plants (Feeny et al. 1989). Fertilised plants tend to contain higher nitrogen content and many insects seek sources. This is true with aphids and other sap-sucking insects which also prefer nitrogen-fertilised plants and quickly build their populations. Nitrogen is an important component of major plant secondary chemical components such as alkaloids, tannins and other insect digestive reducers. Larval stages of insects need higher water contents for their healthy development and plants that have lesser water contents slowed down the growth of the insect. In contrast, desert grasshoppers are attracted to drought-stressed plants since sugars and nitrogen are more concentrated in water-stressed plants, so that insect obtains instant usable food in a short time. In general younger leaves of plants tend to have more nitrogen and water content than older leaves, hence more density of pest populations. Whereas water and nitrogen content of tree leaves are much lower than the low-growing perennial plants, making them less vulnerable to pests.

Not only herbivore insects or plant pathogens induce the production of volatiles in plants but other herbivores; 'root feeders' are also important in this act. Insects and the nematodes are two major groups of organisms which utilise plant roots as their diet, and several insects including chewers, sap suckers and gall makers feed on roots during at least one life stage (Rasmann and Agrawal 2008). Root feeding insect's attraction to root-emitted volatiles is known in several crop-pest systems, such as attraction of larvae of *Sitona hispidulus* to the roots of *Medicago sativa* and *Trifolium pratense* (Wolfson 1987) and volatiles from fresh perennial ryegrass roots to the larvae of *Costelytra zealandica* (Sutherland and Hillier 1972).

These belowground herbivores through physiological and physical changes of roots have the potential to shape plant communities (De Deyn et al. 2003), belowground microorganism and macroorganism communities (Wardle 2006), as well as aboveground arthropod communities (Bezemer and van Dam 2005). At belowground level too, volatile organic compounds have been found to attract the root feeders to locate the food source (Wenke et al. 2010). The major volatile attractant (proved in at least 20 studies examined) is CO_2. Detection of CO_2 seems however to be dose dependent, and soil insects are able to detect very small differences in the concentration of CO_2 (Johnson and Gregory 2006). It is also found that while lower concentrations of CO_2 attract the belowground herbivores, high concentrations may cause disorientation. Monoterpenes are the volatile chemicals, and in many plants these chemicals played a major role in host location by a herbivore. Monoterpenes in Carrot (*Daucus carota* ssp. *sativus*) plants activate the orientation of forest cockchafer larvae, *Melolontha hippocastani*, while fatty acids in oak (*Quercus* sp.) trees perform the same function (Weissteiner and Schütz 2006).

Plant-parasitic nematodes are the other group of organisms that feed on roots of several important plants feeding exclusively on the cytoplasm of living plant cells and cause extensive damage and crop losses. Plant signals are essential for nematodes to locate hosts and feeding sites, however, besides the general signal furnished by carbon dioxide emissions.

Recently, it was shown that phytopathogenic nematodes can follow gradients of herbivore-induced terpene volatile organic compounds. A good example is that *Tylenchulus semipenetrans* nematodes were more attracted to *citrus* roots infested by weevil larvae compared to uninfested plants (Ali et al. 2011). The volatile terpenes from roots, β-pinene, α-pinene, limonene, geijerene and pregeijerene, were found to be attractants to plant parasitic nematode. Another fascinating discovery is that the use of leaf volatiles produced in host location process of parasitic plants is parallel to the way herbivorous insects use the same compounds (Runyon et al. 2006).

Individual chemicals in a blend of volatiles play a role in eliciting a behavioural change in the

insects exposed. Experiments revealed that only appropriate blends or combination of volatiles induces a response in insects rather than single components. The Colorado beetle, *Leptinotarsa decemlineata*, attracted to potato plant volatiles, but none of the individual components ((E)-3-hexen-1-ol, (Z)-2-hexen-1-ol, (E)-2-hexen-1-ol or (E)-2-hexenal) evoked any response (Visser and Ave 1978) (Fig. 27.4). In the case of grape berry moth *Paralobesia viteana*, a mixture of seven components composed of (E)-linalool oxide, (Z)-linalool oxide, nonanal, (E)-4,8-dimethyl-1,3,7-nonatrien,decanal, (E) caryophyllene and germacrene-D elicited equivalent levels of attraction to grape shoots in a wind tunnel bioassay (Cha et al. 2008). Any one compound removed from the blend decreased the female moth response and suggest that these components are essential for the orientation.

It is fascinating to know that certain host-associated compounds are learnt by exposed insects better than others. *Apis mellifera* conditioned to oil seed rape floral volatiles learnt linalool, 2-phenylethanol and methyl salicylate more readily than other compounds (Pham-Delegue et al. 1993). Another interesting phenomenon is the avoidance of insects to certain volatiles from non-host odours as well as to nutritionally unsuitable hosts. Two isothiocyanates, 3-butenyl isothiocyanate and 4-pentenyl isothiocyanates characteristic of the Brassicaceae, upon which *Aphis fabae* cannot feed, repelled them in olfactometers bioassays (Nottingham et al. 1991). For bark beetles, also the avoidance of the non-host volatiles is important for their host location process (Zhang and Schlyter 2004).

27.3 Modification of Plant Volatile Profile: Pest – Natural Enemy Interactions

Plant volatiles play a major and conspicuous role in tritrophic interactions. Many plants release characteristic volatile organic compounds (VOCs) when attacked by a feeding herbivore, and this odour blend is different from the odour of undamaged or mechanically damaged plants. These VOCs guide the parasitoids, predators or other members of the third trophic levels to the herbivore and towards the plant that harbour their host (Turlings et al. 1995; De Moraes et al. 1998) and is considered as an effective plant defence strategy. Induced plant chemicals may directly affect the feeding herbivore performance by reducing its feeding intensity or indirectly influence the herbivore natural enemies by attracting them towards the plant providing the information

Fig. 27.4 Colorado beetle, *Leptinotarsa decemlineata*, feeding on a potato plant

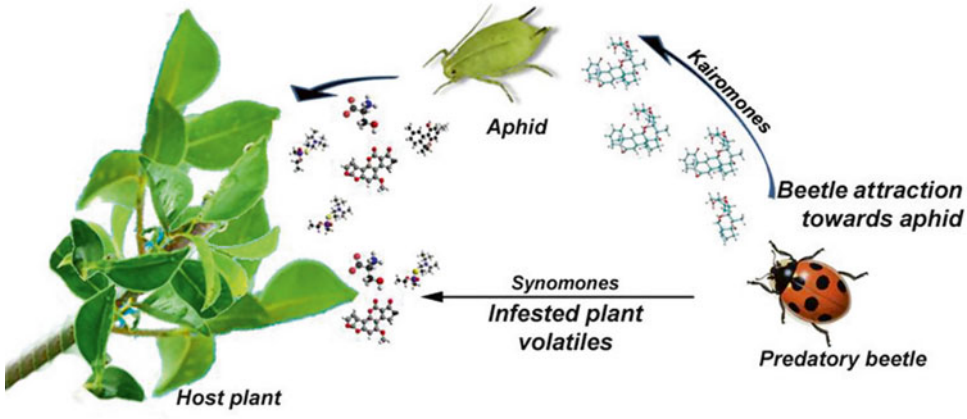


Fig. 27.5 A beetle attraction towards its prey and prey host plant: volatile chemical interactions

about the attacker (herbivore) (Fig. 27.5). The emission of herbivore-induced volatiles has been documented to evoke responses to insects belonging to five orders Lepidoptera, Diptera, Thysanoptera, Coleoptera, Hemiptera and mites (Mumm and Dicke 2010). Making the pest establishment easier, even the plants infested with herbivores or pathogens also emit volatile chemicals. However, these volatile chemicals are different than that of the pest undamaged or normal healthy plants. Among the herbivore-induced plant volatiles, green leaf C6-volatiles are the most important ones as they play major role in mediating the behaviour of herbivores and their natural enemies. The wound-induced ubiquitous (Z)-3-hexenol, a C6-alcohol synthesised in the lipoxygenase pathway, was proved to be the most important info chemical for the herbivore repellence or attractance and natural enemy attraction (Wei and Kang 2011). Insect-specific plant responses are mediated by constituents in the oral secretions and regurgitates of herbivores. Fatty acid-amino acid conjugates (FAC) are found in regurgitate of many insect herbivores and have been shown to elicit herbivore-specific responses.

Such induced indirect responses may enhance the abundance and activity of carnivorous arthropods and consequently make a plan – carnivore dense space. Herbivore feeding (Dicke et al. 1990a, b) and oviposition (Hilker and Meiners 2006) may in turn modify the plants' volatile profiles by the induction of chemical blends associated with tissue damage. Certain C6 alcohols and aldehydes found in green leaves and shoots (GLVs) (Visser et al. 1979) and methyl salicylate, methyl jasmonate and ethylene are involved in stress signalling within and between individual plants (Farmer and Ryan 1990). It is a difficult task to identify the chemicals involved in the blend of induced volatiles that result in carnivorous attraction. These blends are complex mixtures often containing up to 200 components and making the approach of insect natural enemy complicated. The behaviourally active components are totally identified in only a very a few systems, i.e. lima bean, cucumber, potato, tobacco, brinjal, sweet pepper, rose, apple, etc. (Mumm and Dicke 2010).

The relation between the plants and insects is a not only a complex one but very fascinating, and scientists have been exploring these complex interactions since 1980s when Marcel Dicke, professor of insect-plant interactions at Wageningen University in the Netherlands, says he was the first to show that 'plants communicate with the enemies of their enemies'. These responses mediate interactions within organisms on multiple trophic levels leading to complex ecological consequences (Kessler and Halitschke 2007).

Herbivore challenge on plants leads to the expression of defence genes as a direct defence response that stimulates the production of secondary metabolites such as nicotine or protease

inhibitors, and these chemicals make the plant unpalatable or lead to the direct toxic or growth inhibitory changes in the herbivore. More than 100 examples of systemic signalling responses have been found in plants, which bring about production of protease inhibitors that harm the digestive system of insects that chew on the plants. The insect growth and development is delayed, and during which time, they become more prone to parasite attacks. Many a time these chemicals also indicate the presence of the feeding herbivore to the potential herbivore that discovered the plant and prepared to approach, so it has the effect of deterring other herbivores from laying eggs (Kessler and Baldwin 2001). Moths avoid laying eggs on plants that are giving off these volatile signals, either because they want to avoid competition for their offspring or because they don't want to lay their eggs on a plant that is going to attract predators.

Most of the plants consist of long-chain hydrocarbons as part of their emissions, which has profound influence on the approaching insects. Parasitic insects use these chemicals along with other groups of chemicals such as terpenoids, polyphenols and aldehydes and ketones, etc., as host location signals. Bioassay of 11 hydrocarbons, viz., pentadecane, heptadecane, eicosane, heneicosane, docosane, tricosane, tetracosane, pentacosane, hexacosane, octacosane and hexatriacontane with the egg parasitoid, *Trichogramma chilonis* Ishii, was useful in detecting the most active chemicals that took part in the host location of this parasitoid. Octacosane recorded the highest parasitoid activity index followed by docosane and tricosane. However, the tricosane caused the highest percentage of parasitisation, which was at par with octacosane and docosane (Padmavathi and Paul 1998). Similar studies with the maize weevil, *Sitophilus zeamais*, an economically important pest of stored grains in tropical and subtropical regions of the world, also demonstrated the role played by VOCs from the maize (*Zea mays*) plants in host location by this insect. Weevils attracted to the volatile chemicals from the maize plants in Y olfactometers experiments and the gas chromatography (GC), coupled gas chromatography-mass spectrometry (GC-MS), GC peak enhancement and electroantennography (EAG)-identified hexanal, (*E*)-2-heptenal and octanal as biologically active compounds in air entrainment samples and diethyl ether fractions of vacuum distillates (Ukeh et al. 2012).

Previous studies mostly focused on simple interactions between a plant and a herbivore on the above ground. However, recent studies concentrated on the herbivore feeding-induced volatiles and their effects on feeding herbivore itself and also on its parasitic insects. However, roots can also emit volatiles that attract enemies of belowground feeding herbivore (Rasmann et al. 2005; Ali et al. 2010). There is a need to study the more complex networks involving multiple herbivores. It is now proved that it is possible both to reduce herbivory and enhance natural enemy attraction simultaneously. A solitary parasitoid of young noctuid caterpillars, *Cotesia marginiventris* (the braconid wasp), can locate potential hosts from a distance by orienting towards the volatile chemicals released by herbivore-damaged plants (Rostás and Wölfling 2009).

Frati et al. (2013) showed 'footprint-induced synomone' adsorbed onto the epicuticular waxes are exploited by the parasitoid. Induced production of synomones due to feeding punctures, footprints and oviposition activity of *Murganita histrionic* on *Brassica oleracea* plants also attracts the egg parasitoid *Trissolcus brochymenae* (Conti et al. 2010). It is demonstrated that the detection of oviposition-induced synomones by the parasitoid depends on their adsorption by the epicuticular waxes. Epicuticular waxes of broad bean leaves mediated the foraging behaviour of *scelionid egg parasitoid*, *Trissolcus basalis* (*Wollaston*), by absorbing contact kairomones of the host, *Nezara viridula* (L.) (Colazza et al. 2009). Wasps displayed the arrestment posture when intact leaves were contaminated by host female footprints, and it was shown earlier that disturbance of plant cuticular layer by adult insect oviposition or chemical footprints stimulate the volatile chemical production which is further used by insect's natural enemies as cues in finding their host's environment.

Butter flies of *Pieris* spp. are pests on various brassicaceous species characterised by the

content of glucosinolates (Rodman et al. 1998). Using two different herbivores, they tested the response of the specialist butterfly *P. brassicae* and two parasitoids to volatiles of *B. nigra* plants induced by egg deposition by the specialist butterfly and a generalist moth *Mamestra brassicae*. The egg parasitoid *Trichogramma brassicae* attacks eggs of both. Volatiles collected from headspace of uninfested *B. nigra* plants was compared with the headspace of *P. brassicae* egg-infested and *M. brassicae* egg-infested plants, and they detected about 50 plant compounds. According to the response, they produce to volatile chemicals present in the host plant; about 21 types of olfactory receptor neurons were classified in *M. Brassicae*.

Trichogramma chilonis showed varied kairomonal responses towards the dichloromethane extracts of the host plants, *Ricinus communis*, where its hosts *Achaea janata* and *Spodoptera litura* normally occur. The foraging activity, maximum percentage parasitism and emergence of the parasitoid were increased in the presence of these plant volatile chemicals (Usha Rani and Lakshminarayana 2008). Herbivory induces defence responses not only in the wounded regions but also in undamaged regions in the attacked leaves and in distal intact (systemic) leaves. It is interesting to note that apart from the leaf feeding insects, the stem boring pests also induce different primary (Usha Rani and Jyothsna (2010) and secondary metabolites especially terpenoids (Usha Rani and Sandhyarani 2012) and are shown to recruit herbivore's natural enemies. The yellow stem borer (*Scirpophaga incertulas* Walker (YSB))-infested rice plants emitted chemicals through the surface of their stems. Such herbivore-induced plant volatiles (HIPVs) played a vital role in attracting and arrestment of in its egg parasitoid, *Trichogramma japonicum* Ashmead, and enhanced the ovipositional activity compared to volatiles which were released from normal undamaged plants. It is confirmed that *T. japonicum* relays on short-range and long-range HIPV compounds in search of potential host (Usha Rani and Sandhyarani 2012). Pest's natural enemies can also discriminate between host-induced and non-host-induced plant volatiles. The leaf surface chemicals of castor, *Ricinus communis* L, plants damaged due to the feeding of the host, *Achaea janata* (L), had synomonal effects on the parasitoid and induced orientation and oviposition in *T. chilonis*, whereas the surface chemicals from the plant infested with non-host *Liriomyza trifolii* (Burgess) ceased to produce any such effects (Usha Rani et al. 2008a).

Insect parasitoids (such as parasitic wasps) and predators distinguish pest damage-induced terpenoids and other volatile chemicals and discriminate the noninfested plants and thus use these chemicals for host or prey location. These phytodistress signals, which result in an active interaction between herbivore-damaged plants and a third trophic level, have been described for several agroecosystems. Examples include lima bean and apple plants, which produce volatiles that attract predatory mites when damaged by spider mites (Takabayashi and Dicke 1996), and corn and cotton plants, which release volatiles that attract hymenopterous parasitoids that attack larvae of several Lepidoptera species (Tumlinson et al. 1993).

The most important semiochemicals for insects are host plant volatiles, and pheromones and insects have to often encounter these chemicals in their day-to-day life. Also their synergism can modulate insect behaviour. Insect attraction to sex and aggregation pheromones can be amplified by specific plant volatile (Wang et al. 2008). These volatiles are used as host location signals by foraging parasitoids. Turlings et al. (1993) studied the tritrophic system that comprised the parasitoid *Cotesia marginiventris*, the host caterpillar *Spodoptera exigua* sps and maize plants. The volatiles released by maize plants due to the damage by the *S. exigua* larvae contained sesquiterpenes and indole which are attractive to parasitoid. As parasitised larvae consume considerably less plant tissue than unparasitised larvae, this is beneficial to plant. It is proved that the parasitoids can distinguish and show their preference for plants with more herbivores and more herbivore damage, but cannot distinguish between different levels of mechanical damage. This phenomenon was recorded in the parasitoid

Cotesia vestalis. Girling et al. (2011) investigated the behavioural responses of the *C. vestalis* to VOCs from a plant-herbivore complex consisting of cabbage plants (*Brassica oleracea*) and the parasitoid host caterpillar, *Plutella xylostella*, in a Y-tube olfactometer and compared the parasitoid's responses to VOCs produced as a result of different levels of attack by the larvae as well as equivalent levels of mechanical damage. The parasitoid *C. marginiventris* can also differentiate the plant volatiles emitted by the closely related host species *S. exigua* and *S. frugiperda* damaged *Z. mays* (Turlings et al. 1995). *C. marginiventris* responded to a terpenoid blend from the *Z. mays* which provide information regarding the host as well as the host plant.

Insect egg deposition on a plant induces plant volatiles; synomones are semiochemical cues and are used by egg parasitoids during host location. They are considered important elements of plant defence. The specificity and role of oviposition induced plant volatiles and their consequences on insects at different trophic levels has created significant interest following the report of Hilker and Meiners (2002). The experiments conducted by Conti et al. (2010) proved that the oviposition, feeding punctures and chemical footprints left by the host insects *Murgantia histrionic* on *Brassica oleracea* plants induced the production of short-range plant volatiles that have an important role in the host location process of the egg parasitoid *Trissolcus brochymenae*. It is interesting to learn how leaf boundary walls influence egg deposition by adult as well as the embryo development inside the egg. Reports are available on the oviposition-induced changes in the plant in the form of changes in photosynthetic activity and of the plant's secondary metabolism (Hilker and Meiners 2002). So far the egg-induced changes in photochemistry were found to be detrimental to the eggs (Hilker and Meiners 2011). The egg deposition can also change plant's volatile profile and leaf surface chemistry which serve as indirect plant defence and lead to attraction of egg parasitoids and their arrestment on a leaf (Hilker and Meiners 2011). Plants having short life or low biomass show oviposition-induced defence, as they benefit from prevention or reduction of feeding damage by larvae that hatch from eggs.

Interestingly, plant volatiles also function in the belowground atmosphere. The root feeding by various belowground herbivores also are shown to induce volatile chemicals which often trigger the predator attraction in the soil. The tulip bulbs infested by rust mites, *Aceria tulipae*, produce belowground volatile chemical signals that lure predatory mites, *Neoseiulus cucumeris*, but the volatiles of untreated or wounded bulbs are inactive (Aratchige et al. 2004). The root weevil, *Diaprepes abbreviates*, feeds on the roots of citrus trees, and in response to this, the roots emit several terpenes in to the surrounding soil. Using belowground olfactometers, Ali et al. (2010) could show that the entomopathogenic nematode *Steinernema diaprepesi* and were significantly more attracted by citrus roots induced by the insect pest larvae than by roots mechanically damaged or by control empty pots (Ali et al. 2010). Several VOCs such as methyl salicylate, hexanol, heptanol, undecyl acetate or 4,5-dimethylthiazole are shown to provide positive chemotaxis of the two entomopathogenic nematodes *Heterorhabidtis bacteriophora* and *Steinernema carpocapsae* (Hallem et al. 2011). Similar observations were reported recently by Rasmann et al. (2010) with common milkweed, *Asclepias syriaca*, which is generally fed by the specialist root herbivore larvae of the Cerambycidae beetle, *Tetraopes tetraophthalmus*, and can release volatiles in the soil. These increased production of volatiles attracted the entomopathogenic nematode *H. bacteriophora* towards the milk weed roots.

One of the best-known belowground tritrophic interactions is reported in corn ecosystem. Western corn rootworm, *Diabrotica virgifera virgifera*, attacks the roots of European maize varieties and feeds voraciously, leading to the emission of the sesquiterpene (*E*)-β-caryophyllene (EβC) (Rasmann et al. 2005; Kollner et al. 2008) which are highly attractive to entomopathogenic nematodes *H. megidis* in the laboratory as well as in the field. *H. megidis* nematode was also shown to be attracted to sesquiterpenoid aristolene, one of the seven terpenes released by cotton plants induced

by the chrysomelid larva. The roots of cotton (*Gossypium herbaceum*) after feeding by the generalist root feeder larvae of the chrysomelid beetle, *Diabrotica balteata*, emit more than ten VOCs (Rasmann and Turlings 2008). However, in this same study, they noticed that cowpea (*Vigna unguiculata*) plants emitted almost undetectable amounts of volatiles, which also resulted in lower nematode attractions. Though root VOCs are reported to occur in many plant ecosystems, still many of these volatiles needs to be identified, and more studies on belowground tritrophic interactions are underway.

Plant has to expend a lot of energy on defence reactions and therefore learned not to use such signals without cause. In many species, the hormone methyl salicylate is emitted only when the plant is attacked by insects but not when other types of damage occur. Elicitors in insects' oral secretions and compounds in oviposition fluids, adult oviposition stimulate the plants to produce the chemicals signals that dramatically reshape plant's transcriptomes, proteomes and metabolomes (Wu and Baldwin 2010). All these herbivory-induced changes are mediated by elaborate signalling networks, which include receptors/sensors, Ca^{2+} influxes, kinase cascades, reactive oxygen species and phytohormone signalling pathways. The induced volatiles may affect the diversity and composition of plant-associated arthropod communities.

27.4 Adverse Effects of Plant Volatiles on Insects

Though the behavioural responses of various insects to plant volatile chemicals are apparent, their physiological and toxic effects are also evident in many insects. Plants' ability to synthesise toxic chemicals have evolved to ward off herbivores, pathogens and even mammals or to suppress the growth of the neighbouring plants (Bennett and Wallsgrove 1994; Harborne 1999). Some plant extracts or essential oils extracted from medicinal plants have volatile toxicity to several insect pests. Majority of the work reported about the adverse effects of plant volatiles is on stored product insects, as fumigation is ideal in controlled and closed conditions where these pests live. A most serious pest of stored products, namely, khapra beetle *Trogoderma granarium* Everts (Coleoptera: Dermestidae) adult as well as the larval stages, reacts to the volatiles of the essential oils of *Myrtus communis* L. (Myrtaceae) plant in laboratory. The leaf essential oil of the myrtle plant showed a highly toxic effect to all developmental stages of Khapra beetle. The mortality of adult insects was dominant followed by eggs compared with other life stages, and the toxic effects were attributed to the monoterpenes present predominantly in the oil (Tayoub et al. 2012) probably acting against insects as neurotoxins (Ayvaz et al. 2010). It is suggested that natural terpenes isolated from essential oils could act as activators of octopaminergic receptors in larvae of *T. granarium* (Shaaya and Afaeli 2007). The common floral volatile Linalool is toxic to several insect species at high concentrations (Phillips et al. 2010) and often affects mortality, growth, activity and feeding. The other common floral volatiles, geraniol, citronellal, eugenol, anisaldehyde and citral were reported to be repellents to mosquitoes *Aedes albopictus* (Skuse) (Diptera: Culicidae), and different concentrations of the compound elicit different behavioural responses in these mosquitoes (Hao et al. 2013).

Essential oils extracted from the foliage of *Mentha longifolia* L. (Lamiales: Lamiaceae) and *Pulicaria gnaphalodes* Ventenat (Asterales: Asteraceae) and flowers of *Achillea wilhelmsii* C. Koch (Asterales: Asteraceae) were tested in the laboratory for volatile toxicity against two stored product insects, the flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), and the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). The individual chemical components isolated from these essential oils, piperitenon, tripal, oxathiane, piperiton oxide and d-limonene extracted from *M. longifolia*; chrysanthenyl acetate, 2L-4L-dihydroxy eicosane, verbenol, dehydroaromadendrene, β-pinene and 1, 8 cineol from *P. gnaphalodes*; *and* 1, 8 cineole, caranol, alpha pinene, farnesyl acetate and p-cymene from *A. wilhelmsii* were

positive in producing volatile toxicity to *C. maculates* (Khani and Asghari 2012). A study on essential oil constituents isolated from aromatic plants showed that two natural terpenes termed ZP-51 and SEM-76 isolated and cultivated from unidentified cultivated aromatic plants belonging to Labiatae family have outstanding fumigant toxicity against *T. granarium* larvae (Kostyukovsky et al. 2002).

Pathak and Pandey (2011) investigated the combined action of plant volatiles from two common trees, *Azadirachta indica*, A. Juss (neem), and *Eucalyptus* sps. Both are known for their larvicidal, repellent and insecticidal properties against several insect pests (Malhotra and Gujar1984; Moore et al. 2002; Chockalingam et al. 1986; Sharma et al. 1994). Exposure of adult male and female individuals of rice moth, *Corcyra cephalonica*, to the vapours of the essential oils from neem and eucalyptus has stimulated the physiological mechanisms presumably neuroendocrine systems and significantly elevated the glycogen, lipids and proteins while reduced FAA content in the testes and ovaries of these individuals. Interestingly some solanaceous plant leaves contain certain volatile chemicals that act as fumigants to stored product pests in a closed atmosphere. The crude leaf extracts *Lycopersicum esculentum* Mill (tomato) and *Capsicum annuum* (green pepper) both displayed potent fumigation toxicity to the notorious pest of stored products, *Trogoderma granarium* (Khapra beetle), by reducing the survival of the adult beetles when exposed (Usha Rani et al. 2008a, b). These two plants along with another plant belonging to Solanaceae family, *Solanum melongena* L (brinjal), also caused considerable antifeedant activity, while the feeding of the leaf extract-treated diets with lower doses caused significant larval growth inhibition of two major lepidopteran pests, *Spodoptera litura* F. and *Achaea janata* L. (Devanand and Usha Rani 2011). Fruit extract of *S. melongena* plant being the most active of all the other tested solanaceous plants as it interfers with the moulting process of the larvae and producing morphological abnormalities upon ingestion of the treated diet. Herbivorous insects have to use a variety of physiological mechanisms to cope with the harmful chemicals in their food plants which are detrimental to their development. Dietary exposure to toxic compounds can induce production of p 450 detoxication enzymes Glendinning (2002). Apart from this, the serine protease activity was shown to be inhibited in both the larval midguts. The chemicals from the *S. melongena* fruit extracts have been identified as caffeic acid methyl ester (methyl-(E)-3-(3, 4-dihydroxyphenyl) prop-2-enoate), a hydroxycinnamic acid (Usha Rani and Devanand 2013).

Not only the normal plants but the genetically modified plants too are shown to produce volatile chemicals that have some impact on the feeding pests. A best example for this is the caterpillars of the tobacco hornworm, *Manduca sexta*, which are deterred from feeding on transgenic tobacco emitting isoprene (Laothawornkitkul et al. 2008) or patchoulol (Wu et al. 2006).

27.5 Plant Volatiles and Insect Sex Pheromones

Insects rely on olfactory cues for finding their mates. For successful reproduction, an insect has to identify the odorant information released by its mate from a background of multiple plant volatiles. In natural environment, a female virgin moth will emit pheromonal blend containing several components and the potential males are attracted to these volatile chemicals, sometimes ignoring the abundant background information of plant volatiles. Male moths are equipped with a well-developed olfactory system which responds specific compounds. In several moth species, the behavioural responses of male to female sex pheromones are enhanced by host plant odours (Reddy and Guerrero 2004), and thus the response of male to female-produced sex pheromone is directly influenced by host plant volatiles. Another role of plants in sex pheromone production is that, plant secondary metabolites provide essential direct precursors for the biosynthesis of sex pheromones in many insect species which mediate successful mating (Hilker and Meiners 2011). The synergistic action of host plant volatiles with pheromones has been shown in

many species of Lepidoptera and Coleoptera (Landolt and Phillips 1997). Pope et al. (2007) examined the interactions between the sex pheromones of the bird cherry-oat aphid, *Rhopalosiphum padi* (L), and the damson-hop aphid, *Phorodon humuli* (Schrank), which migrate and colonise on Prunus spp. and two of the common plant volatiles benzaldehyde and methyl salicylate. In a series of experiments with water traps baited with both plant volatiles and sex pheromone, they discovered that specific volatiles synergise responses of autumn-migrating aphids to their sex pheromone (Pope et al. 2007). Males of European grapevine moth *Lobesia botrana* perceive and respond to host plant volatiles which serve as olfactory cues that *L. botrana* males can discern places where the chance of encountering females is higher, since they totally rely on female-produced sex pheromone for long-distance mate finding that too in a limited time (Von Arx et al. 2011). Among the 12 host plant volatiles, aliphatic esters, aldehydes and alcohols, aromatic compounds and terpenes, the male *L. botrana* made upwind flights in a wind tunnel towards the identified compounds, 1-hexanol, 1-octen-3-ol and (Z) – 3-hexenyl acetate and (E) b-caryophyllene.

The most prominent example of odour interactions is synergic or inhibitory interaction of sex pheromone and host or non-host plant odours in moths (Allmann and Baldwin 2010; Varela et al. 2011). Pregitzer et al. (2012) explored the molecular mechanisms underlying pheromone-plant odour interactions in antennal sensilla of *Heliothis virescens*.

Another interesting function of plant volatiles is their participation in enhancing the attractiveness of insect sex pheromones (Deng et al. 2004). Apart from affecting the mating signals of the insects, thus leading to their reproductive success, plant chemistry also directly affects the production of the eggs, i.e. oogenesis. One example is males of several arctiid species can convert the plant pyrrolizidine alkaloids to hydroxyl danaidal and release it as courtship pheromone from their coremata (Hartmann et al. 2004). Tephritid flies (Bactrocera spp.) can sequester methyl eugenol from their host plant and store them that serves as male sex pheromone (Hee and Tan 2004). Many herbivorous insects are known to adjust their production and release of sex pheromone to the presence of plant volatile (Reddy and Guerrero 2004). The plant volatiles linalool, (Z)-3-hexenyl acetate and geraniol showed week attraction to the soybean pod borer, *Leguminivora glycinivorella* (Matsumura) males, but significantly reduced mean catches when higher doses were combined with pheromones. These volatiles when mixed with two kinds of its sex pheromones ((E,E)-8,10-dodecadienyl acetate (EE8,10-12:Ac), or a blend of EE8,10-12:Ac and (E)-10-dodecenyl acetate in a 10:1 ratio) significantly increased the mean catch in the field (Hu et al. 2013). Certain other plant volatiles, such as (Z)-3-hexen-1-ol, (Z)-3-hexenyl acetate and (E)-2-hexenyl acetate, failed to attract on their own, but significantly reduced mean catch of *L. glycinivorella* males. The corn earworm (*Helicoverpa zea*) and the codling moth (*Cydia pomonella*) are two major pests of corn and the virgin females secrete sex pheromones to attract the male moths, and it is done while they are on the leaves of their host plant. The capture of adult male moths in female sex pheromone traps is enhanced or synergised by a certain group of host plant chemicals, the 'green leaf volatiles' (GLVs). The male moths preferred and their catches have significantly increased in the traps baited with a mixture of female pheromone and prominent volatiles of their host plant; (Z)-3-hexenyl acetate over traps baited only with sex pheromone (Light et al. 1993). Since the traps containing only plant volatiles did not attract the males, it is concluded that the GLVs act as pheromone synergists.

27.6 Sensory Organs and Plant Chemical Perception

Insects rely heavily on olfaction for sensing their external environment which is strongly influenced by volatile perception. Plant chemistry plays an important role in mediating the interactions between the plant and the insect and is initiated by insect's contact chemosensory system (Bernays and Chapman 1994). Sensory sys-

tem is well developed in herbivorous insects as it is very essential for the insect for identifying a correct and suitable plant for oviposition. Insects generally use their antennal sensilla for sensing the chemicals occurring in and around their host plants, and the insect olfactory receptors aid in plant volatile perception and detection and regulate the behaviour. Understanding the morphological characters of insect sensilla, their sensory innervations and relation to functioning in plant volatile chemical perception is a fascinating subject attaining importance in the past two decades. Perception of the plant volatile compounds and their molecular structures depends on olfactory receptor neurones in sensillae, mostly located on insect antennae. Insects have ability to learn and possess a suitable nervous system for displaying the responses to plant volatiles. Insects use their excellent ability to process the olfactory signals from their hosts/prey and the success depends on this ability. Insect olfactory system is highly sensitive to several plant volatiles and also specific to plant. Insects detect the blends of ubiquitous compounds (Campbell et al. 1993; Bruce et al. 2005). It is shown that when a plant volatile sample contains about 200 compounds, EAG (electroantennography) recordings with insect antenna have shown that only a subset of compounds are detected by insects (Bruce et al. 2005) which indicate that not all components of plant volatiles are needed for an insect to respond but a few of them or a combination of a blend of compounds stimulating insect response. Earlier it is known that insects have highly specific receptors only for pheromone receptors, but now it is confirmed that olfactory receptor neurons tuned to specific components from plant volatiles, suggesting the specificity of receptors for host plant recognition. Insect's behavioural responses to host plant volatiles are more transparent than responses to pheromone blend.

Insect olfactory receptor genes belong to a distinct gene family encoding heteromeric (Lundin et al. 2007) ligand-gated ion channels comprised of a variable sensing component (Sato et al. 2008). Boeckh is one of the earliest entomologists to study the insect sensory system and their role in volatile chemical detection. Electrophysiological studies of Boeckh et al. (1965) reported earlier on olfactory sensory neurons classified them as 'specialists' which responded to pheromone components or 'generalists' which responded to host or plant odours.

The role of olfactory and gustatory sensilla present in different peripheral organs of the larvae of the muga silk moth, *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae), could discriminate the chemicals released by the host plants (*Persea bombycina* Kostermans (Laurales: Lauraceae and *Litseapolyantha* Juss)) over the non-hosts (*Litsea grandifolia* Teschner and *Ziziphus jujuba* Miller (Rosales: Rhamnaceae)) (Bora et al. 2012).

Lei Guo and Guo Qing Li (2009) demonstrated the importance of insect antennae in plant volatile chemical recognition. They studied the plant chemical perception by Asian corn borer moths, *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae), in an electorantennogram towards the common plant chemicals, myristic, palmitic, stearic and oleic acids and their corresponding methyl esters and found that mated females with both antennae amputated, in contrast to intact females and females with one antenna removed, could not discriminate between simultaneously provided control filter papers and filters treated with a blend of oviposition deterring fatty acids. The taste receptors on the oral cavity of the insects are assumed to aide insects detect and reject potentially toxic foods. Ablation of these gustatory sensilla from the larval *Manduca sexta* (Lepidoptera: Sphingidae) prevented them from showing aversive response to plant chemicals (Glendinning 2002). In parasitic or predatory insects, there is a conspicuous sexual dimorphism in antennal sensillary structures, probably functioning in identification of ovipositional substrates in females and as mate locating organs in males. Sen et al. (2005) studied the external morphology and peripheral olfactory responses of antennal chemoreceptors of the generalist parasitoid *Trichogramma chilonis* to host plant-related chemical stimuli using scanning electron microscope (SEM) and EAG techniques. Uniporous sensilla on the antennal tip and numerous multiporous pitted sensilla character-

ise the female antennae. In *Trogossita japonicum* Reitter (Coleoptera: Trogossitidae), a predatory beetle that preys on a Cerambycidae beetle and pine tree pest, *Monochamus alternatus* Hope, too, there is a prominent sexual dimorphism and quantitative and qualitative differences in the morphology of antennal sensilla (Usha Rani and Nakamuta 2001) (Fig. 27.6). Single sensilla at the tip of male antenna indicate its specific role in female pheromone perception. The volatile terpenoids emitted from the pine trees play an important role in prey location by the predator and that females are always highly responsive and faster than males in responding to these volatiles. During orientation towards the terpene chemicals, they wave their antennae vigorously, probably to

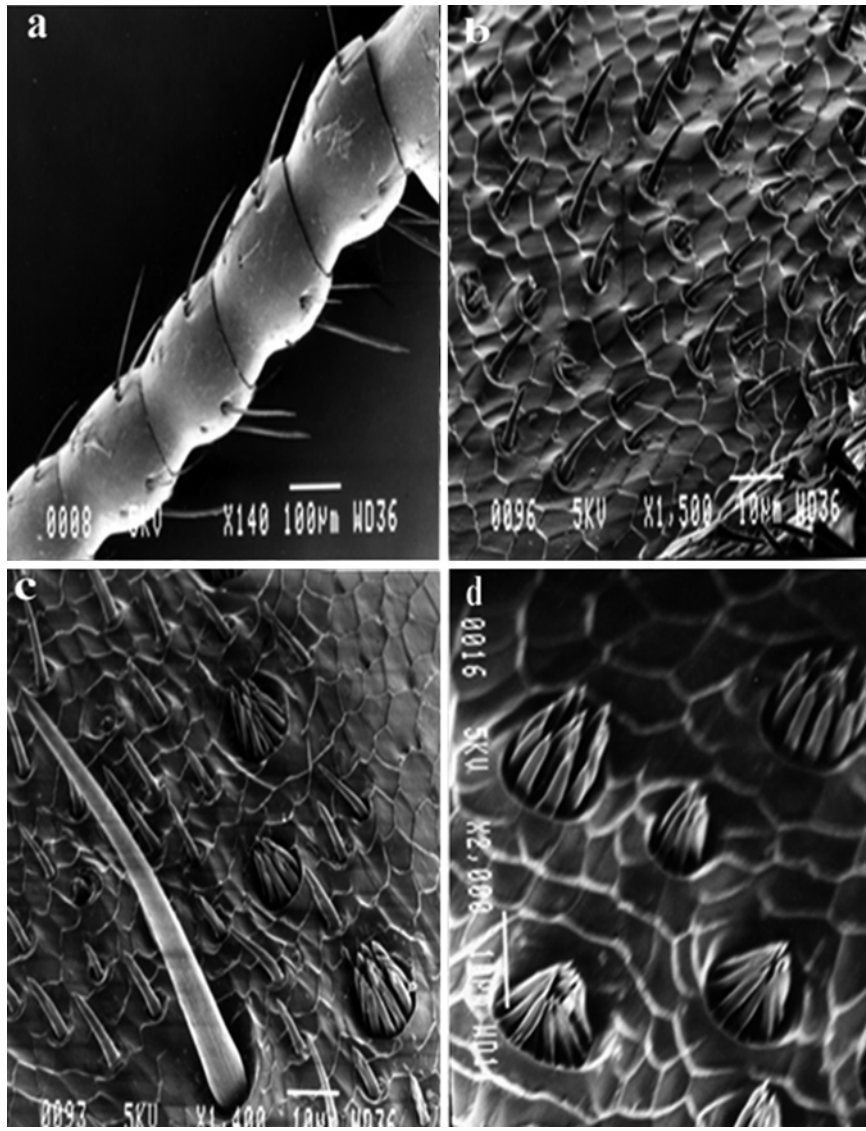


Fig. 27.6 (**a**) Scanning electron microscopic pictures of antenna of a female predatory beetle, *Trogossita japonica*, showing arrangements of sensilla chaetica type 11 (s. ch 2) and sensilla chaetica type 3 (s. ch 3) on fourth to sixth flagellomere (130×). Bar = 10 µm. (**b**) Sensilla on the scape of *T. japonica*. (**c**) SEM antenna – A portion of the terminal club segment of a female showing s. trichodea type 2, clustered sensilla, grooved pegs and s. basiconoca (1,400×). Bar = 10 µm. (**d**) SEM – Clusters of sensilla at the apical antennal segment (1,900×). Bar = 10 µm

monitor the odours, by using chemoreceptive sensilla on the antenna (Usha Rani and Nakamuta 2001). They also use antennae to palpate the surfaces, and the SEM studies showed that olfactory receptor cells appear to be located principally on the terminal club segment. The types and distribution of sensillae are indicative of their way of living (Fig. 27.7).

The plant odour information is encoded in olfactory receptor neurons (RNs), and gas chromatography linked to electrophysiological recordings is the most popular or ideal method for recording this information. Molecular receptive

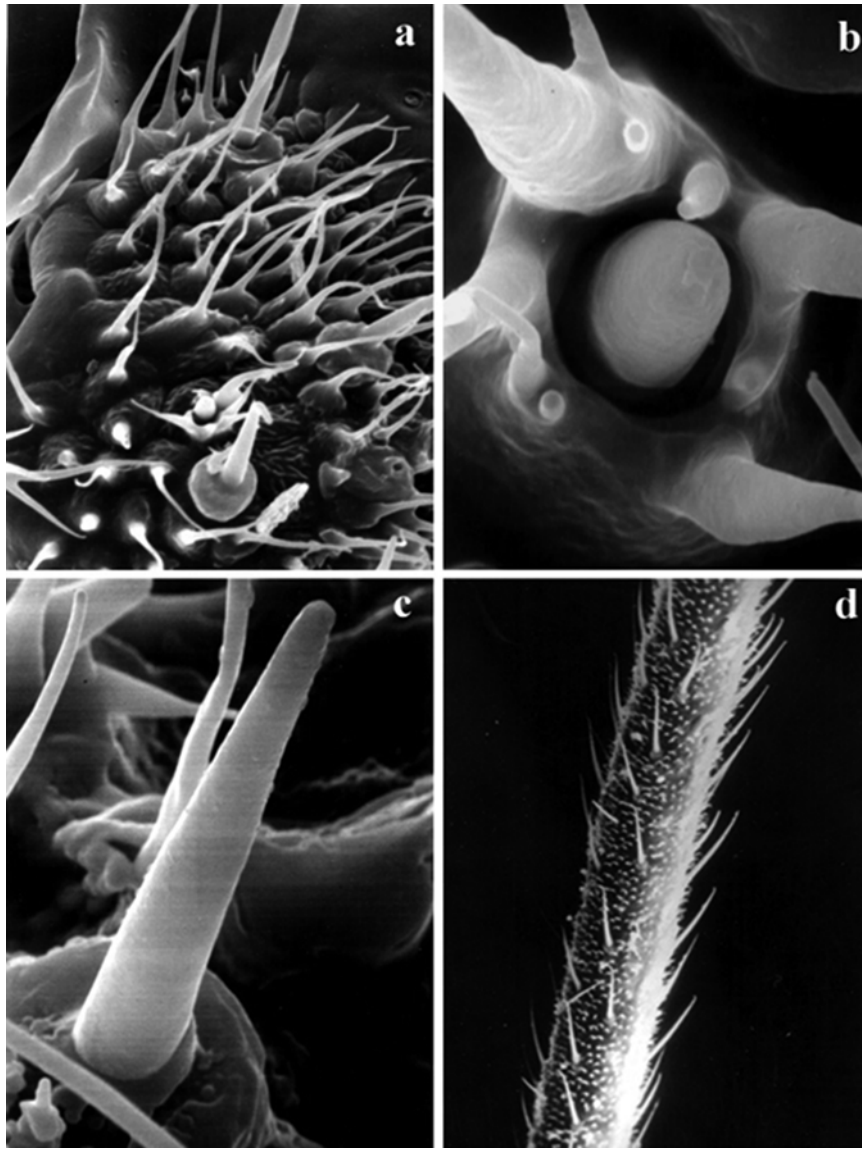


Fig. 27.7 SEM picture of sensillary population on the rostrum of a predatory Hemipteran *Eocanthecona furcellata*. (**a**) Senillae on the rostral tip of *E. furcellata*. s. trichodeum (s.t 2). (**b**) S. basiconica found among the sensilla on the rostral apex of *E. furcellata* surrounded with hair-like protuberances. (**c**) A single sensillum basiconicum type A on the rostral apex of *E. furcellata* having stout shaft (SHA) and a thick socket (SOC). (**d**) Sensilla on the antenna of a plant-feeding Hemipteran

ranges of the neurons for sensitivity towards different naturally produced plant volatile chemicals can be determined using this method.

The sensory system of a mate or oviposition site searching herbivore insect has to meet the challenge of recognising relevant information about volatiles released by conspecifics or sympatric species as well as by plants. Pheromone-receptive sensillae their morphology or function in many insects is thoroughly worked upon, but the sensory organs' reception of plant odours is less known. The use of gas chromatography (GC) linked to electrophysiological recordings from single receptor neurons (GC linked to single-cell recordings [GC-SCR]) provides considerably reliable information about relevant plant odorants in some species of moths, weevils and other beetles (Blight et al. 1995; Larsson et al. 2001; Barata et al. 2002; Stranden et al. 2003; Bichão et al. 2005; Rostelien et al.2005). The insects have to choose their respective host plants from a huge variety of volatile compounds. For this purpose, fortunately insects are well equipped with a good sensory system.

27.7 Conclusions

Plant volatiles have tremendous scope in various disciplines of agriculture. The plants in normal stage without any damage, either mechanical or insect/pathogen wounding, emit volatiles that play a prominent role in attracting the prospective pest insect. Understanding and identifying these host location cues can facilitate the ecofriendly pest control. The volatiles released by plants due to pest feeding damage are also equally important and supplement the biological pest control methods by attracting the pest's natural enemies to the releasing source. The essential oils from various plants almost directly kill the insects and thus are almost already in use. The volatiles given off by flowers and fruits are of interest from the point of view of insect-plant interactions. Over all plant-emitted chemicals have enormous influence on insect behaviour and signify their role in environmentally safer pest control operations.

Acknowledgements I thank Kurra Sandhyarani, Sambangi Pratyusha, Movva Vijaya and Sireesha for their help in various ways in the preparation of this chapter. I am grateful to the director of (CSIR) Indian Institute of Chemical Technology Hyderabad for encouragement.

References

Abel C, Clauss M, Schaub A, Gershenzon J, Tholl D (2009) Floral and insect induced volatile formation in *Arabidopsis lyrata ssp. petraea*, a perennial, outcrossing relative of *A. thaliana*. Planta 230:1–11

Adler LS, Wink M, Distl M, Lentz AJ (2006) Leaf herbivory and nutrients increase nectar alkaloids. Ecol Lett 9:960–967

Agelopoulos NG, Chamberlain K, Pickett JA (2000) Factors affecting volatile emissions of intact potato plants, *Solanum tuberosum*: variability of quantities and stability of ratios. J Chem Ecol 26(2):497–511

Aharoni A, Giri AP, Deuerlein S, Griepink F, De Kogel W, Verstappen FWA, Verhoeven HA, Jongsma MA, Schwab W, Bouwmeester HJ (2003) Terpenoid metabolism in wild-type and transgenic Arabidopsis plants. Plant Cell 15:2866–2884

Ali JG, Alborn HT, Stelinski II (2010) Subterranean herbivore-induced volatiles released by citrus roots upon feeding by *Diaprepes abbreviatus* recruit entomopathogenic nematodes. J Chem Ecol 36:361–368

Ali JG, Alborn HT, Stelinski LC (2011) Constitutive and induced subterranean plant volatiles attract both entomopathogenic and plant parasitic nematodes. J Plant Ecol 99:26–35

Allmann S, Baldwin IT (2010) Insects betray themselves in nature to predators by rapid isomerization of geen leaf volatiles. Science 329:1075–1078

Aratchige NS, Lesna I, Sabelis MW (2004) Below-ground plant parts emit herbivore-induced volatiles: olfactory responses of a predatory mite to tulip bulbs infested by rust mites. Exp Appl Acarol 33(1–2):21–30

Ateyyat MA, Al-Mazra'awi M, Abu-Rjai T, Shatnawi MA (2009) Aqueous extracts of some medicinal plants are as toxic as Imidacloprid to the sweet potato whitefly, *Bemisia tabaci*. J Insect Sci 9:15

Ayvaz A, Sagdic O, Karaborklu S, Ozturk I (2010) Insecticidal activity of the essential oils from different plants against three stored-product insects. J Insect Sci 10(21):13

Barata EN, Mustaparta H, Pickett JA, Wadhams LJ, Araújo J (2002) Encoding of host and non-host plant odours by receptor neurones in the eucalyptus woodborer, *Phoracantha semipunctata* (coleoptera: Cerambycidae). J Comp Physiol A 188:121–133

Beale MH, Birkett MA, Bruce TJA, Chamberlain K, Field LM, Huttly AK, Martin JL, Parker R, Phillips AL, Pickett JA, Prosser IM, Shewry PR, Smart LE, Wadhams LJ, Woodcock CM, Zhang YH (2006) Aphid alarm pheromone produced by transgenic

plants affects aphid and parasitoid behavior. Proc Natl Acad Sci U S A 103:10509–10513
Bennett RN, Wallsgrove RM (1994) Secondary metabolites in plant defence mechanisms. New Phytol 127:617–633
Bernays EA, Chapman RF (1994) Host plant selection by phytophagous insects. Chapman and Hall, New York
Bezemer TM, van Dam NM (2005) Linking aboveground and belowground interactions via induced plant defenses. Trends Ecol Evol 20:617–624
Bichão H, Borg-Karlson A-K, Wibe A, Araújo J, Mustaparta H (2005) Molecular receptive ranges of olfactory receptor neurones responding selectively to terpenoids, aliphatic green leaf volatiles and aromatic compounds, in the strawberry blossom weevil *Anthonomus rubi*. Chemoecology 15:211–226
Bidart-Bouzat MG, Kliebenstein D (2011) An ecological genomic approach challenging the paradigm of differential plant responses to specialist versus generalist insect herbivores. Oecologia 167(3):677–689
Blackmer JL, Rodriguez-Saona C, Byers JA, Shope KL, Smith JP (2004) Behavioral response of *Lygus hesperus* to conspecifics and headspace volatiles of alfalfa in a Y-tube olfactometer. J Chem Ecol 30(8):1547–1564
Blight MM, Pickett JA, Wadhams LJ, Woodcock CM (1995) Antennal perception of oilseed rape, *Brassica napus* (Brassicaceae), volatiles by the cabbage seed weevil *Ceutorhynchus assimilis* (Coleoptera, Curculionidae). J Chem Ecol 21:1649–1664
Blum MS, Hilker M (2002) Chemical protection of insect eggs. In: Meiners T, Hilker M (eds) Chemoecology of insect eggs and egg deposition. Blackwell, Berlin, pp 61–90
Boeckh J, Kaissiling KE, Schneider D (1965) Insect olfactory receptors. Cold Spring Harb Symp Quant Biol 30:263–280
Bora DS, Deka B, Sen A (2013) Host plant selection by larvae of muga silk moth, Antheraea assamensis, and the role of antenna and maxillary palp. J Insect Sci 13:52
Bruce TJA, Pickett JA (2011) Perception of plant volatile blends by herbivorous insects – finding the right mix. Phytochemistry 72:1605–1611
Bruce TJA, Wadhams LJ, Woodcock CM (2005) Insect host location: a volatile situation. Trends Plant Sci 10:269–274
Campbell CAM, Pettersson J, Pickett JA, Wadhams LJ, Woodcock CM (1993) Spring migration of damson hop aphid, Phorodon humuli (Homoptera, Aphididae), and summer host plant derived semiochemicals released on feeding. J Chem Ecol 19:1569–1576
Cha DH, Nojima S, Hesler SP, Zhang A, Linn CE, Roelofs WL, Loeb GM (2008) Identification and field evaluation of grape shoot volatiles attractive to female grape berry moth (*Paralobesia viteana*). J Chem Ecol 34:1180–1189
Chockalingam S, Nalina Sundari MS, Vasntha E (1986) The use of extract of Eucalyptus in the control of *Spodoptera litura* (Noctuidae: Lepidoptera). J Adv Zool 7:79–82
Colazza S, Lo Bue M, Lo Giudice D, Peri E (2009) The response of *Trissolcus basalis* to footprint contact Kairomones from *Nezara viridula* females is mediated by leaf epicuticular waxes. Naturwissenschaften 96:975–981
Conti E, Salerno G, Leombruni B, Frati F, Bin F (2010) Short-range allelochemicals from a plant–herbivore association: a singular case of oviposition-induced synomone for an egg parasitoid. Exp Biol 213:3911–3919
Croteau RB, Davis EM, Ringer KL, Wildung MR (2005) (-)-Menthol biosynthesis and molecular genetics. Naturwissenschaften 92(12):562–577
De Deyn GB, Raaijmakers CE, Zoomer HR, Berg MP, de Ruiter PC, Verhoef HA, Bezemer TM, van der Putten WH (2003) Soil invertebrate fauna enhances grassland succession and diversity. Nature 422:711–713
De Luca V, St Pierre B (2000) The cell and developmental biology of alkaloid biosynthesis. Trends Plant Sci 5:168–173
De Moraes CM, Lewis WJ, Paré PW, Alborn HT, Tumlinson JH (1998) Herbivore-infested plants selectively attract parasitoids. Nature 393:570–573
Degen T, Dillmann C, Marion-Poll F, Turlings CJT (2004) High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. Plant Physiol 135(4):1928–1938
Deng JY, Wei HY, Huang YP, Du JW (2004) Enhancement of attraction to sex pheromones of Spodoptera exigua by volatile compounds produced by host plants. J Chem Ecol 10:2037–2045
Devanand P, Usha Rani P (2011) Insect growth regulatory activity of the crude and purified fractions from *Solanum melongena* L., *Lycopersicum esculentum Mill*. and *Capsicum annuum* L. J Biopest 4(2):118–130
Dicke M, Sabelis MW, Takabayashi J, Bruin J, Posthumus MA (1990a) Plant strategies of manipulating predator-prey interactions through allelochemicals: prospects for application in pest control. J Chem Ecol 16:3091–3118
Dicke M, Van Beek TA, Posthumus MA, Ben Dom N, Van Bokhoven H, De Groot AE (1990b) Isolation and identification of volatile kairomone that affects acarine predator prey interactions. J Chem Ecol 16:381–396
Dudareva N, Negre F, Nagegowda DA, Orlova I (2006) Plant volatiles: recent advances and future perspectives. Crit Rev Plant Sci 25(5):417–440
Farmer E, Ryan A (1990) Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proc Natl Acad Sci 87:7713–7716
Fatouros NE, Lucas-Barbosa D, Weldegergis BT, Pashalidou FG, van Loon JJA, Dicke M, Harvey JA, Gols R, Huigens ME (2012) Plant volatiles induced by herbivore egg deposition affect insects of different trophic evels. PLoS One 7(8):e43607
Feeny P, Stadler E, Ahman I, Carter M (1989) Effects of plant odor on oviposition by the black swallowtail butterfly, *Papilio polyxenes* (Lepidoptera: Papilionidae). J Insect Behav 2:803–827

Frati F, Salerno G, Conti E (2013) Cabbage waxes affect *Trissolcus brochymenae* response to short-range synomones.JInsectSci.doi:10.1111/j.1744-7917.2012.01575.x

Girling RB, Stewart-Jones A, Dherbecourt JT, Wright DJ, Poppy GM (2011) Parasitoids select plants more heavily infested with their caterpillar hosts: a new approach to aid interpretation of plant headspace volatiles. Proc R Soc 278(1718):2646–2653

Glendinning JI (2002) How do herbivorous insects cope with noxious secondary plant compounds in their diet? Entomol Exp Appl 104:15–25

Glinwood R, Pettersson J, Ahmed E, Ninkovic V, Birkett M, Pickett J (2003) Change in acceptability of barley plants to aphids after exposure to allelochemicals from couchgrass (*Elytrigia repens*). J Chem Ecol 29:261–274

Haddad NM, Tilman D, Haarstad J, Ritchie M, Knops JMH (2001) Contrasting effects of plant richness and composition on insect communities: a field experiment. Am Nat 158:17–35

Hallem EA, Dillman AR, Hong AV, Zhang YJ, Yano JM, DeMarco SF, Sternberg PW (2011) A sensory code for host seeking in parasitic nematodes. Curr Biol 21(5):377–383

Hao H, Sun J, Dai J (2013) Dose-dependent behavioral response of the mosquito *Aedes albopictus* to floral odorous compounds. J Insect Sci 13:127

Harborne JB (1999) The comparative biochemistry of phytoalexin induction in plants. Biochem Syst Ecol 27:335–367

Hare JD (2007) Variation in herbivore and methyl jasmonate–induced volatiles among genetic lines of *Datura wrightii*. J Chem Ecol 33:2028–2043

Hartmann T, Theuring C, Beuerle T, Ernst L, Singer MS, Bernays EA (2004) Acquired and partially de novo synthesized pyrrolizidine alkaloids in two polyphagous arctiids and the alkaloid profiles of their larval food-plants. J Chem Ecol 30(2):229–254

Hee AKW, Tan KH (2004) Male sex pheromonal components derived from methyl eugenol in the haemolymph of fruit fly *Bactrocera papaya*. J Chem Ecol 30:2127–2138

Hilker M, Meiners T (2002) Induction of plant responses towards oviposition and feeding of herbivorous arthropods: a comparison. Entomol Exp Appl 104:181–192

Hilker M, Meiners T (2006) Early herbivore alert: insect eggs induce plant defense. J Chem Ecol 32:1379–1397

Hilker M, Meiners T (2011) Plants and insect eggs: how do they affect each other? Phytochemistry 72:1612–1623

Holopainen JK (2004) Multiple functions of inducible plant volatiles. Trends Ecol Evol 9:529–533

Hu D, Feng J, Wang Z, Wu H, Zhang X (2013) Effect of nine plant volatiles in the field on the sex pheromones of *Leguminivora glycinivorella*. Nat Prod Commun 8(3):393–396

Jeong Gwi-Taek, Don-Hee Park (2006) Enhanced secondary metabolite biosynthesis by elicitation in transformed plant root system. Twenty-seventh symposium on biotechnology for fuels and chemicals. ABAB symposium 2006, Humana Press, New York, pp 436–446

Johnson SN, Gregory PJ (2006) Chemically-mediated host-plant location and selection by root-feeding insects. Physiol Entomol 13:1–13

Kaiser R (1993) The scent of orchids: olfactory and chemical investigations. Elsevier, Amsterdam. doi:10.1002/ffj.2730080511

Kessler A, Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. Science 291(5511):2141–2144

Kessler A, Halitschke R (2007) Specificity and complexity: the impact of herbivore-induced plant responses on arthropod community complex. Curr Opin Plant Biol 10:409–414

Khani A, Asghari J (2012) Insecticide activity of essential oils of *Mentha longifolia*, *Pulicaria gnaphalodes* and *Achillea wilhelmsii* against two stored product pests, the flour beetle, *Tribolium castaneum*, and the Cowpea Weevil, *Callosobruchus maculates*. J Insect Sci 12(73):1–10

Knudsen JT, Eriksson R, Gershenzon J, Stahl B (2006) Diversity and distribution of floral scent. Bot Rev 72:1–120, Roche Basel

Kollner TG, Held M, Lenk C, Hiltpold I, Turlings TCJ, Gershenzon J, Degenhardt J (2008) A maize (E)-beta-caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most American maize varieties. Plant Cell 20(2):482–494

Kostyukovsky M, Rafaeli A, Gileadi C, Demchennko N, Shaaya E (2002) Activation of octopaminergic receptors by essential oils constituents isolated from aromatic plants: possible mode of activity against insect pest. Pest Manag Sci 58:1–6

Landolt PJ, Phillips TW (1997) Host plant influences on sex pheromone behavior of phytophagous insects. Annu Rev Entomol 42:371–391

Laothawornkitkul J, Paul ND, Vickers CE, Possell M, Taylor JE, Mullineaux PM, Hewitt CN (2008) Isoprene emissions influence herbivore feeding decisions. Plant Cell Environ 31:1410–1415

Larsson MC, Leal WS, Hansson BS (2001) Olfactory receptor neurons detecting plant odours and male volatiles in *Anomala cuprea* beetles (Coleoptera: Scarabaeidae). J Insect Physiol 47:1065–1076

Lei Guo, Guo Qing Li (2009) Olfactory perception of oviposition – deterring fatty acids and their methyl esters by the Asian corn borer, *Ostrinid furnacalis*. J Insect Sci 9:67

Light DM, Flath RA, Buttery RG, Zalom FG, Rice RE, Dickens JC, Jang EB (1993) Host-plant green-leaf volatiles synergize the synthetic sex pheromones of the corn earworm and codling moth (Lepidoptera). Chemoecology 4(3–4):145–152

Lucas-Barbosa D, van Loon JJA, Dicke M (2011) The effects of herbivore-inducedand flower-visiting insects. Phytochemistry 72:1647–1654

Lundin C, Käll L, Kreher SA, Kapp K, Sonnhammer EL, Carlson JR, Heijne G, Nilsson I (2007) Membrane topology of the Drosophila OR83b odorant receptor. FEBS Lett 581(29):5601–5604

Malhotra KN, Gujar GT (1984) Neem as insect growth inhibitor. Natl seminar on neem in agriculture. IARI Neem Newsl 1:6

