Chittaranjan Kole *Editor*

Wild Crop Relatives: Genomic and Breeding Resources

Tropical and Subtropical Fruits

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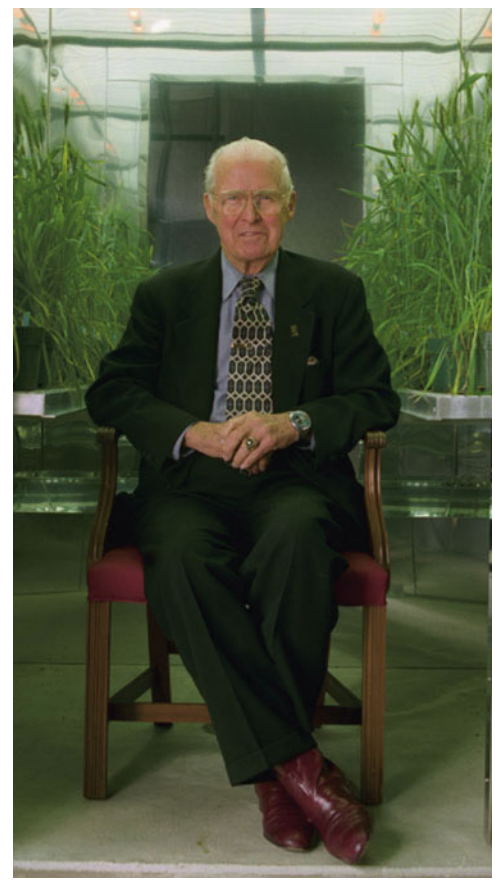
Dedication



Dr. Norman Ernest Borlaug,[1] the Father of Green Revolution, is well respected for his contributions to science and society. There was or is not and never will be a single person on this Earth whose single-handed service to science could save millions of people from death due to starvation over a period of over four decades like Dr. Borlaug's. Even the Nobel Peace Prize he received in 1970 does not do such a great and noble person as Dr. Borlaug justice. His life and contributions are well known and will remain in the pages of history of science. I wish here only to share some facets of this elegant and ideal personality I had been blessed to observe during my personal interactions with him.

It was early 2007 while I was at the Clemson University as a visiting scientist one of my lab colleagues told me that "somebody wants to talk to you; he appears to be an old man". I took the telephone receiver casually and said hello. The response from the other side was – "I am Norman Borlaug; am I talking to Chitta?" Even a million words would be insufficient to define and depict the exact feelings and thrills I experienced at that moment!

[1]The photo of Dr. Borlaug was kindly provided by Julie Borlaug (Norman Borlaug Institute for International Agriculture, Texas A&M Agriculture) the granddaughter of Dr. Borlaug.

I had seen Dr. Borlaug only once, way back in 1983, when he came to New Delhi, India to deliver the Coromandal Lecture organized by Prof. M.S. Swaminathan on the occasion of the 15th International Genetic Congress. However, my real interaction with him began in 2004 when I had been formulating a 7-volume book series entitled *Genome Mapping and Molecular Breeding in Plants*. Initially, I was neither confident of my ability as a series/book editor nor of the quality of the contents of the book volumes. I sent an email to Dr. Borlaug attaching the table of contents and the tentative outline of the chapters along with manuscripts of only a few sample chapters, including one authored by me and others, to learn about his views as a source of inspiration (or caution!) I was almost sure that a person of his stature would have no time and purpose to get back to a small science worker like me. To my utter (and pleasant) surprise I received an email from him that read: "May all Ph.D.'s, future scientists, and students that are devoted to agriculture get an inspiration as it refers to your work or future work from the pages of this important book. My wholehearted wishes for a success on your important job". I got a shot in my arm (and in mind for sure)! Rest is a pleasant experience – the seven volumes were published by Springer in 2006 and 2007, and were welcome and liked by students, scientists and their societies, libraries, and industries. As a token of my humble regards and gratitude, I sent Dr. Borlaug the Volume I on *Cereals and Millets* that was published in 2006. And here started my discovery of the simplest person on Earth who solved the most complex and critical problem of people on it – hunger and death.

Just one month after receiving the volume, Dr. Borlaug called me one day and said, "Chitta, you know I cannot read a lot now-a-days, but I have gone through only on the chapters on wheat, maize and rice. Please excuse me. Other chapters of this and other volumes of the series will be equally excellent, I believe". He was highly excited to know that many other Nobel Laureates including Profs. Arthur Kornberg, Werner Arber, Phillip Sharp, Günter Blobel, and Lee Hartwell also expressed generous comments regarding the utility and impact of the book series on science and the academic society. While we were discussing many other textbooks and review book series that I was editing at that time, again in my night hours for the benefit of students, scientists, and industries, he became emotional and said to me, "Chitta, forget about your original contributions to basic and applied sciences, you deserved Nobel Prize for Peace like me for providing academic foods to millions of starving students and scientists over the world particularly in the developing countries. I will recommend your name for the World Food Prize, but it will not do enough justice to the sacrifice you are doing for science and society in your sleepless nights over so many years. Take some rest Chitta and give time to Phullara, Sourav and Devleena" (he was so particular to ask about my wife and our kids during most of our conversations). I felt honored but really very ashamed as I am aware of my almost insignificant contribution in comparison to his monumental contribution and thousands of scientists over the world are doing at least hundred-times better jobs than me as scientist or author/editor of books! So, I was unable to utter any words for a couple of minutes but realized later that he must been too affectionate to me and his huge affection is the best award for a small science worker as me!

In another occasion he wanted some documents from me. I told him that I will send them as attachments in emails. Immediately he shouted and told me: "You know, Julie (his granddaughter) is not at home now and I cannot check email myself. Julie does this for me. I can type myself in type writer but I am not good in computer. You know what, I have a xerox machine and it receives fax also. Send me

the documents by fax". Here was the ever-present child in him. Julie emailed me later to send the documents as attachment to her as the 'xerox machine' of Dr. Borlaug ran out of ink!

Another occasion is when I was talking with him in a low voice, and he immediately chided me: "You know that I cannot hear well now-a-days; I don't know where Julie has kept the hearing apparatus, can't you speak louder?" Here was the fatherly figure who was eager to hear each of my words!

I still shed tears when I remember during one of our telephone conversations he asked: "You know I have never seen you, can you come to Dallas in the near future by chance?" I remember we were going through a financial paucity at that time and I could not make a visit to Dallas (Texas) to see him, though it would have been a great honor.

In late 2007, whenever I tried to talk to Dr. Borlaug, he used to beckon Julie to bring the telephone to him, and in course of time Julie used to keep alive all the communications between us when he slowly succumbed to his health problems.

The remaining volumes of the *Genome Mapping and Molecular Breeding in Plants* series were published in 2007, and I sent him all the seven volumes. I wished to learn about his views. During this period he could not speak and write well. Julie prepared a letter based on his words to her that read: "Dear Chitta, I have reviewed the seven volumes of the series on *Genome Mapping and Molecular Breeding in Plants*, which you have authored. You have brought together genetic linkage maps based on molecular markers for the most important crop species that will be a valuable guide and tool to further molecular crop improvements. Congratulations for a job well done".

During one of our conversations in mid-2007, he asked me what other book projects I was planning for Ph.D. students and scientists (who had always been his all-time beloved folks). I told him that the wealth of wild species already utilized and to be utilized for genetic analysis and improvement of domesticated crop species have not been deliberated in any book project. He was very excited and told me to take up the book project as soon as possible. But during that period I had a huge commitment to editing a number of book volumes and could not start the series he was so interested about.

His sudden demise in September 2009 kept me so morose for a number of months that I could not even communicate my personal loss to Julie. But in the meantime, I formulated a 10-volume series on *Wild Crop Relatives: Genomic and Breeding Resources* for Springer. And whom else to dedicate this series to other than Dr. Borlaug!

I wrote to Julie for her formal permission and she immediately wrote me: "Chitta, Thank you for contacting me and yes I think my grandfather would be honored with the dedication of the series. I remember him talking of you and this undertaking quite often. Congratulations on all that you have accomplished!" This helped me a lot as I could at least feel consoled that I could do a job he wanted me to do and I will always remain grateful to Julie for this help and also for taking care of Dr. Borlaug, not only as his granddaughter but also as the representative of millions of poor people from around the world and hundreds of plant and agricultural scientists who try to follow his philosophy and worship him as a father figure.

It is another sad experience of growing older in life that we walk alone and miss the affectionate shadows, inspirations, encouragements, and blessings from the fatherly figures in our professional and personal lives. How I wish I could treat my next generations in the same way as personalities like Mother Teresa and Dr. Norman Borlaug and many other great people from around the world treated me!

During most of our conversations he used to emphasize on the immediate impact of research on the society and its people. A couple of times he even told me that my works on molecular genetics and biotechnology, particularly of 1980s and 1990s, have high fundamental importance, but I should also do some works that will benefit people immediately. This advice elicited a change in my thoughts and workplans and since then I have been devotedly endeavoring to develop crop varieties enriched with phytomedicines and nutraceuticals. Borlaug influenced both my personal and professional life, particularly my approach to science, and I dedicate this series to him in remembrance of his great contribution to science and society and for all his personal affection, love and blessings for me.

I emailed the above draft of the dedication page to Julie for her views and I wish to complete my humble dedication with great satisfaction with the words of Julie who served as the living ladder for me to reach and stay closer to such as great human being as Dr. Borlaug and express my deep regards and gratitude to her. Julie's email read: "Chitta, Thank you for sending me the draft dedication page. I really enjoyed reading it and I think you captured my grandfather's spirit wonderfully. . .. So thank you very much for your beautiful words. I know he would be and is honored."

Clemson, USA Chittaranjan Kole

Preface

Wild crop relatives have been playing enormously important roles both in the depiction of plant genomes and the genetic improvement of their cultivated counterparts. They have contributed immensely to resolving several fundamental questions, particularly those related to the origin, evolution, phylogenetic relationship, cytological status and inheritance of genes of an array of crop plants; provided several desirable donor genes for the genetic improvement of their domesticated counterparts; and facilitated the innovation of many novel concepts and technologies while working on them directly or while using their resources. More recently, they have even been used for the verification of their potential threats of gene flow from genetically modified plants and invasive habits. Above all, some of them are contributing enormously as model plant species to the elucidation and amelioration of the genomes of crop plant species.

As a matter of fact, as a student, a teacher, and a humble science worker I was, still am and surely will remain fascinated by the wild allies of crop plants for their invaluable wealth for genetics, genomics and breeding in crop plants and as such share a deep concern for their conservation and comprehensive characterization for future utilization. It is by now a well established fact that wild crop relatives deserve serious attention for domestication, especially for the utilization of their phytomedicines and nutraceuticals, bioenergy production, soil reclamation, and the phytoremediation of ecology and environment. While these vastly positive impacts of wild crop relatives on the development and deployment of new varieties for various purposes in the major crop plants of the world agriculture, along with a few negative potential concerns, are envisaged the need for reference books with comprehensive deliberations on the wild relatives of all the major field and plantation crops and fruit and forest trees is indeed imperative. This was the driving force behind the inception and publication of this series.

Unlike the previous six book projects I have edited alone or with co-editors, this time it was very difficult to formulate uniform outlines for the chapters of this book series for several obvious reasons. Firstly, the status of the crop relatives is highly diverse. Some of them are completely wild, some are sporadically cultivated and some are at the initial stage of domestication for specific breeding objectives recently deemed essential. Secondly, the status of their conservation varies widely: some have been conserved, characterized and utilized; some have been eroded completely except for their presence in their center(s) of origin; some are at-risk or endangered due to genetic erosion, and some of them have yet to be explored. The third constraint is the variation in their relative worth, e.g. as academic model, breeding resource, and/or potential as “new crops.”

The most perplexing problem for me was to assign the chapters each on a particular genus to different volumes dedicated to crop relatives of diverse crops grouped based on their utility. This can be exemplified with *Arabidopsis*, which has primarily benefited the Brassicaceae crops but also facilitated genetic analyses and improvement in crop plants in other distant families; or with many wild relatives of forage crops that paved the way for the genetic analyses and breeding of some major cereal and millet crops. The same is true for wild crop relatives such as *Medicago truncatula*, which has paved the way for in-depth research on two crop groups of diverse use: oilseed and pulse crops belonging to the Fabaceae family. The list is too long to enumerate. I had no other choice but to compromise and assign the genera of crop relatives in a volume on the crop group to which they are taxonomically the closest and to which they have relatively greater contributions. For example, I placed the chapter on genus *Arabidopsis* in the volume on oilseeds, which deals with the wild relatives of Brassicaceae crops amongst others.

However, we have tried to include deliberations pertinent to the individual genera of the wild crop relatives to which the chapters are devoted. Descriptions of the geographical locations of origin and genetic diversity, geographical distribution, karyotype and genome size, morphology, etc. have been included for most of them. Their current utility status – whether recognized as model species, weeds, invasive species or potentially cultivable taxa – is also delineated. The academic, agricultural, medicinal, ecological, environmental and industrial potential of both the cultivated and/or wild allied taxa are discussed.