McCallum EJ, Cunningham JP, Lücker J, Zalucki MP, De Voss JJ, Botella JR (2011) Increased plant volatile production affects oviposition, but not larval development, in the moth *Helicoverpa armigera*. J Exp Biol 214:3672–3677

Miller DR (2006) Ethanol and (-)-α-pinene: attractant Kairomones for some large wood boring beetles in south-eastern USA. J Chem Ecol 32:779–794

Miller JR, Strickler KR (1984) Finding and accepting host plants. In: Bell WJ, Carde RT (eds) Chemical ecology of insects. Chapmann and Hall, London, pp 127–157

Moore SA, Lenglet A, Hill N (2002) Field evaluation of three plants based insect repellents against malaria vectors in VACA diE2 province of the Bolivian Amazon. J Am Mosq Control Assoc 18:107

Mumm R, Dicke M (2010) Variation in natural plant products and the attraction of bodyguards for indirect plant defense. Can J Zool 88:628–667

Neog K, Unni B, Ahmed G (2011) Studies on the influence of host plants and effect of chemical stimulants on the feeding behavior in the muga silkworm, *Antheraea assamensis*. J Insect Sci 11(133)

Ninkovic V, Pettersson J (2003) Searching behaviour of the seven spotted ladybird, *Coccinella septempunctata* – effects of plant–plant odour interaction. Oikos 100:65–70

Ninkovic V, Olsson U, Pettersson J (2002) Mixing barley cultivars affects aphid host plant acceptance in field experiments. Entomol Exp Appl 102:177–182

Nottingham SF, Hardie J, Dawson GW, Hick AJ, Pickett JA, Wadhams LJ, Woodcock CM (1991) Behavioral and electrophysiological responses of aphids to host and non-host plant volatiles. J Chem Ecol 17:1231–1242

Padmavathi C, Paul AVN (1998) Saturated hydrocarbons as kairomonal source for the egg parasitoid, *Trichogramma chilonis* Ishii (Hym., Trichogrammatidae). J Appl Entomol 122(1–5):29–32, Article first published online: 26 AUG 2009. doi:10.1111/j.1439-418.1998.tb01456.x

Pathak PH, Pandey S (2011) Impact of combined action of Neem and Eucalyptus oil volatiles on different stages of *Corcyra cephalonica* (Lepidoptera: Pyralidae). J App Nat Sci 3(2):247–252

Pham-Delegue MH, Bailez O, Blight MM, Masson C, Picard-Nizou AL, Wadhams LJ (1993) Behavioural discrimination of oilseed rape volatiles by the honeybee *Apis mellifera* L. Chem Senses 18:483–494

Phillips AK, Appel AG, Sims SR (2010) Topical toxicity of essential oils to the German cockroach (Dictyoptera: Blattellidae). J Econ Entomol 103:448–459

Piesik D, Dalaney KJ, Wenda-Piesik A, Sendel S, Tabaka P, Buszewski B (2013) *Meligethes aeneus* pollen-feeding suppresses and oviposition induces *Brassica napus* volatiles: beetle attraction/repellence to lilac aldehydes and veratrole. Chemoecology 23:241–250

Pope TW, Campbell CAM, Hardie J, Pickett JA, Wadhams LJ (2007) Interactions between host-plant volatiles and the sex pheromones of the bird cherry-oat aphid, *Rhopalosiphum padi* and the damson-hop aphid, *Phorodon humuli*. J Chem Ecol 33:157–165

Pregitzer P, Schubert M, Breer H, Hansson BS, Sachse S, Krieger J (2012) Front Cell Neurosci 6:42. doi:10.3389/fncel.2012.00042

Quiroz A, Pettersson J, Pickett JA, Wadhams LJ, Niemeyer HM (1997) Semiochemicals mediating spacing behavior of bird cherry-oat aphid, *Rhopalosiphum padi*, feeding on cereals. J Chem Ecol 23:2599–2607

Raguso RA, Levin RA, Foose SE, Holmberg MW, McDade LA (2003) Fragrance chemistry, nocturnal rhythms and pollination "syndromes" in Nicotiana. Phytochemistry 63:265–284

Rasmann S, Agrawal AA (2008) In defense of roots: a research agenda for studying plant resistance to belowground herbivory. Plant Physiol 146:875–880

Rasmann S, Turlings TCJ (2008) First insights into specificity of belowground tritrophic interactions. Oikos 117(3):362–369

Rasmann S, Kollner TG, Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U, Gershenzon J, Turlings TCJ (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 434(7034):732–737

Rasmann S, Erwin AC, Halitschke R, Agarwal AA (2010) Direct and indirect root defences of milkweed (*Asclepias syriaca*): trophic cascades, trade-offs and novel methods for studying subterranean herbivory. J Ecol 99(1):16–25

Reddy GVP, Guerrero A (2004) Interactions of insect pheromones and plant semiochemicals. Trends Plant Sci 9:253–261

Reisenman CE, Riffell JA, Bernays EA, Hildebrand JG (2010) Antagonistic effects of floral scent in an insect-plant interaction. Proc R Soc Lond B 277:2371–2379

Rodman JE, Soltis PS, Soltis DE, Sytsma KJ, Karol KG (1998) Parallel evolution of glucosinolates biosynthesis inferred from congruent nuclear and plastid gene phylogenies. Am J Bot 85(7):997–1006

Rostás M, Wölfling M (2009) Caterpillar footprints as host location kairomones for *Cotesia marginiventris*: persistence and chemical nature. J Chem Ecol 35:20–27

Rostelien T, Stranden M, Borg-Karlson AK, Mustaparta H (2005) Olfactory receptor neurons in two Heliothine moth species responding selectively to aliphatic green leaf volatiles, aromatic compounds, monoterpenes and sesquiterpenes of plant origin. Chem Senses 30(5):443–461

Runyon JB, De Mescher MC, Moraes CM (2006) Volatile chemical cues guide host location and host selection by parasitic plants. Science 313:1964–1967

Sato K, Pellegrino M, Nakagawa T, Nakagawa T, Vosshall LB, Touhara K (2008) Insect olfactory receptors are heteromeric ligand-gated ion channels. Nature 452:1002–1006

Schnee C, Köllner TG, Gershenzon J, Degenhardt J, Held M, Turlings TCJ (2006) The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. Proc Natl Acad Sci U S A 103:1129–1134

Sen A, Raina R, Joseph M, Tungikar VB (2005) Response of *Trichogramma chilonis* to infochemicals: a SEM

and electrophysiological approach. Biocontrol 50:429–447
Shaaya E, Afaeli A (2007) Essential oils as biorational insecticides-potency and mode of action. In: Shaaya I, Nauen R, Horowitz AR (eds) Insecticides design using advanced technologies. Springer, Berlin, pp 249–261
Sharkey TD, Yeh SS (2001) Isoprene emission from plants. Annu Rev Plant Physiol Plant Mol Biol 52:407–436
Sharma RN, Deshpandey SG, Tungikar VB, Tosheph M (1994) Toxicity of natural essential oils to mosquito *Aedes aegypti* and *Culex fatigans*. Geobios 21:162–165
Stadler B (2002) Determinants of the size of aphid–parasitoid assemblages. J Appl Entomol 126:258–264
Stranden M, Røstelien T, Liblikas I, Almaas TJ, Borg-Karlson A-K, Mustaparta H (2003) Receptor neurones in three heliothine moths responding to floral and inducible plant volatiles. Chemoecology 13:143–154
Sun XL, Wang GC, Cai XM, Jin S, Gao Y, Chen ZM (2010) The tea weevil, *Myllocerinus aurolineatus* is attracted to volatiles induced by conspecifics. J Chem Ecol 36:338–356
Sutherland ORW, Hillier JR (1972) Olfactory responses of *Costelytra zealandica* (Coleoptera: Melolonthinae) larvae to grass root odours. N Z J Sci 15(2):165–172
Takabayashi J, Dicke M (1996) Plant-carnivore mutualism through herbivore-induced carnivore attractants. Trends Plant Sci 1:109–113
Takabayashi J, Dicke M, Maarten A (1991) Posthumus variation in composition of predator-attracting allelochemicals emitted by herbivore-infested plants: relative influence of plant and herbivore. Chemoecology 2(1):1–6
Tayoub G, Abu Alnaser A, Ghanem I (2012) Fumigant activity of leaf essential oil from *Myrtus communis* L. against the khapra beetle. Int J Med Aromat Plants 2(1):207–213
Tumlinson JH, Lewis WJ, Vet LEM (1993) How parasitic wasps find their hosts. Sci Am 268:100–106
Turlings TCJ, Tumlinson JH, Lewis WJ (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250:1251–1253
Turlings TCJ, Mccall PJ, Alborn HT, Tumlinson JH (1993) An elicitor in caterpillar oral secretions that induced corn seedlings to emit chemical signals attractive to parasitic wasps. J Chem Ecol 19:411–425
Turlings TCJ, Loughrin JH, McCall PJ, Röse USR, Lewis WJ, Tumlinson JH (1995) How caterpillar-damaged plants protect themselves by attracting parasitic wasps. Proc Natl Acad Sci U S A 92:4169–4174
Ukeh DA, Woodcock CM, Pickett JA, Birkett MA (2012) Identification of host kairomones from maize, Zea mays, for the maize weevil, *Sitophilus zeamais*. J Chem Ecol 38(11):1402–1409
Usha Rani P, Devanand P (2013) Bioactivities of caffeic acid methyl ester (methyl-(E)-3-(3,4- dihydroxyphenyl)prop-2-enoate): a hydroxycinnamic acid derivative from *Solanum melongena* L. fruits. J Pest Sci 86:579–589
Usha Rani P, Jyothsna Y (2010) Biochemical and enzymatic changes in rice plants as a mechanism of defense. Acta Physiol Plant 32:695–701
Usha Rani P, Lakshminarayana M (2008) Defense mechanism in plants – their use as biotechnological approach for the management of insects pests. Pestic Res J 20(2A):33–38
Usha Rani P, Nakamuta K (2001) Morphology of antennal sensilla, distribution and sexual dimorphism in *Trogossita japonica* (Coleoptera: Trogossitidae). Ann Entomol Soc Am 94(6):917–927
Usha Rani P, Sandhyarani K (2012) Specificity of systemically released rice stem volatiles on egg parasitoid, *Trichogramma japonicum* Ashmead behaviour. J Appl Entomol 136:749–760
Usha Rani P, Jyothsna Y, Lakshminarayana M (2008a) Host and non-host plant volatiles on oviposition and orientation behaviour of *Trichogramma chilonis* Ishii. J Biopesticides 1(2):62–68
Usha Rani P, Devanand P, Suresh CH, Sathish K (2008b) Fumigant action of Solanaceae plants against four major species of stored. Uttar Pradesh J Zool Suppl I:165–173
Varela N, Avilla J, Anton S, Gemeno C (2011) Synergism of pheromone and host-plant volatile blends in the attraction of *Grapholita molesta* males. Entomol Exp Appl 141:114–122
Visser JH, Ave DA (1978) General green leaf volatiles in the olfactory orientation of the Colorado beetle, *Leptinotarsa decemlineata*. Ent Expo Appl 24:738–749
Visser JH, Van Straten S, Maarse N (1979) Isolation and identification of volatiles in the foliage of potato, *Solanum tuberosum*, a host plant of the Colorado beetle, *Leptinotarsa decemlineata*. J Chem Ecol 5:13–25
Von Arx M, Schmidt-Büsser D, Guerin PM (2011) Host plant volatiles induce oriented flight behaviour in male European grapevine moths, *Lobesia botrana*. J Insect Physiol 57:1323–1331
Wang ZH, Zhao H, Li JF, Zeng XD, Chen JJ, Feng HL, Xu JW (2008) Synergism of plant volatiles to insect pheromones and related mechanisms. Yingyong Shengtai Xuebao 19(11):2533–2537
Wardle DA (2006) The influence of biotic interactions on soil biodiversity. Ecol Lett 9(7):870–886
Wei J, Kang L (2011) Role of (Z)-3-hexenol in plant – insect interactions. Plant Signal Behav 6(3):369–371
Weissteiner S, Schütz S (2006) Are different volatile pattern influencing host plant choice of belowground living insects. Mitt Dtsch Ges Allg Angew Entomol 15:51–55
Wenke K, Kai M, Piechulla B (2010) Belowground volatiles facilitate interactions between plant roots and soil organisms. Planta 231:499–506
Wiemer AP, Moré M, Benitez-Vieyra S, Cocucci AA, Raguso RA, Sérsic AN (2009) A simple floral fragrance and unusual osmophore structure in *Cyclopogon elatus* (Orchidaceae). Plant Biol 11:506–514

Wolfson JL (1987) Impact of rhizobium nodules on *Sitona hispidulus*, the clover root curculio. Entomol Exp Appl 43:237–243

Wu J, Baldwin IT (2010) New insights into plant responses to the attack from insect herbivores. Annu Rev Genet 44:1–24

Wu S, Schalk M, Clark A, Miles RB, Coates R, Chappell J (2006) Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants. Nat Biotechnol 24:1441–1447

Yasui H, Akino T, Fukaya M, Wakamura S, Ono H (2008) Sesquiterpene hydrocarbons: Kairomones with a release effect in the sexual communication of the white-spotted longicorn beetle, *Anoplophora malasiaca* (Thomson) (Coleoptera: Cerambycidae). Chemoecology 18:233–242

Zhang QH, Schlyter F (2004) Olfactory recognition and behavioural avoidance of angiosperm nonhost volatiles by conifer-inhabiting bark beetles. Agric Entomol 6:1–19

Management of Pollination Services to Enhance Crop Productivity

28

K.R. Shivanna

Abstract

Pollination is a prerequisite for fruit and seed set. As fruits and/or seeds are the economic products of most of our crop species, pollination plays a vital role in realizing optimal yield. Except cereals which are wind pollinated, most of our crop species are pollinated by animals, largely insects. Historically pollination of crop plants was dependent on wild native pollinators present on and around the crop fields. Due to a number of reasons, particularly habitat degradation and monoculture cropping system, wild pollinators have not been able to provide adequate pollination services to crop plants. Various approaches are now available to sustain pollination in crop species. The most effective approach is to use managed pollinators; they are being used routinely in developed countries for over 15 major crops and has developed into a well-organized industry. Managed pollinators include honeybees, particularly *Apis mellifera*, bumblebees and recently a few solitary bees such as species of *Osmia*, *Nomia* and *Megachile*. Greenhouse-grown tomatoes are pollinated exclusively by managed bumblebees. Techniques have also been developed to carry out supplementary pollinations through pollen sprays to overcome pollination constraints in self-incompatible species and also during inclement weather conditions under which bee activity gets reduced. Hand pollination is also followed for production of hybrid seeds in some vegetable crops. Recent trend has been towards integrated use of managed pollinators along with wild pollinators by maintaining pollinator-friendly habitats and agricultural practices. The technology of using managed pollinators to overcome pollination constraints is yet to be exploited in developing countries,

K.R. Shivanna (✉)
Conservation Biology, Ashoka Trust for Research in Ecology and the Environment, Royal Enclave, Srirampura, Jakkur Post, Bengalore 560 064, Karnataka, India
e-mail: shivanna@atree.org

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_28,

largely due to lack of awareness amongst the farmers, insufficient data on crop pollinators and limitation of research backup on the management of pollination services.

Keywords
Bumblebees • Hand pollination • Honeybees • Managed pollinators • Pollination constraints • Pollination of greenhouse crops • Wild pollinators

28.1 Introduction

Pollen grains represent the male partners in sexual reproduction of seed plants. In flowering plants, they develop in the anthers and get exposed following anther dehiscence. Pollination (transfer of pollen grains from the anther to the stigma) is a primary requirement for fertilization and subsequent seed development. As the plants are sedentary, they have to make use of other agents to achieve pollination (Shivanna 2014). As pollen grains are the units of dispersal and gene flow, pollination is the basis of recombination and has played a critical role in the evolutionary success of flowering plants (Endress 1994; Willmer 2011; Shivanna 2003, 2014). Except cereals, which make use of wind to transfer their pollen, a majority of crop plants use animals particularly insects for pollination services (McGregor 1976; Free 1993; Roubik 1995; Delaplane and Mayer 2000; Willmer 2011; Shivanna 2014). Amongst insects bees are the most effective pollinators of a number of crop species (James and Pitt-Singer 2008).

Following successful pollination, pollen grains germinate on the stigma, and the resulting pollen tubes grow through the tissues of the stigma and style and enter the ovary. Each pollen tube enters the ovule, located inside the ovary, and eventually the embryo sac (female gametophyte) and discharges the two sperm cells. One of the sperm cells fuses with the egg to give rise to the zygote that develops into the embryo; the other sperm cell fuses with the central cell that develops into the endosperm, a nutrient tissue for the developing embryo. The ovule develops into the seed and the ovary into the fruit. Development of functional pollen, its transfer to the receptive and compatible stigma (pollination), and successful completion of pollen-pistil interaction are the prerequisites for fertilization and fruit and seed development. Of these, pollination is one of the most critical events. Plants have evolved an amazing variety of devises to attract animals and to use them effectively for pollination services (Endress 1994; Willmer 2011; Shivanna 2014). Biotic pollination (mediated by animals) is largely mutualistic in which both the partners (plants as well as animals) are benefited. It is an example of biological barter; plants exchange their nectar and/or pollen for the pollination services of animals (Ollerton 2006).

Seeds and fruits are the economic products of most of our crop species and pollination failure or limitation reduces crop yield. This review gives a concise account on various approaches available to safeguard pollination services of crop plants.

28.2 Pollination Constraints

Historically pollination needs of crop plants were met by wild pollinators living on and around the farm landscapes. Pollination used to be adequate as the cropping system was less intensive and the habitat was conducive for sustainability of pollinators. However, as the cropping system and associated agricultural practices changed over the years, pollination services with wild pollinators became inadequate (Burd 1994; Larson and Barrett 2000; Wilcock and Neiland 2002; Knight et al. 2005) particularly in developed countries. The following are the major causes for inadequate pollination:

- Reduction in the density and diversity of pollinators as a result of anthropogenic influ-

ences. These include habitat loss (degradation, fragmentation, deforestation, invasion by alien species and elimination of nesting sites of native pollinators), marked increase in the level of pollutants and extensive use of pesticides and herbicides for which insects are very sensitive.
- Monoculture cropping system leading to an enormous increase in the area, often extending to hundreds of hectares, covered by the same crop.
- Climate change resulting in disruption of long-term adaptations between plants and pollinators.

In multiovulate species, fertilization of a minimum number of ovules is necessary to activate fruit development. When the number of ovules fertilized is less than the minimum number, fruit fails to develop. Even when the pollen load is sufficient to initiate fruit development but not enough to fertilize optimal number of ovules, the quality of fruit is affected; the size is reduced and often it is distorted affecting the market value of the fruit. Thus, pollination becomes a limitation when the number of pollen grain deposited on the stigma is less than the number sufficient to fertilize optimum number of ovules. Under conditions of inadequate pollination, application of additional fertilizers or use of improved agronomic practices would not be effective in sustaining or improving crop productivity.

28.3 Use of Managed Pollinators

The problem of insufficient pollination has been tackled effectively by the use of managed pollinators for pollination services. This approach has been used in the USA since 1940s and has grown steadily over the years into a multimillion-dollar well-organized industry (Kearns and Inouye 1993; Currie 1997; Shivanna 2003; James and Pitt-Singer 2008). The technology has subsequently been extended to most of the developed countries: Canada, Mexico, almost all European countries, New Zealand, Australia, Japan and recently China. Adequate pollination enhances the value of crop plants by increasing crop quantity, uniformity and quality (Currie 1997).

28.4 Field-Grown Crops

28.4.1 Honeybees

Honeybees have become the most popular managed pollinators because of the ease of their management and transportation. Also, honeybee colonies contain large foraging populations and display floral constancy. *Apis mellifera*, *A. cerana* and *A. florea* are the most important managed honeybees and have been introduced to many countries for pollination services outside their natural ranges. Managed bees are used to pollinate over 15 crops (James and Pitt-Singer 2008). Almond, apple, pear, blackberries, blueberries, cherries, cranberries, pears, plums, squash, tomatoes, watermelons, canola, clover, alfalfa and sunflower are the important crops in which honeybee colonies are being used routinely for pollination services. Honeybees are not native to North America but were imported from Europe in the early seventeenth century for production of honey and wax (Vergera 2008; Delaney and Tarpy 2008). Since the 1940s, however, their use has shifted largely to pollination services. In the USA, *A. mellifera* forms >90 % managed bees. Contribution of wild pollinators to US fruit and vegetable production has declined to <20 % of managed bees (Kremen 2008). Honeybee colonies are rented by farmers and maintained in their fields during the flowering season of the crop to enhance pollination efficiency. The number of honeybee colonies rented every year by farmers is estimated to be 2–2.5 million in the USA, 70,000 in Canada and 200,000 in Mexico (Vergera 2008). The honeybee colonies are moved from one crop to the other coinciding with the flowering season.

In the USA nearly 60 % of the available beehives are used for pollination services of almond crop in California (Bennett 2013). Honeybees and almonds have become inseparable; together they perform the largest managed pollination show on earth. California is estimated to have 810,000 acres of almond orchards. This huge area requires 1,620,000 beehives (at the rate of two hives per acre) each February for pollination services. Of these, 400,000 hives are available

locally, and the rest have to be trucked from other places as far away as Florida and Texas. Their transportation needs 3,000–6,000 trucks (based on the capacity of 200–400 colonies per trailer) (Delaney and Tarpy 2008; Anonymous 2012; Bennett 2013). After completing pollination services for almond, some of the hives are moved to other crops in California itself, and the rest are moved to other states for pollination services of other crops: Washington for apple and cherry orchards, Texas for vegetable crops, Florida for citrus orchards, Wisconsin for cranberries and Michigan for blueberries (Bennett 2013).

The main income for beekeepers comes from renting the hives for pollination services; honey and wax have become the by-products. The rental value of bee colonies varies from crop to crop depending on the season and the demand. The number of bee colonies available for pollination services in the USA in recent decades has come down by 50 %, from 5 million (1950s) to 2.5 million because of various diseases (James and Pitt-Singer 2008; Bromenshenk et al. 2010; Burgett et al. 2010). At the same time the acreage of almond crop has been steadily expanding, thus increasing the peak-season demand of honeybee colonies for almond. This has resulted in a marked increase in the rental value of bee colonies for pollination services. Average rental value for each hive in 2004 was about US$54 (Burgett et al. 2010). In 2006, the rental value jumped to about US$140 (James and Pitt-Singer 2008). The annual monetary value of honeybees as commercial pollinators in the USA is estimated at about $20 billion (Bennett 2013). Canola is an important crop of Canada and Australia and requires a major share of managed honeybee colonies in these countries. Nearly 500,000 colonies were used for pollination services of canola in Australia in 2005–2006 (Anonymous 2008a).

28.4.2 Bumblebees

Honeybees are not the most efficient pollinators in several crops (Currie 1997; Holm 1966; Greenleaf and Kremen 2006). For example, alfalfa (*Medicago sativa*) is the third largest crop in the USA. It needs tripping for effective pollination. Honeybees are not effective pollinators as they tend to rob the nectar from the side of the flower without tripping. Alkali bee and alfalfa leaf-cutting bee are very effective pollinators of alfalfa. Similarly, bumblebees (*Bombus* spp.) are better pollinators (Holm 1966; Velthuis and van Doorn 2006; Anonymous 2008b) than honeybees for several high-value crops such as blueberries, cranberries and clover because of their large size, their long tongue and their ability to vibrate flowers and to fly at relatively low temperatures (Currie 1997). For cultivated raspberries and blackberries, *Osmia* species are better pollinators than honeybees (Cane 2005). In the absence of any other managed pollinators, farmers continued to rent honeybee colonies for pollination services of these crops also; because of their inefficiency, honeybees had to be maintained in high densities in the field to achieve satisfactory pollination.

Because of the lower pollination efficacy of honeybees for several crops and the danger of dependence on a single managed pollinator, many investigators have been exploring the possibility of commercializing other pollinators, particularly bumblebees, for pollination services. Since the late 1980s, it has become possible to domesticate several bumblebee species and supply them commercially for pollination services for several crop species. Velthuis and van Doorn (2006) have given a comprehensive account on the history of bumblebee domestication and various crops in which bumblebees are being used for pollination services. Belgium was the first to adapt this technology, and gradually it has been extended to other countries in Europe (France, Germany, the UK, Italy, the Netherlands, Turkey, Spain and Russia), New World (the USA, Canada, Mexico and Chile), Asia (Japan, Korea and China), Africa (Israel) and New Zealand. About five species of bumblebees have so far been domesticated. Of these *Bombus terrestris* is the most widely used species. They are being used for about 20 crops of which tomato is the most important. Other crops include apple, pear, blueberries, cranberries, clover, sweet peppers and several cucurbits. The number of bum-

blebee colonies used for pollination services in 2004 was estimated to be one million (Velthuis and van Doorn 2006).

The colonies of bumblebees are raised in nesting boxes and are transported to the fields. The nesting boxes are placed at suitable locations amidst crop species. The colonies adapt readily to the new surroundings and start visiting the flowers.

28.4.3 Solitary Bees

Apart from bumblebees, a few other native solitary bees such as mason bee (*Osmia* spp.), alkali bee (*Nomia melanderi*) and alfalfa leaf-cutting bee (*Megachile rotundata*), which are more efficient for a number of crops, have become available for pollination services (Currie 1997; James and Pitt-Singer 2008). Alfalfa leaf-cutting bees and alkali bees have now become more popular than honeybees for alfalfa pollination (James and Pitt-Singer 2008). Alfalfa leaf-cutting bees are solitary and gregarious. They nest in aboveground tunnels in a number of naturally occurring plant parts such as pithy plant stems, hollow twigs and insect-chewed cavities in tree trunks and also in artificial tunnels such as drilled holes in commercial boards. Canada has monopolized the supply of alfalfa leaf-cutting bees. They are sold as individual cells or in nest-filled bee boards. Alfalfa leaf-cutting bee is also used to pollinate carrots, melons and hybrid canola.

Alkali bee is the only solitary, ground-dwelling bee species that is commercially managed. It is strongly gregarious and builds millions of nests in 1 ha (James and Pitts-Singer 2008). As they forage flowers extending up to 3 km, their artificial nesting sites need not be raised close to each other. In Japan the solitary mason bees, *Osmia cornifrons* and *O. lignaria*, are being reared and managed on a large scale to pollinate apple crops (Batra 1997; Sekita 2001; Osch et al. 2008). Recently the use of *O. lignaria* has been extended to China and Korea (Osch et al. 2008). *O. lignaria* has been commercialized in the USA also, and its use as a commercial pollinator in orchard species is steadily increasing. Another species, *O. aglaia*, has been reported to have the potential to become an efficient managed pollinator in its native distribution range of Western Oregon and California for red raspberries and blackberries (Cane 2005).

28.4.4 Integrated Use of Wild and Managed Pollinators

Many studies in recent years have highlighted the role of wild species in crop pollination (Free 1993; Roubik 1995; James and Pitt-Singer 2008; Obutu 2010). Although over 16,000 bee species are known, it has been possible to domesticate only a few of them. Slaa et al. (2006) have highlighted the potential of many stingless bees such as species of *Melipona* and *Trigona* as alternatives for commercial pollination. They have identified 18 crops which are pollinated efficiently by stingless bees. In the Indian subcontinent, also several species of stingless bees have been documented and are reported to be present in most parts of the country except at higher elevations and drier interior regions (Rasmussen 2013; Rathor et al. 2013).

Several studies in recent years have indicated the role of wild pollinators even in the presence of managed pollinators (Greenleaf and Kremen 2006; James and Pitts-Singer 2008; Mader et al. 2010; Albrecht et al. 2012; Garibaldi et al. 2013; Brittain et al. 2013). Behavioural interactions between wild bees and honeybees have been shown to increase pollination efficiency of honeybees up to fivefold in hybrid sunflower (Greenleaf and Kremen 2006). Based on pollination studies of 41 crops from 600 field sites around the world, Garibaldi et al. (2013) have reported that enhancement of fruit set in crop species by honeybees and wild insects is independent. Managed honeybees supplement pollination services rendered by wild insects rather than substituting wild bees. Thus, managed honeybees cannot compensate fully the role of wild pollinators in pollination of crop species.

Adequate pollination of crop species is going to be more problematic in the coming decades as both the wild pollinators and managed pollinators are facing problems. Native bees and hover flies of Britain and the Netherlands have declined

around 30 % after 1980 (Biesmeijer et al. 2006). Similarly, bumblebees in North America have experienced a significant decline during the last decade, and some of them are on the verge of extinction (Cameron et al. 2011). Apart from habitat destruction, the spread of exotic diseases from introduced non-native pollinators to sympatric wild pollinator guilds seems to be another reason for the decline of wild pollinators. Recent studies have provided strong evidences for the spread of deformed wing virus and the exotic parasite, *Nosema ceranae*, to native bumblebees from honeybees (Furst et al. 2014). This has highlighted the importance of controlling pathogens of managed pollinators to maintain wild pollinators in the habitat.

Dramatic decline in wild pollinators has raised global concerns in safeguarding pollination services (UNEP 2002; Biesmeijer et al. 2006; Potts et al. 2010; Obutu 2010; Burkle et al. 2013; Tylianakis 2013; Winfree et al. 2011; Gemmill-Herren et al. 2014). To manage pollination services of crop species in the coming decades, two approaches have been suggested (Kremen 2008): (i) domestication and commercialization of additional pollinator species and (ii) conservation and enhancement of wild pollinator populations on and around agricultural habitats. There is an urgent need to develop pollinator-friendly farming practices to sustain and increase the density and diversity of wild pollinators and to manage them in a sustainable way (James and Pitts-Singer 2008; Garibaldi et al. 2013). Pollinator-friendly habitats include making the nest sites available by maintaining uncultivated strips along field margins, providing artificial nesting sites and reducing the use of pesticides and other unfriendly agrochemicals. The farmers in the coming decades need to focus on integrated use of wild and managed pollinators to safeguard pollination services of crop plants.

28.5 Greenhouse-Grown Crops

Cultivation of fruits and vegetables in greenhouses permits year-round production. The major crops grown in greenhouses are tomato (*Lycopersicon esculentum*), pepper (*Capsicum annuum*), watermelon (*Citrullus lanatus*), melon (*Cucumis melo*), zucchini (*Cucurbita pepo*), cucumber (*Cucumis sativus*), eggplant (*Solanum melongena*), strawberries and *Fragaria* sp. (Guerra-Sanz 2008). Spain, Turkey, Italy, France, Greece and Israel are some of the leading countries in producing greenhouse-grown vegetables. Total greenhouse acreage in the world is estimated to be over 700,000 ha (Guerra-Sanz 2008).

Tomato is the leading greenhouse-grown vegetable crop in the USA, Canada, Australia and several European countries. Tomato flowers need 'buzz' pollination; under field conditions, wind and insects, particularly bumblebees, are the efficient pollinators. Greenhouse-grown tomatoes were pollinated manually until the mid-1990s by using battery-operated vibrators ('magic bees'). Since the 1990s, managed bumblebees are being used to pollinate greenhouse-grown tomatoes. Bumblebees are very efficient for tomatoes as they bring about buzz pollination, which is necessary for effective pollination in this crop; they also do not get disoriented, the way honeybees are, in confined glasshouses (Banda and Paxton 1991; Straver and Plowright 1991; Kearns and Inouye 1993). Now, bumblebees have turned out to be the most popular pollinators for greenhouse-grown tomatoes. Over 95 % of the sales of bumblebee colonies for pollination services are for greenhouse tomatoes. Over 900,000 bumblebee colonies were sold in Eurasia and 55,000 in North America in 2004 (Guerra-Sanz 2008; Vergera 2008). Only 7–15 colonies are needed per hectare to pollinate greenhouse-grown tomatoes (Velthuis and van Doorn 2006).

Bumblebees were introduced to New Zealand in 1885 and 1905 to enhance pollination of red clover in the country (Velthuis and van Doorn 2006). Australia has no native bumblebee species. An inbred feral population of *B. terrestris* occurs in Tasmania. Australian Government has not permitted the import of bumblebees to the country because of the potential environmental problems. Tomato growers still use vibrating wands to pollinate greenhouse tomatoes. Attempts are being made to develop Australian blue-banded bees (*Amegilla* spp.) as effective

managed pollinators for greenhouse tomatoes (Bell et al. 2006; Hogendoorn et al. 2007).

Initially, the use of managed bumblebees was confined largely to greenhouse-grown tomatoes. Subsequently they are being increasingly used to achieve pollination in other greenhouse-grown crops. Recently *Melipona fasciculata* has been reported to be an efficient pollinator of eggplant (*Solanum melongena*) in greenhouses and reported to form a viable alternative to bumblebees (Nunes-Silva et al. 2013).

28.6 Pollinator Attractants and Repellents

Fidelity of pollinators to the target crop depends on the quantity and quality of the reward the crop offers to the insects in the form of nectar and/or pollen. In the absence of sufficient rewards, bees desert commercial target crops for other attractive pollen- and nectar-yielding co-flowering species (competing species) (Jay 1986; Currie 1997). When other species particularly weed species happen to flower on or around the field, the pollinators may abandon the target crop and move to the weed species. One of the approaches to reduce competition from weeds for pollinators is by mowing them or treating them with herbicides.

A number of investigations have studied the potential of spraying the target plants with bee attractants in the form of sugar solution (Beeline, Bee-Q and Bee-Lure), pheromones (Bee-Here, Bee Scent and QMP) or synthetic plant volatiles isolated from nectar or pollen (linalool) on target crop to attract and/or retain pollinators under suboptimal pollination conditions (Jay 1986; Currie 1997; Delaney and Tarpy 2008; Dobson 1994). Although there are reports of such sprays increasing the abundance of foragers on the target crop, the results are not consistent or cost-effective (Currie et al. 1992a, b; Winston and Slessor 1993; Margalith et al. 1984; Elmstrom and Maynard 1991; Goodwin 1997; Ambrose et al. 1995). These approaches have not yet become regular commercial practices.

Similarly, it becomes necessary to prevent bees visiting target crop when they are sprayed with insecticides. A few studies have indicated that some chemicals such as hexanediol and decylamine and some pyrethroid insecticides have the potential to repel bees.

28.7 Assisted Pollination

Pollination may not be adequate even in the presence of managed pollinators due to several reasons. This is particularly true in self-incompatible (such as almond and apple) and dioecious (pistachio) species (in which male and female plants are separate). Both these situations require interplant movements of pollinators: self-incompatible species require cross-pollination from compatible lines, and for dioecious species, pollen grains have to come from male plants. The orchards may not have suitable proportion of compatible/male plants, or their density and distribution may not be sufficient for effective cross-pollination. Also, the flowering period of pollen donor and receiving lines or male and female plants may not be synchronous. In self-incompatible species, even when the pollination is adequate, most of the pollen deposited on the stigma may belong to the same flower/plant/line and thus does not result in seed set. In cloudy or cool weather conditions, also pollination becomes a limitation as bees do not come out under such conditions.

Assisted pollination is carried out with the pollen of compatible lines under conditions of pollination constraints. One of the easy methods to carry out assisted pollination is by using ‘pollen dispensers’. Compatible pollen is placed in pollen dispensers fitted into the opening of the beehive. The honeybees are forced to walk through pollen dispensers during their exit so that compatible pollen grains from the dispensers are deposited on their body parts (Legge 1976; Ferrari 1990). Some of this pollen eventually gets deposited onto the stigmas of the target crop during the foragers’ visit (Griggs and Iwakiri 1960; Williams et al. 1979). The other method is to spray pollen powder/suspension on to the target crop by using motorized pollen blowers (Brown and Perking 1969;

Williams and Legge 1979; Hopping and Jerram 1980a, b). Assisted pollination requires standardization of protocols for pollen collection, pollen storage and pollination (Wong and Hardon 1971). Sprays of pollen suspension have been tried in several fruit crops (Mizuno et al. 2002, peach; Awad 2010, date; Sakamoto et al. 2009, Japanese pear; Yano et al. 2007, kiwi fruit) with some success. Pollen grains of known varieties/lines of a number of species, pollen dispensers and motorized pollen blowers are sold for commercial application by most of the pollen supply companies (Kearns and Inouye 1993).

Pistachio is dioecious; both hand pollination and spray pollination have been tried (Caglar and Kaska 1994; Kuru 1994; Vaknin et al. 2002; Zeraatkar et al. 2013). Hand pollination used to be a common practice for oil palm plantations in Malaysia before the introduction of its natural pollinator, weevil from Africa (Hartley 1988). Assisted pollination was reported to increase the yield by 20–150 % depending on the age of the palms and weather conditions (Hardon 1973). This approach was found to be unnecessary after the introduction of weevils to Malaysia and was discontinued.

In Maoxian county of the Hengduan Mountains in China, apple and pear are the important crops. Overuse of pesticides on these crops over the years has reduced natural pollinators, and beekeepers are reluctant to rent their bee colonies for pollination services because of excessive use of pesticides even during the flowering period. Hand pollination is a regular practice in this region to sustain productivity of apple crop (Partap and Partap 2000). Almost every member of the family gets engaged in hand pollination during the flowering season.

Hand pollination is also carried out regularly for small-scale production of a few crops such as passion fruit and *Vanilla* orchid (Roubik 1995). *Vanilla* orchid is a native of Southern Mexico and Central America where it is pollinated by euglossine bee, *Eulaema. Vanilla* is being grown extensively in many parts of tropical Asia where the pollinator is absent; hand pollination is routinely carried out to sustain the yield.

28.8 Pollination Management for Hybrid Seed Production

Development of hybrid seed technology has been one of the most important advances in agriculture. It is being used extensively throughout the world in a number of seed crops (corn, sorghum, pearl millet, sunflower), fruit crops (almond, apple, pear) and vegetable crops (tomato, onion, *Capsicum*, brinjal and cucurbits). Hybrid seed production involves prevention of self- and intraline pollinations and effective cross-pollination of the female line with the pollen of the male line in hybrid seed production plots. The success of hybrid seed technology depends on the cost of producing hybrid seeds which is largely determined by the method used for the management of required pollinations. In most of the seed crops, development of male-sterile lines or presence of strong self-incompatibility has facilitated effective prevention of self- and intraline pollinations (Shivanna and Sawhney 1997). Wild and/or managed bees are being used to achieve cross-pollination between male-sterile female line and male-fertile line. As male-sterile female lines do not offer pollen and also the amount of nectar is low in some of the species, the frequency of bee visits to female lines and thus pollination efficiency are often reduced. In such hybrid seed production plots, the density of beehives is maintained at much higher level than that in normal crop fields. For example, in canola as against 0.5 colonies/hectare recommended for canola crop, 5.0 colonies are recommended in hybrid production plots (Anonymous 2008a).

Most of our vegetables available in the market such as tomatoes, eggplant, sweet pepper and cucurbits including melons and vegetable brassicas are hybrids (Tay 2002). They are preferred because of their vigour, consistent high yield, and long shelf life. Most of the vegetables of *Brassica* species such as cabbage, cauliflower, broccoli and Brussels sprout are self-incompatible. Self-incompatibility has been exploited to prevent intraline pollination to produce hybrid seeds. Cross-pollination occurs by wild or managed

pollinators (Pearson 1983; Tay 2002). However, in many of the vegetable crops, suitable male-sterile lines or self-incompatible lines are not available, and thus management of required pollination in hybrid seed production plots has been a problem. In such crops, several alternate methods are being used.

28.8.1 Monoecious Species

Some of the vegetable species such as cucurbits and spinach are monoecious (flowers are unisexual but both male and female flowers develop on the same plant); in most of the species, a few flowers that develop in the beginning are males and all later-developed flowers are females. The ratio of male and female flowers on each plant is highly variable; it depends on the genotype of the plant and environmental conditions. In many monoecious species, hybrid seeds are being produced through manipulation of the ratio of male and female flowers produced (Pearson 1983). Application of various growth substances shifts the sex ratio in favour of male or female flowers. In some cucurbits auxins and ethylene induce female flowers, while gibberellic acid and antiethylene substances such as silver nitrate induce male flowers. In several cucurbits, such as *Cucurbita pepo*, *C. sativus* and *C. melo*, and in spinach, it has been possible to isolate highly gynoecious lines and use them for hybrid seed production. These lines (which produce mostly female flowers) and normal monoecious lines (which produce more male than female flowers) are raised in seed production plots. Any male flowers that happen to develop at the beginning of the flowering of the gynoecious lines are removed manually. Cross-pollination (with the pollen of normal line) is achieved through insect activity (Pearson 1983). When insect pollination becomes a constraint, it is supplemented by hand pollination. The hybrid fruits are harvested from the highly gynoecious female line.

28.8.2 Manual Emasculation and Pollination

In several vegetable crops producing bisexual flowers, however, the absence of suitable male-sterile lines and lack of effective pollinators to transfer pollen from male to female lines continue to be a problem. In such crops hand emasculation and/or pollination is practised (Berke 2000; Tay 2002). This is feasible in only such multi-seeded crops such as tomato, capsicum, eggplant and cucurbits in which each fruit contains a large number of seeds. As this method is labour intensive, hybrid seeds in such crops are produced mostly in developing countries to reduce the cost of labour. Until the 1980s Taiwan was the centre of production of hybrid seeds through hand emasculation and/or pollination. However, as their manufacturing industries started competing for labour, the cost of hybrid seed production became too expensive. The production of hybrid seeds was moved to other countries particularly China, India, Thailand and the Philippines. In India, the hybrid seed production is concentrated in the states of Karnataka and Andhra Pradesh.

Cotton is another crop in which hand emasculation and/or pollination is being followed in hybrid seed production in India and China (Venugopal et al. 2003; Santhy et al. 2008). Cotton is a largely self-pollinated crop, and cross-pollination is limited to about 6 % brought about by bees. Although a few male-sterile lines are available, they have not become popular for hybrid seed production because of several limitations. Most of the hybrid seeds in cotton are produced through manual emasculation and pollination. After the release of genetically transformed Bt cotton hybrids in 2002, conventional hybrids are being replaced rapidly by Bt hybrids.

28.9 Introduction of Pollinators

When crops are introduced from their native region to other regions where natural pollinators are absent, as it often happens when crops are

introduced from one country to the other, pollination becomes a major constraint. In such situations, introduction of pollinators is one of the effective approaches for assured pollination. As introduction of pollinators to areas away from their native regions carries many ecological risks, it involves extensive studies on the biology of the pollinator in the new area and monitoring its establishment. Oil palm (*Elaeis guineensis*), a native of Africa and Central South America is pollinated by wind as well as many insects particularly weevils. Oil palm was introduced to Malaysia and Indonesia in the beginning of the twentieth century, where it established well and is extensively grown at present. Pollinating insects were absent in these countries and the yields were low because of inadequate pollination. Introduction of weevil, *Elaeidobius kamerunicus*, an important pollinator of oil palm from Cameroon to Malaysia during the 1980s, markedly increased the yield (Syed 1979; Syed et al. 1982).

28.10 Pollination Management in Developing Countries

Developing countries in general and India in particular have not been able to exploit the use of managed pollinators for optimization of crop yield. They still depend on natural pollinators for pollination services of crop plants. Several studies since the 1970s have indicated that pollination is a constraint in a range of field and orchard crops (Deodikar and Suryanarayana 1977; Roubik 1995; Kevan 1995; Savoor 1998); however, no serious attempts have been made to utilize this low-tech approach of managed pollinators to increase pollination efficiency and consequent crop yield.

A decline in the production and quality of fruits in orchard crops such as apples, pears and almonds and seed crops such as buckwheat due to pollination constraints have been recorded along the Himalayan ranges (Dulta and Verma 1987; Ahmad et al. 2004; Abrol 2011). A recent study from the University of Calcutta has indicated a reduction in vegetable yields in India as a result of pollination limitation (Kinver 2010). India's vegetable production is estimated to be around 7.5 million tons, making the nation second only to China in the world's vegetable production. Continued decline in pollination services would affect not only its position in vegetable production but also the nutritional quality of the food consumed.

Several studies in India have also indicated that maintaining honeybee colonies in the field increases the yield in several crops such as sunflower, safflower, mustard, cucumber, melon, grape and onion (Prasad et al. 1989; Sihag 1995; Partap et al. 2001; Partap 2010; Anonymous 2013). According to an estimate from the Tamil Nadu Agricultural University, the total area of bee dependant crops in India is around 50 million hectares. This acreage requires, on the basis of three colonies per hectare, 150 million colonies to meet the need of pollinators. At present, only 1.2 million colonies are available in the country. These figures highlight the enormity of the pollination problem and the scope of managed pollinators for pollination services in the country (Anonymous 2013).

So far apple seems to be the only crop in which considerable progress has been made in the use of managed honeybee colonies for pollination services (Partap 1998, 2010). Farmers have started renting honeybee colonies (*Apis cerana*, Indian honeybee, or *A. mellifera*, European honeybee) from the horticulture departments of the government and a few private beekeepers for pollinating their apple orchards. The rental value of a colony is about Rs 300 per colony (Partap 2010). Although there is a demand for additional colonies, enough colonies are not available for renting. The use of honeybee colonies for pollination services in apple is yet to develop into an organized industry. Lack of awareness amongst the farmers and lack of dependable information on pollination ecology of most of our crop species are the major constraints in making use of managed honeybees for pollination services.

There is scope for managed pollinators for several cash crops of the northeastern states of India. For example, large cardamom (*Amomum*

subulatum) is an important crop in Sikkim, Nepal and Bhutan. Pollination efficiency of large cardamom in plantations around Sikkim and Darjeeling areas has been reported to be only about 30 % (Sinu and Shivanna 2007). However, honeybees are not the effective pollinators of this crop, although they visit the flowers regularly and rob the nectar. Bumblebees are the major pollinators of large cardamom (Sinu and Shivanna 2007; Sinu et al. 2011). Although bumblebees have been domesticated and commercially used regularly for pollination services in developed countries, the technology is not yet available in India. There is a need to standardize and exploit the services of managed bumblebees in India.

In recent years, there seems to be an increasing realization of the importance of managed pollinators for many other crops (Mohapatra et al. 2010; Abrol 2011; Anonymous 2013). Global pollination project under the United Nations Environment Programme (UNEP) is being conducted by the Food and Agriculture Organization of the United Nations (FAO) in coordination with the governments of seven countries: Brazil, Ghana, India, Kenya, Nepal, Pakistan and South Africa aiming to harness the benefit of wild pollinators for sustainable agriculture. Hopefully these trends would continue and eventually lead to effective management of pollination services to safeguard crop yield in developing countries.

28.11 Concluding Remarks

A number of approaches are available to overcome pollination constraints in crop plants. These are being exploited effectively in developed countries because of the awareness of agri-horticulturists and beekeepers. Also there is a strong research backup to support and sustain the application of these technologies depending on the need. Nevertheless, the enormous diversity of wild pollinators has not yet been fully utilized for pollination services as highlighted by recent reports on the potential of several solitary bees and stingless bees to develop into commercial pollinators. The limitation of available managed pollinators, their increasing cost and the realization of the risk associated with dependence of a limited number of managed pollinator species have lead to serious efforts to domesticate and commercialize additional pollinator species. More importantly the role of native wild pollinators even in the presence of managed pollinators is being realized. The trend is to develop integrated pollination services by increasing the density and diversity of native pollinators and judicial use of managed pollinators to safeguard pollination services of crop plants in the coming decades. These approaches require sustained efforts to make farm landscape pollinator friendly along with suitable modifications in agricultural practices to sustain wild pollinators.

Unfortunately available technologies to overcome pollination constraints are not being exploited effectively in developing countries. Pollination services of crop plants are still dependent largely on wild pollinators and are being taken for granted. There is a lack of awareness amongst the farmers and the public about these approaches to safeguard crop pollination. More importantly there is lack of relevant information both on pollinators and crop species. For several crop species, we do not have even the base line data on their pollinator/pollinator guilds and their variability over space and time. In contrast to the extensive data available in Western countries on wild pollinators and their decline in recent decades, there is hardly any information on pollinator density and diversity and their possible decline in the developing countries. The degradation of the habitat has been more severe in developing countries when compared to developed countries because of population pressure. Therefore, the decline in pollinator diversity and density on and around the agricultural landscapes is expected to be more drastic than that in developed countries. It is important to generate base line data on pollination scenario of crop plants in developing countries. Different pollinators and their efficacy of various crop species need to be evaluated. There is a need to create awareness amongst the farmers and the public on the importance of wild pollinators in the productivity of crop species, and attractive incentives are needed to make agricultural habitats pollinator friendly.

The focus of beekeepers has to be shifted from honey production to pollination services. Also, there has to be a strong research backup on the maintenance and management of domesticated pollinators and details of their deployment in various crop species. Unless serious efforts are made to safeguard pollination services of crop plants in the developing countries, crop productivity, so vital to feed the growing millions, is going to become worse in the coming decades.

Acknowledgements I thank the Indian National Science Academy, New Delhi, for the award of INSA Senior Scientist (2003–2008) and INSA Honorary Scientist (2009–to date) and Ashoka Trust for Research in Ecology and the Environment (ATREE), Bengaluru, for providing facilities.

References

Abrol DP (2011) Pollination biology: biodiversity, conservation and agricultural production. Springer, Science, Dordrecht

Ahmad F, Partap Uma, Joshi SR, Gurung MB (2004) Indigenous honeybees: allies for mountain farmers. LEISA Magazine, 20.4, Dec 2004, pp 12–13

Albrecht M, Schmid B, Hautier Y, Muller CB (2012) Diverse pollinator communities enhance plant reproductive success. Proc R Soc B 279:4845–4852

Ambrose JT, Schultheis HR, Bambara SB, Mangum W (1995) An evaluation of selected commercial bee attractants in the pollination of cucumbers and watermelons. Am Bee J 135:267–272

Anonymous (2008a) Agricultural commodities: small area data, Australia. Australian Bureau of Statistics 2005–2006 (Reissue), ABS No 7125.0

Anonymous (2008b) Bumble bee conservation: pollination Canada. info@pollinationcanada.ca. Accessed 12 Feb 2014

Anonymous (2012) Almond pollination update. http://scientificbeekeeping.com/2012-almond-pollination-update/. Accessed on 13 Mar 2014

Anonymous (2013) Bee flora and pollination of crops. TNAU Agritech Portal, Tamil Nadu Agricultural University, India. http://agritech.tnau.ac.in/farm_enterprises/fe_api_beefloraapollin.html. Accessed 20 Mar 2014

Awad MA (2010) Pollination of date palm (*Phoenix dactylifera* L."Lulu") with pollen grains water suspension. Acta Horticult 882:337–343

Banda HJ, Paxton RJ (1991) Pollination of greenhouse tomatoes by bees. Acta Horticult 288:194–198

Batra SWT (1997) Solitary bees for orchard pollination. Pennsylvania Fruit News 77(4):89–91

Bell MC, Spooner-Hart RN, Haigh AM (2006) Pollination of greenhouse tomatoes by the Australian bluebanded bee, *Amegilla (Zonamegilla) holmesi* Rayment (Hymenoptera: Apidae). J Eco Entomol 99:437–442

Bennett C (2013) Honey bees to rent? Demand will only grow. http://westernfarmpress.com/blog/honey-bees-rent-demand-will-only-grow. Accessed on 20 Dec 2013

Berke TG (2000) Hybrid seed production in *Capsicum*. In: Basra AS (ed) Hybrid seed production in vegetables, rationale and methods in selected species, Food Products Press, New York, pp 49–67

Biesmeijer JC, Roberts SPM, Reemer M et al (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313:351–354

Brittain C, Williams N, Kremen C, Klein A-M (2013) Synergistic effects of non-*Apis* bees and honey bees for pollination services. Proc R Soc B 280:20122767

Bromenshenk JJ, Henderson CB, Wick CH et al (2010) Iridovirus and Microsporidian linked to honey bee colony decline. PLoS ONE 5(10):e13181

Brown GK, Perking RM (1969) Experiments with aircraft methods for pollinating dates. Date Grow Inst Rep 46:35–40

Burd M (1994) Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. Bot Rev 60:83–139

Burgett M, Daberkow S, Randal RR, Thurman W (2010) U.S. pollination markets: recent changes and historical perspective. Am Bee J 150:35–41

Burkle LA, Marlin JC, Knight TM (2013) Plant-pollinator interactions over 120 years: loss of species, co-occurrence and function. Science 339:1611–1615

Caglar S, Kaska N (1994) A study on the supplemental pollination of pistachio in the Mediterranean region. Acta Horticult 419:55–60

Cameron SA, Lozier JD, Strange JP et al (2011) Patterns of widespread decline in North American bumble bees. Proc Natl Acad Sci U S A 108:662–667

Cane JH (2005) Pollination potential of the bee *Osmia aglaia* for cultivated red raspberries and blackberries (*Rubus*: Rosaceae). Hort Sci 40:1795–1808

Currie RW (1997) Pollination constraints and management of pollinating insects for crop production. In: Shivanna KR, Sawhney VK (eds) Pollen biotechnology for crop production and improvement. Cambridge University Press, New York, pp 121–151

Currie RW, Winston ML, Slessor KN (1992a) Effect of synthetic queen mandibular pheromone sprays on honey bee (Hymenoptera, Apidae) pollination of berry crops. J Econ Entemol 85:1300–1306

Currie RW, Winston ML, Slessor KN, Mayer DF (1992b) Effect of synthetic queen mandibular pheromone sprays on pollination of fruit crops by honey bees (Hymenoptera, Apidae). J Econ Entomol 85:1293–1299

Delaney DA, Tarpy DR (2008) The role of honey bees in apple pollination. Apiculture program, North-

Carolina State University, USA Bee Keeping Notes 3.03. 9/2008: 1–6. http://www.cals.ncsu.edu/entomology/apiculture/PDF%20files/3.03.pdf. Accessed 20 Jan 2014

Delaplane KS, Mayer DF (2000) Crop pollination by bees. CABI Publishing, Cambridge

Deodikar GB, Suryanarayana MC (1977) Pollination in the services of increasing farm production in India. In: Nair PKK (ed) Advances in pollen spore research. Today and Tomorrow Printers and Publishers, New Delhi, pp 60–82

Dobson HEM (1994) Floral volatiles in insect biology. In: Bernays E (ed) Insect plant interactions, vol V. CRC Press, Boca Raton

Dulta P, Verma LR (1987) Role of insect pollinators on yield and quality of apple fruit. Indian J Horticult 44:274–279

Elmstrom GW, Maynard DN (1991) Attraction of honey bees to watermelon with bee attractant. Proc Fla State Hort Soc 103:130–133

Endress SK (1994) Diversity and evolutionary biology of tropical flowers. Cambridge University Press, New York

Ferrari TE (1990) 'Enpollination' of honeybees with precollected pollen improves pollination of almond flowers. Am Bee J 130:801

Free JB (1993) Insect pollination of crops, 2nd edn. Academic, Orlando

Furst MA, McMahon DP, Osborne JL et al (2014) Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature 506:364–366

Garibaldi LA, Steffan-Dewenter I, Winfree R et al (2013) Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science 339:1608–1611

Gemmill-Herren B, Aidoo K, Kwapong P et al (2014) Priorities for research and development in the management of pollination services for agriculture in Africa. J Pollination Ecol 12:40–51

Goodwin RM (1997) Feeding sugar syrup to honey bee colonies to improve pollination: a review. Bee World 78:56–62

Greenleaf SS, Kremen C (2006) Wild bees enhance honey bees' pollination of hybrid sunflower. Proc Natl Acad Sci U S A 103:13890–13895

Griggs WH, Iwakiri BT (1960) Orchard test of beehive pollen dispensers for cross-pollination of almonds, sweet cherries and apples. Proc Am Soc Horticult Sci 75:114–128

Guerra-Sanz JM (2008) Crop pollination in greenhouses. In: James RR, Pitts-Singer TL (eds) Bee pollination in agricultural ecosystems. Oxford University Press, New York, pp 27–47

Hardon JJ (1973) Assisted pollination of oil palm: a review. In: Wasti RL, Earp DA (eds) Advances in oil palm cultivation, Proceeding of international oil palm conference, Incorporated Society Planters, Kuala Lumpur, pp 184–195

Hartley CWS (1988) The oil palm, 2nd edn. Longman, London

Hogendoorn K, Coventry S, Keller MA (2007) Foraging behaviour of a blue banded bee, *Amegilla chlorocyanea* in greenhouses: implications for use as tomato pollinators. Apidologie 38:86–92

Holm SN (1966) The utilization and management of bumble bees for red clover and alfalfa seed production. Ann Rev Entomol 11:155–182

Hopping ME, Jerram EM (1980a) Supplementary pollination of fruit trees I. Development of suspension media. N Z J Agric Res 23:509–515

Hopping ME, Jerram EM (1980b) Supplementary pollination of fruit trees II. Field trials of Kiwi-fruit and Japanese plums. N Z J Agric Res 23:517–521

James RR, Pitts-Singer TL (eds) (2008) Bee pollination in agricultural ecosystems. Oxford University Press, Oxford

Jay SC (1986) Spatial management of honey bees on crops. Ann Rev Entomol 31:49–65

Kearns CA, Inouye DW (1993) Techniques of pollination biologists. University Press Colorado, Niwot Ridge

Kevan PG (ed) (1995) The Asiatic hive bee: apiculture, biology, and role in sustainable development in tropical and subtropical Asia. Enviroquest, Ltd, Cambridge

Kinver M (2010) 'Pollination crisis' hitting India's vegetable farmers. BBC News, Science and Environment, 28 September 2010 (http://www.bbc.co.uk/news/science-environment-11418033). Accessed 12 Dec 2013

Knight TM, Steets JA, Vamosi JC et al (2005) Pollen limitation of plant reproduction: pattern and process. Ann Rev Ecol Evol Syst 36:467–497

Kremen C (2008) Crop pollination services from wild bees. In: James RR, Pitts-Singer TL (eds) Bee pollination in agricultural ecosystems. Oxford University Press, New York, pp 10–26

Kuru C (1994) Artificial pollination of pistachio trees under insufficient pollination condition. Acta Horticult 419:121–124

Larson BMH, Barrett SCH (2000) A comparative analysis of pollen limitation in flowering plants. Biol J Linn Soc 69:503–520

Legge AP (1976) Hive inserts and pollen dispensers for tree fruits. Bee World 57:159–167

Mader E, Vaughan M, Shepherd M, Black SC (2010) Alternative pollinators: native bees. The Xerces Society for invertebrate conservation http://attra.ncat.org/attra-pub/PDF/nativebee.pdf. Accessed 22 Feb 2014

Margalith R, Lensky Y, Rabinowitch HD (1984) An evaluation of Beeline as a honeybee attractant to cucumbers and its effect on hybrid seed production. J Agric Res 23:50–54

McGregor SE (1976) Insect pollination of cultivated crop plants, vol 495, Agricultural Handbook. USDA-ARS, US Govt Print Office, Washington, DC

Mizuno S, Waki K, Todo R (2002) Studies on the labor saving technique for the artificial pollination of peach trees. Pollen storage and pollen spraying methods for the artificial pollination. Bull Fac AgricTamagawa Univ 42:1–14

Mohapatra LN, Sontakke BK, Ransingh N (2010) Enhancement of crop production through bee pollination. Orissa Rev, Sept 2010, pp 44–47

Nunes-Silva P, Hrncir M, de Silva CI et al (2013) Stingless bees, *Melipona fasciculata*, as efficient pollinators of eggplant (*Solanum melongena*) in greenhouses. Apidologie 44:537–546

Obutu GC (2010) Pollination: a threatened vital biodiversity service to humans and the environment. Intl J Biod Conserv 2:1–13

Ollerton J (2006) "Biological barter": patterns of specialization compared across different mutualisms. In: Waser NW, Ollerton J (eds) Plant-pollinator interactions; from specialization to generalization. University of Chicago Press, Chicago, pp 411–435

Osch J, Sgolastra F, Kemp WP (2008) Life cycle ecophysiology of *Osmia* mason bees used as crop pollinators. In: James RR, Pitts-Singer TL (eds) Bee pollination in agricultural ecosystems. Oxford University Press, New York, pp 83–104

Partap U (1998) Successful pollination of apples in Himachal Pradesh. Beekeeping Dev 48:6–7

Partap U (2010) Honeybees as providers of pollination services. Mountain Farm Bull, Jan 2010, pp 42–44

Partap U, Partap T (2000) Pollination of apples in China. Beekeeping Dev 54:6–7

Partap U, Partap T, Yonghua H (2001) Pollination failure in apple crop and farmers' management strategies. Acta Horticult 561:225–230

Pearson CH (1983) Heterosis in vegetable crops. In: Frankel R (ed) Heterosis: reappraisal of theory and practice. Springer, Berlin, pp 138–189

Potts SG, Biesmeijer JC, Kremen C et al (2010) Global pollinator declines: trends, impacts and drivers. Trends Ecol Evol 25:345–353

Prasad D, Hameed SF, Singh R et al (1989) Effect of bee pollination on the quantity and quality of rai crop (*Brassica juncea* Coss.). Indian Bee J 51:45–47

Rasmussen C (2013) Stingless bees (Hymenoptera: Apidae: Meliponini) of the Indian subcontinent: diversity, taxonomy and current status of knowledge. Zootaxa 3647:401–428

Rathor VS, Rasmussen C, Saini MS (2013) New record of the stingless bee *Tetragonula gressitti* from India (Hymenoptera: Apidae: Meliponini). J Melittology 7:1–5

Roubik DW (ed) (1995) Pollination of cultivated plants in the tropics, vol 118, FAO Agriculture Service Bull. FAO, UN, Rome

Sakamoto D, Hayama H, Ito A et al (2009) Spray pollination as a labor-saving pollination system in Japanese pear (*Pyrus pyrifolia* (Burm.f.) Nakai.): development of the suspension medium. Sci Hort 119:280–285

Santhy V, Khadi BM, Singh P et al (2008) Hybrid seed production in cotton, Tech Bull No. 35. Central Institute of Cotton Research, Nagpur (www.cicr.org.in)

Savoor RR (1998) Pollination management: an ecofriendly green revolution eludes India. Curr Sci 74:121–125

Sekita N (2001) Managing *Osmia cornifrons* to pollinate apples in Aomori Prefecture, Japan. Acta Horticult 561:303–308

Shivanna KR (2003) Pollen biology and biotechnology. Science Publishers, Inc, Plymouth

Shivanna KR (2014) Biotic pollination: how plants achieve conflicting demands of attraction and restriction of potential pollinators. In: Ramawat KG, Merillon J-M, Shivanna KR (eds) Reproductive biology of plants. CRC Press, Boca Raton, pp 218–267

Shivanna KR, Sawhney VK (eds) (1997) Pollen biotechnology for crop production and improvement. Cambridge University Press, New York

Sihag RC (ed) (1995) Pollination biology: pollination, plant reproduction and crop seed production. Rajendra Scientific Publishers, Hissar

Sinu PA, Shivanna KR (2007) Pollination biology of large cardamom (*Amomum subulatum*): an important cash crop of Eastern Himalayas. Curr Sci 93:548–552

Sinu PA, Kuriakose G, Shivanna KR (2011) Is the bumblebee (*Bombus haemorrhoidalis*) the only pollinator of large cardamom in central Himalayas, India. Apidologie 42:690–695

Slaa EJ, Sanchez-Chaves LA, Malagodi-Braga KS, Hofstede FE (2006) Stingless bees in applied pollination: practice and perspectives. Apidologie 37:293–315

Straver WA, Plowright RC (1991) Pollination of greenhouse tomatoes by bumblebees. Greenh Can 11:10–12

Syed RA (1979) Studies on oil palm pollination by insects. Bull Entomol Res 69:213–224

Syed RA, Low IH, Corley RHV (1982) Insect pollination of oil palm: introduction, establishment and pollinating efficiency of *Elaeidobius kamerunicus* Faust. Malaysia Planter Kuala Lumpur 58:547–561

Tay D (2002) Vegetable hybrid seed production. In: McDonald M, Contreras S (eds) Proceedings of international seed seminar: trade, production and technology. Pontificia Universidad Católica de Chile, pp 128–139

Tylianakis JM (2013) The global plight of pollinators. Science 339:1532–1533

UNEP (2002) Action for a sustainable future, decisions from the sixth meeting of the conference of the parties to the convention on biological diversity, United Nations, The Hague, http://www.biodiv.org/doc/meetings/cop/cop-06/official/cop-06-20-en.pdf

Vaknin Y, Gan-Mor S, Bechar A et al (2002) Effects of supplementary pollination on cropping success and fruit quality in pistachio. Plant Breed 121:451–455

Velthuis HHW, van Doorn A (2006) A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. Apidologie 37:421–451

Venugopal K, Rathinavel K, Manickam S, Gururajan KN (2003) Cotton. In: Singhal NC (ed) Hybrid seed production in field crops: principles and practices. Kalyani Publishers, New Delhi, pp 137–162

Vergera CH (2008) Environmental impact of exotic bees introduced for crop pollination. In: James RR, Pitts-Singer TL (eds) Bee pollination in agricultural ecosystems. Oxford University Press, New York, pp 145–165

Wilcock C, Neiland R (2002) Pollination failure in plants: why it happens and when it matters. Trends Plant Sci 7:270–277

Williams RR, Legge AP (1979) Pollen application by mechanical dusting in English apple orchards. J Hort Sci 54:67–74

Williams RR, Church RM, Wood DES, Flook VA (1979) Use of an anthocyanin progeny marker to determine the value of hive pollen dispensers in apple orchards. J Hort Sci 54:75–78

Willmer P (2011) Pollination and floral ecology. Princeton University Press, Princeton

Winfree R, Ignasi Bartomeus I, Cariveau DP (2011) Native pollinators in anthropogenic habitats. Ann Rev Ecol Evol Syst 42:1–22

Winston ML, Slessor KN (1993) Application of queen honey bee mandibular pheromone to beekeeping and crop pollination. Bee World 74:111–128

Wong YK, Hardon JJ (1971) A comparison of different methods of assisted pollination in the oil palm. Chemara Res Stn, Seremban

Yano T, Miyata N, Matsumoto H (2007) The use of liquid pollen extender thickened with polysaccharides for artificial pollination of kiwifruit. Acta Horticult 753:415–424

Zeraatkar H, Karimi HR, Shamshiri MH, Tajabadipur A (2013) Preliminary evaluation of artificial pollination in pistachio using pollen suspension spray. Plant Knowl J 2:94–98

Applications of Remote Sensing in Plant Sciences: An Overview

29

C. Sudhakar Reddy

Abstract

Remote sensing and geographical information system techniques are becoming increasingly important in the field of plant sciences. The advantage of integrating the spatial and nonspatial information can be effectively used in various aspects of forests, phytodiversity and conservation. There are many case studies that have illustrated potential application of modern remote sensing to identify areas of high biodiversity, prediction of species distribution, and modeling species responses to environmental and anthropogenic changes. A suite of satellites are available to generate information on vegetation and land covers have been discussed. The present article focused on important contribution of remote sensing for forest canopy density and type mapping, biodiversity assessment – landscape to species, delineation of gregarious species and community types, as stratification base for ground sampling, temporal monitoring, wildlife management, species distribution patterns and modeling, gap areas for biological exploration, gap analysis for protected area network, mapping and monitoring of invasive species, forest fire monitoring, vegetation status, and climate change studies. It was concluded that remote sensing and geographic information systems provide efficient tools for plant sciences and related fields.

Keywords

Remote sensing • Biodiversity • Conservation • Satellites • Sensors • Forest change

29.1 Introduction

Understanding the drivers of species distributions and levels of species richness and how they operate in different geospatial contexts is a fundamental challenge (Turner et al. 2003). Biodiversity is

C. Sudhakar Reddy (✉)
Forestry and Ecology Group, National Remote Sensing Centre, Balanagar, Hyderabad 500 037, Telangana, India
e-mail: drsudhakarreddy@gmail.com

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_29,

expressed at different levels – genetic, species, and landscape. Though biodiversity is generally appreciated at the species level, the understanding at the landscape level has been given emphasis globally as the interaction with the habitat part is very well understood in the latter. Mixture of biotic and abiotic factors influences the structure and functions of forests. Sustainable use of forest resources requires precise assessment of forest stock. Conversion of natural habitats by man is the major cause of the loss of biodiversity that needs to be surveyed, mapped, monitored, and quantified. The conventional methods for collection of information on forests and biodiversity have been found to be costly and time consuming. So there is a need of state-of-the-art technology for a holistic management of forests and biodiversity. Ecologists and conservation biologists are finding new ways to approach their research with the powerful suite of tools and data from remote sensing (Kerr and Ostrovsky 2003).

Remote sensing is being operationally used to monitor and assess the deforestation, commercial logging, fragmentation, and other anthropogenic influences (Roy and Tomar 2000). The application of remote sensing data for assessing biodiversity and identifying the causes of biodiversity depletion has been studied at different spatial scales across the globe (Rashid et al. 2013). The modern technology of remote sensing allows us to collect a lot of spatial data rather easily, with speed and on repetitive basis, and together with GIS helps us to analyze the data spatially, offering various options. Remote sensing and geographic information system (GIS) can be used as important tools separately or in combination for application in studies of forestry and biodiversity. Remote sensing techniques are quite useful for generation of thematic information. It provides a means for obtaining a synoptic view of the status of forest and condition on near real-time basis. Vegetation type and density maps are the primary spatial layers generated from satellite data. Using this information in conjunction with landscape spatial analysis, phytosociological data, bioclimate, soil, and topography, preparation of different kinds of derived maps related to biodiversity, wildlife management, and growing stock assessment is quite possible. Satellite-based remote sensing is probably the only way to inventory the change in global forest ecosystems and prioritize and assess the success of conservation efforts. GIS accommodates large varieties of spatial and nonspatial (attribute) data (Salem 2003). GIS deserves special attention in analysis, measurement, and planning related to biodiversity. The most widely used definition of GIS is "a computer-based system that captures, stores, manages, analyzes, and displays geo-referenced data. Many data relating to environmental and ecological systems have been collected and stored in forms suited to management and analysis using GIS (Aspinall 1995).

The potential of modern remote sensing is to identify areas of high biodiversity, prediction of species distribution, and modeling species/community responses to environmental and anthropogenic changes (Turner et al. 2003). The remote sensing of biodiversity covers two approaches. One is the direct remote sensing of individual organisms, species assemblages, or communities from airborne or satellite sensors. Another approach is the indirect remote sensing of biodiversity through dependence on environmental parameters as proxies (Turner et al. 2003). A suite of remote sensing satellites are available to generate information on vegetation and land cover (Table 29.1). Components of vegetation structure and composition provide a basis for mapping. It is possible to map biodiversity elements, i.e., species composition, number of trees, crown closure, vegetation index, leaf area index, and patch metrics using different image processing techniques, visual image interpretation techniques, algorithms, and object-oriented methods in conjunction with field studies.

Spatial information of vegetation types and land use works as one of the very important contribution for the following:

1. Forest canopy density and type
2. Biodiversity assessment – Landscape to species
3. Delineation of gregarious species and community types

Table 29.1 Commonly used sensors in biodiversity-related studies (Turner et al. 2003 and present review)

Ecological/biodiversity component	Sensor	Spatial resolution	Spectral resolution	Description
Direct approaches				
Species composition	LISS, TM, ETM+, Hyperion, ASTER, IKONOS, QuickBird, AVIRIS, CASI	<1–30 m	V.NIR, SWIR; ASTER also has TIR	Can measure directly canopy community and perhaps species, type based upon unique spectral signatures
Land cover	LISS, AWIFS, MODIS, SPOT, TM, ETM+, ASTER, ALI, IKONOS, QuickBird	<1–1,000 m	V.NIR, SWIR; MODIS and ASTER also have TIR	Can discriminate different land surfaces at various resolutions; l land cover classification is considered a first-order analysis for species occurrence
Indirect approaches				
Primary productivity				
Chlorophyll	OceanSat, SeaWIFS, MODIS, ASTER, LISS, TM, ETM+, SPOT, ASTER, ALI, IKONOS, QuickBird	<1–1,000 m	V.NIR, SWIR; MODIS and ASTER also have TIR	Measure reflectance to assess presence/absence of vegetation and relative greenness for calculating productivity and plant health
Climate				
Rainfall	CERES, AMSR-E	20–56 km	Microwave	Enable detection of precipitation and surface moisture at coarse resolutions
Phenology	OceanSat, SeaWIFS, MODIS, ASTER, AWIFS, LISS, TM, ETM+, ASTER, ALI, IKONOS, QuickBird	<1–1,000 m	V.NIR, SWIR, MODIS, and ASTER also have TIR	Information on leaf turnover can be inferred
Habitat structure				
Topography	CARTOSAT, SRTM, ASTER, IKONOS, SLICER, LVIS, ATM	1–90 m	Microwave SRTM; V/NIR and SWIR for others	Many species are constrained by microhabitats resulting from changes in altitude
Vertical canopy structure	SLICER, LVIS	1–10 m	V/NIR	Provides 3D measurements via laser pulses; provides biomass estimates and information about vegetation structure

4. Stratification base for ground sampling
5. Temporal monitoring
6. Wildlife management
7. Species distribution patterns and modeling
8. Gap areas for biological exploration
9. Gap analysis for protected area network
10. Mapping and monitoring of invasive species
11. Forest fire monitoring
12. Vegetation status
13. Climate change studies

29.2 Forest Canopy Density and Type

Vegetation cover in general and forest cover in particular are indicators for the well-being of the environment. Without comprehensive information on the status, types, dynamics, and responses of the forest ecosystem, it is impossible to evaluate management strategies or to clearly identify and quantify changes in forest resources, including forest areas, and the composition and quality of forests. The vegetation type and forest canopy density layers are the basic thematic layers which serve as primary inputs to the forest working plan preparation. Mapping the distributions of vegetation types provides critical information for managing landscapes. Because vegetation type can link to species composition or habitat types, vegetation maps provide crucial information for conservation planning.

Spatial explicit boundaries of vegetation types are important for studying the patterns of species diversity and long-term monitoring. The delineation of such boundaries for larger spatial extents based on field information would become a time and cost-effective exercise. However, the delineation depends on spatial, spectral, and temporal resolution of the satellite sensor on the one hand and the spatial extent and degree of homogeneity of the species assemblages on the other hand.

The forest cover assessment in India is being carried out by the Forest Survey of India (FSI 2007), biannually on 1:50,000 scale (Fig. 29.1). Forest canopy (crown) in terms of density is the major criterion to identify the ecological status of forest cover. Forest canopy density mapping with three canopy density classes, i.e., very dense (>70 % canopy), dense (>40 % canopy), and open (10–40 % canopy), is being accomplished using IRS LISS III satellite data to facilitate forest cover mapping and assessment at the national/state level. Very high-resolution data (IRS PAN/IKONOS/Cartosat) enable the delineation of five density classes (with an interval of 20 %) to facilitate operational planning and management. Forest stock maps are derived by combining the forest type layer with forest crown density information.

29.3 Biodiversity Assessment: Landscape to Species

A landscape represents a mosaic of interacting ecosystems and consists of patches of different land covers. Mapping the distribution of vegetation types and land covers in a region as landscape elements can be done with the help of remotely sensed data in conjunction with information on topography, climate, and field surveys. Vegetation type data provides stratification base for optimal ground sampling and assessment of biodiversity.

Jha et al. (2005) studied the impact of decreasing patch size of the fragmented forest on species diversity in Indian Vindhyans. Patch dynamics is best understood and explained by analyzing the size, shape, and arrangement in time and space. Forest fragmentation is one of the major factors causing biodiversity loss (Prasad et al. 2009). It is caused by several factors including change in land use (encroachment and deforestation). The existing biodiversity of these patches is again a manifestation of existing environmental conditions and human disturbance. The relationship between the biodiversity and patch size/shape is well known. Therefore, the patch characterization along with landscape parameters enables one to identify disturbance regimes. Besides, the biodiversity characterization of landscapes provides very important inputs for the prioritization of sustainable bioprospecting.

National project on Biodiversity Characterization at Landscape Level (BCLL) using satellite remote sensing (RS) and geographical information system (GIS) has been undertaken by the Department of Space (DOS) and Department of Biotechnology (DBT) as an important initiative to develop baseline database of forest landscapes of India (NRSC 2007).

The primary objectives of the studies are as follows:

- Prepare baseline data on vegetation cover types and land use.
- Study the human influence on landscape as disturbance regimes and habitat characterization and identification of the biologically rich areas.
- Generate web-based data repository and information system (biodiversity information system).

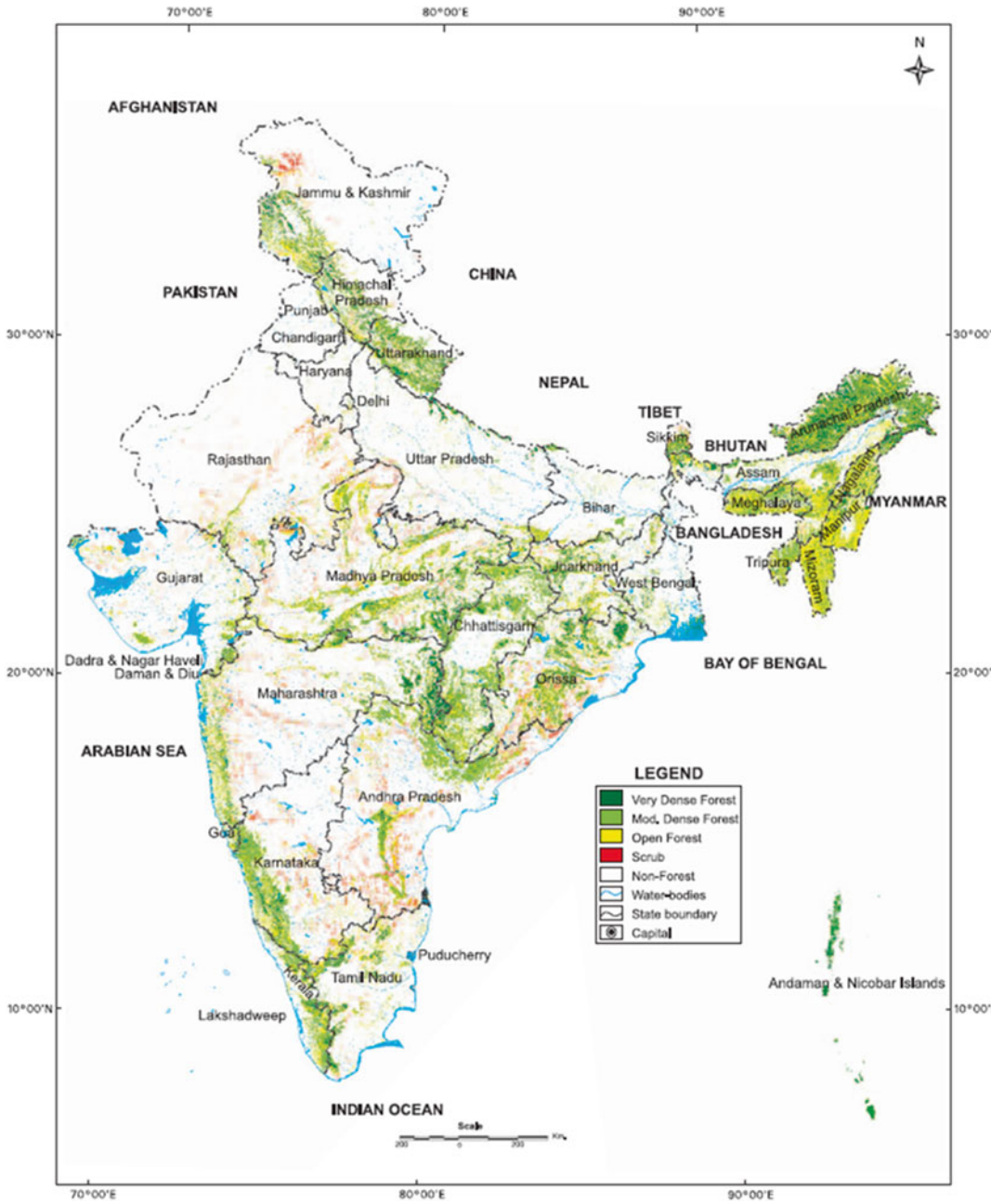


Fig. 29.1 Forest cover map of India prepared based on IRS LISS III data (FSI 2007)

Approach for landscape-level characterization was presented in Fig. 29.2. Fragmentation map provides the size class distribution of the forest patches. The disturbance map provides a qualitative description of the spatial distribution of the relative disturbance regimes based on fragmentation, habitat heterogeneity, and neighborhood influences studied based on vegetation type map, field species data along with anthropogenic pressure information in terms of road net work, villages, forest fires, and grazing. This disturbance index provides an important database on distribution of different disturbance regions and associated sensitive-resistant species.

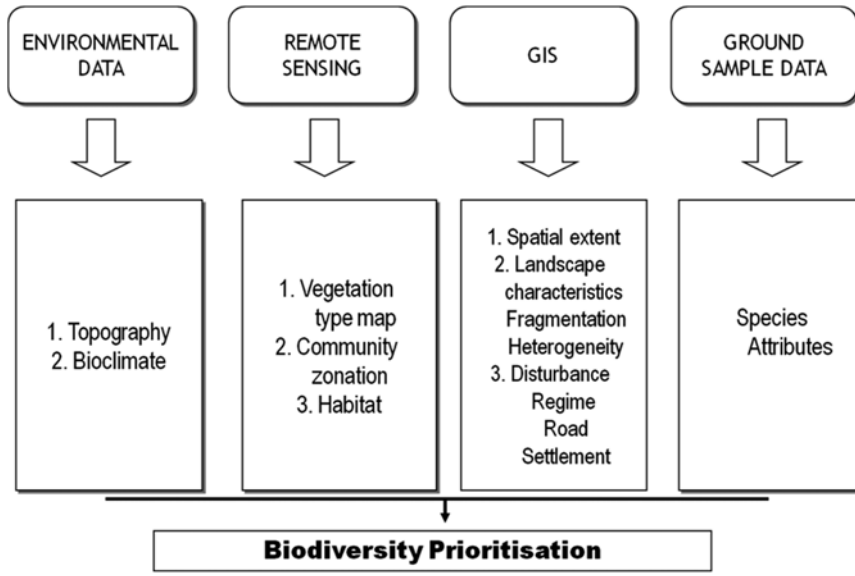


Fig. 29.2 Approach for landscape-level biodiversity characterization (NRSC 2007)

Biological richness map shows spatial distribution of a composite index of biological richness which includes the positive magnitude of biodiversity richness in a patch and negative magnitude of the fragmentation/disturbance. This spatial explicit index map of biological richness is the final outcome of the geospatial modeling of disturbance index, terrain, and field level species database weighted in terms of economic values, biodiversity value, and ecosystem uniqueness. It provides spatial distribution of high, medium, and low biological richness areas (Fig. 29.3).

29.4 Delineation of Gregarious Species and Community Types

The information on different tree species and community types is useful for preparing management plans for conservation and sustainable utilization of these valuable resources. Mapping of species and species assemblages at spatial resolution of more than 1 m and direct identification of certain species (e.g., through the detection of individual tree crowns) and species assemblages are feasible. The IKONOS and the QuickBird multispectral imagery at resolutions of 4 m and 2.4–2.8 m, respectively, and panchromatic imagery at 1 m and 0.6–0.8 m, respectively improved the ability of distinction of tree species. The important tree species communities of *Teak*, *Sal*, *Bamboo*, *Dipterocarpus*, *Anogeissus pendula*, and *Acacia senegal* can be effectively mapped using Landsat data and IRS LISS III, which are having spatial resolutions of 30 m and 24 m, respectively.

29.5 Stratification Base for Ground Sampling

Biodiversity assessment needs spatial perspective, i.e., sampling in space. In natural ecosystems, communities are typically recognized by their location. The satellite remote sensing provides precise stratification in terms of forest crown density, vegetation types, communities, and species formations which can form the basis for reducing the strata variance and make precise estimates. This assumes larger relevance in the context of high degree of variability of spatial vegetation type distribution in the study area. The spatial explicitness in the estimates can be brought out at desired scale and accuracy through GIS by accounting the strata proportions and value of category of interest per unit area for a given strata.

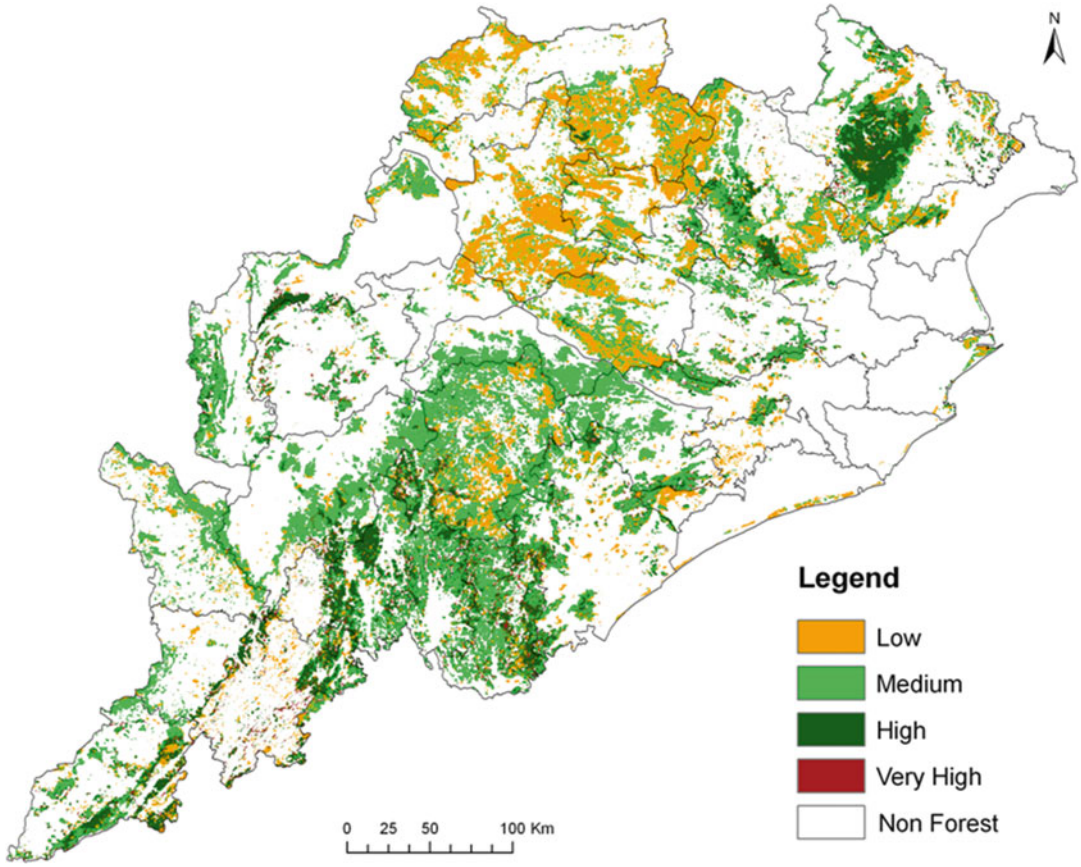


Fig. 29.3 Biological richness map of Odisha, India

29.6 Temporal Monitoring

Deforestation is emerged as a major environmental problem and considered as first worst threat for existence of biodiversity. The rate of deforestation varies from region to region. The Food and Agriculture Organization (FAO) estimates that 137,000 km^2 of tropical forests were destroyed each year. The current rate of tropical forest loss and disturbances has resulted in 5–10 % loss of all forest species in one decade during the last quarter century (Mc Neely et al. 1990). If the current rate of deforestation continues, the world's rain forests will vanish within 100 years – causing unknown effects on global climate and eliminating the majority of plant and animal species on the planet (NASA 1998). The study in Nabarangpur District of Odisha indicates that the forest cover in 1973 accounted for about 2,122 km^2 (40.1 %) out of the total geographical area. Within a period of three decades, by 2004, it was reduced to 793 km^2 (14.8 % of the area) (Reddy et al. 2009a), and it means negative changes (loss of forest area) accounted for 1,338 km^2 (25.3 %) (Fig. 29.4). The Government of Odisha filed more than 12,000 cases against encroachment, but not single hectare of land has been recovered. Mangroves of Bhitarkanika Wildlife Sanctuary also lost forest cover of 1,534 ha in last three decades (Reddy et al. 2007). The recent study by Reddy et al. (2013) on Odisha has used historical topographical maps and Landsat MSS; IRS 1B LISS-I along with the more recent IRS P6 AWiFS (2005 and 2011) imagery to determine the distribution of forest cover and to provide estimates of rates of deforestation (Fig. 29.5).

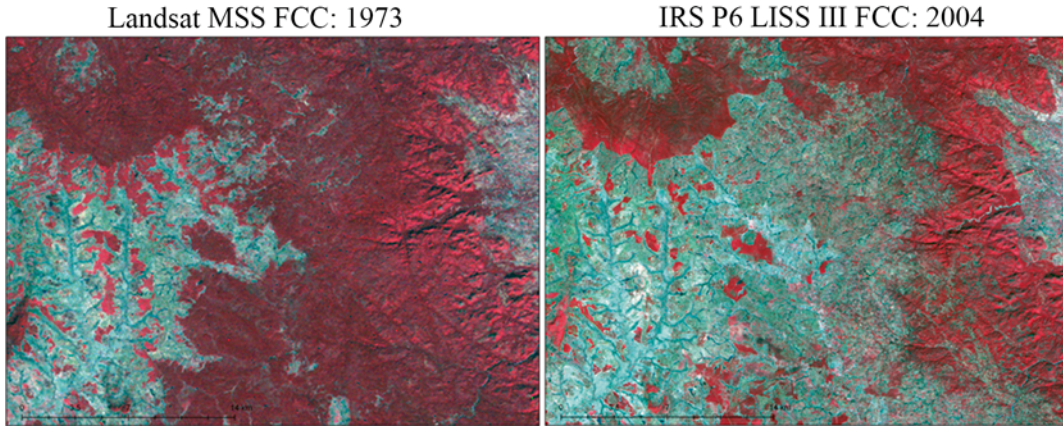


Fig. 29.4 Multi-temporal satellite images showing deforestation (part of Nabarangpur district, Odisha, India)

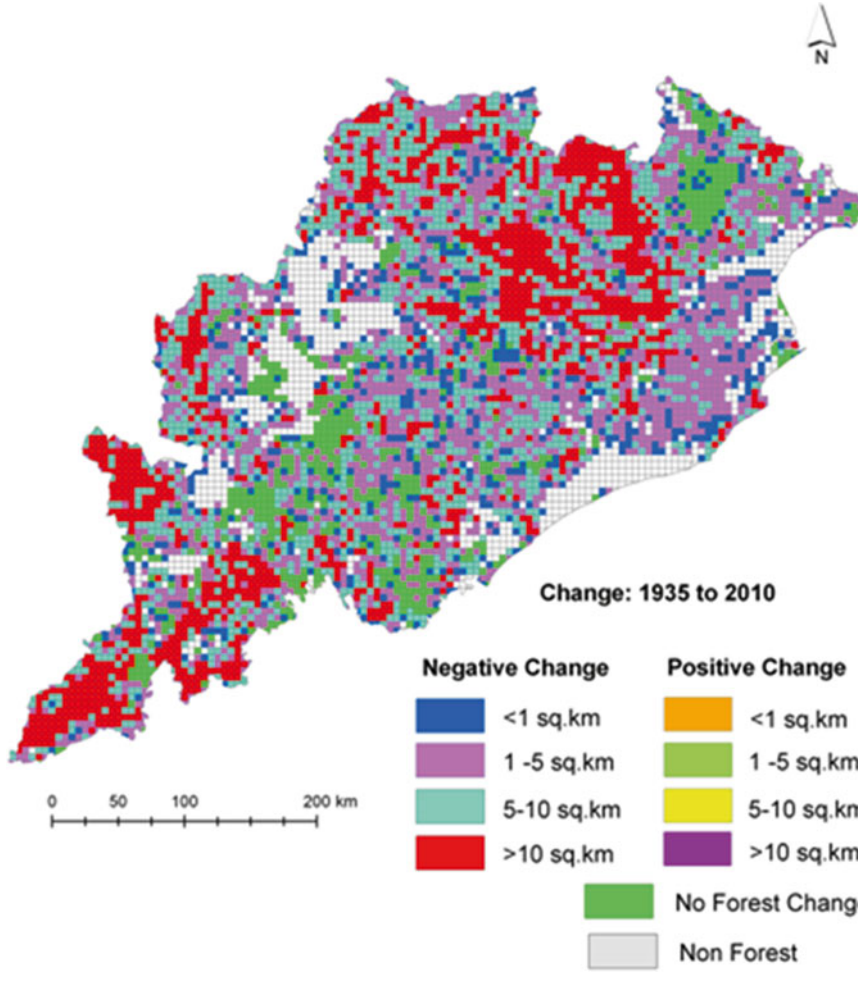


Fig. 29.5 Historical forest cover change map of Odisha, India

29.7 Wildlife Management

Wildlife management is much more than the preservation of certain plant and animal species; it involves management of a complete ecosystem. Quantification and analysis of current impacts on wildlife habitat such as logging agriculture, road developments, etc., are vital phases in the process of formulating sound wildlife management policies. Remote sensing can be applied to wildlife habitat inventory, evaluation, and wildlife census. The role of remote sensing has been highlighted in rapid assessment of habitat attributes, identification of new sites for protected areas, and current status of corridors. Ground survey methods such as counting animals, trapping, collection of droppings, investigations of feeding sites as well as ground mapping of habitats (Kotwal and Parihar 1988) will always be useful. However, in a number of cases, other techniques can supplement or partially replace tedious ground survey methods. Moreover, it is felt that ground methods have limitations as whole area cannot be accessed in one go in many of the cases, and the information collected may not be as accurate as is possible through remote sensing aided by limited ground survey.

GPS-aided wildlife survey in terms of presence/absence data is useful in various studies to understand the habitat distribution patterns. Spatial information generated on disturbance regimes and vegetation habitats stands as baseline information for habitat suitability assessment, prioritization for conservation, exploration for microscale habitat studies, corridor connectivity, and landscape planning (Thulsi Rao et al. 2008).

29.8 Gap Areas for Biological Exploration

India is one of the 12 mega biodiversity countries in the world. There are still many areas where inadequate information on plant wealth is available. Gap area prioritization is of utmost concern to understand the species richness of different parts of India. The recent advances in the remote sensing transfigured (Fig. 29.6), the traditional approaches of inventory and mapping to the spatial survey designs and biodiversity characterization at various levels (Reddy et al. 2008). In this context survey-gap analysis was carried out to assess exploration status and to prioritize areas using integrated approach. In order to highlight the gaps in botanical exploration in the country, the case study was conducted in Adilabad District of Andhra Pradesh. A grid size of 5′×5′ has been prepared to analyze the exploration status. The results indicated that most parts of study area have been underexplored and unexplored. Of the 200 grids, only one grid has been well explored with species collections of above 100. Moist deciduous forests, one of the major forest types which cover an area of 274 km^2, have not received any botanical attention. Comparative analysis with disturbance index and biological richness maps evidently points out that explorations were undertaken only in high disturbed zones and areas of low to medium biological richness. This geospatial analysis clearly points the need for more attention to the exploration of tropical forests in India. There is a need of systematic botanical studies to prioritize the conservation strategies of varied natural ecosystems. It is recommended for national-level survey-gap analysis as an important step to determine the floristic wealth, species representativeness, and distribution (Reddy 2010a). Potential survey sites for inventorization suggested based on integrated spatial analysis of vegetation type, disturbance index (DI), and biological richness (BR).

29.9 Gap Analysis for Protected Area Network

Gap analysis is a technique for identifying vegetation types and species that are not adequately represented in an existing protective network of biological diversity (Spellerberg and Sawyer 1999). Gap analysis helps to locate priority areas for conservation action and research. The technique can therefore be used as a means to prioritize human effort in habitat protection and

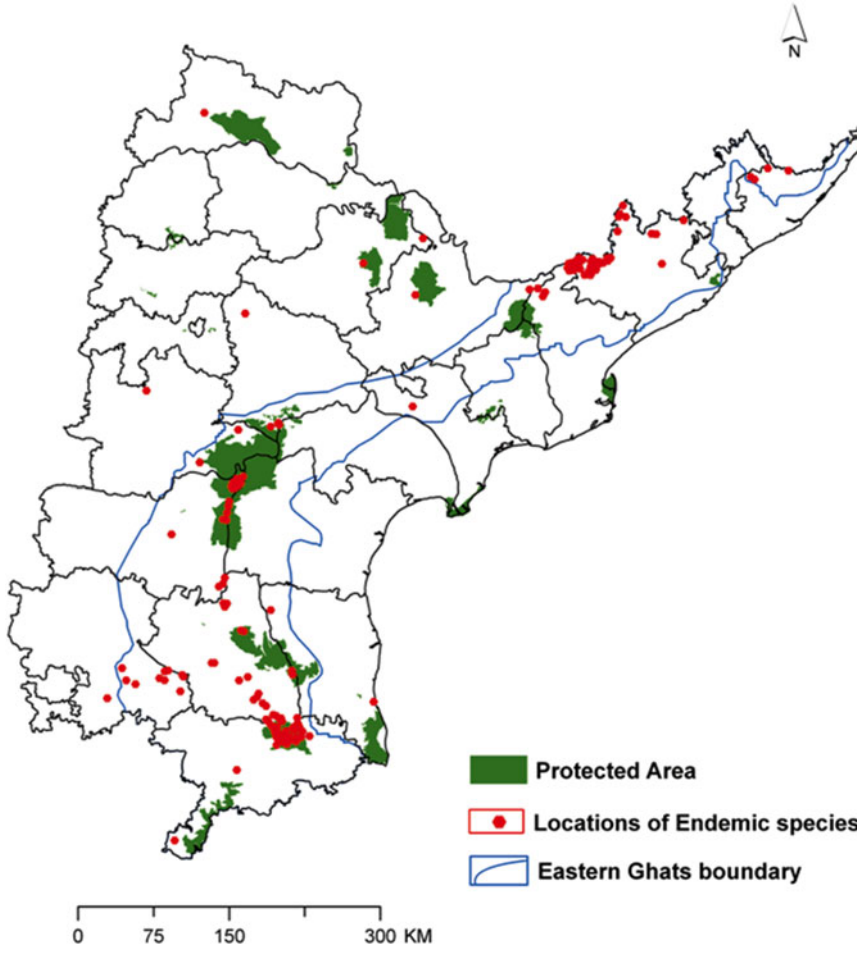


Fig. 29.6 Gap areas for protected area network in Andhra Pradesh, India (Reddy 2010b)

management in order to achieve the conservation of a region's biological diversity (Scott et al. 1996).

India has 590 PAs (ca. 500 wildlife sanctuaries and 90 national parks[9]). PAs of India cover 156,700 km^2, roughly 4.95 % of the total geographical area. There is a pressing requirement to identify gap areas of high biological richness to declare new protected areas in India. A case study was carried out to assess the protected area (PA) network system in Andhra Pradesh, India, using remote sensing and GIS techniques. The decisive factors of vegetation type distribution, elevation, and endemism were used to determine the representativeness of PA system. In Andhra Pradesh, vegetation cover occupies 23.03 % of geographical area and distributed in the coastal plains, Deccan Plateau, and Eastern Ghats. There are 27 PAs for conservation in Andhra Pradesh. The total area protected for biodiversity is about 12,555 km^2 or 4.56 % of geographical area of Andhra Pradesh. Of the three physiographic regions, the Eastern Ghats represents very high area under PAs which was estimated as 7,811.38 km^2 followed by the Deccan Plateau of 3,526.89 km^2. Three main forest types (semi-evergreen forests, thorn forests, and dry evergreen forests) missing in the existing PA network were identified. Moist deciduous forests of Eastern Ghats of northern Andhra Pradesh were underrepresented in PAs. The land area in an elevation range of 900–

1527 m was not included in PA network. Of the 103 species of endemics, 64 species were not included in PA system (Fig. 29.6). Many PAs are experiencing threat from invasive species, forest fires, grazing pressure, etc. There is a need to consider for possible ways for effective conservation and to extend the present PA network system in India (Reddy 2010b).

29.10 Species Distribution Patterns and Modeling

In direct remote sensing approach, hyperspectral sensors slice the electromagnetic spectrum into many more discrete spectral bands, enabling the detection of spectral signatures that are characteristic of certain plant species or communities (Turner et al. 2003). Generally, assessment of biodiversity is based on data on the range of species. A species range is the area occupied by a species and is used to refer to a distribution area. To determine species range, biologists record the geographic location of their observations and collect specimens. These data can be plotted on maps to represent species range using (1) points on a base map (McGranaghan and Wester 1988), (2) synthetic methods where boundaries of vegetation types are delineated with raster or vector formats (Morse et al. 1981) and shading of the entire polygon indicates species presence, or (3) synthetic grid maps (Perring and Walters 1962).

Modeling potential species distribution is becoming a powerful tool for botanist and conservationist in recent years, combining locations from herbarium specimens, desktop modeling software, and geographical information system (GIS) (Skov 2000). To predict a suitable potential species distribution, it requires appropriate scale of environmental data (e.g., temperate and rainfall) and accurately geo-referenced collection data. In recent times, monitoring of rare and endangered populations is increasingly having high priority by most conservation agencies. Species distribution modeling cannot replace fieldwork but can be a useful tool for data exploration to help identify potential knowledge gaps and provide direction to fieldwork design (Engler et al. 2004). By carefully evaluating models and including both species characteristics and sample size in our analyses, our results indicate considerable promise for modeling threatened species. This result should encourage conservation practitioners to explore the use of distribution modeling across a variety of applications (Giriraj et al. 2008) (Fig. 29.7).

29.11 Mapping and Monitoring of Invasive Species

Knowledge of invasive species occurrence, distribution, and potential invasion pathways is important in developing appropriate long-term monitoring protocols. India represents 173 invasive plant species (Reddy 2008). One potentially cost-effective approach in identifying potential occurrences of invasive species is to predict their distributions using remotely sensed data and knowledge of species ecology and environmental tolerances. Remote sensing technology has received considerable interest in the field of biological invasion in the recent years. The key requirement in invasive species mapping is delineation of spatial extent to understand the severity of invasion. Ground surveys to identify the extent of invasive species infestations should be more efficient with the use of remotely sensed data. The data from inventory is essential for prioritizing initiatives for species control, monitoring rate of species spread, and evaluation. Areas with a high potential for invasive spread may require more intensive surveys using Global Positioning System technology to record invasive plant locations, entering that information into a computer-based geographic information system to generate species-specific as well as regional maps. Mapping information is then interpreted to determine the size of invasive colonies, the direction and rate of spread, and other relevant information. Using maps and inventory information, managers can develop strategies focused on removing new and isolated infestations while containing the principle infestation. IKONOS imagery has been used in conjunction with Landsat to map the expansion of a nonnative invasive plant species by different researchers. It is

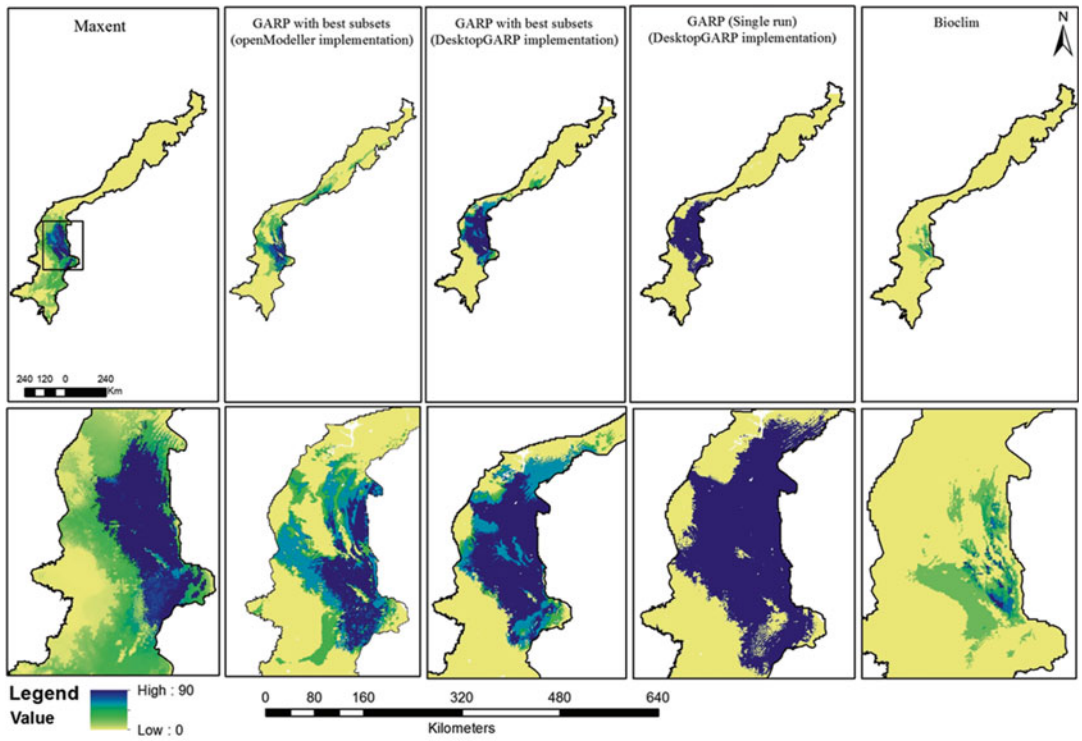


Fig. 29.7 View of the predicted distribution analyzed using multiple ENM models for the *Pterocarpus santalinus* species in Eastern Ghats, India (Giriraj et al. 2008)

easy to map aquatic infestations (e.g., *Eichhornia crassipes*, *Typha angustata*, *Trapa natans*, *Ipomoea carnea*), which dominate the wetland surface and form homogeneous single species stands that extend over larger areas. Several terrestrial invasive species (e.g., *Prosopis juliflora*, *Hyptis suaveolens*, *Chromolaena odorata*, *Parthenium hysterophorus*, *Lantana camara*) can be better delineated if their spread/dominance is more in the open canopies and degraded lands using high-resolution data (e.g., IRS LISS III and Landsat ETM+). The studies for mapping of *Chromolaena odorata* show promising results with the combined use of remote sensing, GIS, and ground surveys (Joshi 2001).

29.12 Impact of Forest Fires on Biodiversity

Forest fires may have profound effects on global climate and cause the extinction of species. Reports from the Ministry of Environment and Forests in 1995 recorded that forest fires affect 37 million ha of forests annually, and about 55 % of the country's forest areas are prone to forest fires each year. If forest fires occur, damage assessment is needed to determine the economical and ecological impact and to improve the fire risk assessment. Optical satellite sensors have become a fast means to cover fire-related issues. Satellite sensors cover fire phenomena if used during three distinct stages: (a) prior to the fire event, through flammability classification; (b) fire risk characterization mapping, during the fire event, through plume and fire core detection and tracking; and (c) after the fire event, through burnt-area mapping and postfire assessment.

Burnt areas have a typical spectral signature, especially if analyzed by a multi-temporal approach because of the different ground coverage between prefire (vegetation) and postfire (ash, bare soil, dead vegetation) conditions. The reflectance of forests will be low in the visible part of spectrum (except for the green region) and

high in the near-infrared part. Thus, the spectral curve of the forest after burning becomes flat, differentiating highly contrast burnt areas from its surroundings (Fig. 29.8). More specifically, fire plumes and burnt areas can be better distinguished in the short-wave IR spectral band (i.e.,1.55–1.70 μm), for detecting high temperature targets (since 1.65 μm in the electromagnetic spectrum is very sensitive to flame and flaming energy). These spatial data on fire occurrence were integrated to examine fire conditions in each forest type (Reddy et al. 2009). Towards forest fire management, the inputs, viz., fire risk maps, fire recurrence maps, and burnt-area maps, provide useful information to forest managers in effective planning for ground control operation.

29.13 Vegetation Status

Reflectance properties of photosynthetic plants detected by multispectral remote sensors form the basis for vegetation indices, in turn, offering estimates of the type and quantity of vegetation on the ground. Healthy leaves absorb red and blue light, capturing energy for photosynthetic reactions. In addition, healthy leaves reflect near-infrared light as a function of their water content and cellular structure. The normalized difference vegetation index (NDVI) is established on these reflective properties of leaves. The NDVI is the difference between near-infrared and red reflectance, normalized by the sum of the reflectance of these wavelength bands. Raw NDVI values are fractional real numbers ranging from −1.0 which means no vegetation, to +1.0 which refers to the maximum vegetation. Besides species composition, factors that affect NDVI values include leaf morphology, leaf position on the tree (sunlit vs. shade), brightness of incoming sunlight, vegetation stress, and biomass. Leaf area index (LAI) is the ratio of leaf to ground area. A high LAI corresponds to a large vegetative biomass. Among stands of a given species in similar environmental conditions, higher NDVI values are associated with higher LAI values.

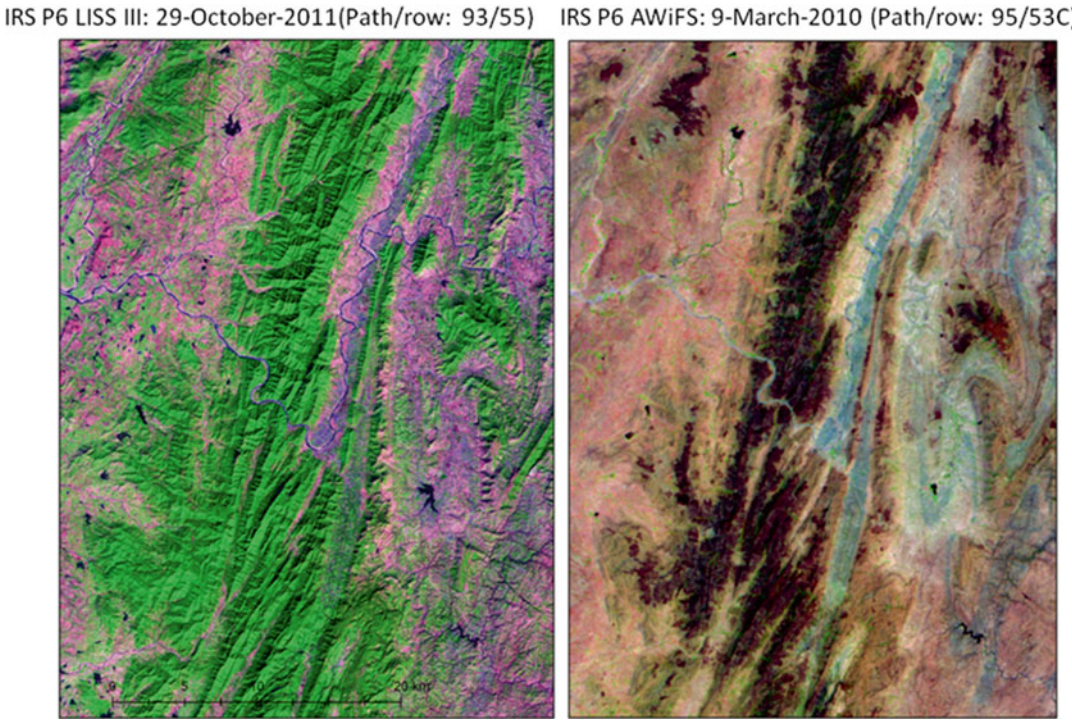


Fig. 29.8 Multi-temporal natural composite images showing forests of Aravallis, Rajasthan, India (*left* Green season image, *right* Dry season image indicating burnt areas in *brownish-black* tone)

However, the relationship between NDVI and LAI is not linear; the rate of change in LAI decreases as NDVI values increase. As a general rule, the curve levels off (becomes saturation) when LAI is greater than 4. Furthermore, the relationship between NDVI and LAI depends on species composition; on the whole, deciduous stands have higher NDVI to LAI ratios than coniferous stands due to differences in leaf morphology, leaf angle, and canopy structure.

Green space analysis classifies land cover into non-greenness, low greenness, medium greenness (moderately vegetated), and high greenness (high-quality green space which has NDVI value >0.5) categories.

29.14 Climate Change Studies

Global change is expected to alter all four filters. Changes are expected in mean and extreme temperatures and rainfall, land use patterns, vegetation flammability, and interactions between plant species (e.g., invasions, Chapin et al. 1998). Changes in global vegetation cover and in the boundaries of the world's biomes are expected to occur in response to climate change.

Using remote sensing techniques to map plant functional types is a recent field of research. Remotely sensed derived information of the phenology (onset and duration of greenness), physiognomy (canopy structure), community composition (i.e., the PFTs and their areal extent), and vegetation structure (e.g., height, leaf area index) are the key inputs to integrate with the variability in precipitation and temperature to map the spatial distribution of plant functional types (PFTs). But the remotely sensed data is alone insufficient to accurately extract the PFTs. In this, a lot of ground measurements need to be integrated. The study of mapping of PFTs from MODIS data in the USA consists of classification scheme with 12 classes including water, evergreen needle leaf trees, evergreen broad leaf trees, deciduous needle leaf trees, deciduous broadleaf trees, shrub, grass, cereal crop, broadleaf crop, urban and built-up, snow and ice, and barren or sparse vegetation (Sun et al. 2008).

It was concluded that remote sensing and geographic information systems (GIS) provide efficient tools for biodiversity and related fields. The use of geospatial techniques enhances the practical approach to species conservation.

References

Aspinall (1995) http://proceedings.esri.com/library/userconf/proc98/proceed/TO800/PAP783/P783.HTM

Chapin S, Sala O, Burke I, Grime P, Hooper D, Lauenroth W, Lombard A, Mooney H, Mosier AR, Naeem S, Pacala SW, Roy J, Steffen WL, Tilman D (1998) Ecosystem consequences of changing biodiversity. Bioscience 48:45–52

Engler R, Guisan A, Rechsteiner L (2004) An improved approach for predicting the distribution of rare and endangered species from occurrence and pseudo-absence data. J Appl Ecol 41:263–274

FAO (2012) State of the world's forests. Food and Agriculture Organisation of the United Nations, Rome

FSI (2007) State of forest report. Forest Survey of India, Dehradun

Giriraj A, Shilpa G, Reddy CS, Sudhakar S, Beierkuhnlein C, Murthy MSR (2008) Mapping the geographical distribution of *Pterocarpus santalinus* L.f. (Fabaceae) – an endemic and threatened plant species using Ecological Niche Modeling. In: Proceedings of the National Seminar on Conservation of Eastern Ghats, EPTRI, Hyderabad, pp 446–457. ISBN: 978-81-7525-955-3

Jha CS, Goparaju L, Tripathi A, Gharai B, Raghubanshi AS, Singh JS (2005) Forest fragmentation and its impact on species diversity: an analysis using remote sensing and GIS. Biodivers Conserv 14:1681–1698

Joshi CM (2001) Invasive Banmara (*Chromolaena odorata*): spatial detection and prediction. International Institute for Aerospace Survey and Earth Sciences, Enschede

Kerr JT, Ostrovsky M (2003) From space to species: ecological applications for remote sensing. Trends Ecol Evol 18(6):299–305

Kotwal PC, Parihar JS (1988) Management plan of Kanha National Park and Project Tiger Kanha for the period 1989–90 to 1998–99, Mandla

Mc Neely JA, Miller KR, Reid WV, Mittermeir RA, Werner TB (1990) Conserving the world biological diversity. IUCN, Gland

McGranaghan M, Wester L (1988) Prototyping a herbarium collection mapping system. Technical Papers of the 1988 ACSM-ASRRS. Annual Convention, 5:232–238. St. Louis, 13–18 Mar 1988

Morse LZ, Henifin MS, Ballman JC, Lawler JI (1981) Geographical data organization in botany and plant conservation: a survey of alternative strategies. In: Morse LE, Henifin MS (eds) Rare plant conservation:

geographical data organization. The New York Botanic Garden, Bronx, pp 9–29

National Aeronautics and Space Administration (NASA), Goddard Space Flight Center, Tropical deforestation (1998) NASA facts, FS-1998-11-120-GSFC

NRSC (2007) Biodiversity characterisation at landscape level in Eastern Ghats and East Coast using remote sensing and geographic information system. National Remote Sensing Centre, Hyderabad

Perring FH, Walters SM (1962) Atlas of the British flora. Thomas Nelson & Sons, London

Prasad PRC, Shringeswara AN, Reddy CS, Nidhi N, Rajan KS, Giriraj A, Murthy MSR, Raza SH, Dutt CBS (2009) Assessment of forest fragmentation and species diversity in North Andaman islands (India) – a geospatial approach. Int J Ecol Dev 14(9):33–46

Rashid I, Rhomshoo S, Vijayalakshmi T (2013) Geospatial modelling approach for identifying disturbance regimes and biodiversity rich areas in North Western Himalayas, India. Biodivers Conserv 22(11):2537–2566

Reddy CS (2008) Catalogue of invasive alien flora of India. Life Sci J 5(2):84–89

Reddy CS (2010a) Survey – gap analysis for botanical research using integrated approach through taxonomical data and geoinformatics. J Indian Soc Remote Sens 38:577–584

Reddy CS (2010b) Gap analysis for protected areas of Andhra Pradesh, India for conserving biodiversity. J Am Sci 6(11):472–484

Reddy CS, Pattanaik C, Murthy MSR (2007) Assessment and monitoring of mangroves of Bhitarkanika Wildlife Sanctuary, Orissa, India using remote sensing & GIS. Curr Sci 92(10):1409–1415

Reddy CS, Pujar GS, Rajasekhar G, Murthy MSR, Raju VS (2008) Identification of gap areas for biological exploration. Curr Sci India 95(5):568

Reddy CS, Ram Mohan Rao P, Pattanaik C, Joshi PK (2009a) Assessment of large scale deforestation in Nawarangpur district, Orissa, India using remote sensing and GIS. Environ Monit Assess 154:325–335

Reddy CS, Navatha K, Rachel B, Murthy MSR, Manikya Reddy P (2009b) Forest fire monitoring in Sirohi district, Rajasthan using remote sensing data. Curr Sci India 97(9):1287–1290

Reddy CS, Jha CS, Dadhwal VK (2013) Assessment and monitoring of long term forest cover changes in Odisha, India using remote sensing and GIS. Environ Monit Assess 185:4399–4415

Roy PS, Tomar S (2000) Biodiversity characterization at landscape level using geospatial modeling technique. Biol Conserv 95:95–109

Salem BB (2003) Application of GIS to biodiversity monitoring. J Arid Environ 54:91–114

Scott JM, Tear TH, Davis FW (1996) Gap analysis: a landscape approach to biodiversity planning. American Society for Photogrammetry and Remote Sensing, Bethesda

Skov F (2000) Potential plant distribution mapping based on climatic similarity. Taxonomy 49:503–515

Spellerberg IF, Sawyer WD (1999) An introduction to applied biogeography. Cambridge University Press, Cambridge, UK, p 243

Sun W, Liang S, Xu G, Fang H, Dickinson R (2008) Mapping of plant functional types from MODIS data using multisource evidential reasoning. Remote Sens Environ 112:1010–1024

Thulsi Rao K, Reddy CS, Murthy MSR (2008) Geospatial databases for wildlife habitat studies. In: Baquer SH, Krishna ISR, Bhakshu LM, Reddy CS (eds) Proceedings of National Seminar on Conservation of Eastern Ghats. EPTRI, Hyderabad, pp 426–428

Turner W, Spector S, Gardiner N, Fladeland M, Sterling E, Steininger M (2003) Remote sensing for biodiversity science and conservation. Trends Ecol Evol 18:306–314

Impact of Climate Change on Agricultural Productivity

30

Anjali Anand and Sangeeta Khetarpal

Abstract

The emerging uncertainties due to climate change and climatic variability are likely to aggravate the problems of future food security by exerting pressure on agriculture. Simulation models project an increase of 1.8–4.0 °C in global surface air temperatures in the next few decades that will result in large yield reductions in many regions. These increases in temperature will probably offset the likely benefits of increasing atmospheric concentrations of carbon dioxide on crop plants. The researchers face an immense challenge of meeting the needs of future generations in the face of both population growth and climate change. The development of climate smart crops needs to be considered on priority as it will make researchers and farmers proactive for the impending adversaries. However, the development of improved cultivars raised to improve yields and enhance adaptation to climate change will have to be complemented by improved crop and agronomic practices. This chapter focuses on the impact of climate change on growth and yield of the food security crops, viz., rice and wheat. Sustainable agronomic and resource management practices that can contribute to climate change mitigation have also been discussed.

Keywords

Crop productivity • Climate change • Plant growth • Adaptation

30.1 Introduction

Climate change is a potential threat to environmental, social and economic sectors of our time at both global and regional level. The current global population can be fed at present by achieving an adequate supply of food, but sustaining this into

A. Anand (✉) • S. Khetarpal
Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi 110 012, India
e-mail: anjuanand2003@yahoo.com; sk16mk@gmail.com

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_30,

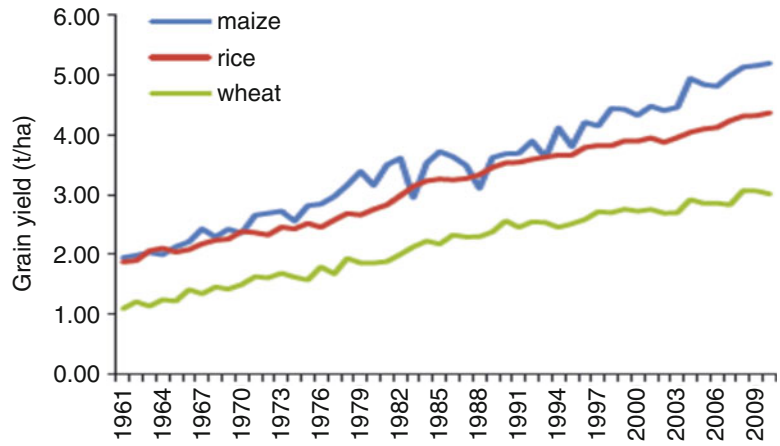


Fig. 30.1 Progress in world average yields for cereals (Source: Hawkesford et al. 2013)

the future will be a major challenge in the face of increasing population and corresponding decline in availability of fertile land and water for agriculture. Supply of food for the projected increase in demand requires it to grow by 2–3 % every year, but in the last decade, the yields of the major cereals—rice, maize, and wheat—have increased at less than half this rate, with wheat showing the lowest rate of increase (Fig. 30.1). Agriculture has a crucial decisive role in influencing the climate change, firstly through greenhouse gas emissions (GHGs)—10–12 % of the total global anthropogenic emissions of GHGs is a result of intensive land use, livestock, and land-use changes (IPCC Working Group III 2007)—and secondly by reducing carbon levels through carbon sequestration by vegetation. Tree-based systems can sequester substantial quantities of carbon into biomass in a short period of time (Lal 2004).