The conservation of wild crop relatives is a much discussed yet equally neglected issue albeit the in situ and ex situ conservations of some luckier species were initiated earlier or are being initiated now. We have included discussions on what has happened and what is happening with regard to the conservation of the crop relatives, thanks to the national and international endeavors, in most of the chapters and also included what should happen for the wild relatives of the so-called new, minor, orphan or future crops.

The botanical origin, evolutionary pathway and phylogenetic relationship of crop plants have always attracted the attention of plant scientists. For these studies morphological attributes, cytological features and biochemical parameters were used individually or in combinations at different periods based on the availability of the required tools and techniques. Access to different molecular markers based on nuclear and especially cytoplasmic DNAs that emerged after 1980 refined the strategies required for precise and unequivocal conclusions regarding these aspects. Illustrations of these classical and recent tools have been included in the chapters.

Positioning genes and defining gene functions required in many cases different cytogenetic stocks, including substitution lines, addition lines, haploids, monoploids and aneuploids, particularly in polyploid crops. These aspects have been dealt in the relevant chapters. Employment of colchiploidy, fluorescent or genomic in situ hybridization and Southern hybridization have reinforced the theoretical and applied studies on these stocks. Chapters on relevant genera/species include details on these cytogenetic stocks.

Wild crop relatives, particularly wild allied species and subspecies, have been used since the birth of genetics in the twentieth century in several instances such as studies of inheritance, linkage, function, transmission and evolution of genes. They have been frequently used in genetic studies since the advent of molecular markers. Their involvement in molecular mapping has facilitated the development of mapping

populations with optimum polymorphism to construct saturated maps and also illuminating the organization, reorganization and functional aspects of genes and genomes. Many phenomena such as genomic duplication, genome reorganization, self-incompatibility, segregation distortion, transgressive segregation and defining genes and their phenotypes have in many cases been made possible due to the utilization of wild species or subspecies. Most of the chapters contain detailed elucidations on these aspects.

The richness of crop relatives with biotic and abiotic stress resistance genes was well recognized and documented with the transfer of several alien genes into their cultivated counterparts through wide or distant hybridization with or without employing embryo-rescue and mutagenesis. However, the amazing revelation that the wild relatives are also a source of yield-related genes is a development of the molecular era. Apomictic genes are another asset of many crop relatives that deserve mention. All of these past and the present factors have led to the realization that the so-called inferior species are highly superior in conserving desirable genes and can serve as a goldmine for breeding elite plant varieties. This is particularly true at a point when natural genetic variability has been depleted or exhausted in most of the major crop species, particularly due to growing and promoting only a handful of so-called high-yielding varieties while disregarding the traditional cultivars and landraces. In the era of molecular breeding, we can map desirable genes and polygenes, identify their donors and utilize tightly linked markers for gene introgression, mitigating the constraint of linkage drag, and even pyramid genes from multiple sources, cultivated or wild taxa. The evaluation of primary, secondary and tertiary gene pools and utilization of their novel genes is one of the leading strategies in present-day plant breeding. It is obvious that many wide hybridizations will never be easy and involve near-impossible constraints such as complete or partial sterility. In such cases gene cloning and gene discovery, complemented by intransgenic breeding, will hopefully pave the way for success. The utilization of wild relatives through traditional and molecular breeding has been thoroughly enumerated over the chapters throughout this series.

Enormous genomic resources have been developed in the model crop relatives, for example *Arabidopsis thaliana* and *Medicago truncatula*. BAC, cDNA and EST libraries have also been developed in some other crop relatives. Transcriptomes and metabolomes have also been dissected in some of them. However, similar genomic resources are yet to be constructed in many crop relatives. Hence this section has been included only in chapters on the relevant genera.

In this book series, we have included a section on recommendations for future steps to create awareness about the wealth of wild crop relatives in society at large and also for concerns for their alarmingly rapid decrease due to genetic erosion. The authors of the chapters have also emphasized on the imperative requirement of their conservation, envisaging the importance of biodiversity. The importance of intellectual property rights and also farmers' rights as owners of local landraces, botanical varieties, wild species and subspecies has also been dealt in many of the chapters.

I feel satisfied that the authors of the chapters in this series have deliberated on all the crucial aspects relevant to a particular genus in their chapters.

I am also very pleased to present many chapters in this series authored by a large number of globally reputed leading scientists, many of whom have contributed to the development of novel concepts, strategies and tools of genetics, genomics and breeding and/or pioneered the elucidation and improvement of particular plant

genomes using both traditional and molecular tools. Many of them have already retired or will be retiring soon, leaving behind their legacies and philosophies for us to follow and practice. I am saddened that a few of them have passed away during preparation of the manuscripts for this series. At the same time, I feel blessed that all of these stalwarts shared equally with me the wealth of crop relatives and contributed to their recognition and promotion through this endeavor.

I would also like to be candid with regard to my own limitations. Initially I planned for about 150 chapters devoted to the essential genera of wild crop relatives. However, I had to exclude some of them either due to insignificant progress made on them during the preparation of this series, my failure to identify interested authors willing to produce acceptable manuscripts in time or authors' backing out in the last minute, leaving no time to find replacements. I console myself for this lapse with the rationale that it is simply too large a series to achieve complete satisfaction on the contents. Still I was able to arrange about 125 chapters in the ten volumes, contributed by nearly 400 authors from over 40 countries of the world. I extend my heartfelt thanks to all these scientists, who have cooperated with me since the inception of this series not only with their contributions, but also in some cases by suggesting suitable authors for chapters on other genera. As happens with a mega-series, a few authors had delays for personal or professional reasons, and in a few cases, for no reason at all. This caused delays in the publication of some of the volumes and forced the remaining authors to update their manuscripts and wait too long to see their manuscripts in published form. I do shoulder all the responsibilities for this myself and tender my sincere apologies.

Another unique feature of this series is that the authors of chapters dedicated to some genera have dedicated their chapters to scientists who pioneered the exploration, description and utilization of the wild species of those genera. We have duly honored their sincere decision with equal respect for the scientists they rightly reminded us to commemorate.

Editing this series was, to be honest, very taxing and painstaking, as my own expertise is limited to a few cereal, oilseed, pulse, vegetable, and fruit crops, and some medicinal and aromatic plants. I spent innumerable nights studying to attain the minimum eligibility to edit the manuscripts authored by experts with even life-time contributions on the concerned genera or species. However, this indirectly awakened the "student-for-life" within me and enriched my arsenal with so many new concepts, strategies, tools, techniques and even new terminologies! Above all, this helped me to realize that individually we know almost nothing about the plants on this planet! And this realization strikingly reminded me of the affectionate and sincere advice of Dr. Norman Borlaug to keep abreast with what is happening in the crop sciences, which he used to do himself even when he had been advised to strictly limit himself to bed rest. He was always enthusiastic about this series and inspired me to take up this huge task. This is one of the personal and professional reasons I dedicated this book series to him with a hope that the present and future generations of plant scientists will share the similar feelings of love and respect for all plants around us for the sake of meeting our never-ending needs for food, shelter, clothing, medicines, and all other items used for our basic requirements and comfort. I am also grateful to his granddaughter, Julie Borlaug, for kindly extending her permission to dedicate this series to him.

I started editing books with the 7-volume series on Genome Mapping and Molecular Breeding in Plants with Springer way back in 2005, and I have since

edited many other book series with Springer. I always feel proud and satisfied to be a member of the Springer family, particularly because of my warm and enriching working relationship with Dr. Sabine Schwarz and Dr. Jutta Lindenborn, with whom I have been working all along. My special thanks go out to them for publishing this "dream series" in an elegant form and also for appreciating my difficulties and accommodating many of my last-minute changes and updates.

I would be remiss in my duties if I failed to mention the contributions of Phullara – my wife, friend, philosopher and guide – who has always shared with me a love of the collection, conservation, evaluation, and utilization of wild crop relatives and has enormously supported me in the translation of these priorities in my own research endeavors – for her assistance in formulating the contents of this series, for monitoring its progress and above all for taking care of all the domestic and personal responsibilities I am supposed to shoulder. I feel myself alien to the digital world that is the sine qua non today for maintaining constant communication and ensuring the preparation of manuscripts in a desirable format. Our son Sourav and daughter Devleena made my life easier by balancing out my limitations and also by willingly sacrificing the spare amount of time I ought to spend with them. Editing of this series would not be possible without their unwavering support.

I take the responsibility for any lapses in content, format and approach of the series and individual volumes and also for any other errors, either scientific or linguistic, and will look forward to receiving readers' corrections or suggestions for improvement.

As I mentioned earlier this series consists of ten volumes. These volumes are dedicated to wild relatives of Cereals, Millets and Grasses, Oilseeds, Legume Crops and Forages, Vegetables, Temperate Fruits, Tropical and Subtropical Fruits, Industrial Crops, Plantation and Ornamental Crops, and Forest Trees.

This volume "Wild Crop Relatives: Genomic and Breeding Resources – Tropical and Subtropical Fruits" includes 11 chapters dedicated to *Actinidia*, *Ananas*, *Citrus*, *Mangifera*, *Morus*, *Musa*, *Passiflora*, *Persea*, *Poncirus*, *Spondias*, and *Vasconcellea*. The chapters of this volume were authored by 42 scientists from 14 countries of the world, namely Australia, Brazil, Belgium, China, Columbia, France, India, Italy, New Zealand, Peru, Taiwan, Turkey, the USA and Venezuela.

It is my sincere hope that this volume and the series as a whole will serve the requirements of students, scientists and industries involved in studies, teaching, research and the extension of tropical and subtropical fruit crops with an intention of serving science and society.

Clemson, USA Chittaranjan Kole

Contents

Abbreviations

2,4-D	2,4-D-dicholorphenoxy acetic acid
ACC	1-Aminocyclopropane-1-carboxylic acid
AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
APG	Angiosperm phylogenetic group
ARS	Agriculture Research Service (of USDA)
BAC	Bacterial artificial chromosome
BAP	Benzyl amino purine
BC1	First backcross
BC2	Second backcross
BIBAC	Binary-BAC
BSA	Bulked segregant analysis
BSV	Banana streak badnavirus
CAAS	Chinese Academy of Agricultural Sciences
CABMV	Cowpea aphid-borne mosaic virus
CAPS	Cleaved amplified polymorphic sequence
CBD	Convention on Biological Diversity
CcGA20ox1	Citrus gibberellin 20-oxidase cDNA gene
cDNA	Complementary-DNA
CiMV	Citrus mosaic virus
CINVESTA	Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (Center for Research and Advanced Studies of the National Polytechnic Institute), Mexico
CIRAD	Centre de coopération Internationale en Recherche Agronomique pour le Développement (Agricultural Research Centre for International Development), France
cM	CentiMorgan
CMA	Chromomycin A 3
CMS	Carboxymethyl-Sephadex
COSEWIC	Committee on Status of Endangered Wildlife in Canada
cpDNA	Chloroplast-DNA
cpSSR	Chloroplast-SSR
CR	Critically endangered
CSGRC	Central Sericultural Germplasm Resources Centre (India)
CTV	*Citrus tristeza* clostero virus
CTV	*Citrus tristeza* virus
CVC	Citrus exocortis viriod

DAPI	4,6-Diamidino-2-phenylindole
DFP	DNA fingerprint
DNJ	Deoxyjirimycin
EAPV	East Asian *Passiflora* Virus
EFN	Extrafloral nectaries
ELISA	Enzyme-linked immunosorbent assay
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária (Brazil)
EN	Endangered
EOO	Extent of occurrence
EST	Expressed sequence tag
F_1	Filial 1 (first filial generation)
F_2	Filial 2 (second filial generation)
F3	Filial 3 (third filial generation)
FAO	Food and Agriculture Organization (of the United Nation)
FHIA	Fundación Hondureña de Investigación Agrícola (Honduras Foundation for Agricultural Research), Honduras
FISH	Fluorescent/ce in situ hybridization
FocR4	Race 4 of Fusarium wilt
FPB	Farmers' participatory breeding
G3pdh	Glyceraldehyde 3-phosphate dehydrogenase
GA	Gibberellin
GA_3	Gibberellic acid 3
GCGN	Global Citrus Germplasm Network
GIS	Geographic information sysytem
GISH	Genomic in situ hybridization
GRIN	Germplasm Resources Information Network (USA)
GS	Glutamine synthetase
HAL2	Halotolerance gene
HAT-RAPD	High annealing temperature-RAPD
HBV	Hepatitis B virus
HCN	Hydrogen cyanide
hEGF	Human epidermal growth factor
IAA	Indole-3-acetic acid
IAPAR	Instituto Agronômico do Paraná (Agronomic Institute of Parana), Brazil
IBA	Indole-3-butyric acid
IEB	Institute of Experimental Botany (Czech Republic)
IGS	Ribosomal gene spacer
IIHR	Indian Institute of Horticultural Research (India)
IITA	International Institute of Tropical Agriculture (Nigeria)
INIA	Instituto Nacional de Investigaciones Agrícolas (Venezuela)
INIBAP	International Network for the Improvement of Banana and Plantain
IPGRI	International Plant Genetics Resource Institute (presently Biodiversity International), Italy
IRAP	Interretrotransposon amplified polymorphism
IRD	Institut de Recherche Pour le Développement (Institute of Research for Development), France
IRS	Intertranslation space of ribosomal-DNA

ISSR	Intersimple sequence repeat
ITC	International Transit Center (of US at Kyrgyzstan)
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
ITS	Internal transcribed spacer
IUCN	International Union for Conservation of Nature
Kn	Kinetin
LANGEBIO	Laboratorio Nacional de Genómica para la Biodiversidad (National Laboratory of Genomics for Biodiversity), Mexico
LD	Linkage disequilibrium
LG	Linkage Group
LRR	Leucine-rich repeat
LTR	Long terminal repeats
MarMV	Maracuja mosaic tobamovirus
MGIS	*Musa* Germplasm Information System
MIPS	Myo-inositol-1L-phosphate synthase
MMP-2	Metallo-proteases-2
MMP-9	Metallo-proteases-9
MS	Mono S Sepharose
MS	Murashige and Skoog (medium)
MSAP	Methylation-sensitive amplification polymorphism
MSY	Specific region of the Y chromosome
mtDNA	Mitochondrial-DNA
Mya	Million years ago
NAA	α-Naphthaleneacetic acid
NAD	Nicotinamide adenine dinucleotide
NBS-LRR	Nucleotide-binding site
NCBI	National Center for Biotechnology Information (USA)
NCEPL	National Committee on Environmental Planning and Coordination (India)
ncpGS	Nuclear copy chloroplast expressed glutamine synthase
NGO	Non-Governmental Organization
NIAS	National Institute of Agrobiological Sciences (Japan)
NISES	National Institute of Sericultural and Entomological Science (Japan)
NK	Natural killer
NT	Near threatened
nt	Nucleotides
ORF	Open reading frame
overgo	Overlapping oligonucleotide probe
PBA	P450-based analogs
PCA	Principal component analysis
PCO	Principal coordinates
PCR	Polymerase chain reaction
PE-ACS1	*Passiflora edulis* ACC synthase-1
PE-ACS2	*Passiflora edulis* ACC synthase-2
Pe-AFP1	*Passiflora edulis* antifungal peptide-1
PE-ERS1	*Passiflora edulis* ethylene receptor-1
PE-ERS2	*Passiflora edulis* ethylene receptor-2

PEG	Polyethylene glycol
Pf-AOS	*Passiflora* allene oxide synthase
PLV	*Passiflora* latent virus
PPO	Polyphenol oxidase
PRI	Pineapple Research Institute (USA)
PRSV	Papaya ringspot virus
prsv-1	Single resistant gene for *PRSV*
PRSV-P	Papaya ringspot virus type P
PWV	Passion fruit woodiness virus
QDPI	Queensland Department of Primary Industries and Fisheries
QRL	Quantitative resistance loci
QTL	Quantitative trait loci
RAF	Randomly amplified DNA fingerprint
RAPD	Random(ly) amplified polymorphic DNA
rDNA	Ribosomal DNA
REMERFI	Red Mesoamericana de Recursos Fitogeneticos [Mesoamerican Network of Plant Genetic Resources]
RFLP	Restriction fragment length polymorphism
RGA	Resistance gene analog
RT	Reverse transcriptase
SAGE	Serial analysis of gene expression
SAMPL	Selective amplification of microsatellite polymorphic loci
SCAR	Sequence characterized amplified region
SD-AFLP	Secondary digest-AFLP
SES	Sericulture Experiment Station (Bulgaria)
SMTA	Standard material transfer agreement
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
STMS	Sequence-tagged microsatellite site
STS	Sequence tagged site
tRNA-Leu	Leucine Transfer-RNA
tRNA-Lys	Lysine Transfer-RNA
tRNA-Phe	Phenylalanine Transfer-RNA
UC	University of California
UNDP	United Nations Development Program
UNESCO	United Nations Educational, Scientific and Cultural Oraganization
USDA	United States Department of Agriculture
UTR	Untranslated region
VNTRS	Variable number tandem repeats
VU	Vulnerable
WGS	Whole genome shotgun
WWF	World Wide Fund
Ω	Omega

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Chapter 1
Actinidia

P.M. Datson and A.R. Ferguson

1.1 Introduction

Kiwifruit in comparison with most other crop plants have a very short history of cultivation.

The two commercially important kiwifruit species are *Actinidia deliciosa* and *A. chinensis*. These are sometimes combined as varieties of the same species (Li et al. 2007a, b), but we retain them here as distinct species. *A. deliciosa* was introduced into cultivation toward the end of the nineteenth century (Ferguson and Huang 2007) and the first commercial orchards were established in New Zealand around 1930. *A. chinensis*, a species closely related to *A. deliciosa*, was successfully introduced into cultivation in China as recently as 1961 (Zhang et al. 1983) and it has been grown commercially in other countries and its fruits have been traded internationally for little more than a decade. At present, *A. deliciosa* provides about 85% of the kiwifruit produced commercially worldwide and *A. chinensis* about 15%. A few other *Actinidia* species are also cultivated, *A. arguta*, *A. eriantha*, *A. kolomikta*, and *A. polygama*, but these are of very minor commercial importance.

Almost all current kiwifruit cultivars are either direct selections from the wild or only a few generations removed from the wild (Ferguson and Huang 2007; Ferguson and Seal 2008; Ferguson 2009; Li et al. 2010). All the early New Zealand cultivars of *A. deliciosa*, including the main commercial cultivar 'Hayward', are selections descended from a very small number of seedlings grown from a single seed introduction into New Zealand from China at the beginning of the twentieth century (Ferguson and Bollard 1990). Very few cultivars of *A. deliciosa* have yet emerged from systematic breeding programs and there are only small commercial plantings of them (Testolin and Ferguson 2009). Only one commercially important cultivar of *A. chinensis* has so far resulted from a controlled hybridization. 'Hort16A' came from the cross of a female, the product of open-pollination of seedlings from a seed accession from the wild, and a male raised from seed collected in the wild (Mugglestone et al. 1998; Ferguson et al. 1999).

- Cultivated kiwifruit are, therefore, generally not greatly different from those in the wild. Some wild individuals of *A. deliciosa* and *A. chinensis* contained genes suitable for domestication and selection has involved recognition of plants with fruit having commercial potential. Comparison of the plants that have been selected with those remaining in the wild contributes to our understanding of the kiwifruit we now grow and the potential for their improvement. We describe studies using both cultivated plants and their wild allies on the origin, evolution, phylogenetic relationships, cytogenetics, and the genetic improvement of kiwifruit employing both classical strategies and advanced tools of genomics and biotechnology.

1.2 Basic Botany

1.2.1 The Genus Actinidia

Kiwifruit belong to the genus *Actinidia* Lindl. Currently, 55 species and about 76 taxa are recognized

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within the genus, with the most recent revision (Li et al. 2007a, b, 2009) having relegated about 25% of the species and 40% of all taxa previously described (Ferguson and Huang 2007). Further changes seem inevitable as many taxa are not well known, and transitional forms, sometimes formally described, suggest considerable hybridization between taxa with overlapping geographical distributions (Liang 1983; Ferguson 1990a, b; Chat et al. 2004; Li et al. 2009).

Female flowers of the genus have a characteristic radiating arrangement of styles, like the spokes of the wheel. This is the basis for Lindley's generic name *Actinidia* from aktis (Greek), ray. In China, the name mihoutao [monkey peach] is used for the whole genus and individual species are now distinguished such as zhonghua [Chinese] mihoutao for *A. chinensis* and meiwei [delicious] mihoutao for *A. deliciosa* (Cui et al. 2002; Li et al. 2007a). In older literature, these two species were usually distinguished by the hairs on the fruit, ruanmao for soft-haired [*A. chinensis*] and yingmao [*A. deliciosa*] for stiff-haired. A variety of names were used traditionally, yangtao [sun peach] being one of the most common (Ferguson 1990c). Outside China, "Chinese gooseberry" became the most popular name (or translations such as groseille de Chine) until it was replaced in New Zealand by kiwifruit (Ferguson and Bollard 1990). This is the most widely used name today, even in China, although in Italy, "actinidia" is often used. Kiwifruit is sometimes incorrectly shortened to "kiwi" or wrongly separated into two words.

1.2.2 Geographic Distribution

Nearly all *Actinidia* species (52 of 55) and taxa (73 of 76) occur in China (Li et al. 2007a) and some extend to adjoining countries, but only three species occur exclusively outside China: *A. strigosa* from Nepal, *A. petelotii* from Vietnam, and *A. hypoleuca* from Japan. The genus has a wide geographic distribution in eastern Asia, from just south of the Equator, to as far north as latitude 50°, with most taxa occurring in south-central and south-west China between the Yangzi (Chang Jiang) and Pearl (Zhu Jiang) Rivers, in a belt between approximately 25 and 30°N (Fig. 1.1). Most *Actinidia* grow best in warm, moist, and sheltered environments; cold, dry conditions are unsuitable. Hence, the northernmost boundary of the area of greatest abundance corresponds to the Qin Ling Mountains in southern Shaanxi, the western boundary, the lower mountains of the eastern border of the Tibetan plateau, the Hengduan Shan (Hengduan Mountains) to the west of Yunnan and Sichuan, and the southernmost, the isotherm that corresponds to a mean annual temperature between 20°C and 22°C (Ferguson and Huang 2007). This area is considered to be the center of diversity of *Actinidia* as well as the center of current evolution (Liang 1983). Taxa tend to vary little in their relative vertical distributions, but the altitude at which a particular *Actinidia* taxon occurs is not absolute but decreases with increasing latitude (Ferguson and Huang 2007). Thus, taxa that grow high in the mountains of southern China can be found at sea level in Siberia.

1.2.3 Vegetative Morphology

All *Actinidia* are climbing plants and under most conditions are deciduous. Vines can be extraordinarily robust and vigorous, capable of smothering large trees (Li 1952), but high in the mountains or in cold, northernmost regions, plants may be reduced to scrambling thickets rather like brambles (Berestova 1970). In cultivation, vine vigor will depend on local growing conditions and genotype, but commercial vines are usually very vigorous and require strong and expensive support structures.

Actinidia species are often very variable in vegetative structures. Even individual plants can show variation in the leaves produced at different times of year or on different types of wood (Dunn 1911). This can cause confusion when single specimens are used to describe taxa. Differences in vegetative morphology between staminate and pistillate plants can also be misleading (Cuong et al. 2007). Widespread species have often been divided into morphologically distinct varieties, which sometimes, but not always, occupy discrete geographical areas. Broader taxonomic concepts with more intrataxal variation seem more realistic, and further collections, especially of taxa based on only a few specimens, are needed (Li et al. 2009).

Key	Taxa
F	1-5
E	6-10
D	11-15
C	16-20
B	21-30
A	31-40

Countries	Taxa
Bhutan	2
Cambodia	1
India	2
Indonesia	1
Japan	5
Korea	4
Laos	1
Malaysia	1
Myanmar	2
Nepal	2
Russia	3
Thailand	2
Vietnam	6

Fig. 1.1 Geographic distribution of *Actinidia* taxa

1.2.4 Flower Morphology and Sex Determination

All *Actinidia* species appear to be dioecious, although functional dioecy has been confirmed in only *A. deliciosa* and *A. polygama* (McNeilage 1991a; Kawagoe and Suzuki 2004). *Actinidia* are cryptically dioecious: female plants produce morphologically perfect flowers with well-developed pistils and stamens, but their stamens produce non-viable pollen; flowers of male plants have small, rudimentary ovaries without viable ovules, but their stamens release viable pollen (Rizet 1945; Schmid 1978; White 1990) (see Fig. 1.2). Failure of pollen maturation or of pistil growth occurs late in flower bud development (Brundell 1975; Schmid 1978; Messina 1993). Dioecy creates problems in commercial cultivation: part of the orchard area (usually about 10%) must be dedicated to pollenizer vines, male and female vines must flower at the same time, and there must be efficient transfer of pollen as fruit size in kiwifruit is largely dependent on the number of seed set. Orchard layout must also encourage honeybee activity to facilitate pollination. Some of these problems are overcome by mechanical pollination of fruiting vines by harvested pollen; a better solution might be the development of hermaphrodite, self-setting vines.

Fig. 1.2 Flowers of female (*left*) and male (*right*) *Actinidia eriantha*

There is an active-Y sex determination system (X_nX/X_nY) in *Actinidia* with the plants that contain

Y-chromosomes being male (Testolin et al. 1995; Harvey et al. 1997b; Fraser et al. 2009). Sex determination in *Actinidia* is probably controlled by two genes: one suppressing pistil development in staminate flowers, and the other stopping pollen development in pistillate flowers (Harvey et al. 1997a). It is likely that the sex chromosomes of extant *Actinidia* have a single origin that pre-dates the radiation of the group (Harvey et al. 1997a; Yan et al. 1997b; Testolin et al. 1999). Disomic sex segregation seems to operate at the different ploidy levels (Testolin et al. 1995).