Agriculture preserves natural resources and cultural landscapes by increasing soil carbon contents and also adapting management practices to conserve carbon sinks. Today, there are three specific challenges facing the agriculturists: increasing yield potential, protecting yield potential, and increasing resource use efficiency to ensure sustainability. Since the green revolution, yields at the farm gate have stagnated in many countries or are increasing at less than half the rate required to meet the projected demand. Delivering increased yields can unlikely be solved by single approach, and a multidisciplinary integrated approach to crop improvement is required. In some countries, large gains can still be achieved by improvements in agronomy, but in many others, the yield gains will only be achieved by further genetic improvement (Hawkesford et al. 2013). The rising temperature and CO_2 and uncertainties in rainfall associated with global climate change have serious direct and indirect consequences for crop production and food security (Sinha and Swaminathan 1991). Therefore, climate change is likely to influence food-producing capacity in many areas. While some areas may experience a reduction in crop yields, others are likely to benefit (Raleigh and Urdal 2007). In this chapter, we present the impact of climate change especially CO_2 and atmospheric temperature on staple crops mainly rice and wheat. Crop growth, development and yield are influenced by climatic variability through linear and nonlinear responses to weather variables. The approach will be to provide a general overview of high CO_2 and temperature effects on plant growth processes and the strategies to manage crops and breed for newer varieties to adapt to changing climate in order to minimize the adverse impacts.

30.2 Crop Response to Climate Change

30.2.1 Impact of Rising CO_2 on Crops

Atmospheric CO_2 has changed from preindustrial levels of 280–384 μmol mol^{-1} in 2009 with a concomitant increase in mean temperature by

0.76 °C over the same time period. Projections up to 2050 suggest a level of 550 and 700 μmol mol^{-1} or more towards the end of this century (IPCC 2007). As a consequence, droughts, water scarcity, and heavy rain events will endanger agricultural productivity (Easterling et al. 2007). There is growing evidence suggesting that many crops, notably C_3 crops, may respond positively to increased atmospheric CO_2 in the absence of other stressful conditions (Long et al. 2004). Yields are estimated to be enhanced by around 15 % in C_3 plants under approximately 200 ppm atmospheric CO_2 increase, although the relative benefit of this effect varies widely in 200 studies and is still a subject of considerable debate in the scientific literature (Long et al. 2006). The rising atmospheric CO_2 concentrations, apart from its fertilization effect, are likely to also modify plant water, nutrient, and belowground dynamics, thus directly impacting plant production and quality. Free-air carbon enrichment experiments confirm that CO_2 enrichment under field conditions consistently increases biomass and yields in the range of 5–15 %, with CO_2 concentration being elevated to 550 ppm. Climate simulation studies have shown that in climate scenarios of MIRO and PRECIS, the irrigated rice yields are projected to reduce by ~4 % in 2020, 7 % in 2050, and ~10 % in 2080 scenarios (Kumar et al. 2012). Elevated CO_2 (627 ppm) increased yields by 23 % in rice with modest increase in grain mass but with a larger increase in panicle and grain number (Ainsworth in 2008). Rising atmospheric CO_2 concentrations provide counteracting tendencies to the otherwise negative impacts of rising temperatures and reduced soil moisture. Higher CO_2 has a fertilization effect in C_3 species such as wheat and rice given that photorespiratory costs in the C_3 photosynthesis pathway are alleviated by higher CO_2. Elevated CO_2 also has the benefit of reducing stomatal conductance and thereby increasing water-use efficiency in both C_3 and C_4 crops (Ainsworth and Long 2005). In this context, Erda et al. (2005) predicted that climate change without carbon dioxide fertilization could reduce the rice, maize, and wheat yields by up to 37 % in the next 20–80 years. Interactions of CO_2 with limiting factors, especially water and nitrogen, are increasingly well understood and capable of strongly modulating observed growth responses in crops. Increasing atmospheric CO_2 directly stimulated photosynthesis under increased levels on nitrogen in rice, resulting in increased growth and yield (Razzaque et al. 2011). Zhu et al. (2013) observed that although elevated CO_2 improved photosynthetic rates and enhanced yields of rice, it also enhanced the risk of stem-lodging for cultivars with strong CO_2 responses. Studies on elevated CO_2 in wheat have also shown a similar response. Yield increase in wheat due to doubling of CO_2 resulted due to increase in grain number rather than larger grains (Wrigley 2006).

Lobell and Gourdji (2012) highlighted the need for studying the influence of climate change on global crop productivity, relative to the many other factors such as O_3 concentration, air pollution, solar dimming, etc., that influence productivity. This question helps to set the challenge of climate adaptation in this context.

30.2.2 Impact of Elevated CO_2 on Photosynthesis

Increasing the availability of CO_2 for photosynthesis can have profound effects on plant growth, and many aspects of plant physiology as carbon, hydrogen, and oxygen assimilated into organic molecules by photosynthesis make up ~96 % of the total dry mass of a typical plant (Marschner 1995). Elevated CO_2 increases the size and dry weight of most C_3 plants and plant components. Relatively more photoassimilate is partitioned into structural components (stems and petioles) during vegetative development in order to support the light-harvesting apparatus. The availability of additional photosynthate enables most plants to grow faster under elevated CO_2, with dry matter production being increased on average by 17 % for the aboveground and more than 30 % for the belowground portions of plants (Ainsworth and Long 2005; Graaff et al. 2006). This increased growth is also reflected in the harvestable yield of crops, with wheat, rice, and soybean all showing increases in yield of 12–14 % under elevated CO_2 in FACE experiments (Ainsworth 2008; Long et al. 2006).

One of the most consistent effects of elevated atmospheric CO_2 on plants is an increase in the rate of photosynthetic carbon fixation by leaves. Across a range of FACE experiments, with a variety of plant species, growth of plants at elevated CO_2 concentrations of 475–600 ppm increased leaf photosynthetic rates by an average of 40 % (Ainsworth and Rogers 2007). As CO_2 concentrations increased, plants could maintain high photosynthetic rates with relatively low stomatal conductance (Ainsworth and Rogers 2007). The increase in photosynthesis is the result of increasing the carboxylation rate of Rubisco and competitively inhibiting the oxygenation of ribulose-1,5-bisphosphate (RuBP) (Drake et al. 1997). Exposure to elevated CO_2 resulted in a 31 % increase in the light-saturated leaf photosynthetic rate and a 28 % increase in the diurnal photosynthetic carbon assimilation when averaged across all FACE experiments and species. Stomatal conductance was reduced by 20 % with growth at elevated CO_2 when averaged for 40 species grown at all 12 FACE experiments (Ainsworth and Long 2005). Decrease in stomatal conductance would be expected to decrease overall plant water use, although the magnitude of the overall effect of CO_2 will depend on how it affects other determinants of plant water use, such as plant size, morphology, and leaf temperature. Thus, a decrease in whole plant water use of 5–20 % under elevated CO_2 was observed. This in turn will have consequences for the hydrological cycle of the entire ecosystems, with soil moisture levels and runoff both increasing under elevated CO_2 (Leakey et al. 2009). Much of our understanding of the effects of CO_2 on plants has been gained from studies with individual leaves. There have been far fewer studies of long-term effects of elevated CO_2 on canopy-scale photosynthesis and transpiration. Most of those reported have been in closed- or open-top chambers, though reports of field-scale exposures are now available. Drake and Leadley (1991) summarized the available data in 1991 and concluded that canopy photosynthesis increased under elevated CO_2 when there was a sink available for the carbon. Elevated CO_2 altered many interacting factors, such as canopy architecture and partitioning of assimilates, which mediate gas exchange of canopies and ecosystems. Lawlor and Mitchell (1991) concluded that yields of C_3 and C_4 crops growing in 700 μmol/mol CO_2 would be approximately greater by 30–40 % and 9 %, respectively, than present yields provided water, nutrients, and pest control are not the limiting factors.

30.2.3 Impact of Elevated CO_2 on Grain Quality

CO_2 fertilization reduces the nutritional quality of crops, especially in nutrient-poor cropping systems, through reduced nitrate assimilation and lower protein concentrations in harvestable yield (Taub et al. 2008). It is known to affect grain chemical composition, which in turn affects milling and cooking quality traits. The amylose content in rice grain that is a major determinant of cooking quality increased under elevated CO_2 (Conroy et al. 1994). The protein content of the grains decreased with combined increases in temperature and CO_2 concentration (Ziska et al. 1997). Total sugars and nonstructural carbohydrates substantially increased in rice grains (Uprety et al. 2007) under high CO_2. Elevated CO_2 caused a reduction in protein content in rice and a slight reduction of aroma of basmati cultivars. The gelatinization temperature increased resulting in firmer cooked rice under high CO_2 (Kumar et al. 2012). Seneweera and Conroy (1997) observed that cooked rice grains from plants grown in high-CO_2 environments were firmer than those from plants grown in ambient CO_2 environments; however, the concentrations of iron and zinc important from nutritional perspective were lower. A substantial reduction in amino acids with essential amino acids reducing between 29 and 38 % was reported under elevated CO_2 conditions (Xu et al. 1998). Studies have shown that higher CO_2 concentrations led to reduced plant uptake of nitrogen (N) and trace elements, such as zinc, resulting in crops with lower nutritional value (Taub and Wang 2008). This would primarily affect people in poorer countries, who cannot compensate this

deficiency by eating more food and have less varied diets (Kaur and Rajni 2012).

In wheat, elevated CO_2 reduces the protein content of grain and flour by 9–13 % (Rogers 1996). Plants grown under elevated CO_2 in field, open-top chambers and free-air carbon dioxide enrichment (FACE) resulted in decrease in protein concentration under elevated conditions (Fig. 30.2) (Hogy and Fangmeier 2008). Grain quality in wheat is reduced at low nitrogen levels that are further exacerbated at high CO_2 (Hatfield et al. 2011; Kimball et al. 2001). Protein concentration is positively correlated with bread making due to its impact on dough strength and is a major determinant of grain prices (Lawlor and Mitchell 2001). The total gluten concentration as well as concentrations of dry and wet gluten also decreased under high CO_2 (Bencze et al. 2004). Increasing CO_2 to twice the preindustrial level and decreasing nitrogen to half the adequate level reduced crude protein by 4–13 % in wheat (Erbs et al. 2010). These adverse effects on grain quality can be minimized by use of ample nitrogen fertilizer (Kimball et al. 2001). At low nitrogen levels, protein content was reduced by 39 % under elevated CO_2 compared to a 33 % reduction under ambient CO_2. In a study on wheat quality at low nitrogen availability, they observed that loaf volume was reduced by 29 % compared to 22 % at elevated vs. ambient CO_2 (Kimball et al. 2001). Elevated CO_2 also brought significant changes in wheat grain-protein composition: gliadins reduced up to 20 %, glutenins up to 15 %, and glutanin macro-polymer up to 19 %, while albumins and globulins fractions were not affected. Within gliadins, ω-5-gliadins and ω-1,2-gliadins were more affected than α-gliadins and γ-gliadins, while within glutenins, high-molecular weight (HMW) subunits were more affected than low-molecular weight (LMW) subunits, thus adversely impacting baking quality (Wieser et al. 2008). Variation in soil N and enriched CO_2 adversely impacted hemicellulose, reducing it by 26 % at low N, while at high N and enriched CO_2, the decline in hemicellulose was only 13 %. Furthermore, starch content increased by 7–8 % under elevated CO_2 irrespective of the variation in soil N, while water-soluble

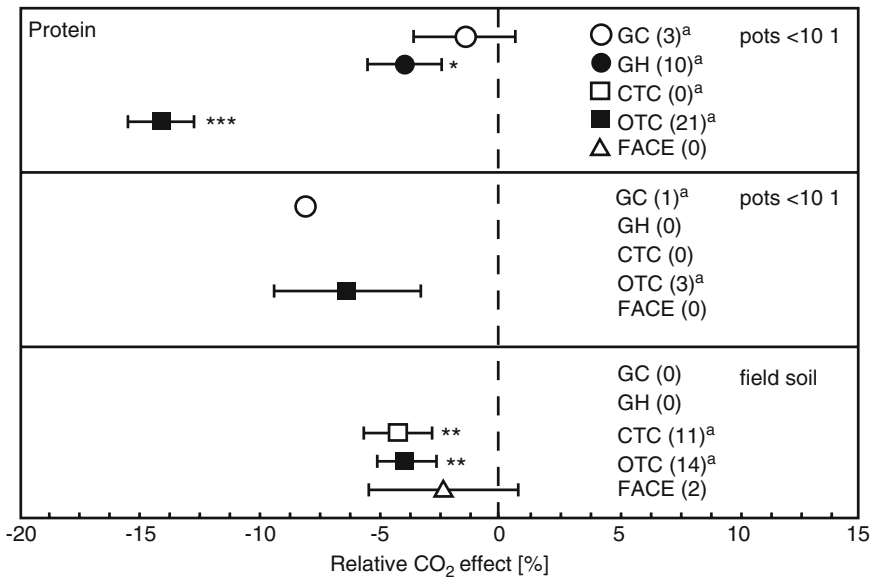


Fig. 30.2 Relative average changes (± SE) in protein concentration of wheat grains due to CO_2 enrichment (550 vs. 380 µmol mol^{-1}) in regard to exposure system and rooting volume. *GC* Growth chamber, *GH* glasshouse/greenhouse, *CTC* closed field chamber, *OTC* open-top chamber, *FACE* free-air CO_2 field enrichment. Significant CO_2 effects are denoted by ***(P,0.001), **(P,0.01), and *($P \leq 0.05$). The number in parenthesis indicates number of studies included (k); letter indicates significant differences between exposure systems (Source: Hogy and Fangmeier 2008)

carbohydrates reduced by 7–15 % at low/high N supply (Porteaus et al. 2009).

Elevated CO_2 results in decrease in the concentration of all microelements by 3.7–18.3 %, whereas macroelements are not affected (Hogy and Fangmeier 2008). AGFACE studies from Australia have shown a decrease of micronutrient content in wheat under elevated CO_2 to 10 % in iron and zinc concentrations (AGFACE 2013).

A proper balance between protein and non-protein components of grain determines the quality of wheat flour. Grains grown under high CO_2 levels produce poor dough of lower extensibility and decreased loaf volume (Blumenthal et al. 1996), but the physiochemical properties of wheat starch during grain filling are not significantly modified (Tester et al. 1995). Grain quality is not only determined by the sum of the contributions of grain constituents such as proteins, starch, and lipids to dough strength and loaf volume but also by an interaction between these components.

30.3 Impact of Rising Temperature on Crops

Although yields of temperate crops increase with enhanced CO_2 levels, this may be offset by the negative effects of warmer temperatures (Wheeler et al. 1996; Batts et al. 1998). The global mean annual temperature at the end of the twentieth century was almost 0.7 °C above that recorded at the end of the nineteenth century, and it is likely to increase further by 1.8–6.4 °C by AD 2100, with an estimate of 1.8–4.0 °C (IPCC 2001, 2007). The predicted increase in air temperature might lead to a 20–40 % decrease in cereal yields, mostly in Asia and Africa (Lele 2010). Warmer daytime temperatures are likely to have decreased wheat yields over a wide range, from 6 to 20 % (Tao et al. 2008), and rice by 10 % (Peng et al. 2004) per °C which stresses the need for regional and crop-specific studies. In the rice-wheat system of eastern India, remote sensing studies revealed that at least 60 % of district wheat areas were suboptimally late planted (Chandna et al. 2004). Crop simulation modeling has predicted a 51 % decrease in the most favorable and high-yielding mega-environment due to heat stress yielding heavy losses in wheat yield (Fig. 30.3a, b) (Ortiz et al. 2008).

A trial was conducted to evaluate the grain yield performance of high yielding, early maturing heat-tolerant CIMMYT wheat lines across two mega-environments: ME1 being the temperate-irrigated locations with terminal high-temperature stress, and ME 5 as warm, tropical, irrigated locations showed that cooler ME1 locations had higher mean grain yield of 5.26 t/ha compared to 3.63 t/ha for ME 5 (Mondal et al. 2013). The optimum temperature for wheat anthesis and grain filling ranges from 12 to 22 °C. Exposure to temperatures above this can significantly reduce grain yield (McDonald et al. 1983; Mačas et al. 2000; Tewolde et al. 2006). Heat stress during the reproductive phase can cause pollen sterility, tissue dehydration, lower CO_2 assimilation and increased photorespiration. Although high temperatures accelerate growth (Kase and Catsky 1984), they also reduce the phenology, which is not compensated for by the increased growth rate (Zahedi and Jenner 2003). High-temperature events in winter wheat occurring at or near to anthesis can increase floret abortion and reduce the number of grains per ear and the subsequent rate of increase in harvest index, resulting in smaller grain yields (Wardlaw and Wrigley 1994; Wheeler et al. 1996). The short period of exposure to high temperature of 31 °C can be considered equivalent to a 2 ± 3 °C increase in the seasonal mean temperature (Wheeler et al. 1996). There are various reports on significant decrease (up to a 23 %) in grain yield in a brief exposure of 4 days to very high temperatures (Hawker and Jenner 1993; Stone and Nicolas 1994). As the high-temperature occurrence is more prevalent around anthesis time, it is likely that pollination process is affected. High temperatures can cause both male and female sterility in wheat (Saini and Aspinall 1982), although production and transfer of viable pollen grains to the stigma, germination of the pollen grains and growth of the pollen tubes down the style, and fertilization to finally development of the zygote can be affected by increased

temperature. Heat stress effects during pre-anthesis, particularly during meiosis and growth of the ovaries, may also be associated with reduced grain numbers (Calderini et al. 1999). The availability of carbohydrates for floret development is one factor determining grain number (Abbate et al. 1995; Demotes-Mainard and Jeuffroy 2004) because inadequate availability of assimilates may cause floret death (Kirby 1988). Wheat root biomass tends to decline around the time of anthesis (Ferris et al. 1998) which coincides with the remobilization of assimilates from pre-anthesis growth and translocation to the developing grain (Hay and Walker 1989). The integration of high temperatures (i.e., accumulated thermal time above 31 °C over 8 days of the treatment during anthesis) was related to a 50 % reduction in grain yield (Fig. 30.4). Thus, the cumulative exposure to high temperatures at anthesis reduces grain number and finally grain yield. This decline in grain numbers per ear (of approx. 40 %) from the coolest to the warmest maximum temperature was associated with a decrease in grain yield from 850 to 500 g m^{-2}.

Grain filling is also seriously impaired at high temperatures (Blum et al. 1994). South Asian wheat-growing areas have witnessed terminal heat stress on several occasions in the last decade that affected wheat productivity (Nagarajan et al. 2008). A loss of 4.6 million tons of grain due to advancement of maturity by 12–20 days and reduction in 1,000 grain weight was reported

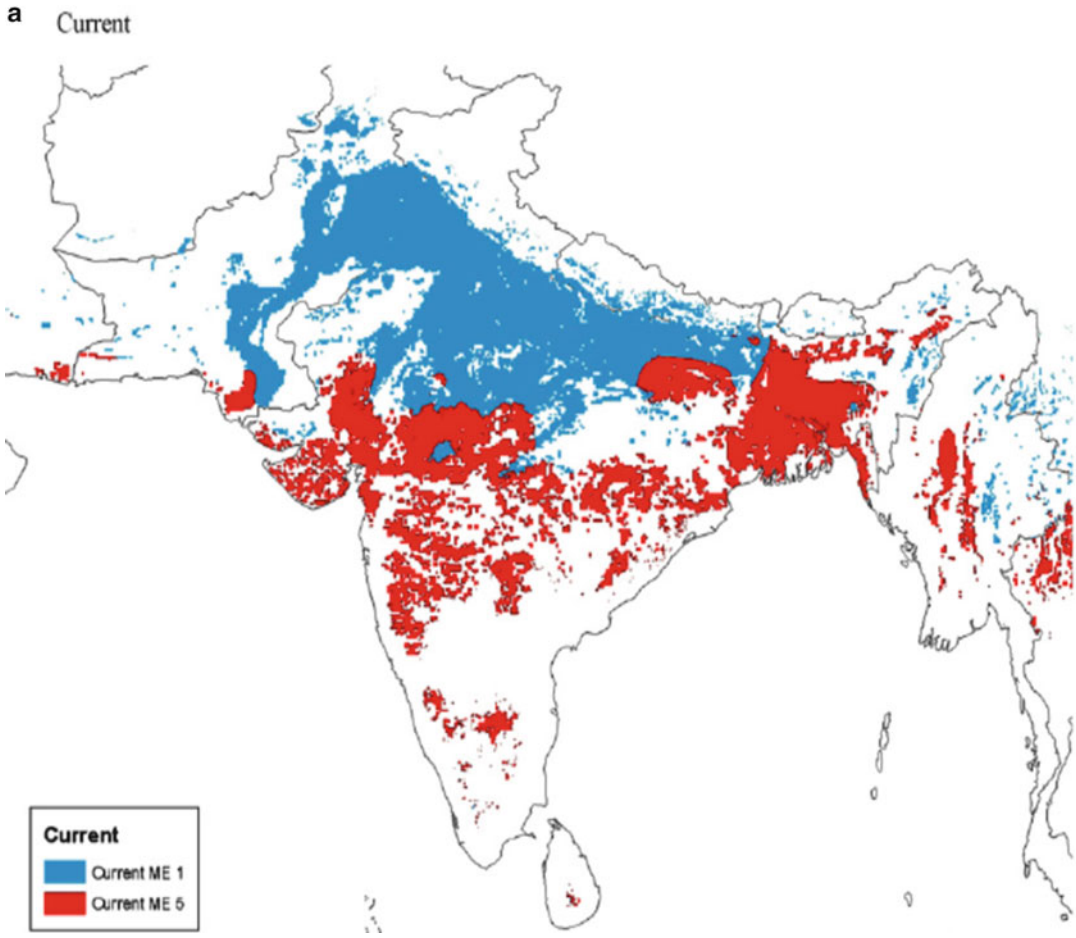


Fig. 30.3 (**a**, **b**) Current and future potential wheat mega-environments in the Indo-Gangetic Plains (Source: Ortiz et al. 2008)

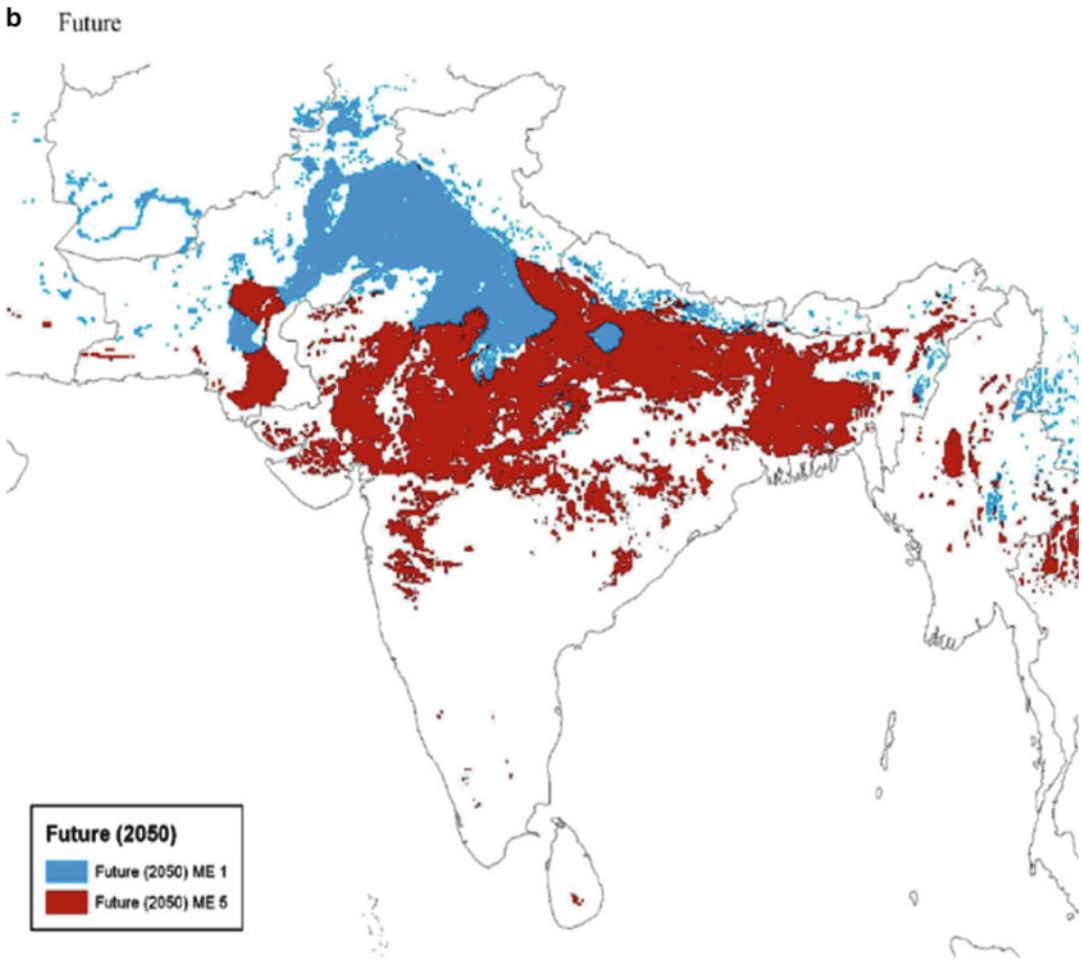


Fig. 30.3 (continued)

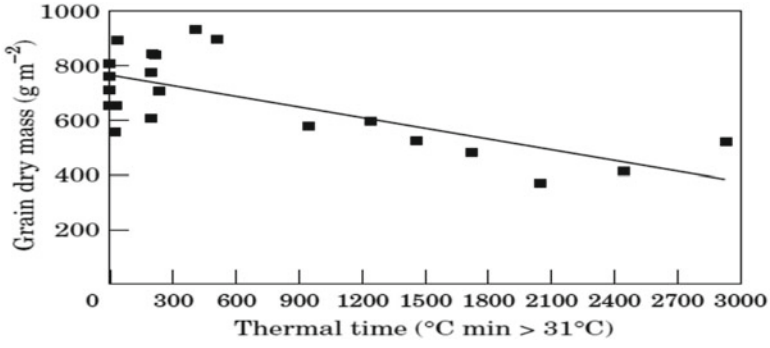


Fig. 30.4 Relationship between grain dry mass at harvest maturity and thermal time (accumulated, in 10-min interval, above a base temperature of 31 °C between days 3 and 10 of the 12-day temperature treatment) (Source: Ferris et al. 1998)

with sudden increase with temperature during grain development phase in the wheat crop season of 2003–2004 (Samra and Singh 2005). Despite favorable weather conditions during the vegetative growth in 2009–2010, an abrupt rise in night temperature during the grain-filling stage in

wheat adversely affected wheat productivity in the Indo-Gangetic Plains (IGP) and other northern states of India (Gupta et al. 2010), resulting in a mean yield penalty of 5.8 % compared with the previous year. Sucrose synthase, soluble starch synthase (SSS), and granule bound starch synthase are the three enzymes which rate limit starch biosynthesis in wheat (Hawker and Jenner 1993). SSS regulates the synthesis of starch and is sensitive to heat stress (Rijven 1986; Keeling et al. 1993, 1994). Heat stress decreases the activity of SSS in wheat, reducing grain growth and starch accumulation (Prakash et al. 2004). Even short periods of episodic temperature over 30 °C slow starch accumulation principally due to heat-induced denaturation of SSS (Jenner 1994).

Rice-growing regions in the temperate zone may be benefitted from temperature increase of 1–2 °C, but in the low-latitude regions in the tropical and subtropical zones, the negative effect is more conspicuous (Rosenzweig and Parry 1994; Easterling et al. 2007). The optimum temperature for growth and development of rice ranges from 27 to 32 °C (Yin et al. 1996). Anther dehiscence is the most susceptible stage during anthesis under high temperature in rice (Matsui et al. 1999). High temperature increases vapor pressure deficit and deprives the crucial moisture needed for pollen grain swelling which is mandatory for anther dehiscence. High temperatures can also affect microsporogenesis (Yoshida et al. 1981) as studies have shown that exposure to 41 °C for 4 h at flowering caused irreversible damage and rice plants became completely sterile (Shah et al. 2011), whereas the same elevated temperature had no effect on spikelet fertility at 1 day before or after flowering (Yoshida et al. 1981). Reciprocal studies with manual shedding of pollen from control plants on to the stigma exposed to high temperature and vice versa showed that the ability of the pistil to be fertilized remained unaffected even over a period of 5 days at 41 °C (Yoshida et al. 1981) (Fig. 30.5).

Therefore, the timing and duration of high temperature are important factors for growth and development of crops with tolerance at one developmental stage which may or may not necessarily lead to tolerance during other stages (Table 30.1). Decreased grain weight, reduced grain filling, higher percentage of white chalky rice, and milky white rice are common effects of high-temperature exposure during ripening stage in rice (Osada et al. 1973; Yoshida et al. 1981). The reduction in grain weight results due to higher grain dry matter accumulation rate along with the decrease in duration of grain-filling period (Kobata and Uemuki 2004). The developmental stage at which the plant is exposed to high temperature determines the severity of its impact on the crop (Wahid et al. 2007) with vegetative development usually having a higher optimum temperature than reproductive development.

Besides studies on impact of high mean temperature at different developmental stages on various crop plants, experimental evidence has also suggested that increasing nighttime temperature has been an important factor contributing to the yield decrease in crops (Peng et al. 2004; Sheehy et al. 2005; Ismail and Hall 1998; Lobell et al. 2005).

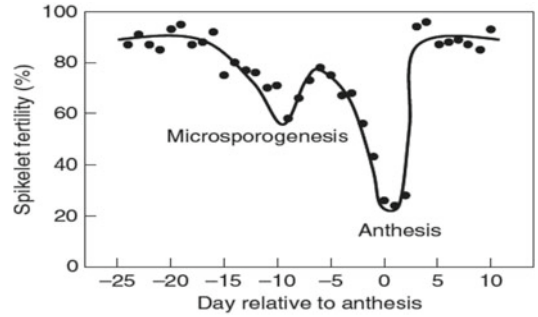


Fig. 30.5 Spikelet fertility of BKN6624-46-2 exposed to high temperature of 35 °C at different stages of panicle development for 5 days (Source: Wassmann et al. 2009)

Table 30.1 Summary of threshold temperatures for wheat and rice for various phenological phases

Phenological phase	Threshold temperatures (°C)	
	Wheat	Rice
Sowing to emergence	32.7	40.0
Tillering	15.7	32.0
Flowering	>20.0	35.0
Anthesis	31.0	33.7
Grain filling	35.4	34.0

Source: Wheat- Porter and Gawith (1999); Rice-Shah et al. (2011)

30.3.1 High Night Temperature

Pathak et al.'s (2003) estimation on the rate of change in the potential yield trend of rice from 1985 to 2000 showed it to be in the range −0.12 to 0.05 Mg ha^{-1} yr^{-1}. This negative yield trends were statistically significant for four out of the nine sites that were analyzed, and decreased radiation and increase in minimum temperature accounted for the decline in yield. Direct evidence about the decrease in rice yield with high nighttime temperature (HNT) was obtained in studies by Peng et al. (2004) where they observed an increase in annual mean maximum and minimum temperatures by 0.35 and 1.13 °C, respectively, from 1979 to 2003, resulting in 10 % decline in rice grain yield for each 1°C increase in minimum temperature in the dry season, whereas the effect of maximum temperature was insignificant. Both high day (34/22 °C) and night (22/34 °C) temperature reduced the duration of grain growth with rate of growth being less under HNT than high day temperature. The decrease in final grain weight of rice under HNT was due to reduction in growth rate in the early or middle stages of grain filling and also reduced cell size midway between the central point and the surface of endosperm (Morita et al. 2005). There was a significant reduction in nitrogen and nonstructural carbohydrates in susceptible rice genotype under HNT (Shi et al. 2013). These studies have been further corroborated by field studies relating HNT from 21 to 32 °C to reduced spikelet fertility and grain weight $plant^{-1}$ (Nagarajan et al. 2010). The effect of HNT has been widely studied in cowpea where high temperature can negatively impact floral bud development, flower development, pod set, grain filling, and even grain quality with the sensitive stage occurring at about 9–7 days before anthesis (Ahmed et al. 1992; Warrag and Hall 1984). This stage occurs after meiosis and coincides with the release of pollen microspores from the tetrads, and high night temperature at this stage causes premature degeneration of the tapetal layer that provides nutrients to developing pollen, resulting in infertile pollen, and also adversely affects anther dehiscence in cowpea genotypes (Mutters et al. 1989). Subjecting cowpea shoot to moderately high night temperature can also damage pod set (Warrag and Hall 1984); however, much hotter day temperatures did not. The reciprocal artificial pollinations between plants grown under high and optimal night temperatures suggested that low pod set was caused by male sterility as the pistils did not appear to be affected in high temperature (Nielsen and Hall 1985). Mutters and Hall (1992) demonstrated that there is a distinct period during the 24-h cycle when pollen development in cowpea is sensitive to high night temperatures. Plants subjected to high temperature in the last 6 h of the night were sensitive than plants exposed during the first 6 h of the night, indicating that specific heat-sensitive processes during pollen development occur in the late night period or at predawn when temperatures are the coolest and are probably under circadian control. For minimum night temperatures greater than 16.5 °C, seed yield decreased by 14 % per °C, associated with a similar decrease (12 % per °C) in number of pods per peduncle, but only a small decrease (6 % per C) in shoot biomass production (Ismail and Hall 1998). Increased daily minimum temperature has also been predicted to have a greater impact on wheat production as grain yield is more strongly negatively correlated with increasing minimum temperatures than maximum temperatures (Lobell et al. 2005). A case study from Mexico showed 10 % decrease in wheat yield for every 1 °C increase in nighttime temperature, although the same increase in daytime temperature had no significant effect (Lobell et al. 2005). Night temperatures >20 °C can reduce spikelet fertility with a concomitant reduction in grain number and size (Prasad et al. 2008). Increased night temperatures of 20 and 23 °C reduced the grain-filling period by 3 and 7 days, respectively. Though there are various reports signifying the impact of high night temperature on yield in crops, research in this area has not been dealt on priority and should be given due consideration with much higher mean night temperatures predicted.

30.3.2 Impact of High Temperature on Photosynthesis

The cumulative photosynthesis of the growing season is the primary determinant of crop biomass provided that other constraints do not become limiting has been demonstrated in free-air CO_2 enrichment experiments that have increased yields (Ainsworth and Long 2005). The cumulative photosynthesis under the changing climatic conditions can be increased by increasing photosynthetic rate, light interception, or its duration (Hawkesford et al. 2013). The duration of photosynthesis may be increased in some regions, but the growing season is most often constrained by high/low temperature. Another feature that can be exploited is improvement in canopy architecture and light capture efficiency (targeting complete canopy closure or maximizing leaf angle for light interception) under the climate change scenario (Murchie et al. 2009; Reynolds et al. 2012). The other opportunities to increase photosynthesis can be improving early vigor or manipulating senescence to delay its onset under increasing temperature, but the best target for enhancing the potential gains in cumulated photosynthesis would be achieved by increasing the photosynthetic rate. It has been observed that 4.6 % of the intercepted radiation is converted to photosynthate in wheat, leaving a lot of scope for improvement (Zhu et al. 2010). A possible approach can also be to modify the CO_2-fixing enzyme, Rubisco, so as it delivers higher photosynthetic rates. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is a key enzyme that regulates carboxylation during photosynthesis (Ogren 1984). An increase in the ratio of oxygenase/carboxylase activities of Rubisco leads to inhibition of net photosynthesis under high temperature; thus, the catalytic turnover rate of Rubisco increases minimally with temperature. The increase in the rate of photorespiration due to high temperature is because the solubility of CO_2 and O_2 and the kinetics of Rubisco are affected under high temperatures (Ogren 1984; Long et al. 2004). Rubisco has low affinity for CO_2 and needs to be activated by a chloroplastic protein, Rubisco activase, at physiological concentration of CO_2 and Mg^{2+} (Portis et al. 1986). Rubisco activase, however, is quite sensitive to high temperature (Salvucci and Crafts-Brandner 2004). High temperature denatures Rubisco activase, leading to formation of high-molecular mass aggregates, consequently not allowing Rubisco to be converted into its active form (Crafts-Brandner and Salvucci 2000). Screening and selection of a germplasm with thermostable Rubisco activase and with better CO_2-concentrating mechanisms can help in activation of Rubisco and also increasing internal CO_2 concentrations, respectively, under high-temperature conditions. A good deal of variation exists in the kinetic properties of Rubisco isolated from different species (Parry et al. 1989; Delgado et al. 1995), and it is more than probable to confer superior characteristics to photosynthesis in crops (Zhu et al. 2010; Parry et al. 2011). Besides the photosynthetic enzyme, Photosystem II appears to be influenced by temperatures above 45 °C (Sharkey 2005) but is not severely affected by moderately high temperatures (<40 °C) (Allakhverdiev et al. 2008).

The grain-filling period can be extended during high temperature by optimization of a crop canopy that includes complete and early canopy closure together with early flowering. Stem and spike compete for assimilates during this critical period; thus, the higher the assimilates partitioned to the spikes, the larger the number of fertile florets at flowering, and thereby the final number of grains is increased (Fischer 1984). Field studies have demonstrated that an extension of the period from terminal spikelet formation to the onset of flowering promoted spike fertility and increased the number of grains per unit area in wheat (Gonzalez et al. 2005). This feature can have a special relevance in overcoming the effect of high temperature during the reproductive phase as it has been seen that increase in spike fertility can reduce the competition between stem and spike, enabling sustained floret development due to a reduced inter-floret competition within the spikelets and allowing more floret primordia to reach the fertile floret stage (Gonzalez et al.

2011). The relationships between grain weight and ovary weight (Hasan et al. 2011), and the similarity between grain size dynamics and expansins (proteins which loosen cell walls) expression (Lizana et al. 2010), may be exploited to develop tools for wheat breeding programs aimed at increasing grain yield under increasing temperatures. Therefore, increasing the genetic gains beyond the current values is a requirement if we aim to cope with the reductions in crop yield caused by climate change.

30.3.3 Impact of High Temperature on Grain Quality

The quality of cereals exerts a large effect on the market value and consumer acceptance (Lapitan et al. 2007), and development of cultivars with good quality is an important objective in crop improvement programs (Pingali et al. 1997). Besides genetic factors, quality is strongly influenced by environmental conditions (Shi et al. 1997). Variations in growth temperature could be a cause of quality variation in rice (Matsue 1995) and wheat (Gooding et al. 2003). High temperature affects cellular and developmental processes leading to reduced grain quality (Barnabas et al. 2008). Decreased grain weight, reduced grain filling, and higher percentage of white chalky rice and milky white rice are common effects of high-temperature exposure during ripening stage in rice (Osada et al. 1973; Yoshida et al. 1981; Yamakawa et al. 2007; Zhu et al. 2005; Ambardekar et al. 2011), further reducing the potential economic benefits farmers can derive from rice cultivation due to depression in farm gate and/or milled grain prices. Rice quality can vary inexplicably from year to year and often from field to field (Cooper et al. 2006). Studies investigating the effect of temperature on grain development have indicated that higher temperatures during grain-filling stage result in decreased milled rice percentage (MRP), increased percentage of chalky rice grains (PCRG) and gelatinization temperature (GT), and loosely packed starch granules (Yoshida and Hara 1977; Shi et al. 2013; Lisle et al. 2000; Zhong et al. 2005). Amyloplast structure is highly correlated with appearance quality of rice (Yang et al. 2007). In general, loosely packed starch granules result in higher percentage of chalky rice grains and degree of chalkiness. Scanning electron microscopy of transverse sections of rice grains revealed that endosperm of chalky grains ripened under high temperature contained loosely packed starch granules with large air spaces, while translucent grains ripened under normal temperature were filled with densely packed granules (Fig. 30.6) (Yamakawa et al. 2007). High temperature during grain-filling period accelerates the demand for more assimilates to avoid milky white kernels (Kobata and Uemuki 2004). The reduced grain weight under high temperature is attributed to

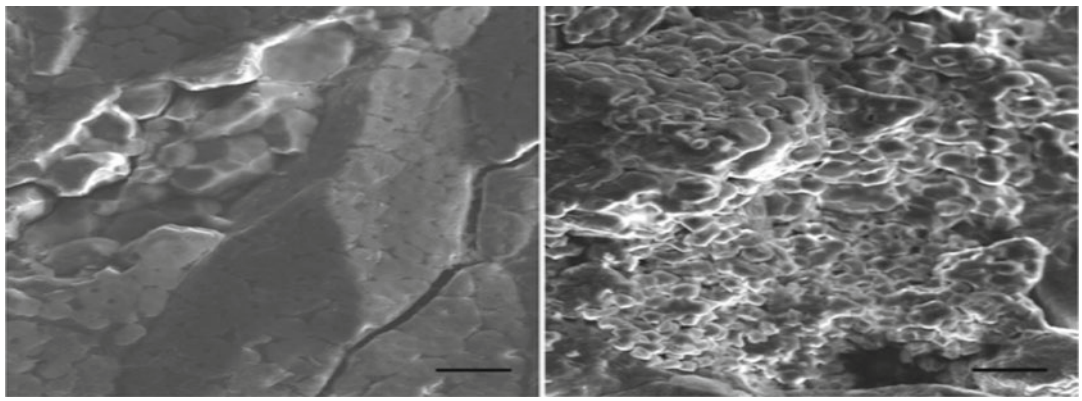


Fig. 30.6 Scanning electron micrographs of 25 °C/20 °C-ripened translucent (*left*) and 33 °C/28 °C-ripened chalky grains (*right*). Bars = 10 mm (Source: Yamakawa et al. 2007)

excessive energy consumption to meet the respiratory demand of the seeds (Tanaka et al. 1995).

The important quality parameters in wheat are grain hardiness, grain size, milling, dough strength, protein, and starch quality. Grain protein and grain size are used as grading factors in cereal trading (Coles et al. 1997). High temperature during grain-filling phase affects the grain protein contents (Wardlaw et al. 2002; Stone and Nicolas 1998; Castro et al. 2007) through reductions in starch deposition, which influences protein concentration by allowing more nitrogen per unit of starch (Stone and Nicolas 1998). Grain protein content is inversely related to grain size (Erekul and Kohn 2006). While grain protein content increases under heat stress, the functionality of protein significantly decreases (Corbellini et al. 1997), affecting market quality. There is decrease in synthesis of glutenin, while synthesis of gliadins remains stable or increases (Majoul et al. 2003). In Australian variety trials extending over 27 years, grain nitrogen concentration was positively associated with the number of hours above 35 °C during grain filling (Blumenthal et al. 1991a). Heat stress also decreases the sedimentation index as an effect associated with increased protein content in grain, but with decreased levels of essential amino acids (Dias et al. 2008). Increases in daily average temperatures above 30 °C, for a period of 5 days, resulted in weakening of dough properties like dough strength and loaf score accompanied by increase in proportion of gliadin proteins (Randall and Moss 1990; Blumenthal et al. 1991b).

30.4 Interactive Studies on Elevated CO_2 and Temperature

The benefit of additional CO_2 to the crops can be nullified by an increase in temperature, and a number of studies have examined the effects of elevated atmospheric CO_2 or combinations of elevated air temperature and CO_2. Table 30.2 presents a summary of rough estimates of the impact of high CO_2 and temperature on calorie supply from major crops, averaged over the next 30 years. It is predicted in the near term that warming will slow global yield growth by about 1.5 % per decade, while increased CO_2 will raise yields by roughly the same amount, but after the mid-century, there is likelihood of CO_2 benefits diminishing and climate effects becoming larger. The net effects of warming and CO_2 are estimated to be as negative as −3 % per decade or as positive as +2 % per decade, depending on how fast temperature and CO_2 change and how responsive crop yields turn out to these modulations (Lobell and Gourdji 2012).

Interactive studies on growth and yield of rice in combination of elevated air temperature and CO_2 during the last several decades have been reported (Ziska et al. 1996; Horie et al. 2000; Kim et al. 2003; Baker 2004; Sasaki et al. 2007). Most results have shown that elevated CO_2 increases yield. Conversely, several studies have shown that high air temperatures can reduce grain yield even under CO_2 enrichment (Baker et al. 1992; Ziska et al. 1996; Matsui et al. 1997;

Table 30.2 Estimates for the response of global average crop yields to warming and CO_2 changes over the next decades

Global crop area	Change in temperature per decade[a]	Change in yield per °C	Change in yield per decade	Change in CO_2 per decade	Change in yield μl L^{-1}[b]	Change in yield per decade
	°C	%		μl L^{-1}	%	
Likely value	0.3	−5	−1.5	25	0.07	1.8
Expected range	0.1 to 0.5	−8 to −3	−4 to −0.3	20–30	0.05–0.09	1.0–2.7

Source: Lobell and Gourdji (2012)

[a]Averaged over crop land areas

[b]Using values for C_3 grains, ignoring differences for C_4 grains and non grain crops, which would be lower and higher, respectively

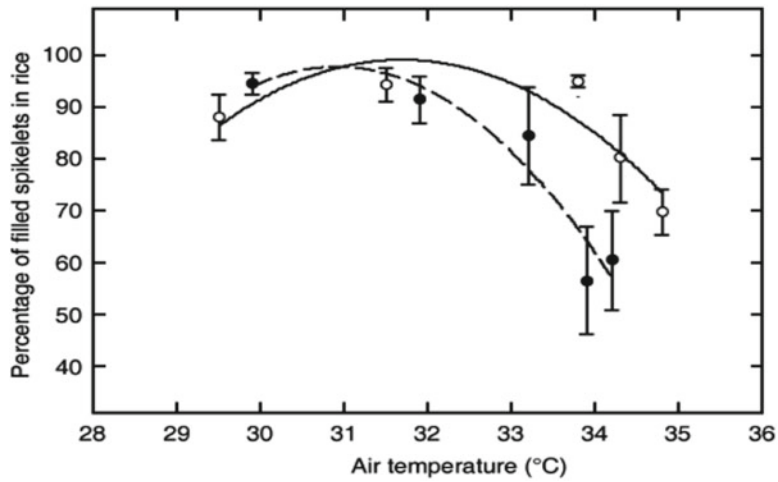


Fig. 30.7 Relationship between percentage of filled spikelets and increasing air temperature in rice (*Oryza sativa* cv. IR 72) grown in open-top chambers in the Philippines at ambient (*open circles*) and elevated (*closed circles*; ambient +300 μmol mol^{-1}) atmospheric CO_2 concentration (Source: Matsui et al. 1997)

Horie et al. 2000, Prasad et al. 2006), owing to increased spikelet sterility (Satake and Yoshida 1978; Kim et al. 1996; Matsui et al. 1997; Moya et al. 1998; Ohe et al. 2007; Jagadish et al. 2007) and reductions in transpirational cooling, higher canopy temperatures, and increased pollen sterility. Increase in CO_2 concentration stimulates increase in Rubisco and reduces photorespiration. Ziska et al. (1996) recorded a significant increase in root/shoot ratio with elevated CO_2 and increasing temperature thereby suggesting that alternative sinks become active recipients with reduced carbon sink capacity of the grains due to spikelet sterility from high-temperature exposure. Matsui et al. (1997) studying the interaction of CO_2 and temperature at reproductive stage of rice recorded an increase in canopy temperature due to stomata closure at high CO_2 concentrations (Fig. 30.7). Hence, this indicates that increasing CO_2 concentration could limit rice yield if average air temperature increased simultaneously. Crop simulation studies have predicted that average yield can reduce by 6–7 % for every 1.8 °C increase in temperature, but an increase in CO_2 concentration up to 700 μmol L^{-1} can lead to average yield increase of 30.7 % (Krishnan et al. 2007). The interaction studies of CO_2 and temperature at vegetative and reproductive stages of crops will provide information on the physiological and biochemical processes that affect final yields in field crops. Higher CO_2 concentration will be beneficial in regions where temperature is optimum, but increase in temperature beyond this will cause a decrease in carbon gain and accelerate crop development.

The interaction of temperature and CO_2 seems more complex for wheat, with a majority of experiments indicating a reduction in yield with elevated CO_2 in combination with warming compared with elevated CO_2 alone (Amthor 2001). A few studies have compared responses of soybean crops grown for the full season under different temperature regimes (Sionit et al. 1987; Baker et al. 1989; Boote et al. 2005; Heinemann et al. 2006) but have found only minor effects of these treatments on the yield response to elevated CO_2.

30.5 Adaptation of Crops to Climate Change

Crop improvement must aim at increase in productivity and resource use efficiency if yields are to double under the climate change scenario. Currently available varieties are not suitable for the changing climatic conditions especially in areas where either or both night and day temperatures are changing. Wahid et al. (2007) suggested that agricultural productivity can be improved under stress by developing cultivars that can tolerate environmental stresses and maintain economic yield.

30.5.1 Development of Climate Smart Crops

A vast amount of research has focused on individual stresses, but in the farmers' fields, the crop is subjected to a combination of stresses. Development of new crop varieties with higher yield potential and resistance to multiple stresses (drought, flood, salinity) is the key to maintain yield stability under such environments. Therefore, genes or quantitative trait loci (QTL) underlying tolerance to these stresses need to be identified and then introgressed in high-yielding cultivars with acceptable grain quality attributes. The breeding process could involve the basic steps such as:

1. Identification of donors from the available germplasm
2. Hybridization and recombination of selected germplasm
3. Phenotypic and/or molecular marker-aided selection of desired genotypes from segregating populations
4. Evaluation of elite breeding lines
5. Multi-environment (both temporal and spatial) testing at a large scale
6. On-farm trials and participatory varietal selection
7. Varietal release and production of breeder, foundation, registered, and certified seeds
8. Frontline demonstration and promotion of the newly approved cultivars

Germplasm of wild relatives and local land races could be used for developing climate-resilient crop cultivars. Due to regional impacts of climate change and time lag between the development of improved cultivars and adoption in farmers' fields, it is crucial to identify future breeding target environments to allow priority setting for both researchers and policy markers. By connecting genotype to phenotype, high-yielding, stress-tolerant plants can be selected far more rapidly and efficiently than is currently possible. Spectacular advances in "next-generation" DNA sequencing are rapidly reducing the costs of genotyping (Jackson et al. 2011). Crop simulation models may also guide the use of genetic manipulation to produce crop varieties that can tolerate the effects of climate change.

Fig. 30.8 High clearance tractor with sets of sensors that allow simultaneous measurement of canopy height, temperature, and spectral reflectance of cotton crop (Source: White et al. 2012)

The progress in breeding depends on genetic variability for the trait of interest, high-selection intensity through screening a large number of genotypes, and high broad-sense heritability for the trait of interest. Next-generation genotyping tools for characterizing sequence variation are capable of meeting the requisite, but modern phenotyping technology lags that of genotyping (White et al. 2012). Improved phenotyping platforms will provide the foundation for the success of conventional, molecular, and transgenic breeding. A field-based phenomics approach is capable of attaining the requisite high levels of throughput (Fig. 30.8). Searching for single indices that correlate strongly with yield is unlikely to provide more information than simply analyzing yield differences. Phenomics requires integrative, interdisciplinary teamwork and meticulous attention to quality control at all stages, starting with field preparation and experimental design, followed by timely processing and analysis of data, and ending with direct application toward finding solutions to major problems currently limiting crop production. A given yield level is often attainable through multiple mechanisms, and the optimal combination of traits for one environment often differs from that required in another, and phenotyping can provide data on these mechanisms. Attaining the phenotyping capability that will allow agriculture to address climate change, food security, and bioenergy requires coordinated and sustained efforts with adequate resources to

test and develop the necessary infrastructure and procedures. Phenotyping system needs to be rapid, flexible, and reliable and characterize multiple traits in a single pass. The system should permit measurements to be made repeatedly throughout the season. Field-based phenomics however does not exclude complementary phenotyping in controlled environments or rapid screening for specific traits (White et al. 2012).

Research efforts should be oriented for improvement in radiation, water, and nutrient-use efficiency of crops as they assume more relevance in the climate change scenarios. For exploiting the beneficial effects of elevated CO_2 concentrations, crop demand for nitrogen is likely to increase. Nitrogen-use efficiency may be reduced under the climate change scenarios because of high temperatures and heavy precipitation events causing volatilization and leaching losses. Cultivars with a broad genetic base and tolerant to drought, heat, and salinity can minimize the risks of climatic aberrations.

30.5.2 Changes in Crop, Land-Use, and Pest Management for Increasing Resilience

Despite the availability of improved crop varieties with increased yield potential, the optimum production is not attained generally because of poor crop management (Reynolds and Tuberosa 2008). Yields in warm environments can be raised by modifying agronomic practices (Badaruddin et al. 1999). Changing land-use practices such as the location of crop and livestock production and reducing the intensity of fertilizer and pesticide application as well as capital and labor inputs can help reduce risks from climate change in farm production. Soil carbon sequestration can be increased by intensifying and altering crop rotations, including perennial forages and reducing bare fallow, by retaining crop residues, and by optimizing agronomic inputs such as fertilizer, irrigation, pesticides, and liming. Although it has limitations in tropical areas because of high temperature, a substantial quantity of C can be sequestered with the adoption of improved agricultural practices. Poor intensification of agriculture in sub-Saharan Africa (low use of fertilizer and irrigation) has resulted in a large expansion of agricultural land within this region (FAO 2003). Changing land use by increasing the area under biofuel-producing crops and agroforestry could help in mitigating GHG emissions with due emphasis on the goal of increasing food production (Venkateswarlu et al. 2011). Management practices to build up soil organic carbon requires increasing the C input, decreasing decomposition, or both (Paustian et al. 1997). The planting of multipurpose trees on degraded lands helps in C sequestration and reduce the effects of extremely high temperatures (Cannell et al. 1996). Biochar is another approach to sequester C in terrestrial ecosystems; several associated products are in the process of being manufactured (Chauhan et al. 2014). Climate change can increase the vulnerability of crops to pest and weed. The strategies to manage this interaction can be (1) developing cultivars' resistance to pests and diseases, (2) adopting integrated pest management, and (3) pest forecasting using recent tools such as simulation modeling. Biocontrol agents can be effectively used in pest management that can aid in reduced use of pesticides and consequently carbon emissions as they promote natural enemies like the release of predators and parasites.

30.5.3 Crop Diversification and Changing Planting Time for Resilience

Cropping systems may have to be changed to include growing suitable cultivars (to counteract compression of crop development), increasing crop intensities (i.e., the number of successive crop produced per unit area per year), or planting different types of crops. Diversification of crop varieties by replacing the current cultivars with new plant types, cultivars, and hybrids and adjustment of the cropping sequence by changing the timing of sowing, planting, and harvesting can improve productivity under the projected increase

in temperature. Farmer adaptation can also involve changing the timing of irrigation or use of other inputs such as fertilizers. Diversification from rice-wheat toward high-value commodities can increase income and result in reduced water and fertilizer use. Manipulation of planting time along with use of cultivars from different maturity groups can be explored as a feasible strategy for increasing crop productivity. To attain high and stable productivity, it is essential to extend the growing season to maximize a crop's productivity. Crop plants produce their dry matter through photosynthesis to fix atmospheric CO_2, using energy from solar radiation; therefore, increasing radiation use by the canopy during the growing season can increase total dry matter production, potentially leading to increased grain yield (Horie and Sakuratani 1985; Nemoto et al. 2011). This can be achieved by manipulating the planting time and by choosing cultivars from a maturity group that is appropriate for a particular region. Krishnan et al. (2007) demonstrated potential outcomes by adjusting the sowing time of rice at two sites (Cuttack and Jorhat in India) by simulating crop growth under different climate change scenarios. Manipulation of planting dates helped in reducing yield instability by keeping flowering from coinciding with the hottest growing season (Mahajan et al. 2009). Shimono et al. (2010) analyzed historical changes in the rice cropping schedule by farmers in northern Japan and found that the transplanting date had advanced from the 1960s to the 1980s in response to temperature increases, but found no further changes thereafter despite apparently increasing temperatures, indicating a potential for increasing rice productivity by adopting earlier planting. Adjustment of planting dates to minimize the effect of temperature increase-induced spikelet sterility can be used to reduce yield instability, by avoiding having the flowering period to coincide with the hottest period. The early-morning flowering advantage of O*ryza glaberrima* has been exploited to advance peak flowering time of the day by 1 h toward early morning (Yoshida et al. 1981). Despite favorable weather conditions during the vegetative growth, sudden increase in temperature during the grain-filling stage in wheat has adversely affected wheat productivity in the Indo-Gangetic Plains (IGP) in the past decade, resulting in significant decrease in the yield (Gupta et al. 2010). Agronomic management of crops, such as method of sowing, can be an effective adaptation strategy under the climate change scenario. Bed planting of crops has proved successful in the wake of climate change as it results in increased water-use efficiency, reduced waterlogging, better access for inter-row cultivation, weed control, banding of fertilizers, better stand establishment, less crop lodging, and reduced seeding rates (Bhardwaj et al. 2009; Chauhan 2012a). In irrigated areas, zero-till system is being considered as a viable alternative to alleviate from adverse effects of climate change as it reduces the demand for water and other resources. Intercropping can also come to rescue in the face of climate change as the failure of one crop can be compensated by second crop, assuring minimum returns for livelihood security (Mittal and Singh 1989).

30.5.4 Adoption of Resource Conserving Technologies

Yields of wheat in heat- and water-stressed environments can be increased by adopting resource-conserving technologies (RCTs) which minimize unfavorable environmental impacts. Soil and water management is essential for the adaptation to climate change. The RCTs in rice-wheat system have pronounced effects on mitigation of greenhouse gas emission and adaptation to climate change (Pathak and Wassmann 2007). These approaches of crop management should be coupled with the measures of crop improvement for wider adaptation to climate change. Farmers should be trained and encouraged for adopting water conservation techniques for better water-use efficiency. Increasing water infiltration with improvement in soil aggregation; decreasing runoff with use of contours, ridges, vegetative hedges, etc.; and reducing soil evaporation with use of crop residues mulch will be useful for management of soil water. Traditional ecological knowledge of farmers can be harnessed and used

for community-based resource management and enhance their capacity to adapt to the impacts of future climate change.

30.5.5 Weather Forecasting and Crop Insurance Programs

Weather forecasting and early warning systems will help minimize the risks of climatic adversaries. Effective crop insurance programs can help reduce income losses as a result of climate-related impacts. Advancement in information technology can help the farmers in receiving SMS-based weather forecasting information and also help in developing the irrigation and other crop management schedules.

30.6 Mitigation Strategies to Climate Change

Adaptation strategies enable the plants to perform optimally under adverse climatic conditions through agronomic and genetic manipulations at the regional or local level, whereas mitigation helps in reducing GHG emissions into the atmosphere, thus benefitting at local and global level. The benefits of mitigation activities will be evident in several decades because of the longer duration of GHGs in the atmosphere, whereas the effects of adaptation measures should be seen immediately or in the near future (Kumar and Parikh 2001; Lal 2011). Reducing or preventing deforestation would have the largest and most immediate impact on reducing atmospheric carbon emissions (IPCC 2007). Maintaining carbon sinks in tropical forests is therefore one of the major climate change mitigation measures.

Conservation agriculture has been proposed as a set of management principles that assure a more sustainable agricultural production and reduce production costs while increasing profitability. It combines reduced tillage and retention of adequate levels of crop residues, maintaining soil surface cover and crop rotations (Cairns et al. 2012). It reduces the CO_2 emissions associated with farming activities by the reduction of tillage operations. Conservation agriculture can improve infiltration and reduce evaporation compared to practices involving conventional tillage and zero tillage without retention of adequate levels of crop residue (Verhulst et al. 2010). Zero-till systems have a direct mitigation effect as they convert GHGs such as CO_2 into O_2 in the atmosphere and C enriches soil organic matter. This practice allows the farmers to sow wheat sooner after rice harvest, so the crop heads and fills the grain before the onset of pre-monsoon hot weather (Fig. 30.9) (Ortiz et al. 2008). In dry-seeded rice, because of

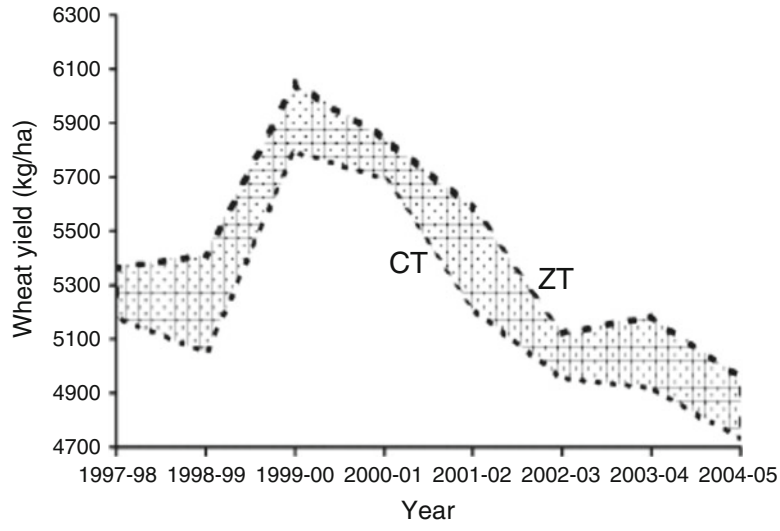


Fig. 30.9 Increase in wheat yield by adopting zero tillage (*ZT*) in the Indo-Ganges: about 247 kg ha^{-1} (*shaded area*) on average were added to land previously under conventional tillage (*CT*) (Source: Ortiz et al. 2008)

minimum anaerobic conditions, improved root growth and diversity of aerobic soil organisms may help in mitigating climate change. An important mitigation strategy for climate change is a reduction on the reliance of chemical inputs without compromising on yields. Research has shown that yields similar to those in puddled-transplanted rice can be achieved with alternate wetting and drying (Mahajan et al. 2011).

Mitigation of CO_2 emission from agriculture can be achieved by increasing carbon sequestration in soil through manipulation of soil moisture and temperature, setting aside surplus agricultural land, and restoration of soil carbon on degraded lands. The strategies for mitigating methane emission from rice cultivation could be alteration in water management, particularly promoting mid-season aeration by short-term drainage; improving organic matter management by promoting aerobic degradation through composting or incorporating it into soil during off-season drained period; using rice cultivars with few unproductive tillers, high root oxidative activity, and high harvest index; and applying fermented manures like biogas slurry in place of unfermented farmyard manure (Pathak and Wassmann 2007). However, alternate wetting and drying may lead to emissions of N_2O, which has greater global warming potential than CH_4 does. Globally, N fertilizers account for 33 % of the total annual creation of reactive nitrogen (Nr) and 66 % of all anthropogenic sources of reactive forms of Nr (Dobermann and Cassman 2005). This problem could be reduced by adopting integrated nutrient management practices, which can help in mitigating climate change. Integrated nutrient management involves a package of organic, inorganic, and biofertilizers in proportions that will keep the soil capable of producing at an accelerated rate without damaging its physical, chemical, and biological characteristics. Management options related to N rate, timing, source, and placement can be used to optimize N uptake (Ortiz-Monasterio et al. 2010). The advantages of integrated nutrient management are increased N-use efficiency and increased yield. In rice fields, the emissions of NO_2 and CH_4 can be reduced by the application of urease, hydroquinone, and nitrification inhibitors, dicyandiamide together with urea. It has also been suggested that certain tropical grasses like *Brachiaria humidicola* inhibit or reduce soil nitrification by releasing inhibitory compounds from roots and suppress *Nitrosomonas* bacteria (Subbarao et al. 2005; Wang et al. 2005). Subbarao et al. (2007) discovered a source for high BNI ability in *Leymus racemosus*—a wild relative of wheat and chromosome containing the relevant gene(s) was introduced into wheat, and biological nitrification inhibitors were produced and productivity increased. However, further studies are needed to characterize and quantify the BNI ability from wild relative that will aid in genetically improving the BNI ability of the cultivated wheat. Improved management of livestock, and their diet could also assist in the mitigation of GHGs released by ruminants. The use of improved food additives, substitution of low-digestibility feeds with high-digestibility ones, concentrate feeding, substituting fibrous concentrate with starchy concentrate, supplementation with molasses, and changing microflora of rumen could help in reducing CH_4 emissions (Aggarwal 2008). The withdrawal of groundwater from deeper layers demands more energy and leads to GHG emissions in agriculture (Hira 2009). If surface storage of rainwater in dugout ponds is encouraged, dependence on withdrawing groundwater might decrease. The conjunctive use of surface water and groundwater is an important strategy to mitigate climate change (Chauhan et al. 2014). Temperature changes may cause an expansion of weeds, with some species moving to higher latitudes and altitudes (Mahajan et al. 2012). Hardy weeds, such as *Rumex* spp., may increase in wheat because of increased adoption of zero tillage in wheat. Higher CO_2 concentration may stimulate belowground growth relative to aboveground growth and may favor rhizome and tuber growth of perennial weeds (Ziska 2003). The risk of herbicide application for weed control may increase as a result of environmental change. Integrated weed management strategies need to be developed as a futuristic climate-resilient strategy that will target weed invasion and reproduction. Such

strategies may include a combination of optimal fertilizer schedule, summer plowing, crop rotation, land preparation, plant geometry modification, stale-seedbed technique, and the use of weed-competitive cultivars (Chauhan 2012b).

30.7 Conclusions

Elevated CO_2 concentration may increase growth and yield of rice and wheat crop due to increased photosynthesis, decreased photorespiration, and decreased stomatal conductance. The increase in temperature, however, may decrease grain yields due to the shorter duration of crop growth. Because of the complexity of crop-environment interactions, the adaptation to climate change requires cross-disciplinary solutions (Howden et al. 2007) that include the development of varieties with increased resilience to abiotic and biotic stresses (Fedoroff et al. 2010). Crop simulation models can be used for modeling crop growth in variable environments, and a better understanding of the relationships between the complex and component traits can be achieved so as to rank the simple traits, for their influence on the complex traits (Semenov and Halford 2009). Conservation agriculture that involves reductions in tillage, improved retention of crop residues, and diversified and economically viable crop rotations also contributes to increase yields and resilience of farming systems. The development of such more sustainable systems and technology demonstration of the existing management practices will enhance income and nutrition for the poor while also contributing to both adaptation and mitigation of climate change. Planning effective policies at regional and global level will be required to ensure the technologies reach the intended beneficiaries to have the desired impacts.

References

Abbate PE, Andrade FH, Culot JP (1995) The effect of radiation and nitrogen on number of grains in wheat. J Agric Sci 124:351–360

AGFACE (2013) The Australian grains free air carbon dioxide enrichment program: crops for a changing climate. A report published by the Primary Industries Climate Challenges Centre (PICCC) www.piccc.org.au/agface

Aggarwal PK (2008) Global climate change and Indian agriculture: impact, adaptation and mitigation. Indian J Agric Sci 78:911–919

Ahmed FE, Hall AE, DeMason DA (1992) Heat injury during floral development in cowpea (*Vigna unguiculata* Fabaceae). Am J Bot 79:784–79

Ainsworth EA (2008) Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. Glob Chang Biol 14:1642–1650

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol 165:351–371

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant Cell Environ 30:258–270

Allakhverdiev SI, Kreslavski VD, Klimov VV, Los DA, Carpentier R, Mohanty P (2008) Heat stress: an overview of molecular responses in photosynthesis. Photosynth Res 98:541–550

Ambardekar AA, Siebenmorgen TJ, Counce PA, Lanning SB, Mauromoustakos A (2011) Impact of field-scale night-time air temperatures during kernel development on rice milling quality. Field Crop Res 122:179–185

Amthor JS (2001) Effects of atmospheric CO_2 concentration on wheat yield: review of results from experiments using various approaches to control CO_2 concentration. Field Crops Res 73:1–34

Badaruddin M, Reynolds MP, Ageeb OAA (1999) Wheat management in warm environments: effect of organic and inorganic fertilizers, irrigation frequency, and mulching. Agron J 91:975–983

Baker JT (2004) Yield responses of southern US rice cultivars to CO_2 and temperature. Agric For Meteorol 122:129–137

Baker JT, Allen LH Jr, Boote KJ, Jones P, Jones JW (1989) Response of soybean to air temperature and carbon dioxide concentration. Crop Sci 29:98–105

Baker JT, Allen LH, Boote KJ (1992) Response of rice to carbon-dioxide and temperature. Agric For Meteorol 60:153–166

Barnabas B, Jager K, Feher A (2008) The effect of drought and heat stress on reproductive processes in cereals. Plant Cell Environ 31:11–38

Batts GR, Ellis RH, Morison JIL, Nkemka PN, Gregory PJ, Hadley P (1998) Yield and partitioning in crops of contrasting cultivars of winter wheat in response to CO_2 and temperature in field studies using temperature gradient tunnels. J Agric Sci Camb 130:17–27

Bencze S, Veisz O, Bedo Z (2004) Effects of high atmospheric CO_2 and heat stress on phytomass, yield and

grain quality of winter wheat. Cereal Res Commun 32:75–82
Bhardwaj V, Yadav V, Chauhan BS (2009) Effect of nitrogen application timings and varieties on growth and yield of wheat grown on raised beds. Arch Agron Soil Sci 56:211–222
Blum A, Sinmena B, Mayer J, Golan G, Shpiler L (1994) Stem reserve mobilisation supports wheat-grain filling under heat stress. Funct Plant Biol 21:771–781
Blumenthal CS, Bekes F, Batey IW, Wrigley CW, Moss HJ, Mares DJ, Barlow EWR (1991a) Interpretation of grain quality results from wheat variety trials with reference to high temperature stress. Aust J Agric Res 42:325–334
Blumenthal CS, Batey IL, Bekes F, Wrigley CW, Barlow EWR (1991b) Seasonal changes in wheat-grain quality associated with high temperatures during grain filling. Aust J Agric Res 42:21–30
Blumenthal C, Rawson HM, McKenzie E, Gras PW, Barlow EWR, Wrigley CW (1996) Changes in wheat grain quality due to doubling the level of atmospheric CO_2. Cereal Chem 73:762–766
Boote KJ, Allen LH Jr, Prasad PV, Baker JT, Gesch RW, Synder AM, Pan D, Thomas JM (2005) Elevated temperature and CO_2 impact pollination, reproductive growth and yield of several globally important crops. J Agric Meteorol Jpn 60:469–474
Cairns JE, Sonder K, Zaidi PH, Verhulst N, Mahuku G, Babu R, Nair SK, Das B, Govaerts B, Vinayan MT, Rashid Z, Noor JJ, Devi P, San Vicente F, Prasanna BM (2012) Maize production in a changing climate: impacts, adaptation, and mitigation strategies. Adv Agron 114:1–58
Calderini DF, Abedelo LG, Savin R, Slafer GA (1999) Final grain weight in wheat as affected by short periods of high temperatures during pre and post-anthesis under field conditions. Aust J Plant Physiol 26:452–458
Cannell MGR, van Noordwijk M, Ong CK (1996) The central agroforestry hypothesis: the trees must acquire resources that the crop would not otherwise acquire. Agrofor Syst 34:27–31
Castro M, Peterson CJ, Rizza MD, Dellavalle PD, Vázquez D, Ibáñez V, Ross A (2007) Influence of heat stress on wheat grain characteristics and protein molecular weight distribution. In: Buck HT, Nisi JE, Salomón N (eds) Wheat production in stressed environment. Springer, Dordrecht, Netherlands, pp 365–371
Chandna P, Hodson DP, Singh UP, Gosain AK, Sahoo RN, Gupta RK (2004) Increasing the productivity of underutilized lands by targeting resource conserving technologies- a GIS/Remote sensing approach. In: A case study of Ballia district, Uttar Pradesh in Eastern Gangetic plains, CIMMYT, Mexico, DF
Chauhan BS (2012a) Can knowledge in seed ecology contribute to improved weed management in direct-seeded rice? Curr Sci 103:486–489
Chauhan BS (2012b) Weed ecology and weed management strategies for dry-seeded rice in Asia. Weed Technol 26:1–13
Chauhan BS, Kaur P, Mahajan G, Randhawa RK, Singh H, Kang MS (2014) Global warming and its possible impact on agriculture in India. Adv Agron 123:65–121
Coles GD, Hartuniansowa SM, Jamieson PD, Hay AJ, Atwell WA, Fulcher RG (1997) Environmentally-induced variation in starch and non-starch polysaccharide content in wheat. J Cereal Sci 26:47–57
Conroy JP, Seneweera S, Basra A, Rogers G, Nissen-Wooley B (1994) Influence of rising atmospheric CO_2 concentrations and temperature on growth, yield, and grain quality of cereal crops. Aust J Plant Physiol 21:741–758
Cooper NTW, Siebenmorgen TJ, Counce PA, Meullenet JF (2006) Explaining rice milling quality variation using historical weather data analysis. Cereal Chem 83:447–450
Corbellini M, Carnevar MG, Mazza L, Ciaffi M, Lafiandra E, Borghi B (1997) Effect of the duration and intensity of heat shock during grain filling on dry matter and protein accumulation, technological quality and protein composition in bread wheat and durum wheat. Aust J Plant Physiol 24:245–250
Crafts-Brandner SJ, Salvucci ME (2000) Rubisco activase constraints the photosynthetic potential of leaves at high temperatures. Proc Natl Acad Sci U S A 97:13430–13435
de Graaff MA, van Groenigen KJ, Six J, Hungate B, van Kessel C (2006) Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. Glob Chang Biol 12:2077–2091
Delgado E, Medrano H, Keys AJ, Parry MAJ (1995) Species variation in Rubisco specificity factor. J Exp Bot 46:1775–1777
Demotes-Mainard S, Jeuffroy MH (2004) Effects of nitrogen and radiation on dry matter and nitrogen accumulation in the spike of winter wheat. Field Crop Res 87:221–233
Dias AS, Bagulho AS, Lidon FC (2008) Ultrastructure and biochemical traits of bread and durum wheat grains under heat stress. Braz J Plant Physiol 20:323–333
Dobermann A, Cassman KG (2005) Cereal area and nitrogen use efficiency are drivers of future nitrogen fertiliser consumption. Sci China 48:745–758
Drake BG, Leadley PW (1991) Canopy photosynthesis of crops and native plant communities exposed to long-term elevated CO_2. Plant Cell Environ 14:853–860
Drake BG, Gonzàlez-Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO_2? Annu Rev Plant Physiol Plant Mol Biol 48:609–639
Easterling W, Aggarwal P, Batima P, Brander K, Erda L, Howden M, Kirilenko A, Morton J, Soussana JF, Schmidhuber J, Tubiello F (2007) Food fibre and forest products. In: Oarry ML, Canziani OF, Palutikof JP, van der Lindin PJ, Hanson CE (eds) Climate change 2007: impacts, adaptation and vulnerability. Cambridge University Press, Cambridge, UK, pp 273–313

Erbs MR, Manderscheid G, Jansen S, Seddig A, Weigel H-J, Pacholski A (2010) Effects of free-air CO_2 enrichment and nitrogen supply on grain quality parameters of wheat and barley grown in a crop rotation. Agric Ecosyst Environ 136:59–68

Erda L, Wei X, Hui J, Yinlong X, Yue L, Liping B, Liyong X (2005) Climate change impacts on crop yield and quality with CO_2 fertilization in China. Philos Trans R Soc Lond B Biol Sci 360:2149–2154

Erekul O, Kohn W (2006) Effect of weather and soil conditions on yield components and bread-making quality on winter wheat (*Triticum aestivum* L.) and winter triticale (*Triticosecale* Wittm.) varieties in North-East Germany. J Agron Crop Sci 192:452–464

FAO (2003) State of the world's forests 2003. Food and Agricultural Organization of the United Nations, Rome

Fedoroff NV, Battisti DS, Beachy RN, Cooper PMJ, Fischhoff DA, Hodges CN, Knauf VC, Lobell D, Mazur BJ, Molden D, Reynolds MP, Ronald PC et al (2010) Radically rethinking agriculture for the 21st century. Science 327:833–834

Ferris R, Ellis RH, Wheeler TR, Hadley P (1998) Effect of high temperature stress at anthesis on grain yield and biomass of field-grown crops of wheat. Ann Bot 82:631–639

Fischer RA (1984) Physiological limitation to producing wheat in semi tropical and tropical environments and possible selection criteria. In: Villareal RL, Klatt A (eds) Wheat for more tropical environments. CIMMYT, Mexico, pp 201–230

Gonzalez F, Slafer GA, Miralles DJ (2005) Floret developmental and survival in wheat plants exposed to contrasting photoperiod and radiation environments during stem elongation. Funct Plant Biol 32:189–197

Gonzalez FG, Miralles DJ, Slafer GA (2011) Wheat floret survival as related to pre-anthesis spike growth. J Exp Bot 62:1–13

Gooding MJ, Ellis RH, Shewry PR, Schafield JD (2003) Effects of restricted water availability and increased temperature on the grain filling, drying and quality of winter wheat. J Cereal Sci 37:295–309

Gupta R, Gopal R, Jat ML, Jat RK, Sidhu HS, Minhas PS, Malik RK, Gupta R, Gopal R, Jat ML, Jat RK, Sidhu HS, Minhas PS, Malik RK (2010) Wheat productivity in Indo-Gangetic plains of India during 2010: terminal heat effects and mitigation strategies. PACA Newsl 14:1–3

Hasan AK, Herrera J, Lizana C, Calderini DF (2011) Carpel weight, grain length and stabilized grain water content are physiological drivers of grain weight determination of wheat. Field Crop Res 123:241–247

Hatfield JL, Boote KJ, Kimball BA, Ziska LH, Izaurralde RC, Ort D, Thomson AM, Wolfe D (2011) Climate impacts on agriculture: implications for crop production. Agron J 103:351–370

Hawker JS, Jenner CF (1993) High temperature affects the activity of enzymes in the committed pathway of starch synthesis in developing wheat endosperm. Aust J Plant Physiol 20:197–209

Hawkesford MJ, Araus JL, Park R, Calderini D, Miralles D, Shen T, Zhang J, Parry MAJ (2013) Prospects of doubling global wheat yield. Food Energy Secur 2:34–48

Hay RKM, Walker AJ (1989) Dry matter partitioning. An introduction to the physiology of crop yield. Longman Scientific &Technical, Harlow, pp 107–156

Heinemann AB, Maia A, Dourado-Neto D, Ingram KT, Hoogenboon G (2006) Soybean (*Glycine max*. (L.L. Merr.)) growth and development response to CO_2 enrichment under different temperature regimes. Eur J Agron 24:52–61

Hira GS (2009) Water management in northern states and the food security of India. J Crop Improv 23:136–157

Hogy P, Fangmeier A (2008) Effects of elevated atmospheric CO_2 on grain quality of wheat. J Cereal Sci 48:580–591

Horie T, Sakuratani T (1985) Studies on crop-weather relationship model in rice. (1) Relation between absorbed solar radiation by the crop and the dry matter production. J Agric Method 40:331–342

Horie T, Baker JT, Nakagawa H, Matsui T, Kin HY (2000) Crop ecosystem responses to climatic change: rice. In: Reddy KR, Hodges HF (eds) Climate change and global crop productivity. CAB Int, New York, pp 81–106

Howden SM, Soussana JF, Tubiello FN, Chhetri N, Dunlop M, Meinke H (2007) Adapting agriculture to climate change. Proc Natl Acad Sci USA 104:19691–19696

IPCC (2001) Climate change 2001: the scientific basis. Contributions of working group I to the third assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge

IPCC Working Group III (2007) Mitigation of climate change. In: Metz B, Davidson OR, Bosch PR, Dave R, Meyer LA (eds) Fourth assessment report. Cambridge University Press, New York

Ismail AM, Hall AE (1998) Positive and potential negative effects of heat-tolerance genes in cowpea. Crop Sci 38:381–390

Jackson SA, Iwata A, Lee SH, Schmutz J, Shoemaker R (2011) Sequencing crop genomes: approaches and applications. New Phytol 191:915–925

Jagadish SVK, Craufurd PQ, Wheeler TR (2007) High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). J Exp Bot 58:1627–1635

Jenner CF (1994) Starch synthesis in the kernel of wheat under high temperature conditions. Funct Plant Biol 21:791–806

Kase M, Catsky J (1984) Maintenance and growth components of dark respiration rate in leaves of C_3 and C_4 plants as affected by leaf temperature. Biol Plant 26:461–470

Kaur R, Rajni (2012) Climate change and its possible impacts on agriculture in India. Indian Farming 62:10–15

Keeling PL, Bacon PJ, Holt DC (1993) Elevated temperature reduces starch deposition in wheat endosperm by

reducing the activity of soluble starch synthase. Planta 191:342–348
Keeling PL, Banisadr R, Barone L, Wasserman BP, Singletary GW (1994) Effect of temperature on enzymes in the pathway of starch biosynthesis in developing wheat and maize grain. Aust J Plant Physiol 32:807–827
Kim HY, Horie T, Nakagawa H, Wada K (1996) Effects of elevated CO_2 concentration and high temperature on growth and yield of rice. Japan J Crop Sci 65:644–651
Kim HY, Lieffering M, Kobayashi K, Okada M, Miura S (2003) Seasonal changes in the effects of elevated CO_2 on rice at three levels of nitrogen supply: a free air CO_2 enrichment (FACE) experiment. Glob Chang Biol 9:826–837
Kimball BA, Morris CF, Pinter PJ Jr, Wall GW, Hunsaker DJ, Adamsen FJ, LaMorte RL, Leavitt SW, Thompson TL, Matthias AD, Brooks TJ (2001) Elevated CO_2, drought and soil nitrogen effects on wheat grain quality. New Phytol 150:295–303
Kirby EJM (1988) Analysis of leaf, stem and ear growth in wheat from terminal spikelet stage to anthesis. Field Crop Res 18:127–140
Kobata T, Uemuki N (2004) High temperatures during the grain-filling period do not reduce the potential grain dry matter increase of rice. Agron J 96:406–414
Krishnan P, Swain DK, Bhaskar BC, Nayak SK, Dash RN (2007) Impact of elevated CO_2 and temperature on rice yield and methods of adaptation as evaluated by crop simulation studies. Agric Ecosyst Environ 122:233–242
Kumar KSK, Parikh J (2001) Indian agriculture and climate sensitivity. Glob Environ Chang 11:147–154
Kumar NS, Singh AK, Aggarwal PK, Rao VUM, Venkateswarlu B (2012) Climate change and Indian agriculture: impact, adaptation and vulnerability – salient achievements from ICAR network project. IARI Publication, New Delhi, p 32
Lal R (2004) Soil carbon sequestration impacts on global climate change and food security. Science 304:1623–1627
Lal M (2011) Implications of climate change in sustained agricultural productivity in South Asia. Reg Environ Chang 11:79–94
Lapitan VC, Brar DS, Abe T, Redo˜na ED (2007) Assessment of genetic diversity of Philippine rice cultivars carrying good quality traits using SSR markers. Breed Sci 57:263–270
Lawlor DW, Mitchell RAC (1991) The effects of increasing CO_2 on crop photosynthesis and productivity: a review of field studies. Plant Cell Environ 14:803–818
Lawlor DW, Mitchell RAC (2001) Crop ecosystems responses to climate change: wheat. In: Reddy KR, Hodges HF (eds) Climate change and global crop productivity. CABI Publishing, Wallingford, pp 57–80
Leakey ADB, Ainsworth EA et al (2009) Elevated CO_2 effects on plant carbon, nitrogen, and water relations; six important lessons from FACE. J Exp Bot 60:2859–2876
Lele U (2010) Food security for a billion poor. Science 326:1554
Lisle AJ, Martin M, Fitzgerald MA (2000) Chalky and translucent rice grains differ in starch composition and structure and cooking properties. Cereal Chem 77:627–632
Lizana XC, Riegel RL, Gomez D, Herrera J, Isla A, McQueen-Mason SJ et al (2010) Expansins expression is associated with grain size dynamics in wheat (*Triticum aestivum* L.). J Exp Bot 61:1147–1157
Lobell DB, Gourdji SM (2012) The influence of climate change on global crop productivity. Plant Physiol 160:1686–1697
Lobell DB, Ortiz-Monasterio IJ, Asner GP, Matson PA, Naylor RL, Falcon WP (2005) Analysis of wheat yield and climatic trends in Mexico. Field Crops Res 94:250–256
Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants face the future. Annu Rev Plant Biol 55:591–628
Long SP, Ainsworth EA, Leakey ADB, Nosberger J, Ort DR (2006) Food for thought: lower-than-expected crop yield stimulation with rising CO_2 concentrations. Science 312:1918–1921
Mačas B, Gomes MC, Dias AS, Coutinho J (2000) The tolerance of durum wheat to high temperatures during grain filling. In: Royo C, Nachit MM, Di Fonzo N, Araus JL (eds) Durum wheat improvement in the Mediterranean region: new challenges. Proceedings of seminar jointly organized by CIHEAM, Centre UdL-IRTA, CIMMYT and ICARDA, Zaragoza, 12–14 April 2000, pp 257–261
Mahajan G, Bharaj TS, Timsina J (2009) Yield and water productivity of rice as affected by time of transplanting in Punjab. India Agric Water Manag 96:525–535
Mahajan G, Timsina J, Singh K (2011) Performance and water use efficiency of rice relative to establishment methods in northwestern Indo-Gangetic Plains. J Crop Improv 25:597–617
Mahajan G, Singh S, Chauhan BS (2012) Impact of climate change on weeds in the rice–wheat cropping system. Curr Sci 102:1254–1255
Majoul T, Bancel E, Triboï E, Ben Hamida J, Branlard G (2003) Proteomic analysis of the effect of heat stress on hexaploid wheat grain: characterization of heat-responsive proteins from total endosperm. Proteomics 3:175–183
Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London
Matsue Y (1995) Influence of abnormal weather in 1993 on the palatability and physicochemical characteristics in rice. Nihon Sakumotsu Gakkai Kiji 64:709–713
Matsui T, Namuco OS, Ziska LH, Horie T (1997) Effect of high temperature and CO_2 concentration on spikelet sterility in indica rice. Field Crop Res 51:213–219
Matsui T, Omasa K, Horie T (1999) Mechanism of anther dehiscence in rice (*Oryza sativa* L.). Ann Bot 84:501–506

McDonald GK, Sutton BG, Ellsion FW (1983) The effect of time of sowing on the grain yield of irrigated wheat in Namoi Valley, New South Wales. Aust J Agric Res 34:224–229

Mittal SP, Singh P (1989) Intercropping field crops between rows of *Leucaena leucocephala* under rainfed conditions in northern India. Agrofor Syst 8:165–172

Mondal S, Singh RP, Crossa J, Huerta-Espino J, Sharma I, Chatrath R, Singh GP, Sohu VS, Mavi GS, Sukaru VSP, Kalappanavarg IK, Mishra VK, Hussain M, Gautam NR, Uddin J, Barma NCD, Hakim A, Joshi AK (2013) Earliness in wheat: a key to adaptation under terminal and continual high temperature stress in south Asia. Field Crops Res 151:19–26

Morita S, Yonemaru JI, Takanashi JI (2005) Grain growth and endosperm cell size under high night temperatures in rice (*Oryza sativa* L.). Ann Bot 95:695–701

Moya TB, Ziska LH, Namuco OS, Olszyk D (1998) Growth dynamics and genotypic variation in tropical, field-grown paddy rice (*Oryza sativa* L.) in response to increasing carbon dioxide and temperature. Glob Chang Biol 4:645–656

Murchie EH, Pinto M, Horton P (2009) Agriculture and the new challenges for photosynthesis research. New Phytol 181:532–552

Mutters RG, Hall AE (1992) Reproductive responses of cowpea to high temperature during different night periods. Crop Sci 32:202–206

Mutters RG, Hall AE, Patel PN (1989) Photoperiod and light quality effects on cowpea floral development at high temperatures. Crop Sci 29:1501–1505

Nagarajan S, Chowdhary HB, Anand A (2008) Impact and adaptation of spring wheat genotypes to chronic high temperature and heat shock conditions during grain growth period in North Western India. Indian J Agric Sci 78(2):117–119

Nagarajan S, Jagadish SVK, Prasad ASH, Thomar AK, Anand A, Pal M, Agarwal PK (2010) Local climate affects growth, yield, and grain quality of aromatic and non-aromatic rice in northwestern India. Agric Ecosyst Environ 138:274–281

Nemoto M, Hamasaki T, Shimono H (2011) Extraordinary hot summer in Hokkaido decrease rice yield and satisfy growing of cultivar in Tohoku region 'Hitomebore'. J Agric Meteorol 67:269–274

Nielsen CL, Hall AE (1985) Responses of cowpea (*Vigna unguiculata* [L.] Walp) in the field to high night temperatures during flowering. II. Plant responses. Field Crop Res 10:181–196

Ogren WL (1984) Photorespiration pathways, regulation, and modification. Annu Rev Plant Physiol 35:415–442

Ohe I, Saitoh K, Kuroda T (2007) Effects of high temperature on growth, yield and dry-matter production of rice grown in the paddy field. Plant Prod Sci 10:412–422

Ortiz R, Sayre KD, Govaerts B, Gupta R, Subbarao GV, Ban T, Hodson D, Dixon JM, Ortiz-Monasterio I, Reynolds M (2008) Climate change: can wheat beat the heat? Agric Ecosyst Environ 126:46–58

Ortiz-Monasterio I, Wassmann R, Govaerts B, Hosen Y, Katayanagi N, Verhulst N (2010) Greenhouse gas mitigation in the main cereal systems: rice, wheat and maize. In: Reynolds MP (ed) Climate change and crop production, CABI Series in Climate Change. CABI, Wallingford, pp 151–176

Osada A, Sasiprada V, Rahong M, Dhammanuvong S, Chakrabandhu M (1973) Abnormal occurrence of empty grains of indica rice plants in the dry, hot season in Thailand. Proc Crop Sci Soc Jpn 42:103–109

Parry MAJ, Keys AJ, Gutteridge S (1989) Variation in the specificity factor of C-3 higher-plant Rubisco determined by the total consumption of ribulose-P2. J Exp Bot 40:317–320

Parry MAJ, Reynolds M, Salvucci ME, Raines C, Andralojc PJ, Zhu X-G, Price GD, Condon AG, Furbank RT (2011) Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. J Exp Bot 62:453–467

Pathak H, Wassmann R (2007) Introducing greenhouse gas mitigation as a development objective in rice-based agriculture: I. Generation of technical coefficients. Agric Syst 94:807–825

Pathak H, Ladha JK, Aggarwal PK, Peng S, Das S, Singh Y, Singh B, Kamra SK, Mishra B, Sastri A, Aggarwal HP, Das DK, Gupta RK (2003) Climatic potential and on-farm yield trends of rice and wheat in the Indo-Gangetic Plains. Field Crop Res 80:223–234

Paustian K, Andre´n O, Janzen HH, Lal R, Smith P, Tian G, Tiessen H, Van Noordwijk M, Woomer PL (1997) Agricultural soils as a sink to mitigate CO_2 emissions. Soil Use Manag 13:230–244

Peng S, Huang J, Sheehy JE, Laza RC, Visperas RM, Zhong X, Centeno GS, Khush GS, Cassman KG (2004) Rice yields decline with higher night temperature from global warming. Proc Natl Acad Sci U S A 101:9971–9975

Pingali PL, Hossain M, Gerpacio RV (1997) Asian rice bowls: the returning crisis? CAB International and Manila (Philippines): International Rice Research Institute, Oxon, p 341

Porteaus F, Hill J, Ball AS, Pinter PJ, Kimball BA, Wall GW, Adamsen FJ, Hunsaker DJ, LaMorte RL, Leavitt SW, Thompson TL, Matthias AD, Brooks TJ, Morris CF (2009) Effect of Free Air Carbon dioxide Enrichment (FACE) on the chemical composition and nutritive value of wheat grain and straw. Anim Feed Sci Technol 149:322–332

Porter JR, Gawith M (1999) Temperatures and the growth and development of wheat: a review. Eur J Agron 10:23–36

Portis AR, Salvucci ME, Ogren WL (1986) Activation of ribulose bisphosphate carboxylase/oxygenase at physiological CO_2 and ribulose bisphosphate concentrations by rubisco activase. Plant Physiol 82:967–971

Prakash P, Sharma-Natu P, Ghildiyal MC (2004) Effect of different temperature on starch synthase activity in excised grains of wheat cultivars. Indian J Exp Biol 42:227–230

Prasad PVV, Boote KJ, Allen JLH, Sheehy JE, Thomas JMG (2006) Species, ecotype, and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. Field Crop Res 95:398–411

Prasad PVV, Pisipati SR, Ristic Z, Bukovnik U, Fritz AK (2008) Impact of nighttime temperature on physiology and growth of spring wheat. Crop Sci 48:2372–2380

Raleigh C, Urdal H (2007) Climate change, environmental degradation and armed conflict. Political Geogr 26:674–694

Randall PJ, Moss HJ (1990) Some effects of temperature regime during grain filling on wheat quality. Aust J Agric Res 41:603–617

Razzaque MA, Haque MM, Khaliq QA, Soliman ARM, Hamid A (2011) Effects of CO_2 and nitrogen levels on yield and yield attributes of rice cultivars. Bangladesh J Agric Res 36:213–221

Reynolds M, Tuberosa R (2008) Translational research impacting on crop productivity in drought-prone environments. Curr Opin Plant Biol 11:171–179

Reynolds M, Foulkes J, Furbank R, Griffiths S, King J, Murchie E, Parry M, Slafer G (2012) Achieving yield gains in wheat. Plant Cell Environ 35:1799–1823

Rijven AHGC (1986) Heat inactivation of starch synthase in wheat endosperm tissue. Plant Physiol 81:448–453

Rogers D (1996) Changes in disease vectors. In: Hulme M (ed) Climate change and southern Africa: an exploration of some potential impacts and implications in the SADC region. Climatic Research Unit, University of East Anglia/World Wildlife Fund International, Norwich/Gland

Rosenzweig C, Parry ML (1994) Potential impact of climate change on world food supply. Nature 367:133–138

Saini HS, Aspinall D (1982) Abnormal sporogenesis in wheat (*Triticum aesti.um* L.) induced by short period of high temperature. Ann Bot 49:835–846

Salvucci ME, Crafts Brandner SJ (2004) Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. Plant Physiol 120:179–186

Samra JS, Singh G (2005) Heat wave of March, 2004: impact on agriculture. Research Bulletin, Indian Council of Agricultural Research, New Delhi

Sasaki H, Hara T, Ito S, Uehara N, Kim HY, Lieffering M, Okada M, Kobayashi K (2007) Effect of free-air CO_2 enrichment on the storage of carbohydrate fixed at different stages in rice (*Oryza sativa* L.). Field Crop Res 100:24–31

Satake T, Yoshida S (1978) High temperature-induced sterility in indica rice at flowering. Jpn J Crop Sci 47:6–17

Semenov MA, Halford NG (2009) Identifying target traits and molecular mechanisms for wheat breeding under a changing climate. J Exp Bot 60:2791–2804

Seneweera SP, Conroy JP (1997) Growth, grain yield and quality of rice (*Oryza sativa* L.) in response to elevated CO_2 and phosphorus nutrition. Soil Sci Plant Nutr 43:1131–1136

Shah F, Huang J, Cui K, Nie L, Shah T, Chen C, Wang K (2011) Impact of high-temperature stress on rice plant and its traits related to tolerance. J Agric Sci 149:545–556

Sharkey TD (2005) Effect of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species and thermo tolerance provided by isoprene. Plant Cell Environ 28:269–277

Sheehy J, Elmido A, Centeno G, Pablico P (2005) Searching for new plants for climate change. J Agric Meteorol 60:463–468

Shi CH, Zhu J, Zhang RC, Chen GL (1997) Genetic and heterosis analysis for cooking quality traits of indica rice in different environments. Theor Appl Genet 9:294–300

Shi W, Muthurajan R, Rahman H, Selvam J, Peng S, Zou Y, Jagadish SVK (2013) Source-sink dynamics and proteomic programming under elevated night temperature and their impact on rice yield and grain quality. New Phytol 197:825–837

Shimono H, Kanno H, Sawano S (2010) Can the cropping schedule of rice be adapted to changing climate? A case study in cool areas of northern Japan. Field Crop Res 118:126–134

Sinha SK, Swaminathan MS (1991) Deforestation, climate change and sustainable nutrition security: a case study of India. Clim Chang 19:201–209

Sionit N, Strain BR, Flint EP (1987) Interaction of temperature and CO_2 enrichment on soybean: photosynthesis and seed yield. J Plant Sci 67:629–636

Stone PJ, Nicolas ME (1994) Wheat cultivars vary widely in their responses of grain yield and quality to short periods of post- anthesis heat stress. Aust J Plant Physiol 21:887–900

Stone PJ, Nicolas ME (1998) Comparison of sudden heat stress with gradual exposure to high temperature during grain-filling in two wheat varieties difference in heat tolerance. II. Fractional protein accumulation. Aust J Plant Physiol 25:1–11

Subbarao GV, Ito O, Wang HY, Nakahara K, Suenaga K, Rondon M, Rao IM, Lascano C, Ishitani M (2005) Root exudates of *Brachiaria humidicola* inhibit nitrification—characterization and quantification of this unique biological phenomenon. In: Li CJ, Zhang FS, Dobermann A, Lambers H, Li XL, Marschner P, Maene L, McGrath S, Oenema O, Peng SB, Rengel Z, Shen QR, Welch R, von Wiren N, Yan XL, Zhu YG (eds) Plant nutrition for food and security, human health and environment protection. Tsinghua University Press, Beijing, pp 444–445

Subbarao GV, Ban T, Kishii M, Ito O, Samejima H, Wang HY, Pearse SJ, Gopalakrishnan S, Nakahara K, Zakir Hossain AKM, Tsujimoto H, Berry WL (2007) Can biological nitrification inhibition (BNI) genes from perennial *Leymus racemosus* (Triticeae) combat nitrification in wheat farming? Plant Soil 299:55–64

Tanaka K, Kasai Z, Ogawa M (1995) Physiology of ripening. In: Matsuo T, Kumazawa K, Ishii KI, Hirata H (eds) Science of the rice plant, vol 2, Physiology. Food and Agriculture Policy Research Center, Tokyo, pp 07–118

Tao LX, Tan HJ, Wang X, Cao LY, Song J, Cheng SH (2008) Effects of high temperature stress on flowering and grain-setting characteristics of Guodao 6. Acta Agron Sin 34:609–674

Taub DR, Wang XZ (2008) Why are nitrogen concentrations in plant tissues lower under elevated CO_2? A critical examination of the hypotheses. J Integr Plant Biol 50:1365–1374

Taub D, Miller B, Allen H (2008) Effects of elevated CO_2 on the protein concentration of food crops: a meta-analysis. Glob Chang Biol 14:565–575

Tester RF, Morrison WR, Ellis RH, Pigott JR, Batts GR, Wheeler TR, Morison JIL, Hadley P, Ledward DA (1995) Effects of elevated growth temperature and carbon dioxide levels on some physiochemical properties of wheat starch. J Cereal Sci 22:63–71

Tewolde H, Fernandez CJ, Erickson CA (2006) Wheat cultivars adapted to post-heading high temperature stress. J Agron Crop Sci 192:111–120

Uprety DC, Bisht BS, Dwivedi N et al (2007) Comparison between open top chamber (OTC) and Free air CO_2 Enrichment (FACE) technologies to study the response of rice cultivars to elevated CO_2. Physiol Mol Biol Plants 13:259–266

Venkateswarlu B, Singh AK, Prasad YG, Ravindra Chary G, Srinivasarao C, Rao KV, Ramana DBV, Rao VUM (2011) District level contingency plans for weather aberrations in India. Central Research Institute for Dryland Agriculture, Natural Resource Management Division, Indian Council of Agricultural Research, Hyderabad

Verhulst N, Govaerts B, Nelissen V, Sayre K, Crossa J, Raes D, Deckers J (2010) The effect of tillage, crop rotation and residue management on maize and wheat growth and development evaluated with an optical sensor. Field Crop Res 120:58–67

Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61:199–223

Wang HY, Subbarao GV, Ito O, Nakahara K (2005) Regulation of nitrification inhibitory activity in the root exudates of *Brachiaria humidicola*. In: Li CJ, Zhang FS, Dobermann A, Lambers H, Li XL, Marschner P, Maene L, McGrath S, Oenema O, Peng SB, Rengel Z, Shen QR, Welch R, von Wiren N, Yan XL, Zhu YG (eds) Plant nutrition for food and security, human health and environment protection. Tsinghua University Press, Beijing, pp 478–479

Wardlaw IF, Wrigley CW (1994) Heat tolerance in temperate cereals: an overview. Aust J Plant Physiol 21:695–703

Wardlaw IF, Blumenthal C, Larroque O, Wrigley CW (2002) Contrasting effects of chronic heat stress and heat shock on kernel weight and flour quality in wheat. Funct Plant Biol 29:25–34

Warrag MOA, Hall AE (1984) Reproductive responses of cowpea (*Vigna unguiculata* Walp) to heat-stress.1. Responses to soil and day air temperatures. Field Crop Res 8:3–16

Wassmann R, Jagadish SVK, Heuer S, Ismail A, Redona E, Serraj R, Singh RK, Howell G, Pathak H, Sumfleth K (2009) Climate Change Affecting Rice Production: The Physiological and Agronomic Basis for Possible Adaptation Strategies. In: Sparks DL (ed) Advances in Agronomy, vol 101. Academic Press, Burlington, pp 59–122

Wheeler TR, Batts GR, Ellis RH, Hadley P, Morison JIL (1996) Growth and yield of winter wheat (*Triticum aestivum*) crops in response to CO_2 and temperature. J Agric Sci Camb 127:37–48

White JW, Sanchez PA, Gore MA, Bronson KF, Coffelt TA, Conley MM, Feldmann KA, French AN, Heun JT, Hunsaker DJ, Jenks MA, Kimball BA, Roth RL, Strand RJ, Thorp KR, Wall GW (2012) Field-based phenomics for plant genetics research. Field Crop Res 133:101–112

Wieser H, Manderscheid R, Erbs M, Weigel H-J (2008) Effect of elevated atmospheric CO_2 concentrations on the quantitative protein composition of wheat grain. J Agric Food Chem 56:6531–6535

Wrigley C (2006) Global warming and wheat quality. Cereal Foods World 51:34–36

Xu Z, Yanzhuo L, Qingni K, Nongron H, Yi C, Daijan P (1998) Growth, grain yield and kernel quality of high-yield rice variety Te-San-Ai2 growing in a simulated CO_2-enriched habitat. Chin J Appl Environ Biol 4:238–242

Yamakawa H, Hirose T, Kuroda M, Yamaguchi T (2007) Comprehensive expression profiling of rice grain filling-related genes under high temperature using DNA microarray. Plant Physiol 144:258–277

Yang J, Chang E, Tang C, Zhang H, Wang Z (2007) Relationships of ethylene evolution rate and 1-aminocylopropane-1-carboxylic acid concentration in grains during filling period with appearance quality of rice. Rice Sci 14:33–41

Yin X, Kroff MJ, Goudriann J (1996) Differential effects of day and night temperature on development to flowering in rice. Ann Bot 77:203–213

Yoshida S, Hara T (1977) Effects of air temperature and light on green filling of an indica and a japonica rice (*Oryza sativa* L.) under controlled environmental conditions. Soil Sci Plant Nutr 23:93–107

Yoshida S, Satake T, Mackill D (1981) High temperature stress. IRRI Res Pap 67:1–15

Zahedi M, Jenner CF (2003) Analysis of effects in wheat of high temperature on grain filling attributes estimated from mathematical models of grain filling. J Agric Sci 141:203–212

Zhong LJ, Cheng FM, Wen X, Sun ZX, Zhang GP (2005) The deterioration of eating and cooking quality caused by high temperature during grain filling in early-season indica rice cultivars. J Agron Crop Sci 191:218–225

Zhu C, Xiao Y, Wang C, Jiang L, Zhai H, Wan J (2005) Mapping QTL for heat-tolerance at grain filling stage in rice. Rice Sci 12:33–38

Zhu D, Cheng S, Zhang Y, Lin X, Chen H (2010) Analysis of status and constraints of rice production in the world. Sci Agric Sin 43:474–479

Zhu CW, Cheng WG, Sakai H, Oikawa S, Laza Rebecca C, Usui Y, Hasegawa T (2013) Effects of elevated [CO_2] on stem and root lodging among rice cultivars. Chin Sci Bull 58:1787–1794

Ziska LH (2003) Evaluation of the growth response of six invasive species to past, present and future atmospheric carbon dioxide. J Exp Bot 54:395–404

Ziska LH, Weerakoon W, Namuco OS, Pamplona R (1996) The influence of nitrogen on the elevated CO_2 response in field-grown rice. Funct Plant Biol 23:45–52

Ziska LH, Namuco O, Moya T, Quilang J (1997) Growth and yield response of field grown tropical rice to increasing carbon dioxide and air temperature. Agron J 89:45–53

31 Alien Crop Resources and Underutilized Species for Food and Nutritional Security of India

N. Sunil and S.R. Pandravada

Abstract

The diversity of crops cultivated across the world ensured food and nutritional security to mankind over the years and in India, the introduced alien crops are still significantly contributing towards that. However, the diversity of the food basket has declined drastically as a consequence of insidious socio-economic compulsions and developments. Even though, variation among and within a crop is also an important factor for nutritional security, in order to overcome threats to biodiversity, viz. climate change, change in dietary habits, globalization, etc., utilization of diverse species ensures food security. Hence, there is a need to relook at the alien crops and underutilized species for sustaining and enhancing food and nutritional security. The alien crop species to be introduced in India have been prioritized based on 'Ecocrop' database and also based on the authors' perceptions, even though the exchange of genetic resources has been affected post CBD. The plant genetic resources for food and agriculture (PGRFA) represent the basis for the establishment of a multilateral system of access and benefit-sharing of important food crops. The introduction of alien crop species should be guided by research efforts which may include crops suitable for multi-cropping systems/designated areas, climate resilience etc. for safe and sustained utilization.

N. Sunil
Winter Nursery Centre, Indian Institute of Maize Research, Rajendranagar, Hyderabad 500 030, Telangana, India

S.R. Pandravada (✉)
National Bureau of Plant Genetic Resources, Regional Station, Rajendranagar, Hyderabad 500 030, Telangana, India
e-mail: pandravadasr@yahoo.com

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_31,

Keywords

Alien crops • Centres of diversity • Climate change • Nutritional security • Underutilized crops

31.1 Introduction

Inter and intraspecific diversity naturally occurring in different agri-horticultural crops is contributing immensely to the food and nutritional security especially in the subsistence farming across the globe. Genetic variation has played an immense role in the evolution and domestication of crop plants. Many plant species originated thousands of years ago in places known as centres of origin basically located between the tropic of Cancer and Capricorn. There is a rich genetic wealth in different agri-horticultural crop species in different centres of diversity. The present landrace diversity is a result of continued selection pressure brought in by the ethnic groups based on their wisdom. Plants cultivated in these centres were later introduced into other regions where they became crops of worldwide importance.

Germplasm diversity in cereals, millets, small millets, pulses, oilseeds, fruits, nuts, vegetables, tubers and medicinal plants encompasses the whole spectrum of diversity under the agri-horticultural genetic resources. It contributes greatly both to food security and to integrate and diversify the dietary needs of mankind.

31.2 Centres of Origin/Diversity of Crops

The origin of crop plants and utilization of wild relatives and related species for new genes especially dominant genes for sources of disease resistance are basic to plant breeding and crop improvement. Knowledge of the origin of crop plants is vitally important to avoid genetic erosion; loss of germplasm; mainly the ecotypes and landraces; destruction of habitats such as rainforests; increased urbanization; and other biotic pressures.

About 250–300 major and minor species of agri-horticultural crops seem to be in cultivation throughout the world. Under these, some are of worldwide importance and some crops are of only local importance. Early evidence of cultivation of crops in the New World dates back to 7,000–5,000 BC. Evidence from a variety of sources suggests that the root crops are the earliest to be domesticated in the humid tropics preceding the seed crops. Many of the present species are so altered as a result of selection and breeding that their wild ancestors can no longer be identified with certainty. Only two vegetable crops (bottle gourd and sweet potato) are known to have been grown in both the Old and New Worlds before 1492. Their presence in both the western and eastern hemispheres suggests possible pre-Columbian agricultural contacts. Based on the evidences, it can be deduced that there was no movement of economic plants by man between the Old and New Worlds in the pre-Colombian times. After the discovery of the New World by Columbus, there had been a rapid exchange of crops among the continents.

The study of the origin and spread of agriculture provides clues to geographical location and activities concerning plant domestication. These centres were primarily located in areas where maximum diversity in crops was found, and only at a later stage, the practice of agriculture had spread to different regions, as a result of which crop diversification took place. The presence of wild relatives was considered an essentiality in designating a place as ‘a centre of origin’. While almost one third of the world’s species originated in Southeast Asia and most of the main fruit and vegetable crops did come from the east and west Asiatic and Mediterranean centres of origin, the roots and tubers and tropical fruit trees did originate from the Central American and Andean centres.

The designated centres of origin and their boundaries were revised and rerevised subsequently by different authors, and more theories and concepts were put forth as more detailed studies were taken up on a number of crop species. The viewpoints based on evidences from different sources and applying logic in pioneering efforts were put forth by different researchers. Vavilov (1951) based on distribution and magnitude of genetic diversity, concentration of nearest wild relatives and supporting/linking evidences from archaeology, linguistics and history postulated eight centres where the crops had originated leading to the development of agriculture independently and named them as 'centres of origin' of crop plants. These centres were also characterized by the accumulation of dominant genes in the core and the recessive genes in the periphery. Harlan (1971) recognized only three main 'centres', each more or less connected but with large diffused non-centres. Harlan also recognized smaller areas/pockets of varietal and/or racial diversity within a Vavilovian centre, and he termed these as 'microcentres' as in Turkey and Africa (Harlan 1975), containing varietal diversity in several crops either in plains/mountains. Zeven and Zhukovsky (1975) by adding Australia, Africa and Siberia and revising the boundaries of Vavilov's eight centres proposed 12 mega gene centres of crop-plant diversity and dealt very elaborately on the range and extent of distribution of genetic/varietal/specific diversity. These 12 regions had wider coverage and more acceptability. Hawkes (1983) opined that agriculture had begun several times, more or less simultaneously in different regions of the world. His concept envisaged centres of agricultural origin from where farming spread to one or more regions which he referred as 'nuclear centres/regions of diversity'. He linked nuclear centres with the archaeological evidences to provide strong proofs of agricultural origins. There are several regions where crops actually did not originate. In these regions crops perhaps had spread from the nuclear centres in the past and due to spatial isolation and intensive human selection led to accumulation of genetic diversity. Hawkes (1983) further identified small 'minor' centres for several crops.

31.3 Indian Subcontinent as a Centre of Origin/Diversity

India is one of the 12 centres/regions of diversity of crop plants in the world (Zeven and de Wet 1982). The antiquity of agriculture and the ethnic diversity in the subcontinent have played a major role in diversification of crop resources in this region. Rich genetic diversity occurs in several crop plants and their wild progenitors. The Indian gene centre possesses about 166 species of agri-horticultural crop plants (Zeven and de Wet 1982) and 320 species of wild relatives distributed in eight phytogeographical/agro-ecological zones (Arora and Nayar 1984). The Indian agriculture has been enriched by a continuous stream of introductions of new crops and their cultivars by man since the ancient times. The Portuguese, the Spaniards and the British brought several new crops in to this country. Prominent among these were maize, potato, tomato, chilli, coffee, cocoa, cashew nut, etc. The current matrix of diversity consists of the gene pool of indigenous crop plants, their wild and/or weedy relatives and the well-adapted introduced crops from practically all over the globe. In the introduced crops also depending on the time, period, degree of variability in the introduced material and the areas of introduction, enormous diversity got built-up within the Indian subcontinent itself. The Indian gene centre is an important region of crop diversity that had strong linkages and contiguity with the Indo-Chinese-Indonesian, Chinese-Japanese and the Central Asian regions. Further, the influx of germplasm in the distant past from the Mediterranean, African and tropical American regions facilitated to build up enormous locally adapted variability. The different agro-ecological/phytogeographical regions of India hold rich diversity in both the cultivated and the wild crop gene pools (Arora 1991; Pandravada et al. 2004, 2008).

31.4 Origin and Distribution of Variability

The potential of landrace diversity and wild relatives in crop improvement programmes cannot be underestimated, and there is a dire need for conservation of crop genetic resources as their value in the future will be immense and indispensable than what can be visualized currently considering the diversified unknown technological and human requirements in future. Presently, the diversity in the Indian subcontinent is composed of rich genetic wealth of native as well as introduced species (Table 31.1), making it a primary as well as a secondary centre of diversity for several crops, and also has rich regional diversity for several South/Southeast Asian crops as described below:

1. Primary centre of diversity for crops: Rice, black gram, moth bean, pigeon pea, cucurbits (smooth gourd, ridged gourd and pointed gourd), tree cotton, *C. capsularis* jute, jackfruit, banana, mango, *Syzygium cumini*/jamun, large cardamom, black pepper, several small millets and medicinal plants like *Rauvolfia serpentina* and *Saussurea costus*.
2. Secondary centre of diversity for African crops: Finger millet, pearl millet, sorghum, cowpea, cluster bean (trans-domesticate), okra, sesame, niger and safflower and tropical American types such as maize, tomato, musk melon/*Cucumis* species, pumpkin/*Cucurbita* species, chayote/chou-chou, chilli and *Amaranthus.*
3. Regional (Asiatic) diversity for crops: Maize, barley, amaranth, buckwheat, proso millet, Italian/foxtail millet, mung/green gram, chickpea, sword bean, tomato, cucumber, bitter gourd, bottle gourd, snake gourd, taro, yams, citrus, small cardamom, ginger, turmeric and some members of tribe Brassicaceae.

India is a primary centre of diversity for crops such as cucurbits (smooth gourd, ridge gourd and pointed gourd), fruits (jackfruit, banana, mango, *Syzygium cumini*/jamun), spices (large cardamom, black pepper) and several medicinal plants (*Rauvolfia serpentina* and *Saussurea lappa*). It is also a secondary centre of diversity for African crops (cluster bean and okra) and tropical American crops (tomato, pumpkin/*Cucurbita* spp., chayote, chilli and *Amaranthus spp.*) and regional (Asiatic) centre of diversity for crops amaranth, buckwheat, cucumber, bitter gourd, bottle gourd, snake gourd and Brassicaceae. Geographical contiguity with the Far East and/or the Indo-Malayan (South/Southeast Asian region) belt is largely responsible for accumulation of more regional diversity in sword bean, tomato, citrus, small cardamom, ginger, turmeric and tuber crops, particularly taros and yams.

The dietary diversity depends not only on diversity of crops but also on diversity within crops. There is an increasing piece of evidence of wide variation in nutrient contents within a

Table 31.1 Some of the main crop groups and cultivated crops of importance in India which are native and/or alien introductions

Crop group	Cultivated crops
Cereals	Maize, paddy, wheat
Millets	Pearl millet, sorghum
Small millets	Barnyard millet, finger millet, italian millet, kodo millet, little millet, proso millet
Oilseeds	Castor, coconut, ground nut, linseed, niger, sesame, safflower
Pulses	Black gram, chickpea, chickling pea, cowpea, green gram, horse gram, pigeon pea, rice bean, broad bean
Vegetables	Bitter gourd, bottle gourd, brinjal, cucumber, dolichos bean, field bean, ivy gourd, okra, pea, pointed gourd, pumpkin, radish, snake gourd, spine gourd, ridged gourd, roselle, smooth gourd, sponge gourd, tomato, yardlong bean
Leafy vegetables	Amaranth, Indian spinach, spinach
Spices	Chilli, coriander, fenugreek, garlic, mustard, onion, turmeric, ginger
Fibres	Cotton (perennial), flax, kapok, coconut, jute, bimli jute
Tubers	*Alocasia indica*, *Alocasia* sp., greater yam, yam, elephant foot yam, tapioca
Fruits	Grapefruit, watermelon, mango, apple, kiwi, kinnow, sapota, custard apple, pineapple
Fodder	Sunn hemp, pillipesara bean, clover

species, but data is lacking on nutrient composition and dietary intake for many underutilized species as well as for cultivars within a species. Such information is needed both to enhance use of more nutritious cultivars in diets and to make them available for use in breeding programmes aimed at increasing the nutrient content of more commonly used varieties of the same species, eliminating the need for transgenic modifications. The utilization of plant genetic resources to enhance the chemical composition of a few staple foods through biotechnology or conventional breeding has led to the development of varieties with enhanced levels of micronutrients, such as enhanced beta-carotene sweet potatoes, quality-protein maize (QPM) and beta-carotene-enriched rice (Johns and Eyzaguirre 2007). Brazil has a good case of harnessing biodiversity to improve nutrition successfully through the nutrient data. In recent years, food producers have become more cognizant of the value of nutrients and bioactive compounds in their produce. For example, lycopene-rich guava varieties, Acerola and Pitanga, which used to be only garden fruits, are now commercially produced and processed. The leafy vegetable rucola (*Eruca sativa*) is also now widely consumed. Foods gathered from the wild, including vegetables and fruits, have been the mainstay of human diets for centuries. Many are a rich source of micronutrients and could make an important contribution to combating micronutrient malnutrition as well as providing food security. Unfortunately, wild foods have been neglected by researchers and policymakers. Their chemical, nutritional and toxicological properties, the bioavailability of micronutrients and their modification by various processing techniques still need to be properly documented. Such information would be of fundamental importance in addressing dietary deficiencies in impoverished rural communities (Flyman and Afolayan 2006).

Information about the centres of origin for different agri-horticultural crops of recent introductions which have become indispensable in the dietary/food habits of the people and have commercial importance is given (Tables 31.2a and 31.2b).

31.5 Diversity and Current Status of the Major Alien Crops Grown in India

The current status of diversity, cultivation pattern and impact of major introduced crops is as follows:

31.5.1 Maize

Maize was introduced in India by the Portuguese during the seventeenth century. It is the third largest produced and consumed food crop after rice and wheat in India. Sikkim is the bedrock of accumulated maize diversity in India with unique landraces that are still conserved and utilized by the farmers for diverse purposes (Prasanna and Sharma 2005; Prasanna 2010). Dhawan (1964) christened them as 'Sikkim Primitives', whose New World progenitors seem to have become extinct. The most important attributes of the 'Sikkim Primitive' maize are tallness with drooping tassels, prolificacy (five to nine ears/plant), lack of apical dominance, uniformity in ear size and pop-type kernels (Dhawan 1964; Singh 1977). There are about 376 released/notified varieties of maize in India (http://202.141.12.149/norv/Reports/Statisyical Result.aspx). The National Gene Bank (NGB) at NBPGR, New Delhi, is currently conserving 8,448 accessions of which 770 accessions are of exotic origin (www.nbpgr.ernet.in).

Maize is cultivated throughout the year in all states of the country for various purposes including grain, fodder, green cobs, sweet corn, baby corn and pop corn (http://www.iimr.res.in). Maize production has seen a regional shift from north to south in the recent past. Bihar, Uttar Pradesh and Madhya Pradesh which were the major maize producing states during the 1990s have been overtaken in the past two decades by the southern states, especially Andhra Pradesh and Karnataka, as the major maize-producing states (Gulati and Dixon 2008). India is the sixth largest producer of maize in the world and contributed about 2 % to the global maize production of 855.7 million tonnes during

Table 31.2a Alien crops which were introduced in India by the Portuguese/British and cultivated on large scale for food security in the country

Crop	Scientific name	Country of origin	Nutritive value*
Maize	*Zea mays* L.	Mexico	Pr-11.1 %, F-3.6 %, C-66.2 %, E-342 Kcal, M-1.5 %, CF-2.7 %, Ca-10 mg, P-348 mg, Fe-2.3 mg
Soybean	*Glycine max* (L.) Merr.	East Asia	Pr-43.2 %, F-19.5 %, C-20.9 %, E-432 Kcal, M-4.6 %, CF-3.7 %, Ca-240 mg, P-690 mg, Fe-10.4 mg
Sunflower	*Helianthus annuus* L.	USA	Pr-19.8 %, F-52.1 %, C-17.9 %, E-620 Kcal, M-3.7 %, CF-1.0 %, Ca-280 mg, P-670 mg, Fe-5.0 mg
Oil palm	*Elaeis guineensis* Jacq.	West Africa	–
Tomato	*Solanum lycopersicum* L.	South and Central America	Pr-1.9 %, F-0.1 %, C-3.6 %, E-23 Kcal, M-0.6 %, CF-0.7 %, Ca-20 mg, P-36 mg, Fe-1.8 mg
Onion	*Allium cepa* L. var. *cepa*	Mediterranean	Pr-1.2 %, F-0.1 %, C-11.1 %, E-50 Kcal, M-0.4 %, CF-0.6 %, Ca-46.9 mg, P-50 mg, Fe-0.6 mg
Chilli	*Capsicum annuum* L.	Semi-tropical Mexico	Pr-2.9 %, F-0.6 %, C-3.0 %, E-50 Kcal, M-1.0 %, CF-6.8 %, Ca-30 mg, P-80 mg, Fe-4.4 mg
Mango	*Mangifera indica* L.	South-east Asia	Pr-6.8 %, F-0.1 %, C-11.1 %, E-50 Kcal, M-0.4 %, CF-0.6 %, Ca-46.9 mg, P-50 mg, Fe-0.6 mg
Banana	*Musa* sp.	South-east Asia/Pacific	Pr-1.2 %, F-0.3 %, C-27.2 %, E-116 Kcal, M-0.8 %, CF-0.4 %, Ca-17 mg, P-36 mg, Fe-0.36 mg
Coconut	*Cocos nucifera* L.	North-western South America	Pr-6.8 %, F-62.3 %, C-18.4 %, E-662 Kcal, M-1.6 %, CF-6.6 %, Ca-400 mg, P-210 mg, Fe-7.8 mg

*Gopalan et al. (1989)

2012–2013. The total production of Maize has increased from 15.1 mt during 2006–2007 to 21.76 mt during 2011–2012. India has become a net maize exporter since 2007–08 and exported 4.3 mt of maize during 2012–13 (Kumar et al. 2013)

31.5.2 Soybean

It has its origin in China and entered the northern parts of India from central China through silk-route (Hymowitz and Kaizuma 1981). It is the third most important oilseed crop in India after ground nut and rapeseed/mustard. Cultivated varieties of soybean along with wild progenitor *Glycine soja* form the primary gene pool (GP-1). There is no secondary gene pool for soybean and the tertiary gene pool (GP-3) comprises of 16 wild perennial species belonging to the subgenus *Glycine* (Tiwari et al. 2004). About 116 varieties of soybean were released/notified for cultivation in India (http://202.141.12.149/norv/Reports/Statisyical Result.aspx). The NGB has 3,308 germplasm accessions and indigenous accessions (http://www.nbpgr.ernet.in). Madhya Pradesh, Rajasthan, Andhra Pradesh and Chhattisgarh are the major soybean-producing states in India. India ranks fifth in the production of soybean with a total production of 11.5 mt (http://faostat3.fao.org).