The large number of *Actinidia* species that contain related sex chromosomes is a valuable tool for studying sex chromosome evolution in plants as it should allow for comparative analysis of the process of sex chromosome evolution across a wide range of related species.

Gender changes are possible. A bud mutation in a mature male vine caused a gender change from male to female (Testolin et al. 2004). Gender inconstancy has also been observed in individuals of *A. arguta*, *A. chinensis*, *A. deliciosa*, and *A. eriantha* (Messina et al. 1990; Tang and Jiang 1995; Testolin et al. 1999; Mizugami et al. 2007). Fruiting ("inconstant" or andromonoecious) males occasionally occur: these have labile sex expression with occasional bisexual flowers occurring among staminate flowers (Hirsch et al. 1990; McNeilage 1991a, b). Hermaphrodite plants of *A. deliciosa* have been produced from crosses involving inconstant males (McNeilage and Steinhagen 1998; McNeilage et al. 2007). Hermaphroditism in *A. deliciosa* is stable and heritable. It is, therefore, likely that hermaphrodite breeding lines could be developed in different *Actinidia* species by identifying and crossing inconstant males. Variants in sex expression, either collected from the wild (e.g., Tang and Jiang 1995; Mizugami et al. 2007) or from commercial orchards (e.g., Hirsch et al. 1990), could be an important resource for kiwifruit improvement programs.

1.2.5 Fruit Characteristics

Interspecific and intraspecific variation in fruit attributes has understandably received most attention and is well described (Li 1952; Huang et al. 1983, 2000a, b, 2004; Liang 1984; Li et al. 1985; Ferguson 1990a; Cui et al. 2002; Han et al. 2003; Ferguson and Huang 2007; Nishiyama 2007). Infructescence size, fruit size, fruit shape, fruit hairs and skin characteristics, external color, flesh color, ripening indicators, flesh texture, flesh flavor, time of maturity and ripening, handling and storage responses, and yield potential all need to be considered in the development of a commercial cultivar (Fig. 1.3). Wild *Actinidia* species have many fruit characteristics that could, with advantage, be introgressed into cultivated kiwifruit. Achieving this requires a good knowledge of the variability that exists in the wild, the collection and preservation of useful diversity, and a good understanding of the reproductive biology of the genus.

The potential health benefits of kiwifruit deserve special attention. Kiwifruit are often promoted for their high vitamin C (ascorbic acid) content, which probably contributes to the health benefits observed. *A. deliciosa* 'Hayward' and *A. chinensis* 'Hort16A' typically contain 85 and 100 mg/100 g (fresh weight), respectively, and a single fruit can provide the recommended daily intake (Ferguson and Ferguson 2002; Kassardjian et al. 2006). Vitamin C levels vary greatly among and within *Actinidia* species from less than 30 mg/100 g fresh weight to more than 1,000 mg/100 g fresh weight (Nishiyama et al. 2004b; Ferguson and Huang 2007; Nishiyama 2007). The health benefits attributed to vitamin C include antioxidant, antiatherogenic, and anticarcinogenic activity, as well as immunomodulation. Human intervention trials validate some of these health benefits for green kiwifruit (*A. deliciosa* 'Hayward') in the areas of "natural protection" (protection from oxidative stress and DNA damage associated with mutation and cancer), gut health (laxation and healthy bowel habits), and cardiovascular health (reduction in platelet aggregation) (Hunter et al. 2009). A diet rich in fruit and vegetables offers health and wellness benefits, and preliminary evidence suggests that other cultivars such as *A. chinensis* 'Hort16A' or other *Actinidia* species may also have health benefits.

New kiwifruit cultivars could also benefit from the absence of compounds present in 'Hayward'. Oxalate raphides in 'Hayward' are probably not a health issue but can affect palatability especially of processed products. Studies of the concentrations in fruit of *Actinidia* germplasm of ascorbate and oxalate (a product of ascorbate catabolism) indicate that it should be possible to select cultivars that are high in ascorbate but low in oxalate (Rassam and Laing 2005). Allergic

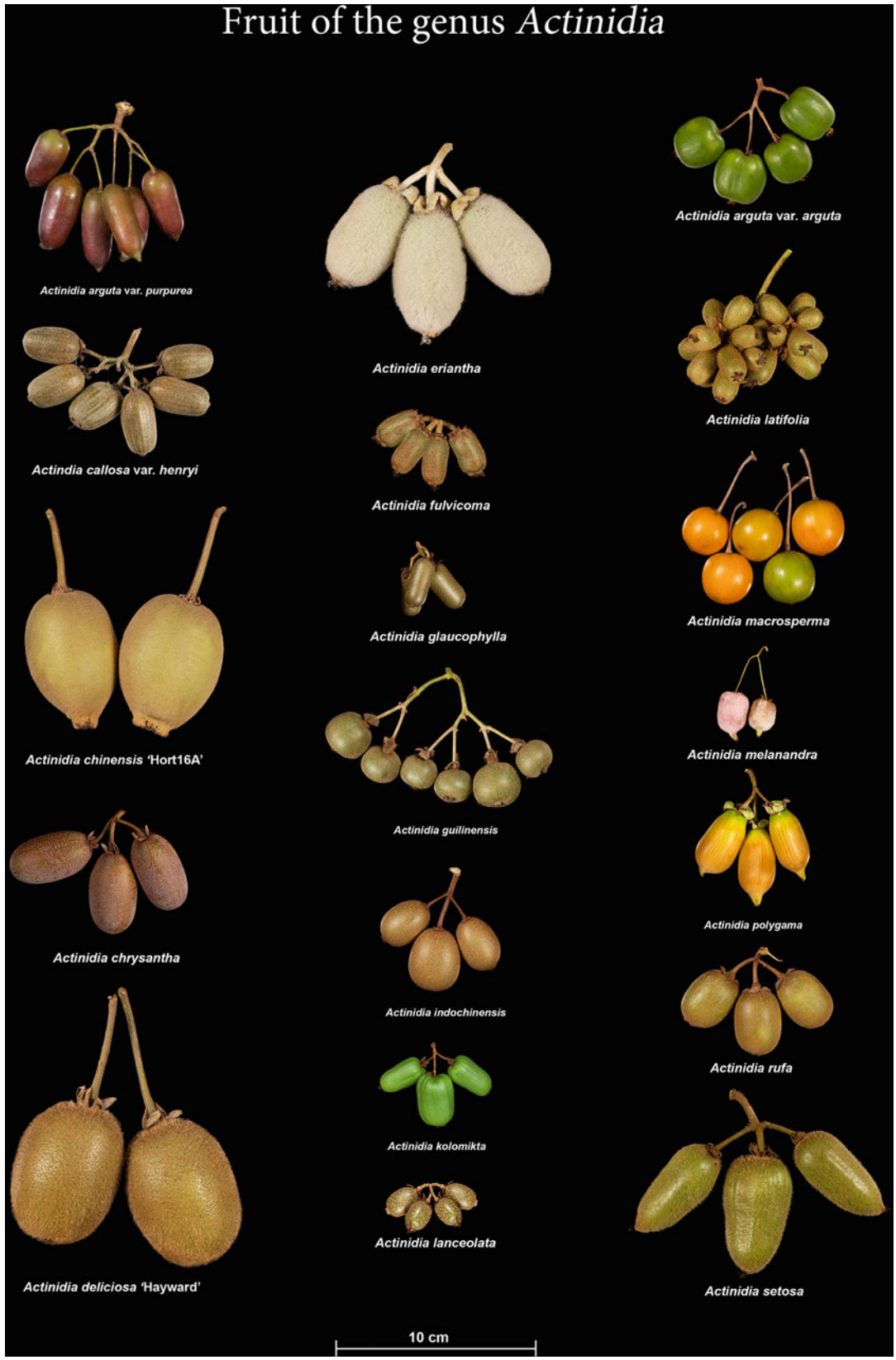

Fig. 1.3 Fruit of 19 taxa of *Actinidia*

responses to kiwifruit have increased with increased kiwifruit production and consumption. Allergenicity has been ascribed to the protease actinidin, which is often present in kiwifruit in large amounts. Actinidin content and protease activity vary in different kiwifruit: fruit of some *A. arguta* cultivars contain more actinidin than *A. deliciosa* 'Hayward', whereas actinidin and protease activity was not detectable or was barely detectable in two *A. rufa* selections (Nishiyama et al. 2004a) and some *A. chinensis* cultivars, including 'Hort16A' (Yamanaka et al. 2004), even though 'Hort16A' fruit still elicit the allergic response (Lucas et al. 2005).

1.2.6 Cytogenetics

The basic chromosome number of *Actinidia* is $x = 29$. Polyploidy in *Actinidia* is common, with diploids, tetraploids, hexaploids, and octoploids occurring in diminishing frequencies (Ferguson and Huang 2007). Although only limited chromosome counts have been made for most taxa, ploidy races have been detected in 15 taxa, suggesting that variation in ploidy may be common. For example, detailed studies have revealed diploid, tetraploid, hexaploid, heptaploid, and octoploid forms of *A. arguta* s.l. in the wild in Japan (Kataoka et al. 2010). The high frequency of polyploids and chromosome races in *Actinidia* is probably due to the production and fusion of numerically unreduced gametes (Yan et al. 1997b). Somatic doubling in bud sports (see Sect. 1.5.1) may also occur in the wild.

Although known ploidy races within taxa are usually separated geographically, they cannot usually be readily distinguished morphologically; e.g., there are no obvious morphological differences between diploid and tetraploid races of *A. chinensis* or between most of the various ploidy races of *A. arguta*. It is likely that within wild populations of *Actinidia* species, there is considerable interspecific hybridization and gene transfer (Liu et al. 2007, 2008), as well as frequent differentiation into ploidy races not yet detected.

The high basic chromosome number of *Actinidia* may be due to chromosome duplication early in the evolution of the genus, followed by rediploidization (McNeilage and Considine 1989; Shi et al. 2010). Comparison of the number and morphology of chromosomes in *Actinidia* with those in the related genera *Clematoclethra* ($x = 12$) and *Saurauia* ($x = 13$) suggests that *Actinidia* ($x = 29$) was derived from a palaeotetraploid ($x = 14$, $2n = 56$) and that an additional chromosome pair was formed through a centromeric chromosome fission (He et al. 2005). An alterative scenario would be the hybridization between $2n = 28$ and $2n = 30$ species followed by a polyploidization event. Frequent duplication of microsatellite loci supports *Actinidia* diploid species being actually polyploid (Huang et al. 1998).

Actinidia chromosomes are relatively small (<1 μm) and it is difficult to observe their morphology. He et al. (2005) characterized the karyotype of diploid *A. chinensis* ($2n = 58$) chromosomes as 38 metacentric + 18 submetacentric (including two satellite chromosomes) + 2 telomeric. The 8th and 9th, and the 11th and 12th pairs of the chromosomes are similar in length and morphology.

1.2.7 Genome Size

Genome size in *Actinidia* has been determined mainly by flow cytometry, with the published 2C-values (holoploid) for hexaploid *A. deliciosa* ranging from 3.8 to 4.5 pg DNA (Hopping 1993, 1994; Ollitrault-Sammarcelli et al. 1994; Ferguson et al. 1997; Start et al. 2007) or 3.7–4.3 $\times$ 10^9 bp (assuming 1 pg DNA = 0.978 $\times$ 10^9 bp), a range that agrees reasonably well with the estimate of Matsunaga et al. (1996) of about 3.5 $\times$ 10^9 bp. *Actinidia* taxa at the one ploidy level vary little in genome size, and polyploidization (increase in ploidy) has not resulted in a loss of nuclear DNA (Ferguson et al. 1997; Zhang et al. 2008). Flow cytometry can, therefore, be used with confidence to determine ploidy in *Actinidia* and the results are consistent with chromosome counts (Ferguson and Huang 2007; A.R. Ferguson unpublished).

1.2.8 *Taxonomic Position of* A. chinensis *and* A. deliciosa

The two commercially important *Actinidia* species, *A. chinensis* and *A. deliciosa*, and a third closely related species restricted to Taiwan, *A. setosa*, have variously been treated either as separate species or as

varieties of the same species. Liang (1975) initially treated *A. chinensis* and *A. deliciosa* as varieties of the same species, but later agreed that there were sufficient morphological differences to differentiate them as separate species (Liang and Ferguson 1984, 1986). *A. setosa*, previously treated as a variety of *A. chinensis*, was also raised to specific status (Liang and Ferguson 1985). The latest taxonomic treatment in the *Flora of China* recombines *A. chinensis, A. deliciosa*, and *A. setosa* into *A. chinensis* (Li et al. 2007a, b, 2009), but we have, for convenience, retained them as separate species. The relationships between *A. chinensis* and *A. deliciosa* and other *Actinidia* species are discussed further in Sect. 1.4.