31.5.3 Sunflower

It is one of the principal oilseed crops of India. The cultivated sunflower is believed to have originated from wild *Helianthus annuus* in the

Table 31.2b Other alien crops that are grown for food security in the country

Crop	Scientific name	Place of origin	Nutritive value*
Pumpkin	*Cucurbita moschata* Duchesne	New World/Mexico	Pr-1.4 %, F-0.1 %, C-4.6 %, E-25 Kcal, M-0.6 %, CF-0.7 %, Ca-10 mg, P-30 mg, Fe-0.44 mg
Squash	*Cucurbita pepo* L.	New World/Mexico	–
Winged bean	*Psophocarpus tetragonolobus* (L.) DC.	Tropical Asia	–
Taro	*Colocasia esculenta* (L.) Schott	Indo-Malayan	Pr-0.3 %, F-0.3 %, C-3.6 %, E-18 Kcal, M-1.2 %, CF-0.6 %, Ca-60 mg, P-20 mg, Fe-0.5 mg
Tannia	*Xanthosoma sagittifolium* (L.) Schott	Northern part of South America	–
Greater yam	*Dioscorea alata* L.		Pr-1.2 %, F-0.1 %, C-18.4 % E-79 Kcal, M-0.8 %, CF-0.8 %, Ca-50 mg, P-34 mg, Fe-0.6 mg
Ginger	*Zingiber officinale* Roscoe	South-east Asia	Pr-2.3 %, F-0.9 %, C-12.3 %, E-67 Kcal, M-1.2 %, CF-2.4 %, Ca-20 mg, P-60 mg, Fe-3.5 mg
Tamarind	*Tamarindus indica* L.		Pr-3.1 %, F-0.1 %, C-67.4 %, E-283 Kcal, M-2.9 %, CF-5.6 %, Ca-170 mg, P-110 mg, Fe-17 mg
Garlic	*Allium sativum* L.	Central Asia	Pr-6.3 %, F-0.1 %, C-29.8 %, E-145 Kcal, M-1.0 %, CF-0.8 %, Ca-30 mg, P-310 mg, Fe-1.2 mg
Sapota	*Achras sapota* L.	South America (Mexico)	Pr-0.7 %, F-1.1 %, C-21.4 %, E-98 Kcal, M-0.5 %, CF-2.6 %, Ca-28 mg, P-27 mg, Fe-1.25 mg
Citrus	*Citrus spp.*		Pr-0.7 %, F-0.3 %, C-7.3 %, E-35 Kcal, M-0.5 %, CF-0.7 %, Ca-30 mg, P-20 mg, Fe-0.7 mg
Custard apple	*Annona squamosa* L.	South America	Pr-1.7 % F-0.6 %, C-25.2 %, E-101 Kcal, M-0.4 %, CF-2.4 %, Ca-3 mg, Fe-4 mg
Pineapple	*Ananas comosus* (L.) Merr. var. *comosus*	South America	Pr-0.4 %, F-0.1 %, C-10.8 %, E-46 Kcal, M-0.4 %, CF-0.5 %, Ca-20 mg, P-9 mg, Fe-2.42 mg
Apple	*Malus domestica* Borkh.	West Asia	Pr-0.2 %, F-0.5 %, C-13.4 %, E-59 Kcal, M-0.3 %, CF-1.0 %, Ca-10 mg, P-14 mg, Fe-0.6 mg
Cashew nut	*Anacardium occidentale* L.	Tropical Americas	Pr-21.2 %, F-46.9 %, C-22.3 %, E-596 Kcal, M-2.4 %, CF-1.3 %, Ca-50 mg, P-450 mg, Fe-5.81 mg
Betel palm	*Areca catechu* L.	Indo-Malayan	–

*Gopalan et al. (1989)
Pr protein, *F* fat, *C* carbohydrate, *E* energy, *M* minerals, *CF* crude fibres, *Ca* calcium, *P* phosphorus, *Fe* iron

South-western USA. There are over 50 species reported in the genus *Helianthus* comprising of both annual and perennials, diploids, tetraploids and hexaploids of which about 32 species are introduced and being maintained in India (Seetharam et al. 2004). A total of 75 varieties including hybrids are released by various state agricultural universities and central institutions (http://202.141.12.149/norv/Reports/StatisyicalResult.aspx). In the National Gene Bank (NGB) at NBPGR, New Delhi, 1,063 accessions are conserved (http://www.Nbpgr.ernet.in:8080/PGRPortal). Karnataka, Andhra Pradesh and Maharashtra are the major sunflower producing

states in India. It was cultivated in 8.7 lakh hectares during 2012–13 with a productivity of 667 kg/ha (http://www.agricoop.nic.in).

31.5.4 Oil Palm

Oil palm is recognized as one of the highest edible oil-yielding crops giving 5–8 tonnes of oil/ha and is seen as the single most important contributor to meet the edible oil needs of the country. At present, oil palm is grown in India to an extent of 2.0 lakh ha. Indian crude palm oil on an average contains total saturated fatty acids up to 45.5 % and unsaturated fatty acids up to 55.5 % of which polyunsaturated fatty acid content was up to 11 %. The important biochemical constituents present in palm oil are carotenoids, vitamin E, squalene, ubiquinones and sterols. All these are marked as either nutritional supplements or drugs for therapeutic use. Due to the presence of antioxidants in many of them, the oil is getting popular as an anti-ageing and anti-cancerous food item (http://www.oilseeds.dacnet.in). Andhra Pradesh (86 %), Kerala (10 %) and Karnataka (2 %) are the major oil palm-producing states in India. Considering the potential of the crop, it is being popularized in new regions of the country including the north-eastern states of India (http://www.dopr.gov.in).

31.5.5 Tomato

Tomato is among the principal vegetable crops grown in the country accounting for 12 % of the area vegetable crops grown in the country (http://agricoop.nic.in). A total of 136 varieties of tomato were released in India for various agro-ecological zones for different culinary purposes. The NGB is conserving 1,734 accessions of tomato of which 467 are exotic (http://www.nbpgr.ernet.in:8080/PGRPortal). It is grown in almost all the states of India year-round. Andhra Pradesh (28.9 %), Karnataka (10.7 %) and Madhya Pradesh (8.3 %) account for nearly 40 % of the tomato production in India. India ranks second in the world with 17.5 mt production (http://faostat3.fao.org/).

31.5.6 Onion

A large genus of about 500 species of herbs occurring mostly in the northern hemisphere with the greatest number reported from former USSR/CIS countries. Onion is not known to occur in wild state. It is believed to have originated in an area encompassing the present Iran, West Pakistan and the adjoining mountainous countries to the north (Purseglove 1972). Onions are under cultivation since the ancient times in the Middle East. Onion was introduced into the New World shortly after its discovery and was under cultivation as early as 1629 and now spread to most parts of the world. Onion is the third most important vegetable crop grown in the country accounting for 11 % of the total vegetables produced. Good diversity exists in India in onion germplasm (Singh et al. 2013; Kamala et al. 2011). There are 69 varieties of onion catering to different climatic zones and different uses (http://www.nbpgr.ernet.in:8080/PGRPortal). Maharashtra, Karnataka, Madhya Pradesh and Gujarat are the major states cultivating onion in the country. India with a production of 16.3 mt is the second largest producer of dry onions in the world (http://faostat3.fao.org).

31.5.7 Chillies

Chilli is the most important spice crop grown all over the world except in the colder parts. The genus *Capsicum* contains 25 wild and five domesticated species, viz. *C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L. and *C. pubescens* Ruiz and Pavon (Bosland and Votava 2000). The primary centre of origin for domesticated chilli (*Capsicum annuum* L.) was semi-tropical region of Mexico (Whitmore and Turner 2002). Zeven and Zhukovsky (1975) and Zeven and de Wet (1982) considered the Central American and Mexican regions as the pockets of diversity of pepper/chilli where the greatest

variability exists in this crop. It was introduced into the Indian subcontinent towards the end of the fifteenth century by the Portuguese (Heiser 1976). Chillies with long history of cultivation, natural selection pressures and adaptability to different environments led to the origin of a great diversity of forms or cultivars differing in their growth habit, size, colour, shape, flavour and pungency and India is considered as the secondary centre of diversity (Mehra and Arora 1982).

In chilli, locally adapted landrace variability occurs throughout the country especially in the eastern and south-eastern uplands, southern peninsula, northern plains, central highlands, Western Ghats and northeast and the Himalayan regions. Good variability occurs for plant type, fruit colour, bearing, shape and size and pungency. Indigenous endemic non-pungent/sweet paprika landraces, the fruits of which are bright red and having more colouring principle, occur in the country. The landraces *Warangal* (tomato chilli) of Andhra Pradesh, *Byadgi* in the Dharwad area of Karnataka and *Degchi* landrace of Kashmir are well known. *C. frutescens* (bird pepper) mainly occurs in the hilly terrain often under semi-domestication by the tribal communities. *C. baccatum* is generally found in the northern hills under cultivation along with *C. annuum. C. chinense* is mainly restricted to Kerala and Western Ghats areas under cultivation either for fruits or for ornamental purposes. Many varieties of chilli have been recorded in India, varying in shape, colour, size and pungency. As such there is a large scope for selection and breeding from *Capsicum* genetic resources for developing genotypes suitable to hot wet tropics and different climates in India (Murthi et al. 2008). Chilli is under wide cultivation with over 78 released varieties in India (http://www.nbpgr.ernet.in:8080/PGRPortal). The most important chilli-growing states in India are Andhra Pradesh (49 %), Karnataka (15 %), Maharashtra (6 %) and Tamil Nadu (3 %) which account for nearly 75 % of the total area (Jagtap et al. 2012). Over 5,000 accessions of chilli germplasm are conserved in the NGB, India (http://www.nbpgr.enrnet.in). With a production of 1.3 mt in 2013, India is the largest producer of dry chilli and peppers (http://faostat3.fao.org).

31.5.8 Other Alien Crops

31.5.8.1 Cucurbits

Many species of Cucurbitaceae were domesticated in prehistoric times in the New and Old Worlds. Among the New World species, pumpkin (*Cucurbita moschata* (Duch. ex Lam.) Duch. ex Poir.) and squash (*Cucurbita pepo* L.) are very ancient which have been recorded in archaeological findings in Mexico dating back to 7000 BC. A number of cucurbits are grown in India, which all put together account for 5.6 % of the total vegetable production in India (Rai et al. 2008).

31.5.8.2 Winged Bean

Under the genus Psophocarpus, five species distributed across Africa, Asia and Madagascar were reported. Tropical Asia might be its probable origin where it is primarily domesticated. Burkill (1935) considers that, it originated on the African side of the Indian Ocean, possibly in Madagascar or Mauritius. Winged bean has excellent nutritional qualities particularly being very rich in protein (Rao and Dora 2002). One hundred sixty four accessions of winged bean are conserved in the NGB of India (http://www.nbpgr.ernet.in). It is grown in the north-eastern states in Assam, Tripura and Meghalaya and in West Bengal, Orissa and to a lesser extent in the southern states.

31.5.8.3 Taro

About seven species are reported which are native to the Indo-Malayan and the Pacific region. *C. esculenta* occurs wild in Southeast Asia (Purseglove 1972). It was spread by introduction to different places which include China, Japan, the Mediterranean region, Africa, Polynesia, Hawaii and New Zealand. The corms and leaves are major sources of carbohydrates, proteins, minerals and vitamins. Leaf blight, acridity of leaves and low yield are some of the production constraints in taro. Lack of diversity is another major constraint in crop improvement (Jain 2009). Uttar Pradesh, Madhya Pradesh, Andhra Pradesh, Odisha and Chhattisgarh are the major taro producing states in India (Srinivas et al. 2012). Nine varieties of taro are released for

cultivation in India (http://www.nbpgr.ernet.in:8080/PGRPortal).

31.5.8.4 Tannia/Cocoyam

About 40 species are reported which are native to tropical America. *Xanthosoma* was domesticated in the New World and was under cultivation in tropical America and the West Indies in the pre-Colombian times. It is a neglected crop and its cultivation is hampered by root rot disease. Apart from the cormels, leaves are also used as a vegetable (Jain 2009).

31.5.8.5 Greater Yam

The genus Dioscorea contains about 600 spp., which occur mainly in the tropics and subtropics throughout the world. A total of 11 species are grown for their stem tubers which were domesticated independently in four centres. Out of the Old World origin species, *D. alata* is very important which is polymorphic and not known to occur in a wild state. The probable centre of origin is in the South-east Asia. The crop must have been developed from *D. hamiltonii* or *D. persimilis* which grow wild in the Assam-Burma region (Alexander and Coursey 1969). New Guinea and Polynesia region is the secondary centre of diversity for this crop which spread to this area probably before 100 BC. In the first millennium AD, it was introduced into Madagascar. During the sixteenth century, the Portuguese and Spanish traders carried this to West Africa and the New World and is now pantropical in distribution. Eight varieties of greater yam were released and notified in India (http://www.nbpgr.ernet.in:8080/PGRPortal).

31.5.8.6 Citrus

The cultivated species of citrus are believed to have originated in South-east Asia probably in eastern India, Burma and southern China (Tanaka 1977). *Citrus* related genera are found in Asia, Australia and Africa. Recent surveys show that considerable genetic diversity exists in the Yunnan province to consider that area as one of the centres of origin and diversity (Gmitter and Hu 1990). Andhra Pradesh, Punjab, Maharashtra and Madhya Pradesh are the top citrus producing states in India (http://agriexchange.apeda.gov.in). Thirteen varieties of citrus have been released and notified in India (http://www.nbpgr.ernet.in:8080/PGRPortal).

31.5.8.7 Sapota

Significant local diversity exists especially in fruit size (small/medium/big) and shape (round/oval), fibrousness, aroma and taste. The popular endemic landraces include *Bengaluru*, *Dwarapudi*, *Jonnavalasa*, *Kirtabarati* and *Vavila Valasa* in South India and *Chhatri* and *Kalipatti* in western India. In India, sapota is mainly grown in Karnataka, Gujarat, Maharashtra, Tamil Nadu, West Bengal and Andhra Pradesh (Ramachandra 2006).

31.5.8.8 Custard Apple

Even though of a South American origin and introduced during the fifteenth century by the Portuguese, custard apple got naturalized in semi-wild state especially in the semi-arid and subhumid climates throughout the country and has good diversity for habit, plant type, fruit and reticulation size, seed size and number.

31.5.8.9 Pineapple

Five species are reported under this genus Ananas which are native to the eastern South America. The centre of origin is probably in the Parana-Paraguay river drainage area, where related seedy species *Ananas bracteatus*, *A. ananassoides* and *A. erectifolius* occur in wild state. It was presumed that the Tupi-Guarani Indians first selected and domesticated *A. comosus* at its centre of origin and later it spread to different parts of the New and Old World. Hawaii is considered as the secondary centre of diversity because of early introduction and somatic mutations. In pineapple *Simhachalam* is a popular landrace adapted to the South Indian hill slopes.

31.5.8.10 Apple

Apple is the most important fruit of the temperate regions and is believed to have originated in the Caucasus mountains of Western Asia where wild apple exists in the forest areas. India is the fifth largest producer of apples with a production of 2.2 mt (http://faostat3.fao.org).

31.5.8.11 Garlic

Garlic is the second most important *Allium* species widely used and cultivated after onions. *A. longicuspis* Regel, which is endemic to Central Asia, is considered as the wild progenitor of garlic. It is well known in Egypt before 3,000 BC and spread to the Mediterranean region in ancient times. It has been in cultivation for a long time in India and China and garlic was introduced into the western hemisphere by the Spanish, Portuguese and the French. Twenty six varieties of garlic are released in India (http://www.nbpgr.ernet.in:8080/PGRPortal and it stands second, after China with a total production of 1.1 mt (http://faostat3.fao.org).

31.5.8.12 Coconut

The species now is very widely cultivated and almost pantropical in distribution. The centre of origin related to coconut where most of the species are still found is in the north-western South America (Purseglove 1968). As of date 23 released varieties are available in India (http://www.nbpgr.ernet.in:8080/PGRPortal) the Indian collections exhibit variation in height, bearing and nut quality. India with a production of 10.5 mt is the third largest producer of coconuts in the world after Indonesia and the Philippines.

31.5.8.13 Cashew Nut

It is a native of tropical Americas from Mexico to Peru and Brazil and also of the West Indies. It has been naturalized in many tropical countries especially in the coastal zone. It was introduced into the Indian subcontinent from Brazil by the Portuguese in the sixteenth century to control soil erosion in the coastal areas. India with a production of 0.6 mt is the third largest producer of cashew nuts in the world.

31.5.8.14 Betel Palm

The genus *Areca* has about 54 spp. of monoecious palms spread from Indo-Malayan region to the Solomon Islands and northern Australia. Several centres of origin have been suggested which include the Sunda Islands, the Philippines and Malaysia (Burkill 1966). It is certainly of very ancient cultivation in Malaysia from where it might have been introduced into the Indian subcontinent very long time ago. The germplasm comparatively is less diverse and productive. Variability occurs for plant height, earliness and yield-attributing characters.

31.6 Need for Identification of Alien and Underutilized Crop Species to Mitigate Food and Nutritional Security Challenges

From 7,000 edible plant species that were cultivated/gathered for food in the human history, the present-day agriculture is based only on 150 species, which are under intensive cultivation. Out of these, about 30 species such as wheat, barley, maize, rice, millet, sorghum, rye, cassava, yams, potato and sweet potato account for 95 % of human food and other requirements. Wheat, rice and maize alone cater to over 50 % of the human protein and dietary energy requirements globally.

In Asia and Africa, the rate of urbanization is at 40 and 38 % respectively in 2005 and is expected to keep growing in the next 25 years at an alarming rate (UNFPA 2007). In addition to the ever-increasing world population, there are national demographic changes affecting the ability of countries to feed and sustain their own population. The crop diversity, which is the spectrum of different crops grown, has declined as landraces are displaced by scientifically developed modern varieties (Chang 1994). Also, the ongoing breeding and selection process has narrowed the genetic base of varieties used in agricultural production (GAO 1997). In the broadest sense, alteration and narrowing of crop genetic diversity began with the first domestication of wild plants. For example, the corn plant is completely dependent on humans for reproduction for thousands of years because, farmer selection has resulted in kernels that can no longer disperse without human intervention. The introduction of the modern high-yielding crop varieties as part of green revolution has led to the erosion of genetic diversity (Fowler and Mooney 1990). The erosion

has been at a rapid pace and the estimates by FAO (1997) indicate that, since the beginning of the twentieth century, about 75 % of the genetic diversity of agricultural crops has been lost. Population growth and extensive farming are often cited as the factors fostering high rates of land conversion to agriculture leading to habitat loss of local landraces/crops/wild species leading to narrowing of variation within the crop species. Habitat loss and land conversion are more pronounced in developing countries than in developed countries (Houghton 1994). Other influences on land conversion include poverty, international trade, land degradation and government policies, particularly where land tenure policies are not clearly defined or enforced (Day-Rubenstein et al. 2000). Climate change is likely to be an additional threat to agricultural biodiversity, increasing genetic erosion of landraces and threatening crop wild relatives (Jarvis et al. 2008). Climate change is predicted to bring about increase in temperatures across the globe in the range of 1.6 °C to as much as 6 °C by 2050. Current varieties will be lost as farmers replace them with other landraces or improved varieties that are better adapted to the new conditions. For example, an analysis of the use of Guinea sorghum varieties in the Sudanian zone of southern Mali showed that the range of varieties grown by families in villages is heavily influenced by climate change, specifically the shortening of rainy season over the last 20 years (Weltzien et al. 2006). Further, Russia's worst drought in over a century during 2010 devastated the country's harvest and grain production by almost 40 % (Hernandez et al. 2010). Thus, climate change will dictate novel and increased requirements for germplasm from gene banks to screen against climate variables for developing suitable cultivars amenable for adaptive agriculture.

Also, rapid dietary change of indigenous people worldwide is leading to the decline in utilization of traditional wild food and traditional knowledge which sustain the balance in the ecosystems (Kuhnlein and Receveur 1996; Kuhnlein et al. 2003; Damman et al. 2008). Use of traditional foods is declining because of several factors: a loss of knowledge about edible local plant species (Pardo-de-Santayana et al. 2007); change in consumption patterns from traditional edible plant species to introduced cultivated crops (Krahn 2005); changes in livelihood patterns that limit the quantity of the time spent gathering for wild foods (Dounias et al. 2007) or processing and preparing traditional food which is time-consuming (Smith 1995); the general shift from gathering to purchasing food as rural communities join the market economy; and non-timber forest products that lose ground to other food sources (Reyes-García et al. 2005). Small markets with local produce/products are being replaced by supermarkets in developing countries like India, which is diversity rich and holds enormous indigenous knowledge focussing on underutilized species. These species hold good promise because of their adaptability to local agroecosystems and farming in degraded and marginal lands. Besides, minor crop species have high genetic diversity, low pest risk and multipurpose uses and scope for value addition. Moreover, they are well tuned to native/traditional farming practices with low inputs and provide food and nutritional security to rural communities. It is time to broaden our approach even further and explore the linkages between agriculture, food production, nutrition, ethnobiology, ethnopharmacology and the resource base of wild and agricultural biodiversity in the context of accelerating global change (Heywood 2011).

31.7 Identification of Important Alien Crops for Food and Nutritional Security

Based on physiographic, climatic and cultural features, India is divided into 20 agroclimatic region and some of the important regions include the following: (1) Arid ecosystem, representing three agroclimatic regions in the country-one in the cold arid zone of western Himalayas and two in hot arid zones in the western India and Deccan Plateau; (2) Semi-arid ecosystem comprising of five hot semi-arid eco-regions of northern plains and central highlands with alluvial-derived soils-Central (Malwa) Highlands, Gujarat Plains and Kathiawar with medium black soils, Deccan (Telangana) Plateau with shallow and medium

black soils, and Deccan (Karnataka) Plateau with red loamy soils; (3) Subhumid ecosystem representing five hot and one warm subhumid ecoregion in the northern central and eastern parts of the country; (4) Humid and prehumid ecosystems comprising three agro-ecological regions in the north-eastern India including Bengal; (5) Coastal ecosystem covering two regions of Western and Eastern Ghats; and (6) Island ecosystem covering Andaman and Nicobar and Lakshadweep. These diverse agro-climatic regions enable cultivation of different crops in India (Fig. 31.1) and offer wide options for the introduction of alien crops to meet the challenges.

Ecocrop, an FAO database having information on more than 2,000 species of crop plants grown the world over, was accessed to get information regarding potential alien crops that can be introduced into the country on priority. In the 'search' option of the 'Description', we queried for 'annual' and under 'plant attributes' 'grown on large scale' for the categories of cereals and pseudo-cereals, pulses, roots and tubers and vegetables which basically form the food crops. Top ten crops that were not present in large scale within the country were listed for introduction. In case of roots and tubers and vegetables perennial species were also queried to arrive at the list (Table 31.3) that can be prioritized for introduction by keeping in view the changing dietary habits, the authors have come up with the list of crops that needs to be prioritized (Table 31.4).

Similarly, new tools such as the 'Climate Analogues' were developed by Climate Change Agriculture and Food Security (CCAFS) programme in collaboration with CIAT, Columbia, to aid in decision support and to make climate change adaptation more practical. This tool also helps in identifying specific locations where sim-

Table 31.3 Alien crop species prioritized for food and nutritional security in India based on Ecocrop database

Cereals and pseudo-cereals	***Avena sativa***, *Secale cereale*, *Fagopyrum esculentum*, *Amaranthus hypochondriacus*, ***Eragrostis tef***
Pulses	***Canavalia ensiformis***, *Pachyrhizus erosus*, *Phaseolus coccineus*, *Phaseolus lunatus*, *Vigna angularis*
Roots and tubers	*Brassica napus var. napobrassica*, *Helianthus tuberosus*, *Manihot esculenta*
Vegetables	*Allium tuberosum*, *Asparagus officinalis*, *Cynara scolymus*, *Metroxylon sagu*, *Morus alba*, *Petroselinum crispum*

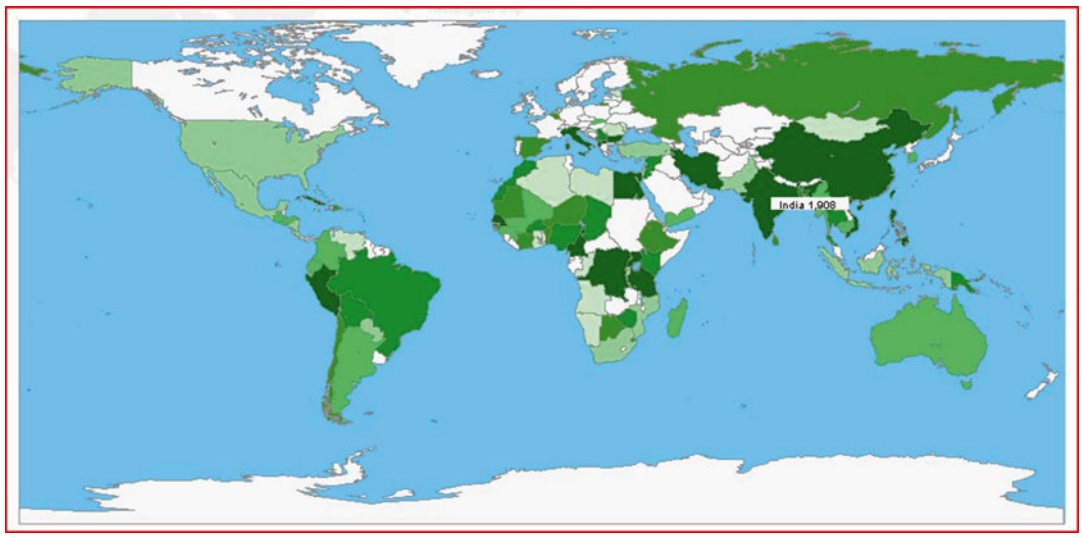


Fig. 31.1 Map showing the wealth of vegetable crops in India (India is one of the richest vegetable-growing countries in the world with the cultivation of 1,908 crops) (*Source*: Hortivar database http://www.fao.org/hortivar/maps/maps.jsp)

Table 31.4 List of alien crops that need to be prioritized for food and nutritional security in India considering the changing dietary habits

Oilseeds	
Olive	*Olea europaea* (low-chilling genotypes)
Cereals	
Tef	*Eragrostis tef*
Oats	*Avena sativa* (low-chilling genotypes)
Pseudo-cereals	
Quinoa	*Chenopodium quinoa*
Nuts	
Hazelnut	*Corylus avellana*
Vegetables	
Asparagus	*Asparagus officinalis*
Broccoli	*Brassica oleracea* var. *botrytis*
Lettuce	*Lactuca sativa*
Avocado	*Persea americana*
Fruits	
Blackberry	*Rubus fruticosus*
Raspberry	*Rubus idaeus*
Blueberry	*Vaccinium corymbosum*
Cranberry	*Vaccinium macrocarpon*
Currants	*Ribes sativum*
Desert apple	*Cereus repandus*
Spices	
Chia	*Salvia hispanica*
Beverages	
Tequila	*Agave tequilana*
	Opuntia spp.

ilar climates might be developing in 2030 (Fig. 31.2).

For example, crops like *Canavalia ensiformis* and *C. gladiata* have been well recognized as important legume crops for nutritional security in India (Rai et al. 2007). Sword bean's potential as a source of nutraceuticals, phyto-pharmaceuticals and other agricultural products has been reported. Also, the cancer-fighting properties of flavonoids and phytochemicals have been discussed (Morris 2007). Although it is reported to contain toxin canavanine, methods to detoxify sword bean seeds using acid and formalin (Tepal et al. 1994) and non-chemical methods such as overnight soaking and boiling in excess water to reduce canavanine content up to 50 % were reported (Ekanayake et al. 2007). Drought tolerance is another qualifying trait of this underutilized vegetable legume species and a drought-tolerant germplasm line was also registered with ICAR (Samadia and Dhandar 2004) (Fig. 31.3).

31.8 Important Underutilized Crops for Popularization for Food and Nutritional Security

In India, underutilized species have received special attention with a coordinated project at the national level. The list of utilizable species identified under the project is given in Table 31.5.

31.9 Underutilized Crops to be Popularized for Food and Nutritional Security

In a study on genetic erosion in maize within small-holder agriculture in southern Mexico, Van Heerwarden et al. (2009) found that despite the dominance of commercial seed, the informal seed system of local farmers persisted. It is estimated that about 60 % of the world's agriculture consists of traditional subsistence farming systems in which there is a high diversity both of crops and species grown and of ways in which they are grown, such as polycropping and intercropping, leading to maintenance of variation within the crops (FAO 2010a; Vigouroux et al. 2011). Experiences from the global UN efforts on Neglected and Underutilized Species (NUS) infer the need for adoption of a new R&D paradigm directed towards cultural-sensitive objectives and not solely towards economic benefits (Padulosi et al. 2013). Considering that these NUS have been part and parcel of livelihood systems, Jaenicke (2013) concluded that in the widest sense, the underutilized crops can make important contributions to food security-better nutrition-and in preparing for future environmental events maintaining the global biodiversity. Crops in which rich diversity occurs in the country include, rice, wheat, maize, barley, pigeon pea, chickpea, small millets, green gram, black gram, horse gram, moth bean, rice bean, cluster

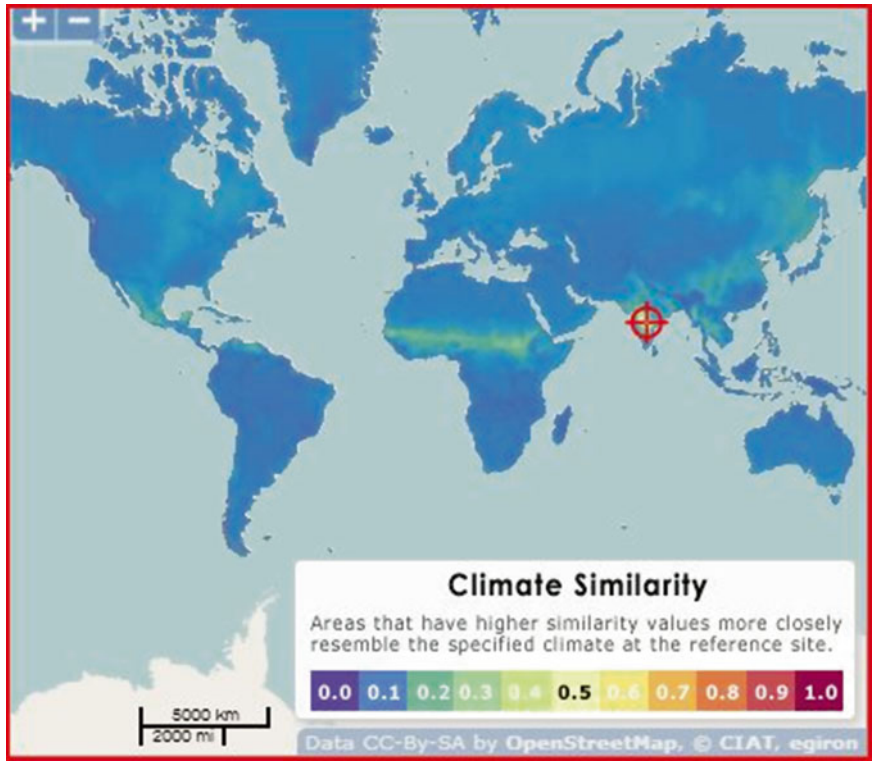


Fig. 31.2 Map generated using 'Climate Analogues' software of CCAFS depicting the areas that would have similar climate of Telangana, India, during 2030

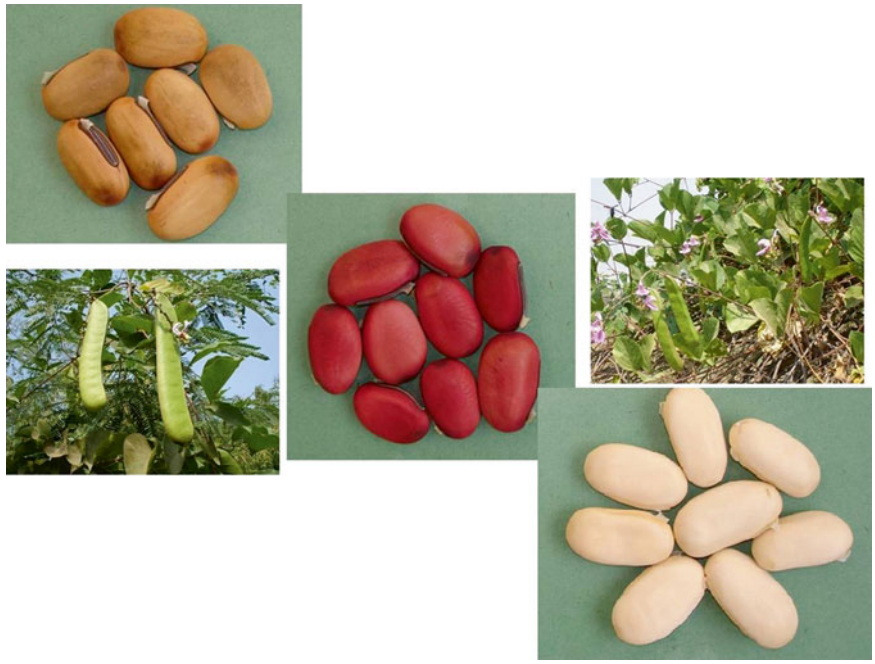


Fig. 31.3 Diversity in Canavalia (Photo courtesy: NBPGR, RS, Hyderabad)

Table 31.5 Underutilized species prioritized under A India Coordinated Research Project

Crop group	Species
Pseudo-cereals	Grain amaranth (*Amaranthus* spp.), buckwheat (*Fagopyrum esculentum* Moench, *F. tataricum* Gaertn.), chenopod (*Chenopodium album* L. and *C. quinoa* Willd.) and Job's tears (*Coix lacryma-jobi* L.)
Legumes	Rice bean (*Vigna umbellata* (Thunb.) Ohwi and Ohashi), adzuki bean (*Vigna angularis* (Willd.) Ohwi and Ohashi), fab a bean (*Vicia faba* L.) and winged bean (*Psophocarpus tetragonolobus* (L.) DC.)
Vegetables	Spine gourd (*Momordica dioica* Roxb. ex Willd.) and winged bean
Oilseeds	Perilla (*Perilla frutescens* (L.) Britton)
Fodder crops	Amaranth, saltbush (*Atriplex* spp.) and casuarina (*Casuarina* spp.)
Industrial plants	Guayule (*Parthenium argentatum* Gray), jojoba (*Simmondsia chinensis* (Link.) Schneid.), purging nut (*Jatropha curcas* L.), colocynth (*Citrullus colocynthis* Schrad.), paradise tree (*Simarouba glauca* DC.) and cuphea (*Cuphea* spp.)

From Arora et al. (2006)

bean, sesame, forage grasses, okra, eggplant, cucumber, melons, citrus, banana and plantains, jackfruit, mango, tamarind, jamun, jute, cotton, ginger, turmeric, pepper, cinnamon and cardamom. Among tuberous crops, rich variability exists in sweet potato, taros and yams. Native resources are also available in *Coleus* species, sword bean, velvet bean and several minor fruits, such as berries and nuts belonging to *Rubus*, *Ribes*, *Juglans*, *Pyrus* and *Prunus*. While their potential may not be fully realized at national level, they are of significant importance locally, being highly adapted to marginal, complex and difficult environments and contributing significantly to diversification and resilience of agro-ecosystems. This means, they are also of considerable interest for future adaptation of agriculture to climate change (Padulosi et al. 2011). Underutilized species also received qualified endorsement in the Commission on Sustainable Agriculture and Climate Change's report to achieve food security in the face of climate change (Beddington et al. 2012).

31.10 Conclusions

Certainly, the alien crops introduced over the years have contributed and continue to do so in the food and nutritional security of the country. However, the challenges of climate change and changing diets etc. warrant continuous scouting for new alien and underutilized crops for sustained and improved food and nutritional security. However, there are some implications on exchange of germplasm in view of international treaties. In any country, among the crops that are under cultivation, some may be of native and some may be of non-native origin from other region(s). For food security, dependence on several crops is the order of the day and exclusive dependence on native crops may not be possible. The enforcement of the CBD from 1993 and provisions under TRIPS led to the apprehension that exchange of germplasm would get restricted. The plant genetic resources for food and agriculture (PGRFA) represents the basis for the establishment of a multilateral system of access and benefit-sharing which applies to a list of crops in Annex I (35 food crops and 29 forage crop species) under an SMTA for food security and interdependence irrespective of the origin of the crops. In the post-CBD era on analysis, it was revealed that there had been a decline by 14.5 % in access and introduction of germplasm globally.

To increase the food production at global level on sustainable basis, dependence on crop genetic resources that originated from different geographical locations through introduction and exchange is inevitable. This holds good more in the case of underutilized crop species in whose cases the centre of origin itself is the centre of diversity as the domestication efforts have just started and still in the evolutionary process. Research efforts towards introduction of alien crops in the designated areas/cropping systems have to be carried out swiftly.

Hence conservation of germplasm variability from the centres of origin and diversity assumes importance as a valuable source of genes for utilization especially for sources of resistance, as the pests and pathogens also co-evolve with the landraces in those centres for improvement of the existing crop species and development of improved varieties.

Disclaimer The views and opinions expressed in this book chapter are those of the authors and do not necessarily reflect the official policy or position of their affiliated institutions/organization.

References

Alexander J, Coursey DG (1969) The origin of Yam cultivation. In: Ucho PJ, Dimbleby GW (eds) The domestication and exploitation of plants and animals. Duckworth, London

Arora RK (1991) Plant diversity in the Indian gene centre. In: Paroda RS, Arora RK (eds) Plant genetic resources conservation and management. IBPGR, New Delhi

Arora RK, Nayar ER (1984) Wild relatives of crop plants in India. NBPGR Sci. Mongr, NBPGR, New Delhi

Arora RK, Sharma GD, Joshi V, Phogat BS, Bhatt KC, Rana JC (2006) Under-utilized crops. In: Dhillon BS, Saxena S, Agrawal A, Tyagi RK (eds) Plant genetic resources: food crops. Narosa Publishing House, New Delhi

Beddington J, Asaduzzaman M, Clark M, Fernández A et al (2012) Achieving food security in the face of climate change: final report from the Commission on sustainable agriculture and climate change. CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS), Copenhagen

Bosland PW, Votava EJ (2000) Peppers vegetable and spice capsicums. Crop production science in horticulture. CAB International Publishing, Wallingford, p 12

Burkill IH (1935) In: Ministry of agriculture (Malaysia) (ed) A dictionary of the economic products of the Malay peninsula. Crown Agents for the Colonies, London, p 839

Burkill IH (1966) A dictionary of the economic products of the Malay Peninsula, vol 2. Ministry of Agriculture and Cooperative, Malaysia

Chang TT (1994) The biodiversity crisis in Asia crop production and remedial measures. In: Peng CI, Chou CH (eds) Biodiversity and Terrestrial Ecosystems, Institute of Botany, Monograph Series No. 14. Academia Sinica, Taipei

Damman S, Eide WB, Kuhnlein HV (2008) Indigenous peoples' nutrition transition in a right to food perspective. Food Pol 33:135–155

Day-Rubenstein K, Stuart M, Frisvold G (2000) Agricultural land use in tropical forested countries: patterns, determinants, and implications for biodiversity loss. World Resour Rev 12(4)

Dhawan NL (1964) Primitive maize in Sikkim. Maize Genet Coop Newsl 38:69–70

Dounias E, Selzner A, Koizumi M, Levang P (2007) From sago to rice, from forest to town: the consequences of sedentarization for the nutritional ecology of punan former hunter-gatherers of Borneo. Food Nutr Bull 28(2):S294–S302

Ekanayake SK, Skog NG, Asp (2007) Canavanine content in sword beans (Canavalia gladiata) analysis and effect of processing. Food Chem Toxicol 45(5):797–803

FAO (1997) The state of the world's plant genetic resources for food and agriculture. FAO, Rome

FAO (Food and Agriculture Organization of the United Nations) (2010) The state of food insecurity in the world. Addressing food insecurity in protracted crises. FAO, Rome

Flyman MV, Afolayan AJ (2006) The suitability of wild vegetables for alleviating human dietary deficiencies. S Afr J Bot 72:492–497

Fowler C, Mooney P (1990) The threatened gene food politics and the loss of genetic diversity. Lutworth Press, Cambridge, UK

GAO (United States General Accounting Office) (1997) US Department of Agriculture: Information on the Condition of the National Plant Germplasm System. Report to Congressional Committees

Gmitter FG Jr, Hu X (1990) The possible role of Yunnan, Chine in the origin of contemporary Citrus species (Rutaceae). Econ Bot 44:257–277

Gopalan C, Rama Sastri BV, Balasubramanian SC (1989) Nutritive value of Indian foods. NIN, Hyderabad

Gulati A, Dixon J (2008) Maize in Asia: changing markets and incentives. Academic Foundation, New Delhi

Harlan JR (1971) Agricultural origins centres and non-centres. Science 174:468–474

Harlan JR (1975) Crops and man. American Society of Agronomy, Madison

Hawkes JG (1983) The diversity of crop plants. Harvard University Press, Cambridge, MA

Heiser CB (1976) Pepper *Capsicum* (Solanaceae). In: Simmonds NW (ed) Evolution of crop plants. Longman, London

Hernandez MA, Robels M, Torero M (2010) Fires in Russia, wheat production, and volatile markets: reasons to panic? IFPRI. Accessed at http://www.ifpri.org/sites/default/files/wheat.pdf

Heywood VH (2011) 'Ethnopharmacology, food production, nutrition and biodiversity conservation towards a sustainable future for indigenous peoples. J Ethnopharmacol 137:1–15

Houghton RA (1994) The worldwide extent of land-use change. Bioscience 44:5

Hymowitz T, Kaizuma (1981) Soybean seed protein electrophoresis profiles from 15 Asian countries or regions; hypothesis on paths of dissemination of soybean from China. Econ Bot 35:10–23

Jaenicke H (2013) Research and development of underutilised plant species crops for the future – beyond food security. Acta Hort 979:33–44

Jagtap PP, Shingane US, Kulkarn KP (2012) Economics of Chilli production in India. Afr J Basic Appl Sci 4:161–164

Jain SM (2009) Mutation induced genetic improvement of neglected crops. In: Tadele Z (ed) New approaches to plant breeding of orphan crops in Africa: proceedings of an international conference. Stämpfli AG, Bern

Jarvis A, Lane A, Hijmans R (2008) The effect of climate change on crop wild relatives. Agr Ecosyst Environ 126:13–23

Johns T, Eyzaguirre PB (2007) Biofortification biodiversity and diet a search for complementary applications

against poverty and malnutrition. Food Policy 32:1–24

Kamala V, Gupta AJ, Sivaraj N, Pandravada SR, Sunil N, Varaprasad KS, Lawande KE (2011) Diversity analysis of onion germplasm collections from northern telangana region of Andhra Pradesh. Indian J Plant Genet Res 24(2):163–171

Krahn J (2005) The dynamics of dietary change of transitional food systems in tropical forest areas of Southeast Asia. The contemporary and traditional food system of the Katu in the Sekong Province, Lao PDR. Agricultural Faculty, University of Bonn, Bonn (Dissertation)

Kuhnlein HV, Receveur O (1996) Dietary change and traditional food systems of indigenous peoples. Annu Rev Nutr 16:417–442

Kuhnlein HV, Johns T, The IUNS Task Force on Indigenous Peoples' Food Systems and Nutrition (2003) Northwest African and Middle Eastern food and dietary change of indigenous peoples. Asia Pac J Clin Nutr 12:344–349

Kumar R, Srinivas K, Sivaramane N (2013) Assessment of the maize situation, outlook and investment opportunities in India. Country Report – Regional Assessment Asia (MAIZE-CRP), National Academy of Agricultural Research Management, Hyderabad

Mehra KL, Arora RK (1982) Plant genetic resources of India their diversity and conservation. NBPGR Sci Mongr No. 4. NBPGR, New Delhi

Morris JB (2007) Swordbean (Canavalia ensiformis (L.) genetic resources regenerated for potential medical, nutraceutical and agricultural traits. Genet Resour Crop Evo 54:585–592

Murthi Anishetty N, Pandravada SR, Abraham Z, Varaprasad KS (2008) Centres of origin and diversity of horticultural crops. In: Peter KV (ed) Biodiversity in horticultural crops, vol II. Daya Publishing House, New Delhi

Padulosi S, Heywood V, Hunter D, Jarvis A (2011) Underutilized species and climate change: current status and outlook. In: Yadav SS, Redden RJ, Hatfild JL (eds) Crop adaptation to climate change. Blackwell Publishing Ltd, Chichester, pp 507–521

Padulosi S, Bala Ravi S, Rojas W, Valdivia R, Jager M, Polar V, Gotor E, Mal B (2013) Experiences and lessons learned in the framework of a global UN effort in support of neglected and underutilized species. Acta Hort 979:517–531

Pandravada SR, Sivaraj N, Varaprasad KS (2004) The changing pattern of plant biodiversity in the Eastern Ghats. In: Dhillon BS, Tyagi RK, Lal A, Saxena S (eds) Plant genetic resource management. Narosa Publishing House, New Delhi

Pandravada SR, Sivaraj N, Kamala V, Sunil N, Varaprasad KS (2008) Genetic resources of wild relatives of crop plants in Andhra Pradesh – diversity, distribution and conservation. Proc AP Acad Sci 12(1 and 2):101–119

Pardo-de-Santayana M, Tardío J, Blanco E, Carvalho AM, Lastra JJ, San Miguel E, Morales R (2007) Traditional knowledge of wild edible plants used in the northwest of the Iberian Peninsula (Spain and Portugal): a comparative study. J Ethnobiol Ethnomed 3:27

Prasanna BM (2010) Phenotypic and molecular diversity of maize landraces: characterization and utilization. Indian J Genet 70:315–327

Prasanna BM, Sharma L (2005) The landraces of maize (Zea mays L.) diversity and utility. Indian J Plant Genet Res 18:155–168

Purseglove JW (1968) The origin and distribution of the coconut. Trop Sci 10:191–199

Purseglove JW (1972) Tropical crops. Monocotyledons. Longman Group Ltd, London

Rai M, Pandey S, Ram D, Rai N, Pandey AK, Yadav DS (2007) Plant genetic resources of legumes and underutilized vegetable crops in India. Acta Hort 752:225–230

Rai M, Pandey S, Kumar S (2008) Cucurbit research in India: a retrospect. In: Pitrat M (ed) Proceedings of the IX EUCARPIA meeting on genetics and breeding of Cucurbitaceae, INRA, Avignon, pp 285–294

Ramachandra VA (2006) Production and marketing of Sapota in Northern Karnataka-an economic analysis. MSc thesis, University of Agricultural Sciences, Dharwad

Rao SM, Dora DK (2002) Less known vegetables for nutritious, health and economic security – Indian context. International conference on vegetables, Bangalore

Reyes-García V, Vadez V, Huanca T, Leonard WR, Wilkie D (2005) Indigenous knowledge and consumption of wild plants: a comparative study of two Amerindian villages in the Bolivian Amazon. Ethnobot Res Appl 3(3):201–207

Samadia DK, Dhandar DG (2004) Sword bean *Canavalia gladiata* (Jacq.) DC germplasm line, a drought tolerant alternative vegetable. Ind J Plant Genet Res 17(3):236

Seetharam A, Giriraj K, Singh R, Chand D et al (2004) Sunflower. In: Dhillon BS, Tyagi RK, Saxena S, Agrawal A (eds) Plant genetic resources: oilseeds and cash crops. Narosa Publishing House, New Delhi

Singh B (1977) Races of maize in India. Indian Council of Agricultural Research, New Delhi

Singh SR, Ahmed N, Lal S, Ganie SA, Amin M, Jan N, Amin A (2013) Determination of genetic diversity in onion (Allium cepa L.) by multivariate analysis under long day conditions. Afr J Agri Res 8(45):5599–5606

Smith IF (1995) The case for indigenous West African food culture. UNESCO Breda Series No. 9. Dakar, Senegal, UNESCO, Breda

Srinivas T, Nedunchezhiyan M, Misra RS (2012) Marketing system of aroids and yams in India. CTCRI, Tech. Bulln. 53. Central Tuber Crops Research Institute, Kerala

Tanaka T (1977) Fundamental discussion of Citrus classification. Stud Citrologia 14:1–146

Tepal JA, Castellanos R, Larios A, Tejada I (1994) Detoxification of jack beans (*Canavalia ensiformis*) I.—Extrusion and canavanine elimination. J Sci Food Agric 66:373–379

Tiwari SP, Singh RV, Patel DP (2004) Soybean. In: Dhillon BS, Tyagi RK, Saxena S, Agrawal A (eds) Plant genetic resources: oilseeds and cash crops. Narosa Publishing House, New Delhi

United Nations Population Fund (2007) State of the World Population 2007: unleashing the potential of urban growth. UNFPA, Geneva. Available at: http://www.unfpa.org/publications/state-world-population-2007. Accessed 20 Feb 2015

van Heerwaarden J, Hellin J, Visser RF, Van Eeuwijk FA (2009) Estimating maize genetic erosion in modernized smallholder agriculture. Theor Appl Genet 119:875–888

Vavilov NI (1951) Phytogeographical basis of plant breeding. The origin, variation, immunity and breeding of cultivated plants (trans: Choster KJ). Chronica Botanica 13:366

Vigouroux Y, Barnauda A, Scarcellia N, Thuilleta AC (2011) Biodiversity, evolution and adaptation of cultivated crops. C R Biol 334:450–457

Weltzien E, Rattunde H, Clerget B, Siart S, Toure A, Sagnard F (2006) Sorghum diversity and adaptation to drought in West Africa. In: Jarvis D, Mar I, Sears L (eds) Enhancing the use of crop genetic diversity to manage abiotic stress in agricultural production systems. International Plant Genetic Resources Institute, Rome

Whitmore TM, Turner BL II (2002) Cultivated landscapes of middle america on the eve of conquest. Oxford University Press, New York

Zeven AC, De Wet JMJ (1982) Dictionary of cultivated plants and their regions of diversity. Centre of Agricultural Publicity and Documentation, Wageningen

Zeven AC, Zhukovsky PM (1975) Dictionary of cultivated plants and their centres of diversity. PUDOC, Wageningen

Microalgal Rainbow Colours for Nutraceutical and Pharmaceutical Applications

32

Tanmoy Ghosh, Chetan Paliwal, Rahulkumar Maurya, and Sandhya Mishra

Abstract

Microalgae**,** one of the largest global primary producers, are a potential source of bioactive compounds. They are unique in producing superfine chemicals that can be used in various industrial sectors like pharmaceuticals, nutraceuticals and cosmeceuticals. The chapter is intended to provide an insight to two of the most important pigments obtained from them, phycobiliproteins and carotenoids having species specificity which can be used as a chemotaxonomic marker. Their unique structural properties play a crucial role in their biological functions. The water-soluble phycobiliproteins are used as fluorescent tags in flow cytometry and immunochemistry, while liposoluble carotenoids are potential alternatives to synthetic dyes in the food industry.

Keywords

Microalgae • Phycobiliproteins • Carotenoids • Fluorescence • Applications

32.1 Introduction

Photosynthesis is an important biochemical reaction responsible for meeting our energy demands directly or indirectly. The solar energy received on earth is converted into chemical energy by means of photosynthesis, which is then stored in various forms. Algae, either unicellular or filamentous, are one of the most primitive photosynthetic organisms found in both freshwater and marine habitats. They are subdivided as macroalgae (seaweeds) and microalgae.

Microalgae are global primary producers which contribute from one-third to more than

T. Ghosh • C. Paliwal • R. Maurya • S. Mishra (✉)
Academy of Scientific and Innovative Research, New Delhi, India

Discipline of Salt & Marine Chemicals, CSIR-Central Salt and Marine Chemicals Research Institute, Bhavnagar 364002, Gujarat, India
e-mail: smishra@csmcri.org

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_32,

Table 32.1 General composition of different algae (% of dry matter)

Strain	Protein	Carbohydrates	Lipids	Nucleic acid
Scenedesmus obliquus	50–56	10–17	12–14	3–6
Botryococcus braunii	22	18	55–60	–
Scenedesmus dimorphus	8–18	21–52	16–40	–
Chlamydomonas reinhardtii	48	17	21	–
Chlorella vulgaris	51–58	12–17	14–22	4–5
Chlorella protothecoides	10–20	12–20	55	–
Spirogyra sp.	6–20	33–64	11–21	–
Dunaliella tertiolecta	55–65	10–15	20	–
Dunaliella salina	57	32	6	–
Euglena gracilis	39–61	14–18	14–20	–
Prymnesium parvum	28–45	25–33	22–38	1–2
Tetraselmis maculata	52	15	3	–
Porphyridium cruentum	28–39	40–57	9–14	–
Spirulina platensis	46–63	8–14	4–9	2–5
Spirulina maxima	60–71	13–16	6–7	3–4.5
Synechococcus sp.	63	15	11	5
Anabaena cylindrica	43–56	25–30	4–7	–

Source: Adapted from Spolaore et al. (2006)

half of the total primary productivity (Van Den Hoek et al. 1995; Miyamoto 1997; Guschina and Harwood 2006).

Because of their high growth rates and ability to mitigate CO_2 from the environment and utilize non-arable land for their cultivation, they are considered as potential energy feedstock for their utilization in biofuel (biodiesel, bioethanol and biogas etc) production. Apart from being an energy feedstock, microalgae are a great store of many different biomolecules such as polyunsaturated fatty acids (PUFAs), sterols, pigments, enzymes, vitamins, minerals, proteins and carbohydrates which are beneficial both economically and medically. The general composition of the algae in terms of carbohydrates, lipids, nucleic acids and proteins is provided in Table 32.1.

They are able to potentially accumulate up to 50 % of their dry weight as carbohydrates, primarily in the form of starch, glucose, cellulose or hemicelluloses or polysaccharides of various kinds (Ho et al. 2012; Yen et al. 2013). Algal polysaccharides are, to a large extent, sulphated polysaccharides with important medical applications. Crude polysaccharide extracts from various microalgae such as *Chlorella vulgaris*, *Chlorella stigmatophora*, *Scenedesmus quadricauda* and *Phaeodactylum tricornutum* have anti-inflammatory, immunomodulatory and antioxidant properties (Guzman et al. 2003; Mohamed 2008).

Microalgae synthesize ω-3 and ω-6 fatty acids including docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), γ-linoleic acid and arachidonic acid (ARA) essential in maintaining the tissue integrity, which humans are not able to synthesize. They have many beneficial properties like they are anti-inflammatory, play a role in brain development, help in functioning of the nervous system and delay ageing. Most of these polyunsaturated fatty acids (PUFAs) are used as health supplements, as baby food additives, in therapeutics and as poultry feed (Ahren et al. 1983; Cohen and Heimer 1992; Gordon and Ratliff 1992; Borowitzka 1993; Barclay and Zeller 1996; Pulz and Gross 2004; Guedes 2010; De Jesus Raposo et al. 2013).

Some species of microalgae are also found to be rich in vitamins and industrially important enzymes. *Porphyridium cruentum* is a good source of vitamins C and E as well as provitamin

A (Sarrobert and Dermoun 1991). *Navicula ostrearia*, a diatom, is a rich source of vitamin E (De Jesus Raposo et al. 2013). *Dunaliella salina*, besides being known for β-carotene production, is also a source of thiamine, pyridoxine, riboflavin, nicotinic acid, biotin and tocopherol (Drokova and Popova 1974). Carbonic anhydrase, a crucial enzyme responsible for the conversion of CO_2 into bicarbonate ions and carbonic acid, is produced by *Isochrysis galbana*, *Amphidinium carterae* and *Prorocentrum minimum* (Yingying and Changhai 2009; De Jesus Raposo et al. 2013). Superoxide dismutase, another enzyme crucial for antioxidant activity in vivo, is produced by *Anabaena* sp., *Porphyridium* sp., *Phaeodactylum tricornutum* and *Synechococcus* sp. (Thepenier et al. 1988; Guzman-Murillo et al. 2007; De Jesus Raposo et al. 2013).

We are witnessing a shift of research interests in functional foods obtained from natural sources, which contain additional nutrients and are beneficial to humans. There has been felt a need to investigate such potentially important high-value products like antioxidants, anti-inflammatory compounds, natural colouring agents, fluorescent dyes and many others, from natural sources (Eisenreich et al. 2004). Microalgae have been exploited for such bioactive compounds for use in pharmaceutical, nutraceutical, food and cosmetic industries.

From an economic point of view, microalgal cultivation is often preferred for the production of high-value compounds like phycobiliproteins and carotenoids (Spolaore et al. 2006; Chu 2012; Markou and Nerantzis 2013). Nevertheless, there still exists a need to improve microalgal cultivation and harvesting technology along with the techniques used for the extraction and purification of the desired molecules (Molina et al. 2003). Currently, different food companies are interested in improving their products through substitution of natural products because of larger profit margins compared to conventional food products and their acceptability to the public in general (Hasler 2002; Siro et al. 2008).

A full description of all these biomolecules would exceed the scope of this chapter which primarily details about the various pigments sourced from these organisms.

Our main focus would be pigments derived from marine microalgae which have potential health and commercial benefits. A number of these pigments have antioxidant, anti-inflammatory and neuroprotective properties which have been conclusively proved through various in vitro and in vivo studies. Apart from these biological properties, they play an important role in diagnostic biosensors and fluorescence analytical techniques. Microalgal pigments are broadly classified into two groups:

- Water-soluble phycobiliproteins sourced from microalgae as well as macroalgae are used for developing fluorescent markers in conjugation with immunoglobulins and other proteins.
- Lipid-soluble carotenoids such as astaxanthin, zeaxanthin and ß-carotene from microalgae which serve as provitamins and antioxidants.

32.2 Phycobiliproteins

Phycobiliproteins are accessory light-harvesting pigments predominantly found in cyanobacteria (blue-green algae), Rhodophyta (red algae), Cryptophyta and Glaucophyta. The phycobiliproteins are further classified on the basis of their spectral properties into three major subgroups: phycocyanin, allophycocyanin and phycoerythrin. Their composition varies with the species and environmental conditions of the source organism (Chu 2012). Due to their fluorescent properties, they were adopted for use in diverse applications such as fluorescence-activated cell sorting, flow cytometry and histochemistry soon after their introduction as pigmented molecules in 1982. They can also be used as markers for electrophoresis, isoelectric focusing and size-exclusion chromatography due to their high absorptivity in visible light wavelengths.

32.2.1 Structure

Phycobiliproteins are composed of apoproteins (α and β subunits) covalently linked to prosthetic

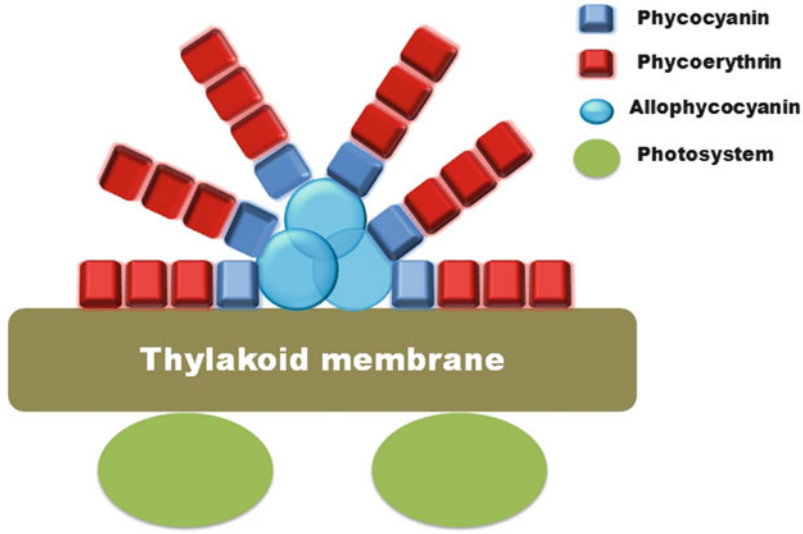


Fig. 32.1 Structure of a phycobilisome

groups called phycobilins. Phycobilins are open-chain, tetrapyrrole chromophores sharing structural similarity with the bile pigment bilirubin (Glazer 1989). The two conserved subunits, α and β, form an (αβ) monomer, which are further aggregated to form trimers $(\alpha\beta)_3$ and disc-shaped hexamers $(\alpha\beta)_6$. The trimeric and hexameric structures form the functional units of PE and PC. In a complete LHC, also termed as a phycobilisome, the central core is occupied with rods of APC joined to disc-shaped hexameric PC and PE which extend outwards as antennae (Fig. 32.1). The light energy is captured by PE and is transferred to chlorophyll for further reaction via PC and APC. The absorption maxima vary from 562 to 568 nm for C-PE, 615 to 620 nm for C-PC and 650 to 652 nm for APC (MacColl 1998) (Table 32.2).

32.2.2 Extraction and Purification of Phycobiliproteins

The extraction of phycobiliproteins chiefly involves cell disruption in a buffered environment after which the crude extract is either centrifuged or filtered to remove cellular debris. Cell disruption is done through ultrasonication, freeze-thaw cycles using liquid nitrogen, cavitation using nitrogen gas, osmotic shock, enzymatic treatments or high-pressure homogenization (Table 32.3).

Wet biomass is directly utilized for the extraction of these proteins as high-temperature drying usually results in a lower-quality product or a lower yield. Usually, 0.05 or 0.1 M phosphate buffer pH 7.0 or 7.2 is used as the extraction buffer although 0.5 M ammonium sulphate is also used.

Purification of these proteins is usually done using ammonium sulphate precipitation, polyethylene glycol precipitation, ion-exchange or size-exclusion chromatography, expanded bed chromatography or membrane filtration to get their purified forms. More often than not, a combination of these techniques is used to reach the desired purity level and the source organism. Drying is usually performed using lyophilization which prevents denaturation of the pigment. The measure of purification is determined by calculating the purity ratio, a ratio of the absorbance of the particular phycobiliprotein at its absorption maxima to that of aromatic amino acids in all proteins at 280 nm. For example, the purity ratio

Table 32.2 Spectral and physical properties of cyanobacterial phycobiliproteins

Phycobiliproteins	Absorbance maxima (nm)	Fluorescence emission (nm)	Molecular weight (kDa)	Absorptivity ($L\ g^{-1}\ cm^{-1}$)	Molar absorptivity $(M\text{-}cm)^{-1}$
C-phycocyanin	615	647	108	7.0	1.54
C-phycoerythrin	566	617	55	8.0	0.44
Allophycocyanin	652	660	100	7.3	0.73

Table 32.3 Different methodologies adopted for the extraction of phycobiliproteins

Extraction method	Phycobiliprotein	Name of species	References
Freeze-thaw and sonication	Phycocyanin	*Spirulina platensis*	Zhang and Chen (1999)
High-pressure homogenization	Phycocyanin	*Spirulina platensis*	Patel et al. (2004), Song et al. (2013) and Seo et al. (2013)
Freeze-thaw	Phycoerythrin, phycocyanin, allophycocyanin	*Spirulina platensis*, *Phormidium* sp. A27DM, *Lyngbya* sp. A09DM, *Halomicronema* sp. A32DM, *Pseudanabaena tenuis*, *Spirulina fusiformis*, *Arthronema africanum*, *Calothrix* sp., *Oscillatoria quadripunctulata*, *Pseudanabaena* sp.	Minkova et al. (2007), Santiago-Santos et al. (2004), Soni et al. (2010), Minkova et al. (2007), Mishra et al. (2008), Su et al. (2010), Cano-Europa et al. (2010), Parmar et al. (2011) and Mishra et al. (2011)
Sonication	Phycoerythrin	*Cyanosarcina* sp. SK40, *Phormidium* sp. PD40-1, *Scytonema* sp. TP40, *Leptolyngbya* sp. KC45	Pumas et al. (2011)
Variable speed stirring	Phycocyanin	*Anabaena marina* ATCC 33047	Ramos et al. (2010)
Nitrogen cavitation	Phycobiliproteins	*Synechococcus* sp.	Viskari and Colyer (2003)
Lysozyme treatment	Phycocyanin	*Synechococcus* sp.	Gupta and Sainis (2010)

of C-PE is calculated by A_{568}/A_{280} and for C-PC using A_{620}/A_{280}. The absorbance values are considered within a range of 0.05–1 at the absorptive maxima of the phycobiliprotein (Bennett and Bogorad 1973). A ratio greater than 1 is generally considered food grade, while a ratio greater than 4 is considered as analytical grade purity.

The purified forms of the proteins are generally stable in phosphate buffer pH 7.0 or 7.2 or in ammonium sulphate suspensions. The latter are usually dialyzed against the corresponding buffer before use. They are stored in temperatures 4–10 °C in the dark to reduce the effects of light.

32.2.3 Applications of Phycobiliproteins

32.2.3.1 As Food Colourants

Natural colouring agents have always held an upper hand when it comes to the food industry. Due to the toxic nature of synthetic colourants and the necessity of colour additives for food processing, there is an increased awareness and curiosity for natural options in this field. However, studies are still underway for the stability of such proteins in pH ranges used in commercial food manufacturing industries worldwide. PBPs are used as natural food colourants in chewing gums,

jellies, ice creams and fermented milk products since many of the synthetic dyes used globally are thought to be possible carcinogens. Their other advantages include their intense colours and high solubility in water (Santiago-Santos et al. 2004; Chakdar et al. 2012). A lower stability to temperature and light has not deterred the food processing industry to use C-PC as an alternative to synthetic dyes such as gardenia and indigo. A study reported that C-PC was insoluble in acidic solutions (pH 3) and denatures at temperatures above 45 °C (Jespersen et al. 2005).

Additionally, the fluorescent properties of PE have been exploited to produce transparent lollipops, cake decorations and soft drinks and alcoholic beverages that fluoresce at pH 5–6. These special effects were tried out to increase the marketability of the respective food items (Dufosse et al. 2005). Although still not approved for use in the USA and European Union (EU), the US Food and Drug Administration (US FDA) has been approached by Desert Lake Technologies in 2012 for grant of generally recognized as safe (GRAS) status to C-PC (CyaninPlus™) developed by them (FDA, GRAS 2012).

32.2.3.2 As Pharmaceutical Agents

PBPs have been recognized as beneficial pharmaceutical agents since many years, and the fact has been reliably established through studies. Oriental cuisine and medicine have traditionally been rich in microalgae since ancient times, but it is only now that their beneficial effects are being investigated scientifically (Bocanegra et al. 2009). The current total market value of PBP products has been estimated to be US $60 million (Borowitzka 2013). The nutritional and therapeutic aspects of *Spirulina*, a blue-green algae, have been critically reviewed which have proved that the beneficial aspects of the cyanobacteria can be attributed to its C-PC content among other things (Kay and Barton 1991; Mishra 2006).

32.2.3.3 As Antioxidants

The antioxidant properties of PC have been well documented over the years. According to published research, C-PC successfully reduced lipid peroxidation and oxidative haemolysis in normal human erythrocytes induced by a free-radical generator, AAPH. C-PC extract from *Aphanizomenon flos-aquae*, a cyanobacterium, was also found to significantly inhibit lipid peroxidation in blood plasma by Cu^{+2} (Benedetti et al. 2004). In vitro studies have established C-PC as an antioxidant, anti-inflammatory, neuroprotective, nephroprotective and hepatoprotective agent (Romay et al. 1998; Farooq et al. 2004; Mishra 2006; Sekar and Chandramohan 2008). Radical scavenging activity of C-PC includes scavenging peroxyl, peroxynitrite and hydroxyl free radicals while preventing or inhibiting lipid peroxidation and DNA damage (Bhat and Madhyastha 2001; Bermejo et al. 2008). C-PC has been demonstrated to significantly reduce hippocampal cell death in gerbils and rats (Thaakur and Sravanthi 2010; Penton-Rol et al. 2011), reduces necrosis and inflammation in hepatic cells (Gonzalez et al. 2003; Sekar and Chandramohan 2008; Kuriakose and Kurup 2010) and decreases Kupffer cell phagocytosis (Remirez et al. 2002). C-PE from *Pseudanabaena tenuis* was examined for its antioxidant ability in mice model fed with mercury and found to reduce the extent of damage in all animals (Cano-Europa et al. 2010).

32.2.3.4 As Anti-inflammatory Molecules

C-PC was also analysed as an anti-inflammant in human models where its ability to effectively inhibit the activity of cyclooxygenase-2, an enzyme involved in the process of inflammation, was studied. It was found that although C-PC effectively inhibited the said enzyme, reduced PC or the isolated PCBs were poor inhibitors without being selective for the enzyme. The results suggest that the apoprotein of C-PC may have an important role to play in the anti-inflammatory activity of C-PC (Reddy et al. 2000). Another study analysed the in vivo effect of excessive C-PE dosage in test mice to evaluate the potential risks due to overdosage. It was found that C-PE had no deleterious effect on the body weight, food intake or toxicity signs even at a dosage of 2,000 mg kg^{-1} body weight C-PE. This is a significant finding for adopting a C-PE based

treatment approach since it indicates no negative health effects through overdosage (Soni et al. 2010).

32.2.3.5 As Anticancer Agents

The anticancer activity of C-PC was studied using human chronic myeloid leukaemia cell line K562. It was observed that as little as a 50 μM dose decreased the proliferation of K562 cells by 49 % for 48 h (Subhashini et al. 2004). Also, C-PC induced apoptosis in prostate cancer (LNCaP) cell line by diminishing the required dosage of topotecan, an anticancer medication which frequently causes adverse side reactions in patients (Gantar et al. 2012). In another study involving human hepatoma cell line (HepG2), C-PC led to a reduction in the proliferation of the cells with the highest reduction observed at a concentration of 7 μg/ml C-PC along with a loss of nuclear entities due to fragmentation (Basha et al. 2008). A separate study has examined the healing effect of C-PC on workers exposed to nuclear radiations in a nuclear power plant. The study was carried out as part of a publication on nuclear power plant operations, safety and environment. It was found that C-PC has the ability to influence repair of damaged DNA, essential for the preservation of genomic integrity. However, the protein also showed DNA lesion in subjects exposed to high doses of radiation; the lesions were not found to be persistent. This may be attributed to the adaptive phenomena due to the chronic adaptation exposure. Although the results are promising, the authors categorically state that the study should be treated as pilot one with the need for further experiments to prove conclusively the role played by PBP such as PC in DNA repair mechanisms (Stankova et al. 2011).

The studies firmly establish the anticancer properties of C-PC that might open up an exciting avenue for medical treatments for various life-threatening cancers. Although the findings are rather sporadic instead of being coherently directed towards a particular type of cancer, the promising results are sure to encourage researchers to focus more on specific types of cancer.

32.2.3.6 As Fluorescing Molecules

The fluorescence properties of PBP have played an important role in the development of various fluorescence-based techniques including fluorescence-activated cell sorting (FACS), flow cytometry, protein-protein conjugation and fluorescence immunoassays and fluorescence microscopy (Mishra 2006). There are many reasons which can explain the advantages of using PBP as fluorescent molecules, such as (1) low interference by other molecules due to absorption and emission at far red end of spectrum, (2) a large Stokes shift of 80 nm or more which minimizes noise due to other phenomena, (3) high solubility in water leading to minimal side reactions, (4) quantum yield independent of pH and (5) protection from quenching by other biological molecules (Kronick and Grossman 1983). An excellent overview of the relevant properties of PBPs has been provided in Glazer (1994). The isoelectric points of the PBP range from 4.7 to 5.3.

32.2.3.7 Bioconjugates

Conjugation of proteins with PBP has attracted much interest due to their highly sensitive detection characteristics and multiparameter detection. A recent US patent application has claimed a process to develop fluorescent kits using PBP and chemical dyes attached together to take advantage of the intermolecular energy transfer phenomena (Mao et al. 2012). However, the conjugation studies and applications are not new. The utility of such conjugated molecules for cell cytometry applications and diagnostic procedures was recognized long time ago (Stryer and Glazer 1985). The only limiting factor has been the molecular weight of the PBP (200 kDa) which hinders their diffusion into cells of interest and hence limits their applicability to antibody conjugates for flow cytometry and enzyme-linked immunosorbent assays (Giepmans et al. 2006). Another example of a conjugated PBP with a suitable dye molecule utilizes R-PE and compares the fluorescence of the pair to that of native R-PE to assess energy transfer from the PBP to the dye. The transfer efficiency was found to be >99 %. The conjugate was used to label streptavidin that retained the fluorescence properties and was

useful in flow cytometry applications (Diwu et al. 2012). The fluorescence properties of B-PE were studied in a nonpolar environment using AOT (sodium bis-(2-ethylhexyl)sulphosuccinate)/water/isooctane micro-emulsions. AOT is an anionic surfactant that can solubilize small quantities of water in various nonpolar solvents. Results indicated that the stability of B-PE in water droplets inside the emulsion is enhanced than the protein that is in the aqueous state. It may be that the protein inside the water droplet retains the same configuration and hence its fluorescence properties, but the chromophores are more protected inside the emulsified environment (Bermejo et al. 2003).

PBPs are extremely amenable to bioconjugation and have bright chromophores. These factors have together contributed to their being used as fluorophores conjugated to various other molecules using standard chemistry. The only drawbacks of the process are that the reactions should be suitable for a biological origin molecule and not disturb the original configuration to avoid a loss of fluorescence. Although limited reports are available for PBP as nanomaterial, commercial applications for the few discovered bioconjugates have already been in use for some time. Commercial ventures are already manufacturing and marketing PBP-conjugated fluorescent dyes and antibodies for flow cytometry and immunolabelling, respectively (Sapsford et al. 2013).

32.3 Carotenoids

Carotenoids are composed of more than 600 natural lipid-soluble pigments with their colour ranging from yellow to red and are found predominantly in plants (Takaichi 2000; Kleinegris et al. 2010). The structures of carotenoids differ in cyclization (one or both ends of the molecule), hydrogenation level and functional groups (Dutta et al. 2005). Some carotenoids are found in both plants and algae, while some are limited only to algae (Takaichi 2011).

Chemical synthesis is a low-cost method for obtaining high-purity carotenoids, but its major drawback is the non-biological reaction precursors/by-products which may have deleterious health effects, and hence, it is suggested to find economical carotenoid production of biological origin. The modern tools of bioprocessing and recombinant DNA technology can significantly increase carotenoid production. Also, it is important to know that there are several disadvantages associated with the production of carotenoids from food such as complicated extraction and purification process, season fluctuation, limited resources, etc. (Asker et al. 2012).

Carotenoids are isoprenoid compounds made up of 40-carbon (C_{40}) backbone synthesized via head-to-head condensation of two geranylgeranyl diphosphate (GGDP, C_{20}) molecules. Naturally occurring carotenoids are generally *trans* in nature (Dutta et al. 2005). They are synthesized in living systems by carotenogenesis pathways, which have been extensively studied in cyanobacteria (Takaichi 2011). Carotenoids exhibit different properties like singlet oxygen quencher, binding affinity for hydrophobic surfaces, antitumor activity, provitamin A activity, anti-inflammatory activity, hepatoprotective activity and antioxidant activity and are also a part of cellular communication, immune-modulation activity such as decrease in UV-induced immune suppression and increase the activity of natural killer cells (van den Berg 1999; Dutta et al. 2005; Vílchez et al. 2011; Han et al. 2012).

Carotenoids are mainly classified into two subgroups (Sergio et al. 1999):

(a) Carotenes: Hydrocarbons consisting of specific end groups, e.g. lycopene and ß-carotene.
(b) Xanthophylls: Oxygenated carotenoids. Xanthophylls are further subdivided depending upon the type of functional groups attached:
    (i) Containing hydroxyl groups, e.g. zeaxanthin and lutein.
    (ii) Containing methoxy group, e.g. spirilloxanthin
    (iii) Containing oxo group, e.g. echinenone
    (iv) Containing epoxy group, e.g. antheraxanthin

Carotenoids can also be classified as primary and secondary carotenoids. Among them, primary

carotenoids play a crucial role in the photosynthetic organisms, while the secondary carotenoids are not essential for photosynthesis and are localized either in plastoglobules or in cytosolic lipid droplets; they are produced under stress conditions and can be accumulated to high levels (Sasso et al. 2012).

32.3.1 Microalgal Sources

Different microalgal strains of research interest are *Dunaliella salina, Sarcina maxima, Chlorella protothecoides, Chlorella vulgaris* and *Haematococcus pluvialis* for the commercialization of carotenoid production (Lordan et al. 2011). The table below depicts different microalgae as sources of carotenoids (Table 32.4).

32.3.2 Factors Affecting Production of Carotenoids

The growth conditions and environmental parameters are important parameters that control carotenoid accumulation in organisms (Walter and Strack 2011). The factors that affect carotenoid production in marine microalgae are described below:

1. Light
   There are different theories on which photostimulation of carotenoid synthesis depends; one describes high light intensity, and other focuses on high illumination time to cause a rise in carotenoid concentration (Bhosale 2004).
2. Temperature
   Temperature plays a crucial role in carotenoid production; a decline in thermal conditions from 34 °C to 17 °C caused a 7.5 times increase in α-carotene content in *Dunaliella* sp. (Bhosale 2004).
3. Nutrients
   Nannochloropsis gaditana deprived of phosphate/sulphur causes an improvement in zeaxanthin concentration due to rapid inhibition of PSII driven by S-limitation that diminishes the primary photosynthetic product formation, i.e. NADPH and Fd_{red} which later on caused insufficient ascorbate supply for the

Table 32.4 Microalgal sources of different carotenoids

Carotenoids	Sources	Reference
α-carotene	*Dunaliella salina*	Christaki et al. (2013)
ß-carotene	*Dunaliella salina*, *Botryococcus braunii*, *Spirulina platensis*, *Chlorococcum* sp., *Synechocystis* sp., *Parietochloris incisa*	Del Campo et al. (2007), Solovchenko et al. (2008) and Ranga Rao et al. (2010)
Lutein	*Muriellopsis sp*., *Scenedesmus almeriensis*, *Chlorella protothecoides*, *Chlorella zofingiensis*, *Botryococcus braunii*, *Neospongiococcus gelatinosum*, *Chlorococcum citriforme*	Fernández-Sevilla et al. (2010), Del Campo et al. (2007), Ranga Rao et al. (2010), Cuaresma et al. (2011) and Durmaz et al. (2009)
	Chlamydomonas acidophila, *Diacronema vlkianum*	
Astaxanthin	*Haematococcus pluvialis*, *Botryococcus braunii*, *Chlorella zofingiensis*, *Scotiellopsis oocystiformis*, *Neochloris wimmeri*, *Diacronema vlkianum*, *Euglena rubida*	Christaki et al. (2013), Ranga Rao et al. (2010), Del Campo et al. (2004), Orosa et al. (2000), Zhang and Lee (1997) and Durmaz et al. (2009)
Zeaxanthin	*Dunaliella salina*, *Spirulina* sp., *Microcystis aeruginosa*, *Botryococcus braunii*, *Chlamydomonas acidophila*	Christaki et al. (2013), Ranga Rao et al. (2010), Sajilata et al. (2008) and Cuaresma et al. (2011)
Fucoxanthin	*Phaeodactylum tricornutum*, *Cylindrotheca closterium*, *Eustigmatos magnus*, *Eustigmatos polyphem*, *Eustigmatos vischeri*, *Vischeria helvetica*, *Vischeria punctata*, *Vischeria stellata*	Kim et al. (2012) and Li et al. (2012a, b)
Canthaxanthin	*Anabaena* spp.	Shahidi and Brown (1998)

xanthophyll cycle and hence reduced xanthophyll biosynthesis (Forján et al. 2007).

4. Metal ions/salts
   Addition of ferrous salt increases the hydroxyl radical which, in turn, promotes cellular carotenoid synthesis in *Haematococcus pluvialis*. This method can substitute high light illumination which is costlier and an energy-intensive process. *Chlorococcum* spp. has provided similar results in the presence of inorganic salts (Bhosale 2004).

32.3.3 Extraction of Carotenoids

The major issue in microalgal biotechnology is the downstream processing where microalgal biomass harvesting remains a prominent research area. Experience suggests that effective harvesting technology is completely dependent on strain characteristics (Del Campo et al. 2007). There is no defined protocol for the extraction of carotenoids as various factors contribute in the transformation or degradation during their extraction; various precautions like dim light, antioxidants need to be taken to prevent photo-damage and oxidation (Oliver and Palou 2000). Extraction can be performed using organic solvents like hexane, methanol and acetone, but they are not recommended due to their toxicity. Green solvents like vegetable oils or supercritical CO_2 are more suitable for this process (Wiltshire et al. 2000; Macías-Sánchez et al. 2008; Guedes et al. 2011; Christaki et al. 2013).

32.3.4 Properties of Carotenoids

32.3.4.1 Provitamin A Activity

Provitamin A activity is the conversion of provitamin A into vitamin A, whose deficiency leads to premature deaths, particularly among children. About 10 % of the natural carotenoids have the ability to get converted into retinol which has provitamin A activity. ß-carotene has 100 % provitamin A activity (Zeb and Mehmood 2004; Vílchez et al. 2011).

32.3.4.2 Antioxidant Activity

Reactive oxygen species (ROS) include both free radicals and non-radical oxidants which are the most reactive molecular species responsible for DNA, protein and lipid degradation (Pérez-Rodríguez et al. 2009). Carotenoids have the ability to scavenge singlet molecular oxygen and peroxyl radicals, which makes them strong antioxidants (Sies and Stahl 2004). These properties help them in preventing chronic and degenerative diseases like cancer. A study shows that the risk of colon cancer gets reduced due to the inclusion of ß-carotene in the diet (Vílchez et al. 2011). High ß-carotene doses show better CD_4 to CD_8 lymphocyte ratio (Christaki et al. 2013).

32.3.4.3 Membrane Stabilization

Researchers have reported mechanical stabilization of liposomal membranes by carotenoids such as zeaxanthin at higher temperatures (Hara et al. 1999). They form a complex molecular structure with lipid membranes and control the dynamics and physical properties of lipid membranes, protecting lipid peroxidation (Popova and Andreeva 2013). It was also found that incorporation of carotenoids into membrane decreases their permeability, whereas polar carotenoids on phospholipids mimic as cholesterol and play important role in the modulation of membranes which does not contain cholesterol (Gruszecki and Strzalka 2005).

32.3.5 Practical Applications

32.3.5.1 Molecular Photovoltaic Nanomaterial Precursors

The total energy, dipole moment, isotropic polarizability and molecular structure of the carotenoids make them eligible candidates for applications in dye-sensitized solar cells (DSSC). There has been a comparative study on the ionization potential and electron affinity of the carotenoids to validate them for the above purpose (Ruiz-Anchondo et al. 2010).

32.3.5.2 Food Industry/Food Colourants

Carotenoids are the precursors of various flavouring and odouring agents. They also function as colour enhancers and hence have a wide use in the food and feed industry. ß-carotene can be of use in food and beverages such as fruit juices, soft drinks and confectionery to improve their appearance and also because of their antioxidant properties (Christaki et al. 2013). The application of carotenoids in the food industry is limited due to their poor water solubility and low bioavailability which can be overcome through their encapsulation into nano-emulsions (Qian et al. 2012).

32.4 Conclusions

Microalgal pigment production is the most significant area of research in the field of blue biotechnology. Classically, pigments have been produced synthetically, but a rising demand for natural pigments has promoted large-scale cultivation of microalgae for pigment production. The enzymes and genes required for the regulation and control of biosynthesis of pigment production need to be investigated along with their applications to enhance their productivity. The extraction process of the pigments can be improved by simultaneous extraction of lipids or other bioactive molecules to offset the single product production cost. Further to this, the interaction of pigments with other biological molecules and pigment-based nanostructure is an area which is still unravelled and can be explored in more detail.

Acknowledgements CP, TG and RM wish to thank AcSIR for Ph.D. enrolment and CSIR for Senior Research Fellowship. Authors would also like to thank Dr. P.K. Ghosh, Director, CSIR-CSMCRI, and Prof. Bir Bahadur for encouraging and providing an opportunity to gain an in-depth knowledge on the subject while formulating the chapter. Sincere thanks are also due to Dr. Arvind Kumar (DC, SMC) for providing financial support through SIP Project (CSC-0203) and Dr. Basil George (DST Young Scientist) along with all the present and ex-laboratory colleagues for their continuous support.

References

Ahren TJ, Katoh S, Sada E (1983) Arachidonic acid production by the red alga *Porphyridium cruentum*. Biotechnol Bioeng 25:1057–1070

Asker D, Awad TS, Beppu T, Ueda K (2012) Isolation, characterization, and diversity of novel radiotolerant carotenoid-producing bacteria. In: José-Luis Barredo (ed) Microbial carotenoids from bacteria and microalgae. Humana Press, NY, US, pp 21–60

Barclay W, Zeller S (1996) Nutritional enhancement of n-3 and n-6 fatty acids in rotifers and *Artemia nauplii* by feeding spray-dried *Schizochytrium* sp. J World Aquacult Soc 23:314–322

Basha O, Hafez R, El-Ayouty Y, Mahrous K, Bareedy M, Salama A (2008) C-Phycocyanin inhibits cell proliferation and may induce apoptosis in human HepG2 cells. Egypt J Immunol 15:161–167

Benedetti S, Benvenuti F, Pagliarani S, Francogli S, Scoglio S, Canestrari F (2004) Anti-oxidant properties of a novel phycocyanin extract from the blue-green alga *Aphanizomenon flos-aquae*. Life Sci 75:2353–2362

Bennett A, Bogorad L (1973) Complementary chromatic adaptation in a filamentous blue-green alga. J Cell Biol 58:419–435

Bermejo R, Tobaruela DJ, Talavera EM, Orte A, Alvarez-Pez JM (2003) Fluorescent behavior of B-phycoerythrin in microemulsions of aerosol OT/water/isooctane. J Colloid Interface Sci 263:616–624

Bermejo P, Pinero E, Villar AM (2008) Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of *Spirulina platensis*. Food Chem 110:426–435

Bhat VB, Madhyastha KM (2001) Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from *Spirulina platensis*: protection against oxidative damage to DNA. Biochem Biophys Res Commun 285:262–266

Bhosale P (2004) Environmental and cultural stimulants in the production of carotenoids from microorganisms. Appl Microbiol Biotechnol 63:351–361

Bocanegra A, Bastida S, Benedi J, Rodenas S, Sanchez-Muniz FJ (2009) Characteristics and nutritional and cardiovascular-health properties of seaweeds. J Med Food 12:236–258

Borowitzka MA (1993) Products from microalgae. Infofish Int 5:21–26

Borowitzka MA (2013) High-value products from microalgae – their development and commercialisation. J Appl Phycol 25:1–14

Cano-Europa E, Ortiz-Butrón R, Gallardo-Casas C, Blas-Valdivia V et al (2010) Phycobiliproteins from *Pseudanabaena tenuis* rich in C-phycoerythrin protect against $HgCl_2$-caused oxidative stress and cellular damage in the kidney. J Appl Phycol 22:495–501

Chakdar H, Jadhav SD, Dhar DW, Pabbi S (2012) Potential applications of blue green algae. J Sci Ind Res 71:13–20

Christaki E, Bonos E, Giannenas I, Florou-Paneri P (2013) Functional properties of carotenoids originating from algae. J Sci Food Agric 93:5–11

Chu WL (2012) Biotechnological applications of microalgae. Intl E-J Sci Med Edu 6:S24–S37

Cohen Z, Heimer YM (1992) Production of polyunsaturated fatty acids (EPA, ARA and GLA) by the microalgae *Porphyridium* and *Spirulina*. In: Kyle DJ, Ratledge C (eds) Industrial applications of single cell oils. AOCS Publishing, CRC Press, Champaign, pp 243–273

Cuaresma M, Casal C, Forján E, Vílchez C (2011) Productivity and selective accumulation of carotenoids of the novel extremophile microalga *Chlamydomonas acidophila* grown with different carbon sources in batch systems. J Ind Microbiol Biotechnol 38:167–177

De Jesus Raposo MF, de Morais RMSC, de Morais AMMB (2013) Health applications of bioactive compounds from marine microalgae. Life Sci 93:479–486

Del Campo JA, García-González M, Guerrero MG (2007) Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. Appl Microbiol Biotechnol 74(6):1163–1174

Del Campo JA, Rodríguez H, Moreno J, Vargas M, Rivas J, Guerrero MG (2004) Accumulation of astaxanthin and lutein in *Chlorella zofingiensis* (Chlorophyta). Appl Microbiol Biotechnol 64:848–854

Diwu Z, Zhang J, Tang Y, Guobing X (2012) Fluorometric analysis kit. U.S. Patent No. 8,105,829. U.S. Patent and Trademark Office, Washington, DC

Drokova TG, Popova RJ (1974) On the content of tocopherol in the alga *Dunaliella salina* Teod. Ukr Bot Zhou 31:229–231

Dufosse L, Galaup P, Yaron A, Arad SM, Blanc P, Murthy KNC, Ravishankar GA (2005) Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? Trends Food Sci Technol 16:389–406

Durmaz Y, Donato M, Monteiro M, Gouveia L, Nunes ML, Pereira TG, Gökpinar S, Bandarra NM (2009) Effect of temperature on α-tocopherol, fatty acid profile, and pigments of Diacronema vlkianum (Haptophyceae). Aquac Int 17(4):391–399

Dutta D, Chaudhuri UR, Chakraborty R (2005) Structure, health benefits, antioxidant property and processing and storage of carotenoids. Afr J Biotechnol 4:510–1520

Eisenreich W, Bacher A, Arigoni D, Rohdich F (2004) Biosynthesis of isoprenoids via the non-mevalonate pathway. Cell Mol Life Sci 61:1401–1426

Farooq SM, Asokan D, Sakthivel R, Kalaiselvi P, Varalakshmi P (2004) Salubrious effect of C-phycocyanin against oxalate-mediated renal cell injury. Clin Chim Acta 348:199–205

Fernández-Sevilla J, AciénFernández FG, Molina Grima E (2010) Biotechnological production of lutein and its applications. Appl Microbiol Biotechnol 86:27–40

Forján E, Garbayo I, Casal C, Vílchez C (2007) Enhancement of carotenoid production in *Nannochloropsis* by phosphate and sulphur limitation. In: Méndez-Vilas A (ed) Communicating current research and educational topics and trends in applied microbiology. Formatex, Spain, pp 356–364

Gantar M, Dhandayuthapani S, Rathinavelu A (2012) Phycocyanin induces apoptosis and enhances the effect of topotecan on prostate cell line LNCaP. J Med Food 15:1091–1095

Giepmans BNG, Adams SR, Ellisman MH, Tsien RY (2006) The fluorescent toolbox for assessing protein location and function. Science 312:217–224

Glazer AN (1989) Light guides directional energy transfer in a photosynthetic antenna. J Biol Chem 264:1–4

Glazer A (1994) Phycobiliproteins – a family of valuable, widely used fluorophores. J Appl Phycol 6:105–112

Glazer AN, Stryer L (1985) Phycobiliprotein fluorescent conjugates. U.S. Patent No. 4,542,104. U.S. Patent and Trademark Office, Washington, DC

González R, Gonzalez A, Remirez D, Romay C, Rodriguez S, Ancheta O, Merino N (2003) Protective effects of phycocyanin on galactosamine-induced hepatitis in rats. Biotecnol Apl 20:107–110

Gordon PT, Ratliff V (1992) The implication of omega−3 fatty acids in human health. In: Flick GJ Jr, Martin RE (eds) Advances in seafood biochemistry—composition and quality. Technomic Publishing, Lancaster

Gruszecki WI, Strzałka K (2005) Carotenoids as modulators of lipid membrane physical properties. Biochim Biophys Acta 1740:108–115

Guedes ACA (2010) Production, extraction and characterization of selected metabolites from microalgae and cyanobacteria. PhD thesis, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto

Guedes AC, Amaro HM, Malcata FX (2011) Microalgae as sources of carotenoids. Mar Drugs 9:625–644

Gupta A, Sainis J (2010) Isolation of C-phycocyanin from *Synechococcus* sp., (*Anacystis nidulans* BD1). J Appl Phycol 22:231–233

Guschina IA, Harwood JL (2006) Lipids and lipid metabolism in eukaryotic algae. Prog Lipid Res 45:160–186

Guzman S, Gato A, Lamela M, Freire-Garabal M, Calleja JM (2003) Anti-inflammatory and immunomodulatory activities of polysaccharide from *Chlorella stigmatophora* and *Phaeodactylum tricornutum*. Phytother Res 17:665–670

Guzmán-Murillo MA, López-Bolaños CC, Ledesma-Verdejo T, Roldan-Libenson G, Cadena-Roa MA, Ascencio F (2007) Effects of fertilizer-based culture media on the production of exocellular polysaccharides and cellular superoxide dismutase by *Phaeodactylum tricornutum* (Bohlin). J Appl Phycol 19:33–40

Han R-M, Zhang J-P, Skibsted LH (2012) Reaction dynamics of flavonoids and carotenoids as antioxidants. Molecules 17:2140–2160

Hara M, Yuan H, Yang Q, Hoshino T, Yokoyama A, Miyake J (1999) Stabilization of liposomal membranes by thermozeaxanthins: carotenoid-glucoside esters. BBA-Biomembr 1461:147–154

Hasler CM (2002) Functional foods: benefits, concerns and challenges—a position paper from the American council on science and health. J Nutr 132:3772–3781

Ho SH, Chen CY, Chang JS (2012) Effect of light intensity and nitrogen starvation on CO_2 fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N. Bioresour Technol 113:244–252

Jespersen L, Stromdahl LD, Olsen K, Skibsted LH (2005) Heat and light stability of three natural blue colorants for use in confectionery and beverages. Eur Food Res Technol 220:261–266

Kay RA, Barton LL (1991) Microalgae as food and supplement. Crit Rev Food Sci Tech 30:555–573

Kim S, Jung Y-J, Kwon O-N, Cha K, Um B-H, Chung D, Pan C-H (2012) A potential commercial source of fucoxanthin extracted from the microalga *Phaeodactylum tricornutum*. Appl Biochem Biotechnol 166:1843–1855

Kleinegris DM, Janssen M, Brandenburg W, Wijffels R (2010) The selectivity of milking of *Dunaliella salina*. Mar Biotechnol 12:14–23

Kronick MN, Grossman PD (1983) Immunoassay techniques with fluorescent phycobiliprotein conjugates. Clin Chem 29:1582–1586

Kuriakose GC, Kurup MG (2010) Hepatoprotective effect of *Spirulina lonar* on paracetamol induced liver damage in rats. Asian J Exp Biol Sci 1:614–623

Li Z, Sun M, Li Q, Li A, Zhang C (2012a) Profiling of carotenoids in six microalgae (Eustigmatophyceae) and assessment of their β-carotene productions in bubble column photobioreactor. Biotechnol Lett 34:2049–2053

Li Z, Ma X, Li A, Zhang C (2012b) A novel potential source of β-carotene: *Eustigmatos* cf. *polyphem* (Eustigmatophyceae) and pilot β-carotene production in bubble column and flat panel photobioreactors. Bioresour Technol 117:257–263

Lordan S, Ross RP, Stanton C (2011) Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. Mar Drugs 9:1056–1100

MacColl R (1998) Cyanobacterial phycobilisomes. J Struct Biol 124:311–334

Macías-Sánchez MD, Mantell Serrano C, Rodríguez Rodríguez M, Martínez de la Ossa E, Lubián LM, Montero O (2008) Extraction of carotenoids and chlorophyll from microalgae with supercritical carbon dioxide and ethanol as cosolvent. J Sep Sci 31:1352–1362

Mao F, Leung W, Cheung C, Hoover HE (2012) Fluorescent dyes, fluorescent dye kits and methods of preparing labelled molecules. US Patent Application 2012/0329068 A1

Markou G, Nerantzis E (2013) Microalgae for high-value compounds and biofuels production: a review with focus on cultivation under stress conditions. Biotechnol Adv 31:1532–1542

Minkova K, Tchorbadjieva M, Tchernov A, Stojanova M, Gigova L, Busheva M (2007) Improved procedure for separation and purification of *Arthronema africanum* phycobiliproteins. Biotechnol Lett 29:647–651

Mishra S (2006) Phycobiliproteins from microalgae. In: Tewari A (ed) Recent advances on applied aspects of Indian marine algae with reference to global scenario. CSMCRI Publications, Bhavnagar, Gujarat, India, pp 273–284

Mishra SK, Shrivastav A, Mishra S (2008) Effect of preservatives for food grade C-PC from *Spirulina platensis*. Process Biochem 43:339–345

Mishra SK, Shrivastav A, Mishra S (2011) Preparation of highly purified C-phycoerythrin from marine cyanobacterium *Pseudanabaena* sp. Protein Express Purif 80:234–238

Miyamoto K (1997) Renewable biological systems for alternative sustainable energy production. Food and Agricultural Organization, United Nations, Rome

Mohamed ZA (2008) Polysaccharides as a protective response against microcystin – induced oxidative stress in Chlorella vulgaris and *Scenedesmus quadricauda* and their possible significance in the aquatic ecosystem. Ecotoxicology 17:504–516

Molina GE, Belarbi EH, Robles Medina A, Acien Fernandez FG, Chisti Y (2003) Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol Adv 20:491–515

Oliver J, Palou A (2000) Chromatographic determination of carotenoids in foods. J Chromatogr A 881:543–555

Orosa M, Torres E, Fidalgo P, Abalde J (2000) Production and analysis of secondary carotenoids in green algae. J Appl Phycol 12:553–556

Paiva SAR, Russell RM (1999) β-Carotene and other carotenoids as antioxidants. J Am Coll Nutr 18:426–433

Parmar A, Singh NK, Kaushal A, Sonawala S, Madamwar D (2011) Purification, characterization and comparison of phycoerythrins from three different marine cyanobacterial cultures. Bioresour Technol 102: 1795–1802

Patel A, Mishra S, Pawar R, Ghosh PK (2004) Purification and characterization of C-Phycocyanin from cyanobacterial species of marine and freshwater habitat. Protein Express Purif 40:248–255

Pentón-Rol G, Marín-Prida J, Pardo-Andreu G, Martínez-Sánchez G, Acosta-Medina EF, Valdivia-Acosta A, Lagumersindez-Denis N, Rodríguez-Jiménez E, Llópiz-Arzuaga A, López-Saura PA et al (2011) C-Phycocyanin is neuroprotective against global cerebral ischemia/reperfusion injury in gerbils. Brain Res Bull 86:42–52

Pérez-Rodríguez L (2009) Carotenoids in evolutionary ecology: re-evaluating the antioxidant role. Bioessays 31:1116–1126

Popova AV, Andreeva AS (2013) Carotenoid-lipid interactions. Adv Planar Lipid Bilayers Liposomes 17:215–236

Pulz O, Gross W (2004) Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol 65:635–648

Pumas C, Vacharapiyasophon P, Peerapornpisal Y, Leelapornpisid P, Boonchum W, Ishii M, Khanongnuch C (2011) Thermostability of phycobiliproteins and antioxidant activity from four thermotolerant cyanobacteria. Phycol Res 59:166–174

Qian C, Decker EA, Xiao H, McClements DJ (2012) Nanoemulsion delivery systems: influence of carrier oil on β-carotene bioaccessibility. Food Chem 135:1440–1447

Ramos A, Acién FG, Fernández-Sevilla JM, González CV, Bermejo R (2010) Large-scale isolation and purification of C-phycocyanin from the cyanobacteria *Anabaena marina* using expanded bed adsorption chromatography. J Chem Technol Biotechnol 85:783–792

Ranga Rao A, Raghunath Reddy RL, Baskaran V, Sarada R, Ravishankar GA (2010) Characterization of microalgal carotenoids by mass spectrometry and their bioavailability and antioxidant properties elucidated in rat model. J Agric Food Chem 58:8553–8559

Reddy CM, Bhat VB, Kiranmai G, Reddy MN, Reddanna P, Madyastha KM (2000) Selective inhibition of cyclooxygenase-2 by C-phycocyanin, a biliprotein from *Spirulina platensis*. Biochem Biophys Res Commun 277:599–603

Remirez D, Fernández V, Tapia G, González R, Videla LA (2002) Influence of C-phycocyanin on hepatocellular parameters related to liver oxidative stress and Kupffer cell functioning. Inflamm Res 51:351–356

Romay C, Ledon N, Gonzalez R (1998) Further studies on anti-inflammatory activity of phycocyanin in some animal models of inflammation. Inflamm Res 47:334–338

Ruiz-Anchondo T, Flores-Holguín N, Glossman-Mitnik D (2010) Natural carotenoids as nanomaterial precursors for molecular photovoltaics: a computational DFT study. Molecular 15:4490–4510

Sajilata MG, Singhal RS, Kamat MY (2008) The carotenoid pigment zeaxanthin—a review. Compr Rev Food Sci Food Saf 7:29–49

Santiago-Santos MC, Ponce-Noyola T, Olvera-Ramirez R, Ortega-Lopez J, Canizares-Villanueva RO (2004) Extraction and purification of phycocyanin from *Calothrix* sp. Process Biochem 39:2047–2052

Sapsford KE, Algar WR, Berti L, Gemmill KB, Casey BJ, Oh E, Stewart MH, Medintz IL (2013) Functionalizing nanoparticles with biological molecules: developing chemistries that facilitate nanotechnology. Chem Rev 113:1904–2074

Sarrobert B, Dermoun D (1991) Extraction et valorisation de molecules à haute valeur ajoutée chez la microalgae *Porphyridium cruentum*. Premier Colloque Scientifique Français sur la Biotechnologie des Microalgues et des Cyanobacteries Appliquée au Thermalisme. Centre d'Études Nucléaires de Cadarache, Amélie-les-Bains, pp 109–114

Sasso S, Pohnert G, Lohr M, Mittag M, Hertweck C (2012) Microalgae in the postgenomic era: a blooming reservoir for new natural products. FEMS Microbiol Rev 36:761–785

Sekar S, Chandramohan M (2008) Phycobiliproteins as a commodity: trends in applied research, patents and commercialization. J Appl Phycol 20:113–136

Seo YC, Choi WS, Park JH, Park JO, Jung K-H, Lee HY (2013) Stable isolation of phycocyanin from *Spirulina platensis* associated with high-pressure extraction process. Int J Mol Sci 14:1778–1787

Shahidi F, Brown JA (1998) Carotenoid pigments in seafoods and aquaculture. Crit Rev Food Sci 38:1–67

Sies H, Stahl W (2004) Carotenoids and UV protection. Photochem Photobiol Sci 3:749–752

Siro I, Kapolna E, Kapolna B, Lugasi A (2008) Functional food. Product development, marketing and consumer acceptance—a review. Appet 51:456–467

Solovchenko AE, Khozin-Goldberg I, Didi-Cohen S, Cohen Z, Merzlyak MN (2008) Effects of light and nitrogen starvation on the content and composition of carotenoids of the green microalga Parietochloris incisa. Russ J Plant Physiol 55(4):455–462

Song W, Zhao C, Wang S (2013) A large-scale preparation method of high purity C-phycocyanin. Int J Biosci Biochem Bioinforma 3:293–297

Soni B, Visavadiya NP, Dalwadi N, Madamwar D, Winder C, Khalil C (2010) Purified C-phycoerythrin: safety studies in rats and protective role against permanganate-mediated fibroblast-DNA damage. J Appl Toxicol 30:542–550

Spolaore P, Cassan CJ, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101:87–96

Stankova K, Ivanova K, Nikolov V, Minkova K, Gigova L, Georgieva R, Boteva R (2011) The biliprotein C-phycocyanin modulates the DNA damage response in lymphocytes from nuclear power plant workers. In: Tsvetkov P (ed) Nuclear power – operation, safety and environment. InTech, Doi:10.5772/18334, pp 16

Su H-N, Xie B-B, Chen X-L, Wang J-X, Zhang X-Y, Zhou B-C, Zhang Y-Z (2010) Efficient separation and purification of allophycocyanin from *Spirulina (Arthrospira) platensis*. J Appl Phycol 22:65–70

Subhashini J, Mahipal S, Reddy M, Mallikarjuna Reddy M, Rachamallu A, Reddanna P (2004) Molecular mechanisms in C-Phycocyanin induced apoptosis in human chronic myeloid leukemia cell line-K562. Biochem Pharmacol 68:453–462

Takaichi S (2000) Characterization of carotenes in a combination of a C18 HPLC column with isocratic elution and absorption spectra with a photodiode-array detector. Photosynth Res 65:93–99

Takaichi S (2011) Carotenoids in algae: distributions, biosyntheses and functions. Mar Drugs 9:1101–1118

Thaakur S, Sravanthi R (2010) Neuroprotective effect of *Spirulina* in cerebral ischemia–reperfusion injury in rats. J Neural Transm 117:1083–1091

Thépenier C, Chaumont D, Gudin C (1988) Mass culture of *Porphyridium cruentum*: a multi-product strategy for the biomass valorisation. In: Stadler T, Mollion J, Verdus MC, Karamanos Y, Morvan H, Christiaen D (eds) Algal biotechnology. Elsevier Applied Science Publishers, London, pp 413–420

van den Berg H (1999) Carotenoid interactions. Nutr Rev 57:1–10

van den Hoek C, Mann DG, Jahns HM (1995) Algae. An introduction to phycology. Cambridge University Press, Cambridge

Vílchez C, Forján E, Cuaresma M, Bédmar F, Garbayo I, Vega JM (2011) Marine carotenoids: biological functions and commercial applications. Mar Drugs 9:319–333

Viskari PJ, Colyer CL (2003) Rapid extraction of phycobiliproteins from cultured cyanobacteria samples. Anal Biochem 319:263–271

Walter MH, Strack D (2011) Carotenoids and their cleavage products: biosynthesis and functions. Nat Prod Rep 28:663–692

Wiltshire K, Boersma M, Möller A, Buhtz H (2000) Extraction of pigments and fatty acids from the green alga *Scenedesmus obliquus* (Chlorophyceae). Aquat Ecol 34:119–126

Yen HW, Hu I, Chen CY, Ho SH, Lee DJ, Chang JS (2013) Microalgae-based biorefinery – from biofuels to natural products. Bioresour Technol 135:166–174

Yingying S, Changhai W (2009) The optimal growth conditions for the biomass production of *Isochrysis galbana* and the effects that phosphorus, Zn^{2+}, CO_2 and light intensity have on the biochemical composition of *Isochrysis galbana* and the activity of extracellular CA. Biotechnol Bioproc Eng 14:225–231

Zeb A, Mehmood S (2004) Carotenoids contents from various sources and their potential health applications. Pak J Nutr 3:199–204

Zhang Y-M, Chen F (1999) A simple method for efficient separation and purification of C-phycocyanin and allophycocyanin from *Spirulina platensis*. Biotechnol Tech 13:601–603

Zhang DH, Lee YK (1997) Enhanced accumulation of secondary carotenoids in a mutant of the green alga, *Chlorococcum* sp. J Appl Phycol 9:459–463

Vegetable Oil-Based Nutraceuticals

33

M.S.L. Karuna and R.B.N. Prasad

Abstract

Nutraceutical is a part of food that provides medicinal or health benefits including the prevention and treatment of diseases in addition to the common nutritional values of the foodstuff. Nutraceuticals provide all the essential substances which are supposed to be present in human diet. It is one of the fastest-growing segments of the global food industry and may range from isolated nutrients, dietary supplements, antioxidants, minerals, functional fibers, probiotics and prebiotics, marine products, functional foods and beverages, phytochemicals, dairy-based ingredients, confectionary products, and cosmeceuticals. Vegetable oil is a rich source of nutraceuticals in addition to essential fatty acids. Triglyceride is the major component of the vegetable oil, and its fatty acids play a vital role in human health and nutrition. Minor components of vegetable oil such as lecithin, tocopherols and tocotrienols, phytosterols and phytostanols, oryzanol, policosanol, squalene, carotenoids, and lignans also play a significant role in human health. This chapter describes the importance of vegetable oil-based nutraceuticals.

Keywords

Vegetable oil • Nutraceuticals • Functional foods • Triglyceride • Tocopherols • Carotenoids • Antioxidants • Minerals • Fibers • Probiotics

33.1 Introduction

The term "nutraceutical" was first coined by Stephen DeFelice from the words "nutrition" and "pharmaceutical" in 1989 (De Felice 1995). According to DeFelice, nutraceutical is a part of food that provides medicinal or health benefits including the prevention and treatment of diseases.

M.S.L. Karuna (✉) • R.B.N. Prasad
Centre for Lipid Research, CSIR-Indian Institute of Chemical Technology,
Tarnaka, Hyderabad 500007, Telangana, India
e-mail: mslkaruna@gmail.com; rbnprasad@iict.res.in

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6_33,

Nutraceuticals provide all the essential substances which are supposed to be present in the human diet (Stauffer 1999). Nutrition is required for health, and pharmaceutical gives remedy for sickness, and hence the food scientists and the food industry have created the "nutraceutical" category to describe foods that have both nutritional and pharmaceutical attributes. Nutraceuticals are one of the fastest-growing segments of the food industry and range from isolated nutrients, dietary supplements, and specific diets to genetically engineered designer food and herbal products (Schilter and Andersson 2003).

The concept of nutraceutical was started from a consumers' survey in some European countries which concluded that diet is rated as very important compared to exercise or hereditary factors to achieve good health. In the early 1900s, food manufacturers in the United States began adding iodine to salt in an effort to prevent goiter representing one of the first attempts at creating a functional component through fortification. In the United States, nutraceutical was commonly used, but there was no regulatory definition that existed in the early days of its introduction into the market. The Health Ministry of Canada has modified the meaning of nutraceutical as a product isolated or purified from the food generally sold in medicinal form and not associated with food and demonstrated to have a physiological benefit and provide protection against chronic diseases (Taylor 2004). These concepts may be distinguished by their description from different points of view, e.g., functional food is a more general term to emphasize foods with specific or strong purposes. Nutraceuticals have already become part of the dietary landscape in several developed and developing countries. Dietary supplements such as vitamins, minerals, herbs or other botanicals, amino acids, and other dietary substances intended to supplement the diet by increasing the total dietary intake of these ingredients (Halsted 2003). Dietary supplements are not intended to treat or cure diseases, whereas nutraceuticals emphasize more on prevention and treatment of diseases (Zeisel 1999). Functional foods such as omega fatty acid-fortified foods, probiotic fortified foods, branded iodinated salt, branded wheat flour market, and functional beverages covering energy drinks, sports drinks, and fortified juices also come under the category of nutraceuticals.

All therapeutic areas such as antiarthritic, pain killers, cold and cough, sleeping disorders, digestion and prevention of certain cancers, osteoporosis, blood pressure, cholesterol, depression, and diabetes have been covered by nutraceuticals with a variety of natural and synthetic products (Parasuram and Rawat 2011). Phytochemicals and antioxidants, which are known as nutraceuticals, are being used for preventing diseases and oxidative damage in the biological processes. Credible scientific research indicates many potential health benefits for nutraceuticals.

In the pharmaceutical development process, it is a requirement to have clinical test results from animal studies for verification of the effects. In the case of nutrition, there is no verification method for foods in preventing diseases in the past. In recent years, however, as food composition has been scientifically proven to cause lifestyle-related diseases and has become as social issue.

Even though large numbers of nutraceuticals are being available in the market, oils and fats occupy a significant place as they are a good source for essential fatty acids and also several nutritionally rich minor constituents. Hence this chapter covers the importance of vegetable oil-based nutraceuticals.

33.1.1 Global Nutraceutical Market

There has been a boom in the sale of nutraceuticals because adverse effects of pharmaceuticals increased the tendency of patients for self-medication and aging population. A number of nutraceuticals are available for self-medication or for sale for the last 20 years. The global nutraceutical market is defined as aggregate sales of functional foods, beverage, and supplements fortified with bioactive ingredients including fiber, probiotics, protein and peptides, phytochemicals, vitamins, and minerals (Abduallah and Waquar 2013). The global nutraceutical market covers three main cat-

egories, namely, nutraceutical beverages, foods, and supplements. Vitamins, minerals, and nutrients constitute about 85 % of the market, while antioxidants 10 % and other segments such as herbal extracts occupy 5 % of the global market. As per one of the global market report released by Transparency Market of New York, the global nutraceutical market which accounted for US$ 142.1 billion in 2011 is expected to reach US$ 204.8 billion by 2017 (http://www.marketwatch.com). Another market research report revealed that the global nutraceutical market would reach about US$ 250 billion by 2018 (http://www.transparencymarketresearch.com/global-nutraceuticals-product-market.html). Rising health concerns, improving economic conditions, growth of key demographics, and increased focus on e-commerce among consumers are the key factors to market success. In addition, more mergers and acquisitions, new prebiotic/probiotic product launches, and heart health-enhancing nutraceuticals will augment market growth.

Nutraceutical is a scientific area generated all over the world as they are good food supplements and have high nutrition value. The present junk foods will not provide any nutritional value; rather they adversely affect the body. Many nutraceutical functional foods and naturally occurring compounds that have been investigated and reported in various studies revealed that these products are extremely active and have profound effects, on cell metabolism, and often have little adverse effect. It is natural that human focus is shifting to a positive approach for the prevention of diseases to stay healthy. In many cases, nutraceuticals offer an advantage over the synthetic drugs under development by the pharmaceutical industry. Thus, nutraceuticals can be recommended as a regular part of the diet.

Nutraceutical products are collaborative research efforts of pharma, food, and chemistry as nutraceuticals play an important role in development of future therapeutics, however, depending on the control of their purity, efficiency, and safety. Now "nutraceutical a day may keep the doctor away" is replacing the old proverb "an apple a day will keep the doctor away," and accordingly people are turning massively to nutritious food. Nutraceuticals are generally categorized into four main subgroups, namely, nutrients, herbals, dietary supplements, and lipid-based nutraceuticals. This chapter describes the importance of lipid (vegetable oil)-based nutraceuticals.

Table 33.1 Nutraceutical components available in the global market along with their function

Components in the products	Function
Omega-3 fatty acids, zinc, antioxidants, and lutein	Improves vision
Vitamins and trace elements	Nutritional supplement
Blend of vitamins and minerals	Brain health
Protein, vitamins, dietary fiber, xylitol, and trace elements	Meal replacement drink mix
Phytosterols: beta-sitosterol, campesterol, and stigmasterol from soybean oil	Maintains healthy cholesterol levels and maintains healthy joints
Vitamin supplement with natural antioxidants	Improves nerve health
Vitamin D and lignans	Easier menopausal transition, cardiovascular health
Antioxidants, vitamins, lycopene, resveratrol	Immune supplement

The data presented in Table 33.1 represents some of the nutraceutical components available in the market and their potential human health benefits.

33.2 Vegatable Oil-based Nutraceuticals

Typical fats and oils provide approximately 9 kcal/g of metabolizable energy compared to 4 kcal/g for protein or carbohydrate. In addition to the caloric and nutritional value, fats have many functions in the diet. Several nutritionally beneficial compounds are present in plant lipids and particularly in vegetable oils and play a very important role in human diet. Most of the vegetable oils contain phospholipids, tocopherols, phytosterols, and phytosteryl esters, and these components enrich the nutritional properties of vegetable oils. Some of the selected oils contain

tocotrienol, oryzanol, lignans, etc. Lipids perform a variety of functions within the human body, for storing body fats in adipose tissue, providing cell membranes with a structure under normal physiological conditions. More than 95 % of the ingested lipids in the human diet are processed and absorbed. Vegetable oils basically contain saturated, monounsaturated, and polyunsaturated fatty acids (Table 33.2). Coconut and palm kernel oils are the most common oils rich in saturated fatty acids upto 85–95 %. Though saturated fat is responsible for the increase in low-density lipoprotein (LDL) and decrease in high-density lipoprotein (HDL) leading to heart attacks and strokes, all the saturated fats are not harmful. For example, medium-chain triglyceride (MCT) in coconut oil is found to exhibit healing properties (Gopalakrishna et al. 2010) and is being used in medicinal and food products. Olive, high-oleic sunflower oil, and avocado oils are rich in monounsaturated fatty acid, namely, oleic acid (18:1) up to 55–87 %, and mustard oil also contains 40–55 % of erucic acid (22:1). Monounsaturated fats have a beneficial effect on health when consumed in moderation and are used to replace saturated fats or *trans* fats. These fats help reduce LDL levels in the blood and lower the risk of heart disease and stroke and also provide nutrients to help develop and maintain body cells. Polyunsaturated fats include those oils rich in linoleic acid (LA, omega-6 fatty acid), alpha linolenic acid (ALA, omega-3 fatty acid), and gamma linolenic acid (GLA, omega-6), and these fatty acids cannot be made in the body and also act as precursors for the synthesis of other fatty acids, namely, arachidonic acid (AA, omega-6), eicosapentaenoic acid (EPA, omega-3), and docosahexaenoic acid (DHA, omega-3). These fatty acids are termed as essential fatty acids (EFAs) and are obtained primarily in plant-based (LA, ALA, and GLA) and fish (EPA and DHA) oils. Dietary polyunsaturated fatty acids (PUFA) aid in eicosanoid metabolism which regulate many functions of platelets, arterial and endothelial cells, monocytes, and macrophages, which have been implicated in atherogenesis and thrombosis (Fritsche 2006).

33.2.1 Essential Fatty Acids

The main essential omega-3 fatty acid is ALA, and the main essential omega-6 fatty acid is LA. Both omega-3 and omega-6 essential fatty acids have a role in functioning of the brain growth and development, bone health, stimulation of skin and hair growth, regulation metabolism, and maintenance of reproductive processes. However, the two classes of these fatty acids are metabolically and functionally separate and often have important opposing physiological functions. ALA and LA are termed essential as they are required by the body for maintaining health, and these form precursors for other essential AA, GLA, EPA, and DHA (Fig. 33.1).

LA is abundant in many vegetable oils (Table 33.2), comprising over half (by weight) of safflower, sunflower, soybean, and corn oils. LA is converted to GLA which is in turn is used in the biosynthesis of arachidonic acid (AA) and thus some prostaglandins (Zora 2011).

GLA is also an essential fatty acid synthesized in the body by the action of the delta-6 desaturase (D6D) enzyme on LA. As the activity of D6D diminishes with aging, due to stress, pollution, diet, smoking, drinking, and other activities, our body produces suboptimal levels of GLA. GLA once ingested is converted into dihomo-γ-linolenic acid (DGLA; 20:3, n-6) which is a precursor to two important anti-inflammatory metabolites: prostaglandin PGE_1 and 15-OH-DGLA. Many of the favorable effects of GLA are attributed to increased tissue levels of PGE which suppresses chronic inflammation. Specifically omega-6 fatty acids with high content of GLA are helpful in reducing the aches and pains of rheumatoid arthritis and relieve the discomforts of PMS, endometriosis, and fibrocystic breasts. GLA has also been clinically indicated to have therapeutic benefits in many other health conditions including cardiovascular disease, diabetic neuropathy, cancer, and skin diseases such as eczema skin dehydration and psoriasis. Some of the vegetable oils like evening primrose, black current seed oil, and borage oil are good sources of GLA up to 20 %. Safflower plant high in GLA

Table 33.2 Fatty acid composition (wt%) of selected vegetable oils

Vegetable oil	Caprylic (8:0)	Capric (10:0)	Lauric (12:0)	Myristic (14:0)	Palmitic (16:0)	Palmitoleic (16:1)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)	Arachidic (20:0)	Behenic (22:0)
Saturated-rich oils												
Palm olein[a]	0.1–0.2	–	0.2–0.5	0.8–1.0	36–44	–	4–6	39–49	11–12	0.2–0.3	0.2–0.4	0.1–0.2
Palm	0.2–0.4	–	–	0.3–0.5	35–44	–	4–6	36–39	9–12	–	–	0.1–0.2
Coconut oil	6–8	5–7	46–48	14–21	7–9	–	2–3	6–8	1–2	–	–	–
Monounsaturated-rich oils												
Olive	–	–	–	–	10–11	1–2	1–2	72–75	5–7	1–2	0.2–0.4	2–3
Mustard[b]	–	–	–	0.05–0.1	2.1–4.3	0.2–0.4	1.2–2.6	6–12	10–15	11–15	1–3.2	1–2.6
Peanut		–	–	–	0.05–0.1	10–12	0.1–0.2	3–4	46–48	30–32	–	1–2
Avocado	–	–	–	–	12–20	2–10	0.5–2	55–75	9–17	0.1–2	–	–
Hazelnut	–	–	–	–	4–8	0.1–0.6	1–3	68–85	7–15	0.1–0.5	0.1–0.5	1–3
Almonds	–	–	–	–	3–9	0.1–2	0.5–3	65–75	20–30	0.3–0.4	0.1–0.2	0.1–0.2
Pistachio	–	–	–	–	11–12	1–2	1–2	70–76	7–15	0.2–0.4	–	–
High-oleic sunflower oil	–	–	–	–	3–4	–	4–5	80–82	8–9	–	–	–
Polyunsaturated-rich oils												
Flaxseed	–	–	–	–	6–8	–	5–7	22–25	11–13	50–54	–	–
Hemp	–	–	–	–	5–7	–	3–10	11–13	54–56	24–26	–	–
Walnut	–	–	–	–	6–8	–	1–2	20–22	50–53	8–10	–	–
Soybean	–	–	–	0.1–0.2	10–11	0.5–1	2–4	22–34	43–56	6–8	–	0.1–0.2
Poppy seed	–	–	–	–	8–10	–	1–2	10–11	70–72	3–5	–	–
Sunflower	–	–	0.5–2	0.1–0.2	4–7	0.1–0.2	3–5	16–22	60–68	0.5–1	–	–
Rice bran	–	–	–	0.4–1	12–18	0.2–0.4	1–3	40–50	29–32	0.5–1	–	–
Cotton	–	–	–	0.2–0.4	20–27	–	2–3	18–20	42–52	0.1–0.2	–	–
Safflower	–	–	–	–	7–10	–	2–3	12–14	60–75	–	–	–
Wheat germ	–	–	–	–	11–16	–	1–6	8–30	44–65	–	–	–
Sesame	–	–	–	–	7–9	–	4–6	35–45	40–50	–	–	–
Corn	3–4	7–8	–	0.2–1	8–12	–	2–5	19–49	34–62	–	–	–
Peanut	–	–	–	0.1–0.2	10–12	0.1–0.2	2–3	40–46	30–32	–	1–2	1–3

Also contains, [a]24:0 (0.09–0.1); [b]20:1 (4–6.7), 22:1 (40–46), 24:0 (0.5–0.2)

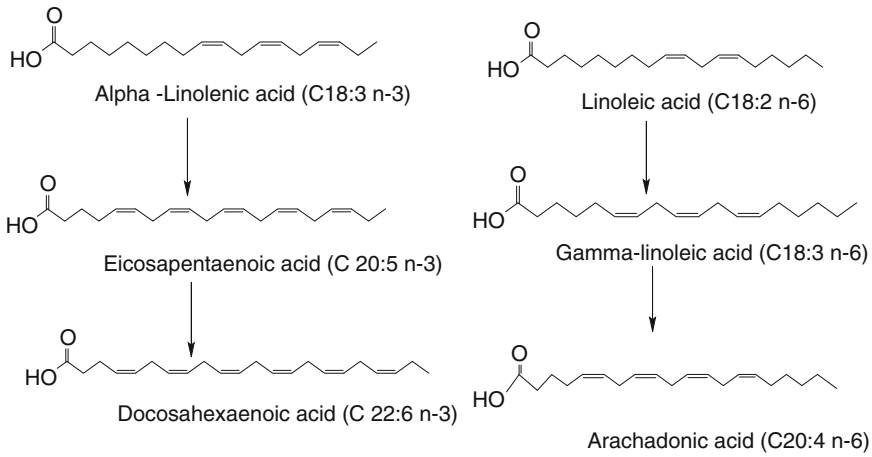


Fig. 33.1 Biochemical pathways of essential fatty acids

(35 %) was developed through a combination of plant breeding and biotechnology by Arcadia Biosciences, Inc., USA (Julianne et al. 2006). Similarly canola oil varieties containing as much as 43 % of GLA were developed by Calgene LLC, USA (Frank 2005).