1.2.9 Agricultural Status

Each year, 100,000–150,000 tons of *Actinidia* fruit are collected from the wild in China (Huang and Ferguson 2001; Chen 2003). Most of these fruit would be of *A. chinensis* or *A. deliciosa*, but other *Actinidia* species are also harvested. The total quantity of fruit collected indicates that millions of plants must remain in the wild. Many *Actinidia* species establish readily, especially in cutover or regenerating forests, and they grow so vigorously that they can envelop large trees: *Actinidia* thus have the potential to become serious weeds in those areas in which the climate allows successful kiwifruit cultivation. In New Zealand, in the main growing area of the Bay of Plenty, naturalized kiwifruit have become so abundant that plant pest control programs have been initiated (Sullivan et al. 2007). New Zealand is possibly unusual in that there are often native forests or commercial plantations close to kiwifruit orchards and these provide suitable conditions for the establishment and spread of weed kiwifruit. Occasional wild kiwifruit have been recorded in Germany (Kasperek 2003) and the United Kingdom (Payne 1997) presumably from seed from imported fruit. In other parts of the world in which kiwifruit are grown commercially, they could easily become serious weeds.

In China, *Actinidia* fruit have been collected from the wild for many centuries. Such fruit are generally small and are of poor quality. The success of *A. deliciosa*, first in New Zealand, and then in other countries, stimulated Chinese interest in kiwifruit cultivation. Now fruit from commercial orchards of *A. chinensis* and *A. deliciosa* in China are much more important than fruit collected from the wild, and China has a greater area in kiwifruit orchards than any other country.

Commercial plantings of kiwifruit around the world now amount to about 150,000 ha and total annual production to about 1.8 million tons (Belrose Inc. 2010). Outside China, the *A. deliciosa* cultivar 'Hayward' and its associated males still predominate. 'Hayward' is the traditional "green" kiwifruit well known to consumers for its bright green fruit flesh. 'Hayward' was favored over other *A. deliciosa* cultivars because its fruit have a better flavor, are larger, and store for much longer. Newer cultivars of "green" kiwifruit, usually sports or seedlings of 'Hayward', are now emerging, particularly in Italy (Testolin and Ferguson 2009), although none is yet widely grown. "Gold" kiwifruit, which are yellow-fleshed cultivars of *A. chinensis*, are becoming increasingly important. In New Zealand, gold kiwifruit from the cultivar 'Hort16A' now account for around 30% of the total production of kiwifruit. In Europe and South America, 'Hort16A' and another yellow-fleshed cultivar, 'Jintao', are being planted. 'Jintao' is a selection from the wild; 'Hort16A' is only a couple of generations removed from germplasm introduced from the wild. This demonstrates the importance of germplasm resources for kiwifruit improvement.

In China, kiwifruit plantings are genetically much more diverse, but apart from cultivars of New Zealand origin, they are nearly all essentially selections from the wild (Ferguson and Huang 2007; Li et al. 2010). About one quarter would be of *A. chinensis* and the remainder of *A. deliciosa*.

1.3 Conservation Initiatives

Cultivated kiwifruit are still very similar to plants in the wild. Breeders of crops with little history of improvement tend to make the greatest use of collections of raw germplasm, and horticultural crops in general are noted for having greater intrataxon variation than do agricultural crops (Engels and Thormann 2007). Since nearly all kiwifruit cultivars grown in China are direct selections from the wild, they themselves represent a valuable resource of genetic diversity (Zhen et al. 2004).

Many of the existing kiwifruit cultivars available outside China come from a very narrow genetic base, and although *A. chinensis* and *A. deliciosa* are highly heterozygous (Ferguson and Huang 2007), it is likely that the development of really novel types of kiwifruit will come from the introgression of genes from other *Actinidia* species. Recognition and acquisition from the wild and the maintenance and evaluation of *Actinidia* germplasm are therefore essential for kiwifruit improvement programs.

1.3.1 Genetic Erosion

Although *Actinidia* species are widely dispersed throughout much of China, they are found mainly in relatively inaccessible or mountainous areas. Intensified land use means that most wild *Actinidia* populations are now mainly remnant populations and this could lead to genetic drift. Even these remnant populations are under increasing threat because of land clearance and with vines often being cut down to collect the fruit (Li and Lowe 2007). Nearly 20 *Actinidia* taxa are currently considered endangered (Zhang 2000; Zhang et al. 2000). Many of the wild *Actinidia* resources recorded only 25 years ago in Sichuan have now dwindled considerably and some taxa have disappeared completely (Li and Lowe 2007). Such losses are probably common throughout the rest of China and both in situ and ex situ conservation are required to protect genetic resources.

1.3.2 Sampling Genetic Diversity

Even large germplasm collections can conserve only a very small part of the wild diversity, especially as *Actinidia* appears to be such a variable genus. It is most unlikely that just a few genotypes will give an adequate representation of the variation within any given taxon. Yet, apart from *A. chinensis*, *A. deliciosa*, and, possibly, *A. arguta*, most *Actinidia* germplasm collections contain only a small number of representatives of any taxon. Furthermore, many of the genotypes held in different germplasm collections have been obtained by exchange with other collections and the sampling of the wild diversity is even more limited than might be first thought. Huang (2003) proposed that the sampling and conservation of *Actinidia* germplasm be made more systematic by considering geographic distribution (since isolated populations may be undergoing genetic drift), gender variation, ploidy races, and chloroplastic and mitochondrial DNA variation, as well as nuclear genome diversity. This is ambitious: in the meantime he recommended that at least five female and four male plants be conserved for each taxon. Patterns of genetic variation within wild populations of *A. chinensis* and *A. deliciosa* suggest that when making ex situ collections from a particular area, plants more than 100 m apart should be sampled first (Liu et al. 2007, 2008). Molecular markers are proving useful data for determining genetic diversity between and within species and within accessions of a particular species (Zhang et al. 2007; LG Fraser personal communication). Considerable diversity can be expected within seed accessions because *Actinidia* are obligatorily outcrossing.

Molecular markers could then be used when choosing genotypes for retention in germplasm collections to maintain maximum diversity.

Viruses have recently been detected in some *Actinidia* genotypes (Nitta and Ogasawara 1997; Clover et al. 2003; Pearson et al. 2007). The implications for commercial production are not clear, but as a precaution, new accessions of *Actinidia* material (both seed and budwood) should be checked for possible infection.

1.3.3 Ex Situ Germplasm Resources

1.3.3.1 China

The repositories in China have the greatest representation in terms of numbers of taxa. The national germplasm repository for *Actinidia* at the Wuhan Institute of Botany, Chinese Academy of Sciences, has as its goal the conservation of natural resources of *Actinidia* in China and the development of superior cultivars (Huang 2003). Nearly 60 taxa of more than 40 species, comprising more than 200 accessions from the wild or from exchange, are conserved as a living collection in the Wuhan Botanic Garden, Moshan, Wuhan, Hubei (Wang et al. 2003). The fruit characteristics have been summarized in Huang et al. (2003, 2004). Most of the

plants come from Hubei, Guangxi, Hunan, Jiangxi, and Fujian and there are 63 named cultivars or selections. To cover the range of growing conditions required by *Actinidia*, complementary collections are held under cooler conditions at Lushan, Jiangxi, or the moister, more subtropical conditions of the Guilin Botanic Garden, Guangxi. The collection at the Guangxi Institute of Botany, Guilin, occupies 0.3 ha and contains representatives of 74 taxa and 69 clones or cultivars (Li et al. 2000b). Phenological and fruit characteristics of plants in the collection have been described in a series of papers (e.g., Huang et al. 1983; Li et al. 1985, 1996). Another important collection is that of the Sichuan Provincial Natural Resources Research Institute, Chengdu, which has a collection of vines of eight species from the Three Gorges region of the Yangzi River and surrounding areas of Sichuan and Chongqing (Li and Lowe 2007).

1.3.3.2 New Zealand

The Plant & Food Research (formerly HortResearch) *Actinidia* germplasm collection occupies 6.2 ha at three research orchards at Kerikeri, Te Puke, and Riwaka. It currently contains about 3,500 genotypes from 310 accessions of 24 taxa (Ferguson 2007), mostly of *A. chinensis* and *A. deliciosa*, but in addition to these two species there are 372 genotypes from 92 accessions of other species. There is a collection of 80 cultivars or named selections, many of them originating outside New Zealand. A few taxa are represented by only a single genotype or genotypes of one gender so, in total, 18 species are fruiting.

1.3.3.3 Europe

The most important collection in Europe is that of the Dipartimento di Scienze Agrarie e Ambientali, University of Udine, Italy. This occupies 0.5 ha and has about 138 accessions of 23 *Actinidia* taxa (mainly separate species) from introductions of budwood or seed and named cultivars or selections (R. Testolin personal communication). The collection is particularly strong in *A. chinensis* cultivars (directly or indirectly from China), and *A. deliciosa* cultivars and selections mainly of Italian or New Zealand origin. This collection includes 48 accessions of taxa other than *A. chinensis*, *A. deliciosa*, or *A. arguta*, including some interesting species seldom found in other repositories.

1.3.3.4 United States of America

The United States Department of Agriculture National Clonal Germplasm Repository for *Actinidia* is mainly at Corvallis, Oregon, with some additional material held at Davis, California. The repository has a particularly good collection of cold-hardy *Actinidia* including 103 genotypes of *A. arguta* and *A. arguta* var. *purpurea* and 48 of *A. kolomikta*; these are mainly named selections. Most other taxa are represented by only a few genotypes, although there are 32 of *A. chinensis* and 15 of *A. deliciosa*, again mostly cultivars or selections. There is also a good collection of cold-hardy *Actinidia* at the University of Minnesota (Start et al. 2007).

1.3.3.5 Korea

Sung Kyun Kwan University in Suwon has collected 20 *Actinidia* species and 18 cultivars (Shim and Ha 1999) and the Subtropical Fruits Experimental Station in Haenam and the Forest Genetics Research Institute in Suwon have collected wild germplasm of *A. arguta* as well as representatives of other taxa.

1.3.3.6 Japan

The Kagawa Prefectural Experiment Station has a collection of cultivars of *A. chinensis*, *A. deliciosa*, *A. rufa*, *A. arguta*, and interspecific hybrids (Kokudo et al. 2003; Mizugami et al. 2007). Kagawa University also has a collection particularly rich in species native to Japan, such as *A. arguta*, *A. hypoleuca*, and *A. rufa*, and selections of these (Phivnil et al. 2005; I. Kataoka personal communication).

1.3.4 Maintenance of Germplasm

Actinidia germplasm vines are usually grown on the support structures and following the procedures of commercial orchards. Vines should preferably be on their own roots as some species are graft-incompatible

(Chartier and Blanchet 1997). Climatic requirements mean that not all *Actinidia* can be grown well at any one site. Thus, more subtropical species, such as *A. indochinensis*, do not survive the winters of Udine, Italy, whereas the warmer conditions of Wuhan, China, or Te Puke, New Zealand, do not suit cold-hardy species such as *A. kolomikta*. Plants of some taxa, e.g., *A. arguta* var. *purpurea*, will grow well at Te Puke but do not flower or fruit adequately: any such requirement for winter chilling will probably vary according to the genotype and possibly the provenance. Vine vigor may also vary in different repositories. *Actinidia* vines are usually long-lived under cultivation – one notable cultivated vine of *A. arguta* in Korea is at least 600 years old (Shim and Ha 1999). Some species, however, are less vigorous and mature plants of *A. polygama* will occasionally suddenly die back to their roots (New Zealand) as can plants of *A. arguta*, *A. hemsleyana*, *A. kolomikta*, and *A. polygama* in Udine, Italy (R. Testolin personal communication).

1.3.5 In Vitro Preservation

Both slow-growth systems, in which conditions are modified so that cultures can be held for extended periods, and cryopreservation have been successful with kiwifruit (Monette 1995). Tips of micropropagated shoots have been held at low temperatures (8°C) for a year (Monette 1995), and stem segments (Jian and Sun 1989), calli derived from hypocotyls (Hakozaki et al. 1996), seedling shoot tips (Suzuki et al. 1995), and encapsulated shoots (Bachiri et al. 2001) have been stored in liquid nitrogen and successfully regenerated. Such systems avoid the considerable maintenance requirements of extensive germplasm repositories of living plants. Nevertheless, these procedures have not been adopted for long-term storage of germplasm by any of the major *Actinidia* collections. Seed is routinely stored at −18 to −25°C, sometimes under vacuum, but it is not known for how long it remains viable.

1.4 Origin and Evolution of Kiwifruit

Actinidia are often so variable vegetatively that using morphological characters to delimit taxa is not always satisfactory (Dunn 1911; Huang et al. 1999; Li et al. 2009). Application of biochemical and molecular markers has added greatly to our understanding of phylogenetic relationships within the genus and of the relationships of wild taxa to cultivated kiwifruit.

1.4.1 Phylogenetic Relationships Within Actinidia

Molecular studies using genotypes of known provenance from the wild strongly suggest that the current infrageneric subdivision of *Actinidia* based on morphological characters is not natural (Li et al. 2000a, 2002). The *Leiocarpae*, which have glabrous ovaries and fruit, are probably monophyletic (see Ferguson and Huang 2007), perhaps excluding *A. kolomitka* (Testolin et al. 1997; Cipriani et al. 1998), but the remaining three traditional sections seem artificial. *Actinidia* taxa within China are more easily grouped if their geographic distributions are also considered: north China, the Yangzi River Valley, south-eastern China, southern China, and south-western China (Huang et al. 1999; Li et al. 2000a, 2002, 2003; Huang et al. 2002). *Actinidia* should perhaps be divided into two sections, *Leiocarpae* and *Maculatae*, the latter being further divided into four series containing taxa from the Yangzi Valley and from the three southern parts of China (Ferguson and Huang 2007). This may help to resolve the delimitation of many species within *Actinidia*.

Evidence from morphological, biochemical, and molecular approaches should be considered together. It is likely that there has been frequent polyploidization, hybridization, and reticulate evolution, particularly as there often seems incongruence between nuclear, mitochondrial, and chloroplastic markers (Chat et al. 2004). Many species appear to be polyphyletic in their mitochondrial DNA, in chloroplastic DNA, or in both (Chat et al. 2004), demonstrating the dangers of studying only a limited number of genotypes in each taxon. Molecular studies are like morphological studies in that any conclusions drawn will be more reliable the more extensive the germplasm resources examined (Li et al. 2009).

1.4.2 *Origin of Tetraploid* A. chinensis *and* A. deliciosa

A. chinensis and *A. deliciosa* are by far the most important *Actinidia* in cultivation. Understanding better the relationships between these closely related taxa should facilitate kiwifruit improvement programs. *A. chinensis* has both diploid and tetraploid chromosome races (Xiong 1992; Yan et al. 1994), whereas *A. deliciosa* is hexaploid (Zhang 1983; McNeilage and Considine 1989). A basic question, as yet unresolved, is whether tetraploid *A. chinensis* and *A. deliciosa* are allopolyploid or autopolyploid in origin. Morphologically similar taxa warrant further study: an example is *A. setosa* from Taiwan which was originally included as a variety of *A. chinensis* s.l. (Li 1952), was subsequently elevated as a distinct species (Liang and Ferguson 1986), and then again reduced to a variety of *A. chinensis* (Li et al. 2007b).

Tetraploid forms of *A. chinensis* are apparently restricted to a limited geographic area in eastern China (Xiong 1992; Yan et al. 1994). Many of the most important Chinese cultivars of *A. chinensis* are tetraploid and were originally selected from this area (Fujian and Jiangxi provinces) (Li et al. 2010). Tetraploid cultivars identified as *A. chinensis* have been selected from other provinces, but at least some may be hybrids between *A. chinensis* and *A. deliciosa* (Li et al. 2010). Tetraploid wild resources of *A. chinensis* appear therefore to be a particularly valuable genetic resource.

Morphologically, tetraploid forms of *A. chinensis* are very similar to diploid forms and the small differences in phenology may simply indicate adaptation to the climate of where they were collected. Molecular evidence suggests that at least some genotypes of tetraploid *A. chinensis* are allopolyploid in origin (Atkinson et al. 1997), whereas allelic segregation at ten isozyme loci in hybrids of *A. chinensis* ($4x$) × *A. eriantha* ($2x$) indicated that those particular tetraploid genotypes of *A. chinensis* used were autoploid (Huang et al. 1997). Such differing conclusions as to the nature of tetraploid *A. chinensis* may simply be a result of different genotypes having been studied. It is possible that tetraploid *A. chinensis* has evolved several times, despite being apparently limited to a restricted part of the species distribution.

The presence of a DNA repeat in *A. deliciosa* and in some tetraploid *A. chinensis* accessions but not in any diploid accessions of *A. chinensis* tested or other tetraploid accessions (Crowhurst and Gardner 1991; Yan et al. 1997a) has likewise been taken as indicating that there may be tetraploid *A. chinensis* groups with differing evolutionary histories (Murray 2002). However, the DNA repeat was also detected in *A. chrysantha*, not thought to be closely related to *A. deliciosa* or *A. chinensis* (Liang 1984), suggesting that the repeat may have been horizontally transferred within the genus or may have been lost from some lineages. It is, therefore, difficult to make firm conclusions about evolutionary history from the presence or absence of the repeat. Further studies with a wider range of genotypes are required.

There is likewise no clear consensus whether *A. deliciosa* is an autopolyploid derived solely from *A. chinensis* or whether any other *Actinidia* species might have contributed. The holoploid genomes of *A. chinensis* and *A. deliciosa* are undoubtedly similar (Testolin and Ferguson 1997; Li et al. 2002; Zhen et al. 2004) and it is likely that *A. chinensis* is the paternal progenitor of *A. deliciosa* (Cipriani et al. 1998; Li et al. 2002). Some molecular evidence suggests that *A. deliciosa* is allopolyploid (Crowhurst et al. 1990; Atkinson et al. 1997; Huang et al. 1997), whereas sequencing of nuclear ribosomal DNA internal transcribed spacers in *A. deliciosa* showed no evidence of *A. deliciosa* being allohexaploid (Li et al. 2002). This is not necessarily conclusive, however, as homogenization of such tandem repeats can obscure evidence of hybridism (Fuertes Aguilar et al. 1999).

If genomes were contributed from another species, then *A. deliciosa* and *A. chinensis* would be better treated as separate species. After the initial polyploidization event or hybridization, the newly formed *A. deliciosa* would have evolved independently from its progenitor species. The presence in *A. deliciosa* of isoyzme or microsatellite alleles absent from *A. chinensis* could thus be interpreted either as indicating subsequent independent evolution or as evidence that another progenitor species was involved. However, this second progenitor species might have been closely related to *A. chinensis* and would therefore share genomic markers with it through coancestry. Recurrent polyploidization or hybridization could add further complications.

Patterns of chromosome association during meiosis are sometimes used to deduce whether a plant is alloploid or autoploid in origin, bivalent pairing being

taken as evidence of alloploidy. The chromosomes of *A. deliciosa* predominantly form bivalents during pollen mother cell meiosis (McNeilage and Considine 1989), but this could be due to "diploidization" after allopolyploid formation or to the existence of a meiotic pairing control system. It is not known whether chromosomes that form bivalents show preferential pairing for a particular partner, or randomly pair with any of their five potential partners.

The morphological differences between *A. chinensis* and *A. deliciosa* are considerably greater than those between diploid and tetraploid races of *A. chinensis*, suggesting that another species morphologically similar to *A. deliciosa* might have contributed a genome to *A. deliciosa*. Possible candidate species, considered to be morphologically close to *A. chinensis* or *A. deliciosa*, include *A. chengkouensis*, *A. hubeiensis*, *A. obovata*, *A. setosa*, *A. sorbifolia*, and *A. stellatopilosa*. Both *A. hubeiensis* (He et al. 1998) and *A. setosa* (Yan et al. 1997c) are diploid, but the ploidy of the other species is not known. Most of these species have not been used in molecular studies of *Actinidia* except that chloroplastic and mitochondrial DNA studies make it unlikely that *A. setosa* is a progenitor of *A. deliciosa* (Chat et al. 2004).

Further studies using a greater range of genotypes from such species should help to determine possible origins of *A. deliciosa*. Detailed studies of sympatric populations of diploid and tetraploid *A. chinensis* and that of *A. chinensis* and *A. deliciosa* would also be useful (Li et al. 2010).

1.5 Cytogenetic Stocks

Variation in ploidy between taxa and within taxa can create obvious difficulties when trying to introgress characters from the different *Actinidia* species into the current commercially important species. The ploidy of parents to be used in crosses can be manipulated or progeny from crosses can be screened by flow cytometry to select for progeny at the desired ploidy. By choosing parents of different ploidies, offspring at a whole range of ploidies can be produced. However, the plants produced by interploidy crosses are often not at the expected ploidy (Ferguson 2009). Tissue culture can be used to regenerate plants from a variety of tissues, but the procedures used may result in somaclonal variation (Marino et al. 1998).

1.5.1 Ploidy Variants

Pollination of *A. deliciosa* (6*x*) female flowers with lethally irradiated pollen stimulated parthenogenetic development of unfertilized egg cells (Pandey et al. 1990; Chalak and Legave 1997). The resulting trihaploid plants, which had 87 chromosomes (Yan et al. 1997c), were unthrifty, grew poorly, and carried very few fruit. It seems that parthenogenesis can also be stimulated by interploidy pollination, as shown by the production of diploid plants from *A. arguta* (4*x*) × *A. deliciosa* (6*x*) (Chat and Dumoulin 1997) and that of triploid plants from *A. deliciosa* (6*x*) × *A. chinensis* (2*x*) (A.G. Seal and A.R. Ferguson, unpubl.). Development of individuals, which display parthenogenesis in interspecific hybrid populations, could enable greater fruit production from hybrids that normally have poor fruit set owing to low fertility; parthenogenetic individuals would also eliminate the requirement of males for pollination.

Crossing 4*x* × 2*x* *A. chinensis* (or in the reverse direction) produces large numbers of triploid plants, which grow vigorously but usually produce no fruit. Some triploid plants ($3x = 87$) have also been regenerated from endosperm of *A. chinensis* ($2x = 58$) (Huang and Tan 1988; Gui et al. 1993), but many of the plants produced were aneuploid. Endosperm cultures from seed produced by interspecific crosses can give rise to plants with a range of ploidies (Mu et al. 1990; Fraser et al. 1991), as can interploidy crosses.

Antimitotic agents have been successfully used in *Actinidia* to double chromosome numbers. Trihaploids have been efficiently doubled using oryzalin (Chalak and Legave 1996). Calli of triploid embryos arising from an interspecific cross between a diploid and a tetraploid (*A. chinensis* × *A. melanandra*) were treated with colchicine and at least some hexaploids regenerated, as well as plants at other ploidy levels (Harvey et al. 1995). Autotetraploid plants of *A. chinensis* 'Hort16A' have also been produced by treatment with colchicine and some of these plants produced carry fruit that are on average much larger (Wu et al. 2009).

Spontaneous doubling can occur. Some of the trihaploid plants of Chalak and Legave (1996) spontaneously doubled in chromosome number. Bud mutations of diploid *A. chinensis* 'Hort16A' in commercial orchards have been recognized because shoots carry fruit that are much larger than normal (Martin 2005). When such shoots are propagated by grafting, some of the plants seem cytologically unstable. Others appear to be stable and can be vegetatively propagated. At least some of these large-fruited mutants are mixoploid (2*x*, 4*x*) or tetraploid (Ferguson 2009). 'Hort16A' has proved to be a good parent in breeding programs (A.G. Seal personal communication) and these mutants allow it to be used at the tetraploid level giving new breeding opportunities.

1.5.2 Unreduced Gametes

Production of numerically unreduced (2*n*) gametes has probably played an important role in the evolution of polyploidy in *Actinidia* (Yan et al. 1997b). It appears that diploid *A. chinensis* plants introduced from the wild in different parts of China produce unreduced gametes to varying extents (A.G. Seal and A.R. Ferguson, unpubl. Unreduced gametes can be very useful in kiwifruit breeding as they could facilitate the transfer of the genomes of selected female genotypes to higher ploidy levels. Even if only a small proportion of the gametes were unreduced, flow cytometric screening of the offspring would allow selection of plants of the desired ploidy. It is likely that other *Actinidia* species also produce unreduced gametes.

1.6 Classical and Molecular Genetic Studies

Wild *Actinidia* have been used in a limited number of genetic studies. Offspring from the cross between *A. chinensis* and *A. callosa* were used to construct the first genetic map of kiwifruit using a combination of microsatellites and amplified fragment length polymorphism (AFLP) markers (Testolin et al. 2001). Recently, a second, more comprehensive genetic map of *A. chinensis* has been produced from a F_1 family resulting from an intraspecific cross between two distantly related *A. chinensis* genotypes, which came from the wild in different parts of China and were expected to differ in important characters (Fraser et al. 2009).

Genetic markers that can be amplified across many *Actinidia* species are potentially much more useful, especially when interspecific hybrid populations are being studied. Testolin et al. (2001), using microsatellites derived from genomic libraries, found insufficient cross-species amplification between two taxonomically distant *Actinidia* species. Expressed sequence tag (EST)-derived microsatellites should be more conserved because of the selection pressure operating on functional and regulatory genes: such markers that were developed in an *A. chinensis* mapping family showed at least some level of cross amplification over 26 different *Actinidia* taxa (Fraser et al. 2005; Tsang et al. 2007). This should enable the development of genetic maps in other *Actinidia* species using a standard set of microsatellite markers.