ALA is found in good amounts in some common vegetable oils like flaxseed (35–60 %), canola (9–11 %), perilla (13–15 %), soybean (6–8 %), and walnut (8–10 %) oils. ALA is a precursor for the longer-chain omega-3 fatty acids like EPA and DHA (Fig. 33.1). These longer-chain fatty acids can also be provided directly from dietary sources. The positive effects of ALA have been documented in areas such as high blood cholesterol, high blood pressure, immune system function, male infertility, and cancer. Dietary ALA has been assessed for its role in cardiovascular health, and a dietary consumption of ALA (2–3 g/day) is essential for the primary and secondary prevention of coronary heart disease (Mozaffarianc 2005). It is widely believed that several diets tend to have too much omega-6, particularly in relation to omega-3 fatty acids, and that this imbalance can increase risk of cardiovascular diseases, cancer, osteoporosis, and other inflammatory disorders. For example, flaxseed oil (linseed oil) contains over 50 % of fatty acids as omega-3 with a ratio of 0.3:1 omega-6/omega-3 fatty acids. Hempseed oil contains about 20 % omega-3 with a ratio of approximately 3:1 omega-6/omega-3 fatty acids. Walnut oil is often recommended as a good source of EFA, with a ratio of approximately 5:1 omega-6/omega-3 fatty acids.

AA, EPA, and DHA are mostly found in animal sources like fish oil and are essential for keeping good health. DHA and EPA are important for the prevention of cardiovascular disease and resulted in decreased cardiac mortality in a large secondary prevention study (Doughman et al. 2007). Recent epidemiologic and preclinical studies also suggested that DHA may protect against Alzheimer's disease and other types of dementia (Calon and Cole 2007), and long-chain n−3 fatty acids may protect against advanced age-related macular degeneration which suggests a continued role of these fats in brain and eye health in adults and the elderly. DHA is known to exhibit beneficial neurological effects in developing infants. Keeping in view the health effects of DHA, health authorities like FDA and EU have recommended DHA fortification of infant formulations.

Though PUFA, namely, LA and ALA, are available through several vegetable and animal sources, biosynthesis of other essential fatty acids like AA, GLA, EPA, and DHA may not be possible due to factors like aging, stress, pollution, smoking, diet, drinking, and other activities of daily living. Hence, there is need to have supplements which can provide these essential fatty

acids directly, for healthy living. The value of the EPA/DHA nutritional supplement market is expected to reach $4.6 billion in 2016, reflecting a 5-year CAGR of 7.3 %. Asia-Pac will post the highest regional growth at 13 %, trailed by North America at 5.5 % (http://www.transparencymarketresearch.com). Some of the commercial plant, animal, and microbial essential fatty acid-based products available in the market are those obtained from natural oils, essential fatty acid rich triglycerides, etc.

33.2.2 Conjugated Linoleic Acid (CLA)

CLA is reported as a family of at least 28 isomers of linoleic acid (Banni 2002), and out of which *cis*-9, *trans*-11 and *trans*-10, and *cis*-12 isomers (Fig. 33.2) are most abundant. CLA is a naturally occurring trans fatty acid made from omega-6 essential fatty acids in the rumens or guts of pastured ruminants such as cows, goats, and sheep. Even though trans fatty acids are found to be harmful, CLA, however, is a naturally occurring trans fatty acid and is exempted from the labeling requirement because it is not considered to be harmful. CLA is generally present in ruminant lipid sources or derived from thermal processing of oils with the capability of preventing cancer and heart disease, improve immune function, and alter body composition for treating obesity or building lean body mass (Whigham et al. 2000). The United States Food and Drug Administration categorized CLA as generally recognized as safe (GRAS) status. Due to its growing commercial importance, several researchers have been attempted to synthesize CLA from different raw materials like linoleic acid, castor oil, safflower oil, soybean oil, etc., employing enzymatic or chemical approaches (Shigenobu et al. 2002; Jyotstna 2013).

33.2.3 Structured Lipids

No single vegetable oil or fat can meet all the nutritional and dietetic requirements, and the only alternative to obtain an ideal fat is to design structured lipids (SLs) with the desired fatty acid composition. SLs are modified triacylglycerols with improved nutritional or functional properties employing enzymatic interesterification methodologies. SLs provide an effective means for producing tailor-made lipids with desired physical characteristics, chemical properties, and/or nutritional benefits. SLs may provide the most effective means of delivering desired fatty acids for nutritive or therapeutic purposes and for targeting specific diseases and metabolic conditions (Lee and Akoh 1998). Some of the examples for SLs are medium-chain triglycerides (MCTs), infant formulations, reduced calorie fats, trans-free plastic fats, cocoa butter equivalents, etc. Some of the SLs were designed to provide simultaneous delivery of beneficial long-chain fatty acids (LCFAs) at a slower rate and medium-chain fatty acids (MCFAs) at a quicker rate (Babayan 1987; Akoh 1998). Several reduced calorie fats are reported in the literature, for example, Akoh and Yee (1997) interesterified tristearin with tricaprin (C10:0) or tricaprylin (C8:0) with sn-1,3-specific immobilized lipase to produce a low calorie SL. Kanjilal et al. (1999) synthesized an SL with plastic nature by incorporating behenic acid into sn-1 and sn-3 positions of natural vegetable oils so that the SL contains essential fatty acids and natural antioxidants. The synthesized SL delivered 5.36 kcal/g with the potential food applications since it is a

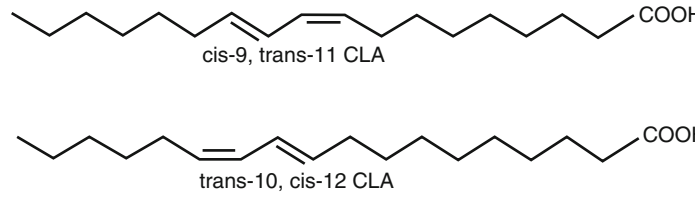


Fig. 33.2 Structures of conjugated linoleic acid (CLA) isomers

trans-free solid fat. Several such SLs are being prepared for a variety of applications.

33.2.4 Lecithin

Lecithin is an important by-product of vegetable oil processing obtained during the degumming step. The gums are removed from vegetable oils as they interfere with subsequent processing steps and degrade during the final deacidification/deodorization process imparting dark colors and off-flavors to the final processed oil. The percentage of lecithin in the oils varies widely from one oil to another. The major commercial source for lecithin is soybean, which contains 2.5–3.5 % of oil, whereas other oils contain from 0.1 to 2 %. Saturated type of oils like palm oil and palm kernel oil contains very small amounts (≈0.05 %) of phospholipids. Nevertheless, lecithin from other vegetable oilseeds, i.e., corn, cottonseed, linseed, sunflower, rice bran, peanut, rapeseed, and safflower has also been studied for their compositions, and major phospholipids are found to be phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI). Commercial lecithin is currently available in more than 40 different formulations. The world lecithin market for all grades of lecithin is about 150,000–160,000 MT (Krawezyh 1996). It is a complex mixture and comprises of phospholipids and triglycerides with minor amounts of other constituents like phytoglycolipids, phytosterols, tocopherols, and fatty acids. Industrially, lecithin is recovered by treating the crude oil with water, and under these conditions the gums are precipitated and separated by centrifugation and dried. Wet gums require immediate processing as they are high in moisture (about 50 %). The wet gums are dried and quickly cooled to obtain crude lecithin. The soybean oil-dried gum is a magic source for different grades of lecithin, modified lecithins, and individual phospholipids. Different grades of lecithin are being prepared, as soybean lecithin does not possess the optimum composition for every potential application.

Soybean lecithin consists of three major phospholipids, namely, PC, PE, and PI (Fig. 33.3). Lecithin is utilized in a wide variety of food and industrial applications. The French scientist, Maurice Gobley, first discovered the substance in 1850 and named it "lekithos," the Greek term for egg yolk. The commercial term "lecithin" is very general and describes a composition of lipid constituents and surface active compounds present in the product rather than a chemical entity of a

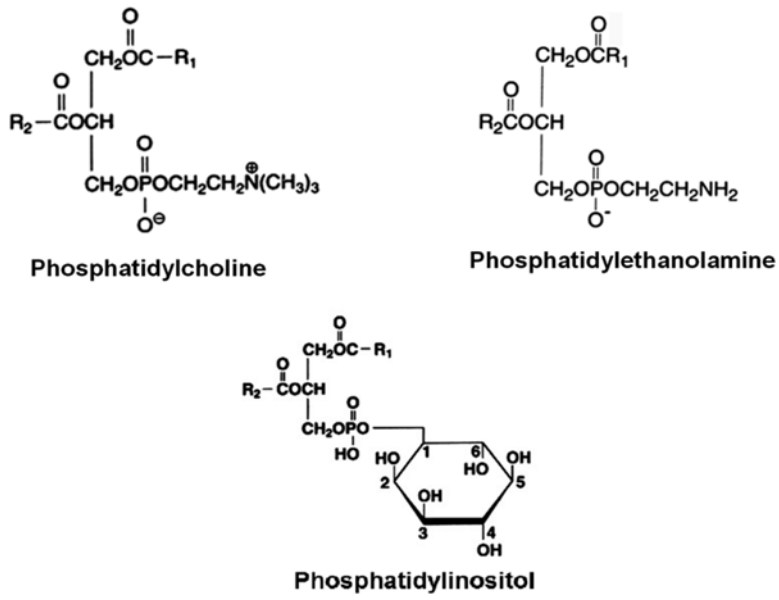


Fig. 33.3 Structures of phospholipids

single phospholipid. A typical dried commercial soy lecithin composition (Sipos and Bernard 1996) is as follows: oil, 35 %; phosphatidylcholine, 16 %; phosphatidylethanolamine, 14 %; phosphatidylinositol, 10 %; phytoglycolipids and other minor phosphatides and constituents, 17 %; carbohydrates, 7 %; and moisture, 1 %.

However, the phospholipid portion of lecithin that is mainly responsible for giving form and function to lecithin. Lecithin is being sold as such and also in modified forms for various applications. Modification of lecithin on an industrial scale is performed according to three principal methods: (a) by physical means, (b) acetone deoiling to prepare granular phospholipid powder, (c) spray drying with proteins to get low cost powdered products, and (d) compounded lecithin by incorporating additives like fatty acids, oils, polysorbates, monoglycerides, solvents, plasticizers, or other surfactants (Sipos and Bernard 1996).

Crude lecithin contains a number of functional groups that can be hydrolyzed, hydrogenated, hydroxylated, ethoxylated, halogenated, sulfonated, acetylated, and ozonized to name just a few possibilities (Stainely 1951). Lecithin provides an excellent source of choline, which is essential to every living cell in the body and is one of the main components of cell membranes. Dietary choline is important for the synthesis of the phospholipids in cell membranes, and it is also necessary for methyl metabolism, cholinergic neurotransmission, transmembrane signaling, and lipid-cholesterol transport and metabolism. Different classes of phospholipids in pure form are useful for very specific pharmaceutical applications and biological research. For example, phosphatidylserine (PS) is essential to the functioning of all body cells, and presently it is being sold as a "memory enhancer." Phosphatidylcholine with definite fatty acid composition has many applications in pharmaceuticals. For example, a suspension of dipalmitoyl phosphatidylcholine (DPPC) is being used for the prevention and treatment of neonatal respiratory distress syndrome (RDA) in newborns. Highly purified phospholipids can be used as raw materials for the preparation of lipid vesicles, liposomes, which are vehicles of choice in drug delivery systems (Juneja et al. 1988). Liposomes could also be used in agricultural chemicals to promote the mixing of compounds. Phosphatidylcholines with high content of polyunsaturated fatty acids contribute towards the regulation of epidermal proliferation (Krawezyh 1996). Alkyl-lysophosphatidylcholine analogues, which are derivatives of natural lyso-phosphatidylcholine, are reported to exhibit anticancer activity (Namba 1993).

Phospholipids are naturally occurring lipids found in nearly every living cell. It is a vital, multifunctional, active substance used in manufacturing variety of food products, nutraceuticals and healthcare products, cosmetics, and various feed formulations. Lecithin has potential as a multifunctional additive for food, pharmaceutical, and industrial applications. Lecithin is used in food products such as cake mixes, cheese, candy and chewing gum, chocolate, dehydrated foods, ice creams, instant foods, bakery products, and margarine. The primary usage of lecithin in food is an emulsifier. For other food uses, it softens and retains moisture, reduces viscosity, stabilizes, and disperses. Lecithin complexes with gluten proteins as a dough conditioner and acts as a wetting agent in baking applications. Lecithin is also used as a release agent through its role as a surfactant. Lecithin's edible nature makes it suitable for use on both cooked products (pan release) and release of food products from conveyor belts in commercial operations. Release agents are used to prevent sticking in finished food products, such as cheese slices. Lecithin is also used for products other than food such as adhesives, adsorbents, animal food, catalysts, soaps, cosmetics, paints and coatings, waxes and polish, ink and dyes, computer printing and photocopier toners, metal processing, explosives, pesticides, fertilizers, plastic and rubber moldings, concrete curing, masonry and asphalt products, dust control agents, magnetic media, leather tanning, lubricants, oil spill control, textile, paper production, wood preservatives, and releasing agents. Lecithin is also well known for nutraceutical properties like cholesterol-lowering property, source of polyunsaturated fatty acids, and

choline and protects against fatty degeneration of the liver/brain and improves memory function.

33.2.5 Tocopherols and Tocotrienols

A group of eight naturally occurring compounds alpha-, beta-, gamma-, and delta-tocopherols and tocotrienols are normally known as vitamin E (Fig. 33.4), and all or some of these are present in different sources of edible oils. The members of each family are designated as α, β, γ, and δ depending on the number and position of methyl groups on a chromane ring. The α- isomer is 5,7,8-trimethyl; β, a 5,8-dimethyl; γ, a 7,8-dimethyl; and δ, 8-methyl derivative of tocopherol or tocotrienol. The contents of tocopherols and tocotrienols are generally less than 0.3 % in the oils, and in some cases they are present in very minor quantities. Most vegetable oils contain an abundance of α, γ, and δ tocopherols, while the β-isomer is less prevalent, except for wheat germ oil that contains high amounts of this isomer. However, even in a particular source of oil, the content and nature of tocopherols and tocotrienols may depend on the geographical area, seasonal effects, degree of processing, and process variables employed.

Tocopherols are most common in several plant materials. Tocopherols are predominantly found in maize, sunflower, soybean, and olive oils; tocotrienols are found at relatively high concentrations

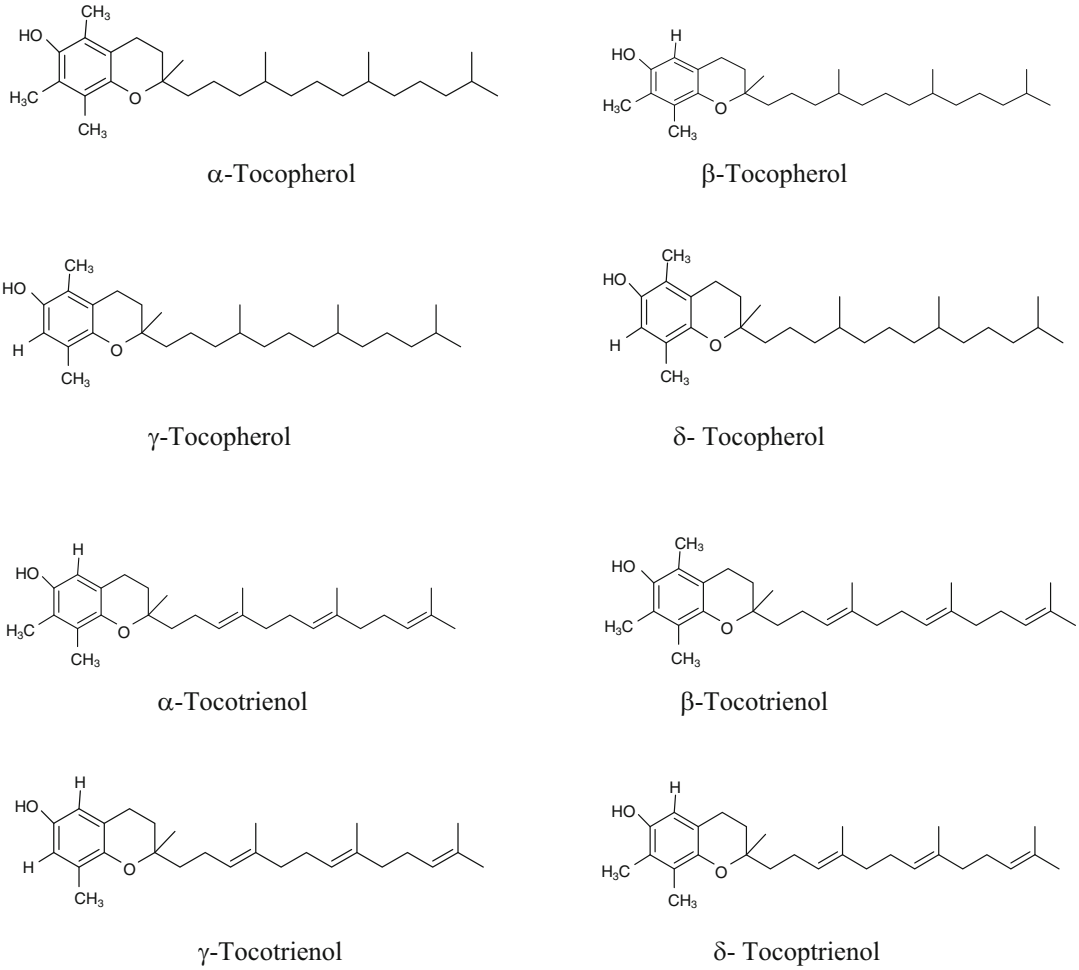


Fig. 33.4 Structures of tocopherols and tocotrienols

in oils extracted from the fruit of the palm tree, and other sources include rice bran oil, wheat germ, and barley. However soybean oil is predominantly rich in γ-tocopherol and sunflower oil is rich in α-tocopherol. The volatile fraction obtained from the deodorization of vegetable oils forms the best commercial source of natural tocopherols, tocotrienols, and sterols. Vegetable oils such as soybean, sunflower, groundnut, and rapeseed can produce deodorizer distillates that are high in unsaponifiables with high tocopherol and sterol contents. Palm oil and rice bran oil contains in addition tocotrienols. The composition of the distillates varies depending on the oil source as well as deodorization conditions. The deodorizer distillate is a mixture of fatty acids, glycerides, unsaponifiable components including tocopherols, sterols, aldehydes, ketones, hydrocarbons, and other volatile materials. Typical samples of the distillates from soybean available in the country contain about 30–40 % of glycerides, 40–45 % free fatty acids, and about 25–30 % unsaponifiables. Two to 8 % of tocopherols and 2–8 % of sterols are typically found in these distillates. While the volumes produced during processing are small, they are undoubtedly a by-product with a high value.

Tocopherols which are physiologically active as vitamin E are considered as natural antioxidants and find extensive application in food, cosmetics, and pharmaceutical industries (Traber and Packer 1995). It is suggested that vitamin E decreases the occurrence of several age-related degenerative diseases (Serbinova and Packer 1991). Tocopherols are the major free radical chain-breaking antioxidant in body tissues and are considered the first line of body defense against lipid peroxidation protecting cell membranes at an early stage of free radical attack. Of the four tocopherols, vitamin E activity is attributed only to alpha-homologue, and hence the price of the product is decided by the alpha-homologue content. The naturally occurring α-tocopherol is about 35 % more active than the synthetic all racemic α-tocopherols (Shahidi and Shukla 1996). Tocopherols were reported to prevent coronary heart diseases and cataract formation; reduce plasma triglycerides and cholesterol; prevent or delays aging, cancer, and arthritis; reduce oxidative stress and blood glucose levels in diabetic animals and humans; and prevent or treat allergic manifestations such as skin allergy, bronchial asthma, or inflammatory manifestations. Tocopherols can also be used in the manufacture of dried green vegetables with superior color retention and in cosmetics where the photoprotection property is taken advantage of.

Tocotrienols are rarely seen in vegetable oils except in palm and rice bran oils. Crude palm oil contains 600–1,000 ppm of tocopherols and tocotrienols, and this is reduced to 350–630 ppm following refining. Tocotrienols have been exclusively studied with particular interest in breast cancer, cardiovascular disease, skin health and aging, and their cholesterol-lowering capabilities (Schaffer and Muller 2005; Theriault and Chao 1999). The palm tocotrienols have unique physiological and health properties that are not apparent with tocopherols. α-Tocotrienol is 40–60 times more potent than normal tocopherol making it one of the most powerful lipid-soluble antioxidants available. It is 6.5 times active at defending cytochrome P-450 against oxidative damage. The efficiency of α-tocotrienol in scavenging peroxyl radicals in liposomes is 1.5-fold increased over α-tocopherol (Demonty and Ras 2009). Tocopherols are being used as multivitamin capsules, single-dose nutrient capsules, and liquid dietary supplement as vitamin E, in the formulations of baby foods, cereals, naturally flavored foods, and as an antioxidant in fried/baked goods, powdered soup, candy, nuts, fruit drinks, spices, flavors, and cosmetics (in ointments, sunscreen agents, etc.). Tocotrienols are several times more potent than tocopherols and are used in multivitamin capsules, single-dose nutrient capsules, liquid dietary supplement as vitamin E, and baby foods, cereals, and naturally flavored foods. Other physiological affects attributed to tocotrienols are decreasing serum and LDL cholesterol and significant antitumor activity.

33.2.6 Phytosterols and Phytostanols

Phytosterols and their esters are a group of steroid alcohols and esters that occur naturally in plants, and in particular they comprise a major

portion of the unsaponifiable matter in most vegetable oils. The steroidal moiety of phytosterols is unsaturated in the five to six position and is saturated in phytostanols (Fig. 33.5). The phytosterols are 28 or 29 carbon compounds which are distinct from cholesterol, which is a 27-carbon substance and is a predominant sterol in animal fats and marine oils. Some of the major phytosterols are brassicasterol, campesterol, stigmasterol, β-sitosterol, fucosterol, Δ^5-avenasterol, Δ^7-stigmasterol, Δ^7-avenasterol, and α-spinasterol. The major sterols of oils are generally β-sitosterol, stigmasterol, and campesterol. Most oils contain 100–500 mg sterols/100 g sample. Notable exceptions are corn, rapeseed/canola, rice bran, and sesame oils which may contain up to 2 % of sterols. The composition of sterols in selected oils has been reported by several researchers, and the major sterols were found to be β-sitosterol (50–90 %), stigmasterol (5–30 %), and campesterol (5–30 %). Phytosterols are isolated in high purity from deodorizer distillate (a by-product of vegetable oil refining) by a combination of processing steps like distillation, extraction, crystallization, and washing. Phytosterol blends derived from vegetable oil deodorizer distillates are converted into the corresponding phytostanols by catalytic hydrogenation. Esters are produced by reacting the phytosterols/phytostanols with saturated and unsaturated fatty acids derived from food grade vegetable oils. Phytosterols, phytostanols, and their esters are commercially available in the market. The major phytosterols present in vegetable oils are beta-sitosterol, campesterol, and stigmasterol.

Sterols are heat-stable molecules and have no flavor or taste activity of their own. Sterols are known as antipolymerization factors in vegetable oils during frying. Many researchers have used the sterol pattern of vegetable oils in order to characterize and fingerprint them or to detect adulteration. In addition, Δ^5-avenasterol, fucosterol, and citrostadienol have been shown to exhibit antioxidant properties (Boskou and Morton 1976). Phytosterols and phytostanols are structurally similar to cholesterol. Intake of phytosterols or phytostanols at the level of 1.5–3.0 g/day was documented to reduce blood LDL cholesterol by 10 % (Law and Wald 1994) which would decrease coronary heart disease risk by 27 %. Several studies have demonstrated that consumption of phytosterols and phytostanol reduces cholesterol absorption and lowers serum total and LDL cholesterol levels in animals and humans. The efficacy and safety of phytosterol- and phytostanol-enriched food products has been reviewed by several regulatory agencies, and the sale of phytosterol- and phytostanol-enriched food products has been allowed as a means to reduce blood cholesterol levels by many European countries, the United States, Australia, and New Zealand. The average dietary intake of phytostanols, in western diets, is roughly one

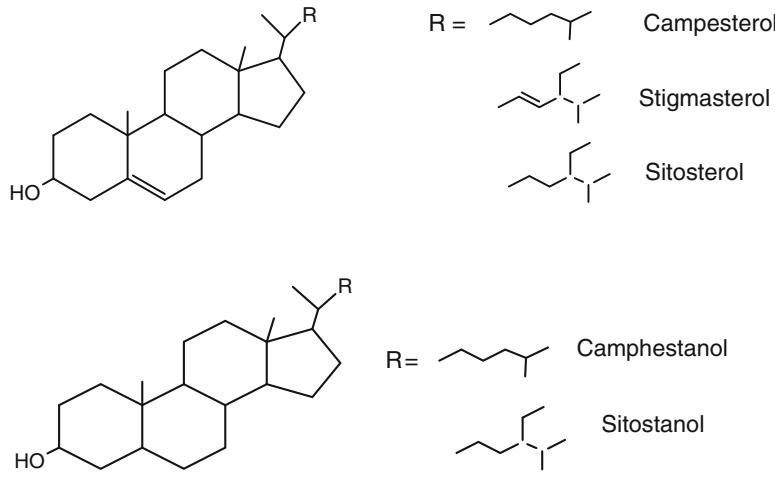


Fig. 33.5 Structures of phytosterols and phytostanols

tenth of the consumed phytosterol amount 250 mg/day. The estimated daily phytosterol content in the vegetarian diet is about 500 mg/day.

Malcolm Law of the Wolfson Institute of Preventive Medicine in London reported that 2 g of plant sterol esters or stanol added to an average daily portion of margarine can reduce the risk of heart disease by 25 % (Law 2000). Reports show that plant sterol esters and stanol esters differentially lower circulating total and LDL cholesterol levels by suppression of cholesterol absorption in hypocholesterolemic subjects. Cholesterol absorption was reduced to 36 % by stanol ester and 26 % by stanol esters (Jones and Parsons 2000). The US Food and Drug Administration (FDA) has approved the use of health claim labels on food products containing plant sterols and stanol esters in 2000.

33.2.7 Oryzanol

Oryzanol is an important component of crude rice bran oil, and its content ranges from 1 to 3% in rice bran oil. γ-Oryzanol was first isolated from rice bran oil and presumed to be a single component. Later, it was found to be a mixture containing ferulic acid (4-hydroxy-3-methoxy cinnamic acid) esters of triterpene alcohols and phytosterols (Rogers et al. 1993). Individual components were identified as ferulic acid esters of cycloartanol (**a**), cycloartenol (**b**), campesterol (**c**), 24-methylene cycloartanol (**d**), and β-sitosterol (**e**) (Fig. 33.6).

The most economically viable raw material for the isolation of γ-oryzanol is the soap stock/acid oil obtained as a by-product during alkali refining of the rice bran oil. A process was developed for the isolation of γ-oryzanol from the soap stock/acid oil obtained during alkali refining of the rice bran oil (Das et al. 1998; Rao et al. 2002). Over the past decade, a number of investigations have demonstrated the beneficial physiological effects associated with the intake of γ-oryzanol. The important biological activity of oryzanol is its cholesterol-lowering property (Sharma and Rukmini 1986). Cholesterol-lowering property is the most important biological activity of γ-oryzanol, and this is confirmed by several studies using rice bran oil. Relative to control animals, oryzanol administration resulted in significant reduction of plasma total cholesterol levels (28 %), non-HDL cholesterols (34 %), and a 25 % reduction in percentage cholesterol adsorption found that the serum total, free, esterified, and LDL+VLDL cholesterol levels of rats maintained on a 10 % rice bran oil diet were lower than those maintained on a 10 % groundnut oil diet. γ-Oryzanol was also found to exhibit antiatherosclerotic effect in addition to hypocholesterolemic activity. Lipid peroxidation has been shown to be prevented in the retina by γ-oryzanol because of its antioxidant property. Oryzanol-containing pharmaceutical formulations are used in preventing motion sickness and in the treatment of nervous system disorder. A plethora or oryzanol-containing transdermal pharmaceutical and moisturizing cosmetic preparations have been prepared for the treatment of skin disorders. Oryzanol emulsions are used as antioxidants and preservatives for cosmetics and foods, and such emulsions are also effective in preventing color changes in the products. γ-Oryzanol is also known for its protective role in UV light-induced lipid per oxidation and is being used in the sunscreen formulations. Ferulic acid and its esters stimulate hair growth and prevent skin aging. Such preparations are claimed to accelerate cell differentiation and to reduce wrinkles in aged women. Soft capsules containing oryzanols with or without riboflavin butyrate can be used to prevent arteriosclerosis (Ito and Hsu 1992). Oryzanols have been shown to be highly effective against lipogenic liver cirrhosis in spontaneously hypertensive rats, an animal having natural abnormalism in lipid metabolism (Gregory and Kelly 1999). Oryzanol is also effective in treating a broad range of gastrointestinal disorders including stress-induced gastric and duodenal ulcers. In some of the western countries, oryzanol is being sold as an agent for bodybuilding in humans, especially athletes and animals such as racehorses and dogs.

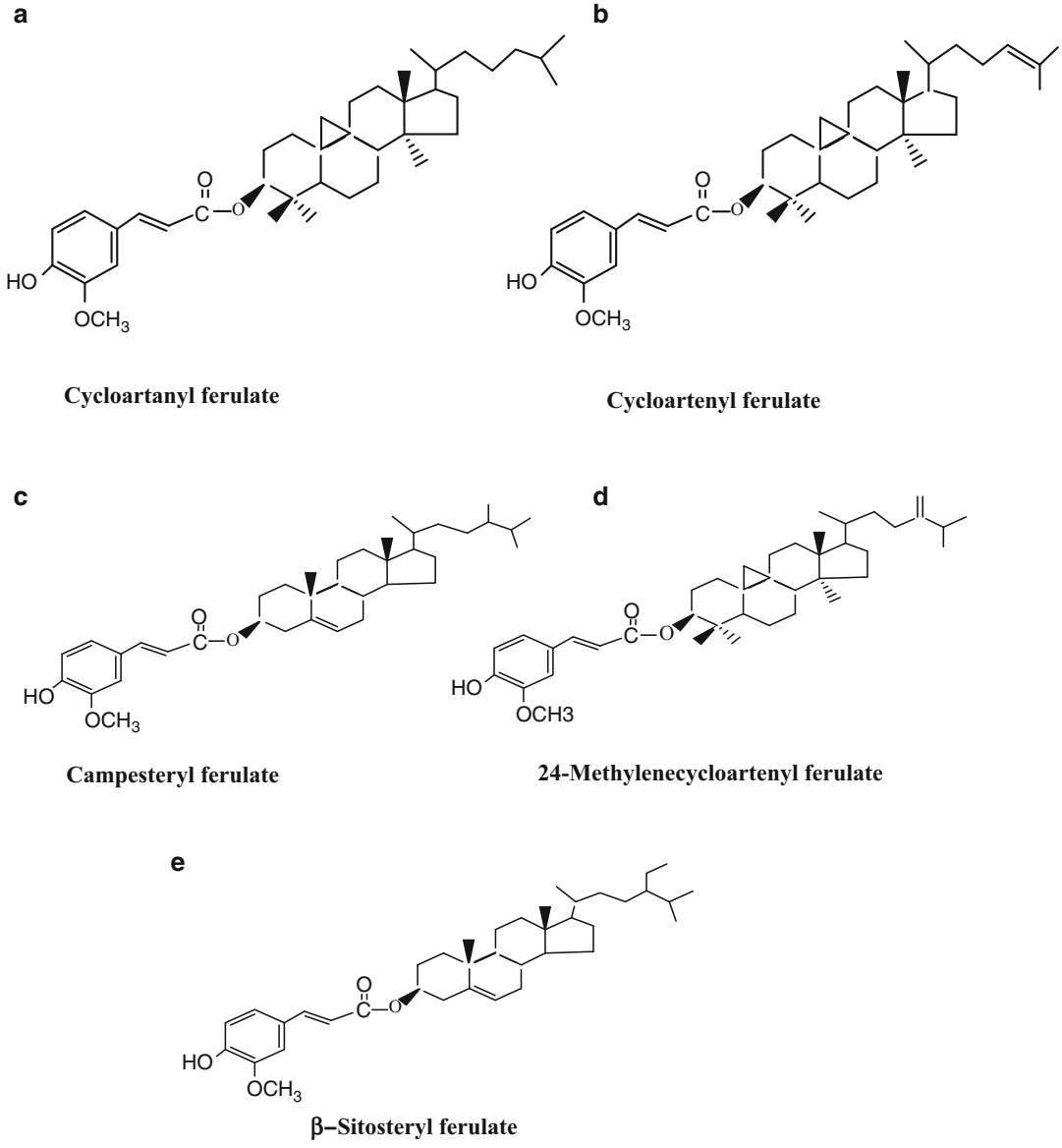


Fig. 33.6 Structures of γ-oryzanol

33.2.8 Squalene

Squalene (Fig. 33.7) is an isoprenoid intermediate used in cholesterol biosynthesis and is widely distributed in nature with reasonable amounts found in olive oil, palm oil, wheat germ oil, amaranth oil, and rice bran oil in less than 1 % quantities. Squalene is not very susceptible to peroxidation and appears to function as a quencher of singlet oxygen in the skin and protecting it from damage. New mark et al. in 1997 has proposed that squalene might be a contributing factor in the epidemiologic observation of reduced risk for several cancers associated with olive oil intake since this oil contains high level of squalene. Squalene is an unsaturated hydrocarbon typically found in human serum and deep-sea shark liver oil. It has a natural affinity for the skin and acts as a natural emollient and skin-soothing agent. Squalene is a precursor for

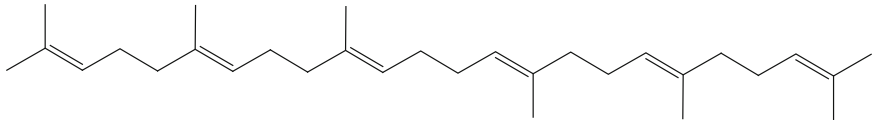


Fig. 33.7 Structure of squalene

Fig. 33.8 Structure of octacosanol (*1*) and triacontanol (*2*)

the production of important lipids and is a key component in the maintenance of youthful skin.

Squalene appears to be critical for reducing free radical oxidative damage to the skin. Serum squalene originates partly from endogenous cholesterol synthesis and partly from dietary sources, especially in populations consuming large amounts of olive oil or shark liver.

Squalene is used as an active ingredient in traditional medical preparations as it is believed to enhance immunity and promote wound healing. Squalene is said to help hydrate and protect the skin, fight wrinkle formation, encourage regeneration of healthy skin cells, and prevent premature aging of the skin. It is also believed to be an essential ingredient in formulations for eczema and psoriasis where relief from dry, itchy, and damaged skin is needed. Its saturated form is used in pharmaceutical creams and lotions and also as a carrier of lipid-soluble drugs. Squalene is considered as an excellent industrial lubricant because of its high resistance to oxidation. Shark liver oil containing up to 89 % squalene is a traditional source of squalene.

33.2.9 Policosanol

Policosanol is a mixture of high molecular weight primary aliphatic alcohols derived from plant waxes, in particular from sugarcane wax and rice bran wax although a number of other sources have been reported including wheat germ, beeswax, perilla seeds, and potato pulp (Adhikari and Hwang 2006; Irmak and Dunford 2005; Nuissier and Bourgeois 2002). The chemical formula for policosanol is CH_3-(CH_2)n-CH_2OH with varying carbon chain length from C_{24} to C_{36} carbon atoms. The major alcohol components are octacosanol (Fig. 33.8), triacontanol, tetracosanol, and hexacosanol with minor traces of other alcohols.

Policosanol is a natural cholesterol-lowering supplement that has been extensively studied globally. It has been reported as a safe and effective alternative for lowering blood cholesterol when fed in low (pharmacological) doses (5–20 mg/day) to experimental animals, healthy volunteers, and patients with type II hypercholesterolemia (Menéndez and Arruzazabala 1997; Canetti and Morera 1995). Beyond the cholesterol-lowering effect, policosanol is also reported to reduce the susceptibility of the LDL particle from lipid oxidation and inhibits platelet aggregation and increases prostacyclin (Pg I2) levels in the serum of rodents (Arruzazabala et al. 1992).

In case policosanol is rich in triacontanol (25–30 %), this fatty alcohol mixture is normally called as triacontanol and is known to be useful for stimulating growth in a wide variety of plants, including agricultural crops such as corn, soybean, wheat, rice, and tomatoes. Formulations are prepared by dissolving the triacontanol in organic solvents and then adding the solution to water with an emulsifying agent. Oral and parenteral preparations containing 0.5–5 % of a mixture of higher fatty alcohol (C_{24}–C_{38}) formulations were reported to be useful for the treatment of hypercholesterolemia and hyperlipoproteinemia.

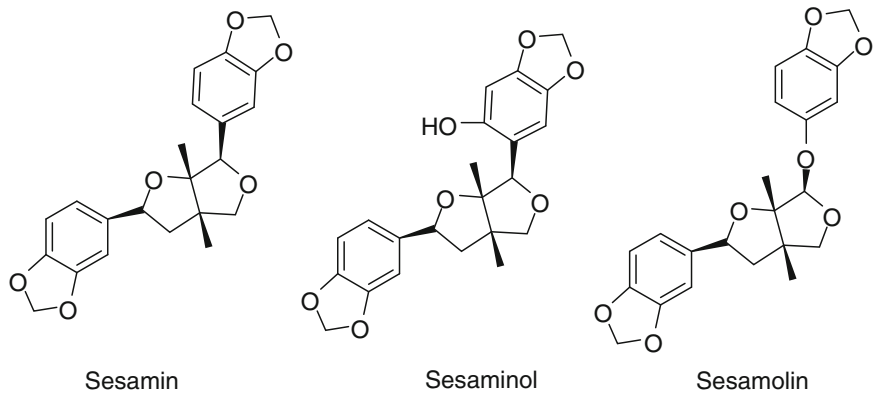


Fig. 33.9 Structures of lignans

Kaimal et al. (2001) developed an economically viable process to isolate long-chain fatty alcohols from the defatted wax in about 30–35 % yields with triacontanol varying from 25 % to 30 % in the fatty alcohol mixture.

33.2.10 Lignans

Sesame oil (SO) contains a class of unique compounds known as lignans. Lignans comprise sesamin, sesamolin, and a small amount of sesamol (Fig. 33.9). They have multiple physiological functions, such as decreasing blood lipids and arachidonic acid levels, increasing antioxidative ability and γ-tocopherol bioavailability, and providing anti-inflammatory function (Alhassane and Xu 2010). Sesame oil effectively attenuates oxidative stress triggered by endotoxin lipopolysaccharide (LPS) in rats (Prasad et al. 2012). Although, the exact mechanism by which dietary SO reduces oxidative stress is not very clear, it is strongly believed that the protective effect is due to the presence of lignans (sesamin, sesamol, and sesamolin) and vitamin E. The nutraceutical effect of each lignan is as described here.

33.2.11 Sesamol

Sesamol is an effective antioxidant found mainly in roasted sesame or in processed sesame oil (Budowski 1964). It was observed that antioxidant efficacy of sesame cake extract is due to the presence of sesamol and other compounds. Sesamol has been shown to inhibit the excessive production of nitric oxide in the lipopolysaccharide-/gamma-interferon-stimulated C6 astrocyte cells (Soliman and Mazzio 1998). It also inhibits the formation of carcinogenic imidazoquinoxaline-type heterocyclic amines through the unstable free radical maillard intermediate. Studies have shown that sesamol can act as a metabolic regulator and possesses chemopreventive, antimutagenic, and antihepatotoxic properties (Zhen et al. 2006). The biological effects of sesamol on health include its inhibitory effects on lipid peroxidation of liposomes when induced by Fe^{2+} on the lipid peroxidation of rat liver microsomes and also when induced by ascorbate/Fe^{3+} ions on carbon tetrachloride and NADPH of lipid peroxidation on the mitochondria (Uchida et al. 1996). An in vitro study indicated that sesamol inhibited the mutagenicity of mutagens in various strains of *Salmonella typhimurium* (Kaur and Saini 2000).

33.2.12 Sesamin

Sesamin is the most abundant lignan in sesame oil and was shown to cause an increase in γ-tocopherols in the plasma and the liver and a reduction in liver cholesterol of rats contrary to secoisolariciresinol diglucoside, the major lignan glucoside in flaxseed (Frank and Kamal-Eldin

2004). Sesamin enhances hepatic detoxification, reduces the incidence of chemically induced tumors, protects against oxidative stress, and inhibits Δ5-desaturase in polyunsaturated fatty acid (PUFA) biosynthesis (Zhen et al. 2006). The inhibition of delta-5 desaturase activity by sesamin results in an accumulation of dihomo-γ-linolenic acid (DGLA) that can displace arachidonic acid and decrease the formation of proinflammatory mediators, such as prostaglandin E2 (PGE2) and leukotriene B4 (Chavali et al. 1998). Sesamin strongly influences lipid metabolism in animals and in humans. Hirose et al. (1992) showed that serum and liver cholesterols were reduced in rat-fed diet containing 0.5 % sesamin. Consumption of 32 mg sesamin capsules for 4 weeks followed by 65 mg sesamin capsules for 4 weeks reduced total cholesterol (TC) by 9 %, low-density-lipoprotein cholesterol (LDL-C) by 16.5 % and apoprotein B by 10.5 % in 12 males with hypercholesterolemia. HDL-C was unchanged in all mentioned human studies after intervention of sesamin or sesame seed (Chen et al. 2005). Since the diet can be an effective means to lower blood levels of total and LDL cholesterol, drug therapy may be reserved for patients who are at high risk for CHD.

33.2.13 Sesaminol

Among the sesame lignans, sesaminol was shown to have the most effective antioxidative activity in vitro experimental systems (Kang et al. 2000). It is known that sesaminol triglucoside is the major lignan glucoside in sesame seeds and that almost 32 % of total lignans in sesame seeds are in glucosylated form. Although the sesaminol glucosides (SGs) directly have no role in antioxidative defense system against various oxidative damages, they could be hydrolyzed to form sesaminol by intestinal beta-glucosidase after ingestion of sesame seeds, thereby working as antioxidants (Katsuzaki et al. 1994). In a recent study, it was confirmed that dietary sesaminol glucosides (SGs) inhibited the development of colonic precancerous lesions in vivo (Sheng et al. 2007). The beneficial effect of SGs might be attributed to the antioxidative property and/or downregulation of serum triglycerides. They also help the elevation of intracellular calcium level, 8-oxodG formation, and inhibition of apoptotic related gene expressions as well as nuclear factor-κB (NF-κB) and extracellular signal-regulated kinase (ERK) signal activation. Consequently, sesaminol glucosides might have beneficial effects on the neuronal cell survival through reduced inflammatory reaction which may eventually prevent the formation of inflammatory complex of the neuronal plagues in Alzheimer's disease (Lee et al. 2006). Sesaminol glucosides have a protective effect on Aβ-induced neuronal cell death via antioxidant property and could be useful as a therapeutic agent for treatment of oxidative stress-induced neuronal degeneration diseases such as Alzheimer's disease (Kim et al. 2003). Sesaminol glucosides were known to have protective effects against Aβ25–35-induced deficit in learning and memory in mice. They prevent beta-amyloid (Aβ)- and H_2O_2-induced cell death of pheochromocytoma (PC12) cells accompanied by the suppression of Aβ25–35-induced ROS generation. Sesamin strongly influences lipid metabolism in animals and in humans. Hirose et al. (1992) showed that serum and liver cholesterols were reduced in rat-fed diet containing 0.5 % sesamin. Consumption of 32 mg sesamin capsules for 4 weeks followed by 65 mg sesamin capsules for 4 weeks reduced total cholesterol (TC) by 9 %, low-density-lipoprotein cholesterol (LDL-C) by 16.5 %, and apoprotein B by 10.5 % in 12 males with hypercholesterolemia. Wu et al. (2006) also observed similar reductions in TC and LDL-C in 24 postmenopausal subjects following a 5-week intervention with 50 g pulverized roasted sesame seed. HDL-C was unchanged in all mentioned human studies after intervention of sesamin or sesame seed.

33.2.14 Carotenoids

Carotenoids are a group of fat-soluble unsaponifiables of edible oils, of which over 500 different molecules have been identified so far in a variety

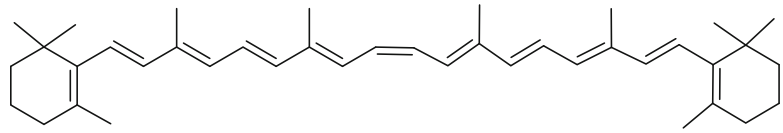


Fig. 33.10 Structure of β-carotene

of natural products. β-carotene (Fig. 33.10) is a main precursor of vitamin A (retinol), and its conversion to vitamin A occurs via cleavage of the molecule at the central double bond by the action of the enzyme carotene dioxygenase which is present in the human intestinal mucosa and liver. Each 6 μg of β-carotene provides 1 μg of retinol because of poor extraction and intestinal conversion of β-carotene in humans (Thompson 1964). Research has shown that vitamin A is important for maintaining good skin, eye, bone, and reproductive health. The protective action of β-carotene and its oxygenated derivatives, known as xanthophylls, against deleterious effects of radiation on light-sensitized cells has been well recognized. This effect has been attributed to the action of carotenoids as quenchers of singlet oxygen and is independent of the vitamin A activity of the molecules (Jung and Min 1991). In addition, β-carotene is particularly effective at low partial pressures of oxygen and those experienced under physiological conditions in most tissues; this may indicate its complementary role in the chain-breaking mechanism of vitamin E in the lipid phase (Burton and Ingold 1984).

Although carotenoids especially β-carotene are present in some crude vegetable oils in small amounts, the richest sources of carotenoids are crude palm oil and fiber oil from palm fruits. Corn oil is reported to contain up to 90 mg/kg carotenoids in the form of β-carotene, β-zeacarotene, zeinoxanthin, cryptoxanthin, lutein, and zeaxanthin (Weber 1987), and olive oil contains β-carotene and lutein at 5 mg/kg level (Stancher et al. 1987). Most of the carotenoids are removed during bleaching and deodorization. Hence some β-carotene may be added to edible vegetable oils in order to extend their shelf life during storage under supermarket conditions to inhibit photosensitized oxidation. Few companies developed a process to produce a red palm oil for culinary use.

33.3 Conclusions

There has been a boom in the sale of nutraceuticals over the last 20 years due to adverse effects of pharmaceuticals, increased tendency of patients for self-medication, and aging population. The world is shifting more towards a positive approach for the prevention of diseases to stay healthy. A number of nutraceuticals are available for self-medication or for sale in the market today. It is expected that the global nutraceutical market will reach US$204.8 billion by 2017. Due to the nutraceutical revolution, the food industry is now becoming a research-oriented sector similar to the pharmaceutical industry. In this context, based on the extensive work reviewed in this chapter, it can be concluded that vegetable oil-based nutraceuticals play a vital role in human health and nutrition. Different components of the vegetable oils (triglycerides and other minor components) exhibit a variety of physiological effects based on their functionality and hence are being used extensively in food, cosmetic, and pharmaceutical industries. Thus nutraceuticals occupy a major place in the development of future therapeutics. However the success depends on the control of purity, efficiency, and safety. Today food manufacturers and the government are taking steps to address these issues through collaborative research between pharma, food, and chemistry sectors. This not only helps the common man, but also strengthens the economy of the vegetable oil industry.

References

Abduallah BJ, Waquar A (2013) Consumption pattern of nutraceutical products among consumers in Delhi. Indian J Appl Res 3:169–172

Adhikari P, Hwang KT (2006) Policosanol content and composition in perilla seeds. J Agric Food Chem 54:5359–5362

Akoh CC (1998) Structured lipids. In: Akoh CC, Min DB (eds) Food lipids chemistry, nutrition, and biotechnology. Marcel Dekker, New York, pp 699–727

Akoh CC, Yee LN (1997) Enzymatic synthesis of position-specific low-calorie structured lipids. J Am Oil Chem Soc 74:1409–1413

Alhassane T, Xu X (2010) Flaxseed lignans: source, biosynthesis, metabolism, antioxidant activity, bio-active components, and health benefits. Compr Rev Food Sci Food Saf 9:261–269

Arruzazabala ML, Carbajal D, Más R, García M, Fraga V (1992) Effects of policosanol on platelet aggregation in rats. Thromb Res 69:321–327

Babayan VK (1987) Medium chain triglycerides and structured lipids. Lipids 22:417–420

Banni S (2002) Conjugated linoleic acid metabolism. Curr Opin Lipidol 13:261–266

Boskou D, Morton ID (1976) Effect of plant sterols on the rate of deterioration of heated oils. J Sci Food Agric 27:928–932

Budowski P (1964) Recent research on sesame, sesamollin and related compounds. J Am Oil Chem Soc 41:281–285

Burton GW, Ingold KV (1984) β-carotene: an unusual type of lipid antioxidant. Science 224:569–573

Calon F, Cole G (2007) Neuroprotective action of omega-3 polyunsaturated fatty acids against neurodegenerative diseases: evidence from animal studies. Prostaglandins Leukot Essent Fat Acids 77:287–293

Canetti M, Morera M (1995) One year study on the effect of policosanol (5 mg-twice-a-day) on lipid profile in patients with type II hypercholesterolemia. Adv Ther 12:245–254

Chavali SR, Zhong WW, Forse RA (1998) Dietary alpha-linolenic acid increases TNF-alpha, and decreases IL-6, IL-10 in response to LPS: effects of sesamin on the delta-5 desaturation of omega6 and omega3 fatty acids in mice. Essent Fat Acids 58:185–191

Chen PR, Chien KL, Su TC, Chang CJ, Liu T, Cheng H, Tsai CE (2005) Dietary sesame reduces serum cholesterol and enhances antioxidant capacity in hypercholesterolemia. Nutr Res 25:559–567

Das PK, Kaimal TNB, Bhale Rao UT, Chaudhuri A (1998) Isolation of γ-oryzanol through calcium ion induced precipitation of anionic micellar aggregates. J Agric Food Chem 46:3073–3080

De Felice LS (1995) The nutraceutical revolution, its impact on food industry. Food Sci Technol 6:59–61

Demonty I, Ras RT (2009) Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake. J Nutr 139:271–284

Doughman SD, Krupanidhi S, Sanjeevi CB, Curr (2007) Omega-3 fatty acids for nutrition and medicine: considering microalgae oil as a vegetarian source of EPA and DHA. Diabetes Rev 3:198–203

Frank JF (2005) GLA: uses and new sources. Inform 16:279–282

Frank J, Kamal-Eldin AMT (2004) Consumption of sesame oil muffins decreases the urinary excretion of γ-tocopherol metabolites in humans. Ann N Y Acad Sci 1031:365–367

Fritsche K (2006) Fatty acids as modulators of the immune response. Annu Rev Nutr 26:45–73

Gopalakrishna AG, Raj G, Bhatnagar AS, Kumar PK, Preeti C (2010) Coconut oil: chemistry, production and its applications. Indian Coconut J July:15–27

Gregory S, Kelly ND (1999) Squalene and its potential clinical uses. Altern Med Rev 4:29–36

Halsted CH (2003) Dietary supplements and functional foods: 2 sides of a coin? J Am Clin Nutr 77:1001–1007

Hirose N, Doi F, Ueki T, Akazawa K, Chijiiwa K, Sugano M, Akimoto K, Shimizu S, Yamada H (1992) Suppressive effect of sesamin against 7,12- dimethyl benz[a]-anthracene induced rat mammary carcinogenesis. Anticancer Res 12:1259–1265

Irmak S, Dunford NT (2005) Policosanol contents and compositions of wheat varieties. J Agric Food Chem 53:5583–5586

Ito M, Hsu CT (1992) Effect of gamma-oryzanol on experimental liver cirrhosis in spontaneously hypertensive rats (SHR). J Clin Biochem Nutr 12:193–199

Jones PJ, Parsons WE (2000) Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. J Lipid Res 41:697–705

Julianne L, Alexander M, Frank JF (2006) GLA oils: a study on consumer purchasing behaviors. Inform 17:342–344

Juneja LR, Kazuoka T, Amane K, Shimizu S (1988) Studies on the enzymic conversion of phospholipids (VI). Conversion of phosphatidyl choline to phosphatidyl serine by various phospholipase D in the presence of L- or D-serine. Biochim Biophys Acta 1000(3):277–283

Jung MY, Min DB (1991) Effects of quenching mechanisms of carotenoids on the photosensitized oxidation of soybean oil. J Am Oil Chem Soc 68:653–658

Jyotstna SW (2013) Conjugated linoleic acid: a significant review. Indian J Sci 2:8–12

Kaimal TNB, Prasad RBN, Swamy PAR, Bhale Rao UT (2001) A process for the preparation of fatty alcohol fraction mainly containing 1-triacontanol from rice bran wax. Indian Patent 184307

Kang MH, Naito M, Sakai K, Uchida K, Osawa T (2000) Mode of action of sesame lignans in protecting low-density lipoprotein against oxidative damage in vitro. Life Sci 66:161–171

Kanjilal S, Prasad RBN, Kaimal TNB, Ghafoorunissa RSH (1999) Synthesis and estimation of caloric value of structured lipid-potential reduced calorie fat. Lipids 34:1045–1055

Katsuzaki H, Kawakishi S, Osawa T (1994) Sesaminol glucosides in sesame seeds. Photochemistry 35:773–776

Kaur IP, Saini A (2000) Sesamol exhibits antimutagenic activity against oxygen species mediated mutagenicity. Mutat Res 470:71–76

Kim SR, Um MY, Ahn JY, Hong JT, Ha TY (2003) The protective effects of sesaminol glycosides against

cognitive deficits and oxidative stress induced by beta-amyloid protein in mice. Physiol Func Dis Risk Reduct 3:120–127
Krawezyh T (1996) Lecithin. Inform 7:1160–1167
Law M (2000) Plant sterol and stanol margarines and health. Brit Med 320:861–864
Law MR, Wald NJ (1994) By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischaemic heart disease? Br Med J 308:367–372
Lee KT, Akoh CC (1998) Structured lipids: synthesis and applications. Food Rev Int 14:17–34
Lee SY, Son DJ, Lee YK, Lee JW, Lee HJ, Yun YW, Ha TY, Hong JT (2006) Effect of sesaminol glucosides on beta-amyloid-induced PC12 cell death through antioxidant mechanisms. Neurosci Res 52:330–341
Menéndez R, Arruzazabala ML (1997) Cholesterol-lowering effect of policosanol on rabbits with hypercholesterolemia induced by a wheat starch-casein diet. Br J Nutr 77:923–932
Mozaffarianc D (2005) Does alpha-linolenic acid intake reduce the risk of coronary heart disease? A review of the evidence. Altern Ther Health Med 11:24–31
Namba Y (1993) Medical applications of phospholipids. In: Gregor C (ed) Phospholipids handbook. Marcel Dekker, New York, pp 879–894
New mark HL (1997) Squalene, olive oil, and cancer risk: a review and hypothesis. Cancer Epidemiol Biomark Prev 6:1101–1103
Nuissier G, Bourgeois P (2002) Composition of sugarcane waxes in rum factory wastes. Phytochemistry 61:721–726
Parasuram RR, Rawat BMS (2011) Nutraceuticals: an area of tremendous scope. Int J Res Ayurveda Pharm 2:410–415
Prasad MNN, Sanjay KR, Prasad DS, Vijay N, Kothari R, Nanjunda SS (2012) A review on nutritional and nutraceutical properties of sesame. J Nutr Food Sci 2:127
Rao KVSA, Rao BVSK, Kaimal TNB (2002) An improved process for the isolation of oryzanol from rice bran oil soap stock. US Patent 6410762, Jap Pat 293793
Rogers EJ, Rice SM, Nicolosi RJ, Carpenter DR, Mc-Clelland CA, Romanczyk LJ (1993) Identification and quantitation of γ-oryzanol components and simultaneous assessment of tocopherols in rice barn oil. J Am Oil Chem Soc 70:301–307
Schaffer S, Muller WE (2005) Tocotrienols: constitutional effects in aging and disease. J Nutr 135:151–154
Schilter B, Andersson C (2003) Guidance for the safety assessment of botanicals and botanical preparations for use in food and food supplements. Food Chem Toxicol 41:1625–1649
Serbinova E, Packer L (1991) Free radical recycling and intramembrane mobility in the antioxidant properties of alpha-tocopherol and alpha-tocotrienol. Free Radic Biol Med 10:263–275
Shahidi F, Shukla VKS (1996) Nontriacyl glycerol constituents of fats, oils. Inform 7:1227–1232
Sharma RD, Rukmini C (1986) Rice bran oil and hypocholesterolemia in rats. Lipids 21:715–717
Sheng HQ, Hirose Y, Hata K, Zheng Q, Kuno T, Asano N, Yamada Y, Hara A, Osawa T, Mori H (2007) Modifying effect of dietary sesaminol glucosides on the formation of azoxymethane-induced premalignant lesions of rat colon. Cancer Lett 246:63–68
Shigenobu K, Jun O, Yoriko O, Kenji M, Sakayu S (2002) Conjugated linoleic acid production from linoleic acid by lactic acid bacteria. J Am Oil Chem Soc 79:159–163
Sipos FE, Bernard SF (1996) Lecithin. In: Hui YH (ed) Bailey's industrial oil and fat products. Wiley, New York, pp 311–396
Soliman KF, Mazzio EA (1998) In vitro attenuation of nitric oxide production in C6 astrocyte cell culture by various dietary compounds. Proc Soc Exp Biol Med 218:390–397
Stainely J (1951) Production and utilization of Lecithin. In: Markley KS (ed) Soybean and soybean products, vol 2. Interscience, New York, pp 593–647
Stancher B, Zonta F, Bogoni P (1987) Determination of olive oil carotenoids by HPLC. J Micronutr Anal 3:97–106
Stauffer JE (1999) Nutraceuticals. Cereal Foods World 44:115–117
Taylor C (2004) Regulatory frameworks for functional foods and dietary supplements. Nutr Rev 62:55–59
Theriault A, Chao JT (1999) Tocotrienol: a review of its therapeutic potential. Clin Biochem 32:309–319
Thompson SY (1964) Factors affecting the absorption of carotene and its conversion into vitamin A. Exp Eye Res 3:392–404
Traber MG, Packer L (1995) Vitamin E: beyond antioxidant function. J Am Clin Nutr 62:1501–1509
Uchida M, Nakajin S, Toyoshima S, Shinoda M (1996) Antioxidative effect of sesamol and related compounds on lipid peroxidation. Biol Pharm Bull 19:623–626
Weber EJ (1987) Lipids of the kernel. In: Watson SA, Ramsted PE (eds) Corn chemistry and technology. American Association of Cereal Chemists, St. Paul
Whigham LD, Cook ME, Atkinson RL (2000) Conjugated linoleic acid: implications for human health. Pharmacol Res 42:503–510
Wu WH, Kang YP, Wang NH, Jou HJ, Wang TA (2006) Sesame ingestion affects sex hormones, antioxidant status, and blood lipids in postmenopausal women. J Nutr 136:1270–1275
Zeisel SH (1999) Regulation of nutraceuticals. Science 285:1853–1855
Zhen L, Niina MS, Lilian UT (2006) Sesamin is one of the major precursors of mammalian lignans in sesame seed (*Sesamum indicum*) as observed in vitro and in rats. J Nutr 136:906–912
Zora D (2011) The mediterranean diet: effects on proteins that mediate fatty acid metabolism in the colon. Nutr Rev 69:730–744

Index

B. Bahadur et al. (eds.), *Plant Biology and Biotechnology: Volume I: Plant Diversity, Organization, Function and Improvement*, DOI 10.1007/978-81-322-2286-6,

Q

R