A selection of microsatellite markers representing genes in the anthocyanin pathway and some of their controlling elements have been tested in an interspecific *Actinidia* population, the result of a cross between an orange-fruited species, *A. macrosperma*, and a red-fruited species, *A. melanandra*. A single marker was found to segregate with the red fruit color phenotype (Fraser et al. 2006).

1.7 Crop Improvement

Although existing kiwifruit cultivars are highly heterozygous, they still have a fairly narrow genetic base, especially cultivars that have been developed outside China. Within China, most of the more important *A. chinensis* cultivars are tetraploid and most of these come from a limited part of the total geographic range of the species (Li et al. 2010). There is thus potential to improve cultivars further through crossing with wild material. There are also many traits within non-cultivated *Actinidia* species that could have commercial potential if transferred into cultivated kiwifruit. By incorporating fruit traits such as different colors, ripening indicators, edible or peelable skins, different flavors, and nutrient compositions (Ferguson 1990b; Testolin and Costa 1994; Huang et al. 2004; Ferguson and Huang 2007) into the currently cultivated species,

there is potential to develop many new kiwifruit cultivars (Ferguson 2007, 2009; Ferguson and Seal 2008). It should also be possible to introduce new vine growth characteristics, such as a reduced requirement for winter chilling, greater cold hardiness, or disease resistance.

1.7.1 Interspecific Crosses

Many successful interspecific *Actinidia* crosses have been described (e.g., Fairchild 1927; Pringle 1986; Wang et al. 1989, 1994, 2000; Ke et al. 1992; Testolin and Costa 1994; An et al. 1995). Some crosses are very successful allowing thousands of hybrid plants to be raised, e.g., *A. chinensis* × *A. eriantha* (Wang et al. 2000); others, especially interploidy crosses, may set only a few fruits containing a very small number of viable seed. Embryo-rescue enables certain cross combinations that would otherwise fail (Harvey et al. 1995; Hirsch et al. 2001). Most F_1 hybrids are unlikely to have immediate commercial potential and further crossing or backcrossing of the hybrids is therefore necessary. Hybrid fertility requires good pairing of the chromosomes and the production of balanced gametes. In polyploid hybrids, chromosome pairing can be allosyndetic or autosyndetic. The type of pairing that does occur can be determined by genomic in situ hybridization (GISH) on the hybrid meiocytes (Datson et al. 2006). Good chromosome pairing between parental genomes is also required for the introgression of characters. In *Actinidia* hybrids, chromosome pairing is usually better the more closely are the parents related. Knowing the type of chromosome pairing in hybrids helps in making decisions as to whether the production of F_2 hybrids is a better breeding strategy than backcrossing to one or other of the parents. The ability of many *Actinidia* species to hybridize should enable transfer of many traits into cultivated species.

1.7.2 Somatic Hybridization

Although somatic hybridization has great potential, it has so far proved difficult in *Actinidia*. Protoplasts have been successfully isolated and cultured in *A. arguta* var. *arguta*, *A. arguta* var. *purpurea*, *A. chinensis*, *A. deliciosa*, *A. eriantha, A. kolomikta*, and *A. polygama* and many plants were obtained (Oliveira and Pais 1991, 1992; Derambure and Hirsch 1995; Zhang et al. 1995; Xiao and Hirsch 1996, 1997). There are reports of successful somatic fusion and regeneration of protoplasts from *A. chinensis* and *A. deliciosa* and of *A. chinensis* and *A. kolomikta* (Xiao and Han 1997; Xiao et al. 2004). Chromosome counts, ploidy measurements, and DNA analyses confirmed that at least one of the plants raised was a somatic hybrid between *A. chinensis* and *A. kolomikta*, apparently with chilling tolerance similar to that of *A. kolomikta* (Xiao et al. 2004).

1.7.3 Genetic Transformation

Research on transgenics in *Actinidia* started when Rugini et al. (1989) reported genetic transformation and plant regeneration from leaf disks of *A. deliciosa* with the *rol* genes of *A. rhizogenes*, and subsequently the field evaluation of transgenic *rolABC* plants (Rugini et al. 1991, 1997). Furthermore, resistant fruits to *Botrytis cinerea* have been evaluated from 10-year-old transgenic plants overexpressing the *osmotin* gene (Gutiérrez-Pesce and Rugini 2008). Subsequently, *Agrobacterium*-mediated transformation has been reported in *A. arguta*, *A. chinensis*, *A. deliciosa*, *A. eriantha*, and *A. kolomikta* and transgenic plants of *A. chinensis*, *A. deliciosa*, and *A. eriantha* have been grown to flowering and fruiting maturity to demonstrate transmission of transgenes to progeny (Firsov and Dolgov 1997; Fung et al. 1998; Wang et al. 2006). *A. eriantha* has advantages over the commercial species for transformation studies because flowering and fruiting can be achieved under containment conditions less than 2 years after inoculation with *Agrobacterium*. *Actinidia* have also been transformed using PEG-mediated transfection and electroporation (Oliveira et al. 1991; Raquel and Oliveira 1996). Electroporation involves the formation of transient pores in biological membranes by the discharge of altering electrical current. The use of protoplasts in these systems is necessary, since they have an exposed plasmalemma. Hence, highly efficient plant regeneration protocols are necessary.

At present, transformation is being used experimentally and there are no genetically modified commercial kiwifruit. However, transformation of

kiwifruit is relatively easy and there are good prospects for developing kiwifruit with improved agronomic traits by using biotechnological techniques. Current genetic transformation programs are focused on improving kiwifruit by transferring one or more foreign genes, or by limiting the expression of endogenous genes.

1.8 Genomic Resources

Plant & Food Research, New Zealand, has available a large database of more than 130,000 expressed sequence tags (ESTs) from kiwifruit (Crowhurst et al. 2008). Most ESTs (>80%) have been developed from cDNA libraries from *A. chinensis* and *A. deliciosa*, but there are also ESTs from *A. arguta*, *A. eriantha*, *A. hemsleyana*, *A. polygama*, and *A. setosa* (Atkinson and MacRae 2007). The ESTs have been used in the preparation of a genetic map (Fraser et al. 2009), in the cloning, ordering, and sequencing of genomic DNA libraries (Hilario et al. 2007), and in studies on genome duplication in the evolution of *Actinidia* (Shi et al. 2010). A microarray of >17,000 kiwifruit genes has also been developed to monitor patterns of gene expression (Walton et al. 2007).

More than 240 compounds have been detected in the volatile components of flowers and fruit from nine *A. arguta* genotypes. Around 60–70 different compounds were extracted from individual tissues of each genotype (Matich et al. 2003).

1.9 Future Prospects

The process of domestication of the green kiwifruit, *A. deliciosa*, started about 100 years ago and the first kiwifruit orchards were planted about 70 years ago. After another quarter of a century came the hesitant beginnings of international trade in kiwifruit. Now green kiwifruit are familiar to most western consumers. Only in the last few years have such consumers begun to realize that not all kiwifruit are green or have a sharp acid flavor, but that some kiwifruit are sweet and aromatic and have golden-yellow flesh. Plants of *A. chinensis* were first successfully established outside China about 30 years ago and commercial trade in yellow kiwifruit began only during the last years of the twentieth century. The arrival of yellow-fleshed kiwifruit was the biggest change to the industry since kiwifruit were first introduced to consumers (Ferguson 2009). The establishment of orchards outside China and the successful development of trade in fruit of *A. chinensis* and *A. deliciosa* were based on tentative explorations of the wild germplasm and a few remarkably limited accessions of seed. The kiwifruit orchards of China are genetically more diverse, but even so are based almost entirely on good selections from the wild. Exploitation of the diversity in the wild germplasm has only just begun.

The world kiwifruit industries were developed primarily to supply fresh fruit to consumers. Kiwifruit are enjoyable to eat. However, expansion of production and increased consumer demand will depend on new cultivars meeting some of the important requirements of new food product development: enhanced pleasure, convenience, and health. Kiwifruit that lack hair, have edible or peelable skins, have different flavors or different flesh colors, store better and ripen more consistently or change color as they ripen, and are different and novel should appeal to consumers. From the diversity that exists in the wild germplasm, we know these are realistic possibilities.

Most consumers recognize that kiwifruit are high in vitamin C and that kiwifruit are, therefore, good for their heath. The fruits of some wild *Actinidia* contain much higher concentrations of vitamin C, and therefore breeding of commercial kiwifruit cultivars that are much richer in vitamin C is possible. However, in addition to vitamin C, kiwifruit contain many compounds that may provide health benefits beyond contributing to basic nutrition (Hunter et al. 2009). There is increasing evidence that eating green kiwifruit (*A. deliciosa* 'Hayward') has positive effects on cardiovascular and gut health. The immune system may also benefit. As we understand better the contribution that kiwifruit consumption makes to health, we will probably find that the wild germplasm can add to the health benefits provided by new cultivars.

Kiwifruit were domesticated and much of the initial development of kiwifruit as a crop occurred long before much was known of kiwifruit in the wild or of the diversity that exists within *Actinidia*. We now know much more, but there remains much we do not know about the genus and about how to exploit its diversity. There is still a need for greater effort on

conserving wild germplasm. We need to know more of the genetics of the commercial cultivars and of the wild species. Genomics and biotechnology may help us to identify the genes controlling traits currently absent from cultivars. We need to know how to transfer these desirable qualities into the kiwifruit of the future.

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Chapter 2
Ananas

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2.1 Introduction

Pineapple can be counted among the major New World contributions to global well-being, along with major staples (maize, cassava, potato, and sweet potato), legumes (beans), and vegetables (tomato, peppers). It was a major Amerindian crop, being widely cultivated in neotropical areas (Coppens d'Eeckenbrugge et al. 1997), and Colón (Columbus) observed it as early as 1493 in his second voyage. From then on, its unique and impressive characteristics, as well as the drought resistance of its propagules, ensured its rapid diffusion throughout the tropics, so it became a pantropical crop in less than two centuries and accounted for significant greenhouse production in Europe. Its economic importance further developed along with efficient preservation and transportation (Rohrbach et al. 2003).

The importance of the international pineapple trade has often overshadowed the even more important local and national markets. Indeed, major producing countries are also major consumers. National markets have mostly differed in the predominance of the fresh products and their wider genetic basis. While the international market was long dominated by canned pineapples of the "Smooth Cayenne" cultivar, national and regional markets allowed the maintenance of some cultivar diversity. Recently, the introduction of new cultivars on the international market has boosted global production up to 18 million tons in 2007, increased market share for fresh fruit, and maintained an interest in genetic diversity of this crop (Loeillet 2008). In addition, a small market for ornamental pineapple has developed on the basis of small, colorful fruit types (Souza et al. 2006, 2009; Sanewski 2009).

Other products derived from the pineapple are a very resistant silk-like fiber, processed into luxury clothes or specialty paper, both of remarkable thinness, smoothness, and pliability (Collins 1960; Montinola 1991). Bromelain, a proteolytic enzyme complex, is used as a meat-tenderizer and as a nutraceutical with potential therapeutic activity on inflammatory changes, blood coagulation, debridement of severe burns, drug absorption, and tumors (Taussig and Batkin 1988).

2.2 Basic Botany of the Species

2.2.1 Morphology

The pineapple, *Ananas comosus* (L.) Merr., is a perennial, herbaceous monocot of the family Bromeliaceae. Pineapple leaves are spirally organized in a dense rosette, around a short stem (Fig. 2.1). They are generally spiny; however, many cultivars show a partial or complete absence of spines. The adult plant is 0.8–2 m high and wide, depending mostly on leaf length. The hormonal shift from vegetative to generative growth is triggered by climatic factors, mainly day length and temperature, the plant receptivity increasing with its size and stress conditions. Flowering may be induced artificially, by the application of ethylene or ethylene-producing chemicals, to ensure crop uniformity. The terminal inflorescence develops into a multiple fruit or sorose, composed of 50–200 fruitlets, disposed around its fibrous axis in a 5/13 (small fruits) or 8/21 (medium

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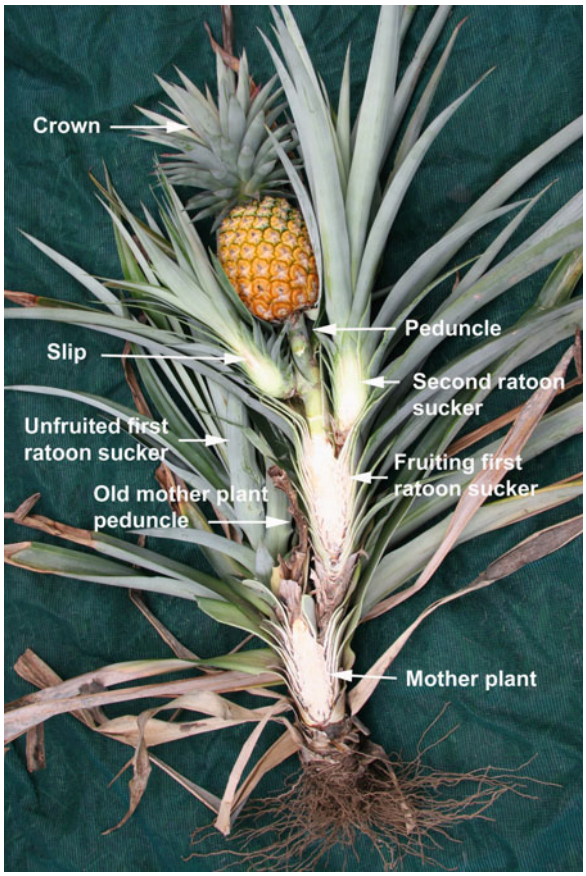

Fig. 2.1 Structure of a pineapple plant (*A. comosus* var. *comosus*) showing the succession of vegetative cycles and different types of planting materials for further vegetative propagation (stem sucker, slip, and crown) (photograph courtesy Garth Sanewski)

to large fruits) phyllotaxy, and borne on a peduncle of variable length. The species reproduces mostly from vegetative propagules that develop on the stem (stem shoots and ground suckers), the peduncle (slips), and the fruit top (crown). The type of planting material determines the initial development of the root system and the duration of the first crop cycle, which usually varies between 12 and 24 months, depending on cultivars and temperature. After fruit maturity, slips can be replanted or suckers may be left on the plant, providing new growth axes for a further production cycle. The latter is cheaper and shorter, as the plant is already established; however, fruit size is reduced and less uniform, so commercial cultivation is generally limited to two or three production cycles.

As with other bromeliads, pineapples have many adaptations related to water economy: CAM metabolism; leaf shape and arrangement, together with aerial roots, favoring rain water collection and absorption; a thick cuticle; and stomata disposed in longitudinal furrows on the abaxial leaf surface, covered with dense shield-shaped trichomes. In addition, the root system is weak, which is also common in a family dominated by epiphytism.

Pineapple flowers are hermaphroditic and trimerous, with three sepals, three petals, six stamens in two whorls, and one tricarpellary pistil. The anthers are bilobed, introrse, and dorsifixed. The style is hollow, trilobed, trifid, almost as long as the petals and equal or longer than the stamens. Petals are free, generally white at their base to violet-blue at their tip. The placentae and numerous ovules are located in the three deep locules of the inferior ovary. These are separated by three nectary glands, whose generous production attracts a range of potential pollinators including hummingbirds, the natural pineapple pollinators. The adjacent ovaries, bracts, and the inflorescence axis coalesce to form the fleshy compound fruit.

2.2.2 Sexual Reproduction, Genome, and Cytology

A. comosus possesses a gametophytic self-incompatibility system, expressed by the inhibition of pollen tube growth in the upper third of the style (Kerns 1932; Majumder et al. 1964; Brewbaker and Gorrez 1967). The most important cultivars of *A. comosus* var. *comosus* are strongly self-incompatible; however, many other cultivars have a weaker self-incompatibility and may produce a few self-seeds. This phenomenon, called pseudo-self-compatibility, is much more common in the other botanical varieties of *A. comosus*, and the self-fertility of some clones is only slightly lower than their cross-fertility (Coppens d'Eeckenbrugge et al. 1993; Muller 1994). *Ananas macrodontes* is highly self-fertile, and self-progenies are homogenous, suggesting that this species is homozygous and autogamous.

The pineapple is diploid, with 50 minute chromosomes. Rare triploids, with 75 chromosomes, have also been observed in *A. comosus* var. *comosus* and *A. comosus* var. *ananassoides*. The meiosis of diploids is normal, with the formation of 25 bivalents, resulting in normal tetrads (Heilborn 1921; Collins and Kerns 1931;

Capinpin and Rotor 1937; Lin et al. 1987; Dujardin 1991; Brown et al. 1997; Gitaí et al. 2005). The formation of a few giant unreduced gametes may result in the production of natural triploids, which produce mostly sterile pollen, and tetraploids (Collins 1933, 1960). Pollen viability is highly variable between varieties, cultivars, and even between clones from the same cultivar (Coppens d'Eeckenbrugge et al. 1993; Muller 1994). Male and female fertility are correlated, and generally lower in pineapples cultivated for fruit (*A. comosus* var. *comosus*), with 0–5 seeds per flower, than in other botanical varieties, where a single ovary may give up to 18 seeds (Coppens d'Eeckenbrugge et al. 1993; see also Leal and Coppens d'Eeckenbrugge 1996). The closest pineapple relative, *A. macrodontes* Morren is a tetraploid ($n = 100$; Collins 1960; Lin et al. 1987). It can be crossed with *A. comosus*, yielding a few fertile seeds that give a majority of tetraploids and some smaller and sterile triploids, whose phenotype is intermediate between parental species (Collins 1960).

Arumuganathan and Earle (1991) estimated the haploid genome size at 444 Mbp for *A. comosus* var. *bracteatus* and 526 Mbp for var. *comosus*.

2.2.3 Taxonomy

The genus *Ananas* belongs to the family Bromeliaceae, a large family of 56 genera and ca. 2,600 species, whose distribution is essentially American, the only exception being the African species of *Pitcairnia feliciana* (Aug. Chev.) Harms & Midbr., native to Guinea. It is further classified in the subfamily Bromelioideae, where it is unique in merging the whole inflorescence into a massive compound fruit. Pineapple is also the most economically important bromeliad. *Aechmea* and *Bromelia*, two other genera of the Bromelioideae, also include species yielding edible fruits, such as *A. bracteata* (Swartz) Grisebach, *A. kuntzeana* Mez, *A. longifolia* (Rudge) L.B. Smith & M.A. Spencer, *A. nudicaulis* (L.) Grisebach, *B. antiacantha* Bertoloni, *B. balansae* Mez, *B. chrysabtha* Jacquin, *B. karatas* L., *B. hemisphaerica* Lamarck, *B. nidus-puellae* (André) André ex. Mez, *B. pinguin* L., *B. plumieri* (Morren) L.B. Smith, and *B. trianae* Mez (Rios and Khan 1998). These minor fruits are consumed locally, under names such as *cardo*, *banana-do-mato* (bush banana), *piñuela* (small pineapple), *karatas*, *gravatá*, or *croata* (generic vernacular names for terrestrial bromeliads). In addition, many bromeliads are cultivated or gathered as ornamentals, fiber extraction, or used in traditional medicine (Corrêa 1952; Purseglove 1972; Reitz 1983; Rios and Khan 1998).

Pineapple taxonomy was recently revised and simplified by Leal et al. (1998) and Coppens d'Eeckenbrugge and Leal (2003), on the basis of new data on reproduction (Coppens d'Eeckenbrugge et al. 1993), morphological (Duval and Coppens d'Eeckenbrugge 1993), biochemical (García 1988, Aradhya et al. 1994), and molecular (Duval et al. 2001b) diversity. The two genera and seven valid species of the previous classification (Smith and Downs 1979) were downgraded to two species and five botanical varieties. Their correspondence is given in Table 2.1.

The tetraploid *A. macrodontes* (Fig. 2.2) is mainly differentiated from *A. comosus* by the lack of a crown at the top of the syncarpic fruit and by its vegetative reproduction by stolons, although it will rarely exhibit a rudimentary crown or produce shoots from the stem. Its strong spines are retrorse at the leaf base, or even

Table 2.1 Correspondence between the current classification (Coppens d'Eeckenbrugge and Leal 2003) and the former one (Smith and Downs 1979)

Coppens d'Eeckenbrugge and Leal (2003)	Smith and Downs (1979)
***Ananas comosus* (L.) Merril**	
A. comosus var. *ananassoides* (Baker) Coppens & Leal	*A. ananassoides* (Baker) L.B. Smith
	A. nanus (L.B. Smith) L.B. Smith
A. comosus var. *erectifolius* (L.B. Smith) Coppens & Leal	*A. lucidus* Miller
A. comosus var. *parguazensis* (Camargo & L.B. Smith) Coppens & Leal	*A. parguazensis* Camargo & L.B. Smith
A. comosus var. *comosus*	*A. comosus* (L.) Merrill
Invalid (Leal 1990)	*A. monstrosus*
A. comosus var. *bracteatus* (Lindl.) Coppens & Leal	*A. bracteatus* (Lindley) Schultes f.
***Ananas macrodontes* Morren**	*Pseudananas sagenarius* (Arruda da Câmara) Camargo

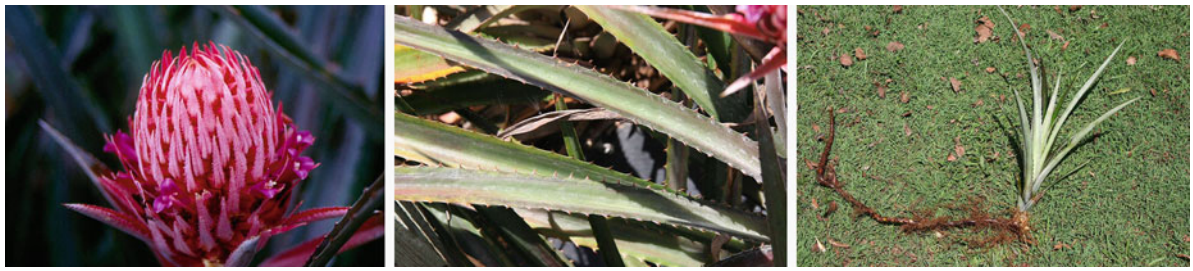

Fig. 2.2 Main distinctive traits of *Ananas macrodontes*: absence of a crown at the apex of the inflorescence and long floral bracts, presence of retrorse spines on the leaves, and vegetative reproduction by stolons (photographs courtesy Garth Sanewski)

higher. The fruit flesh is low in acid and it contains numerous seeds. The plant appears to be highly self-fertile. The natural habitat of *A. macrodontes* corresponds to humid forest areas, under semi-dense shade, in coastal and southern Brazil and in the drainage of the Paraguay and Paraná rivers, from southeastern Paraguay and northeastern Argentina up to Mato Grosso (Coppens d'Eeckenbrugge et al. 1997). The species even tolerates short periods of flooding (Bertoni 1919). Baker and Collins (1939) reported little variation for this species, while Bertoni (1919) distinguished five varieties and Camargo (cited by Reyes-Zumeta 1967) considered the possible distinction between three botanical varieties. Ferreira et al. (1992) reported appreciable variation in a limited sample from Paraguay and southern Brazil, which was confirmed by the restriction fragment length polymorphism (RFLP) study of Duval et al. (2001b). On the other hand, Duval et al. (2003) observed only one chlorotype among six accessions of the species. When selfed, clones of *A. macrodontes* produce uniform progenies (Collins 1960, and field observations by the authors).

Vernacular names for *A. macrodontes* include *yvira* (fiber) in Paraguay, and *gravatá de rede* (fishing net bromeliad), *gravatá de cerca brava* (wild fence-bromeliad), or *nana caçaba* (strong-spine pineapple) in Brazil, thus referring to traditional uses of the plant, mostly as a source of long and strong fibers.

A. comosus var. *ananassoides* is the most common and diverse form of wild pineapple, and it is the most likely ancestor of the cultivated pineapple. It is found in most tropical regions of South America east of the Andes, generally in savannahs or clear forest, growing on soils with limited water-holding capacity (sand, rocks) and forming populations of variable densities. In the Guianas, it can also be found, although rarely, thriving in dense rain forest. In contrast, it is not found in the seasonally flooded lands along the Amazon and main southern tributaries, which seem to act as a barrier dividing its distribution in two main areas: a northern one corresponding to the Guiana shield, Orinoco Basin, and northern drainage of Rio Negro (i.e., from the Brazilian state of Amapá to eastern Colombia), and a southern one roughly corresponding to the Brazilian shield and northeastern Brazil (from the Brazilian states of Acre, Mato Grosso up to Pernambuco and down to Paraguay, and northern Argentina). A higher morphological diversity is observed in the northern area (Fig. 2.3), where habitats also appear more variable for the wild pineapples (Leal and Medina 1995). In the south, they are mostly restricted to wide areas providing an open and markedly dry habitat (grass savannahs and low open forests) (Fig. 2.4).

Most populations of *A. comosus* var. *ananassoides* are monoclonal, but some are polyclonal, with variation of probably recent sexual origin (Duval et al. 1997). *A. comosus* var. *ananassoides* is characterized by long and narrow leaves, up to 2 m long and less than 4 cm wide, subdensely serrate with wholly antrorse spines. The fruit peduncle is elongate (most often more than 40 cm), slender (usually less than 15 mm wide). Its inflorescence is small to medium in size, globose to cylindrical, and it shows little growth after anthesis, so it has little flesh. The pulp is white or yellow, firm and fibrous, and palatable, with a high sugar and acidity content, with numerous seeds. In contrast, the crown resumes fast growth after fruit maturation, looking disproportionate in comparison to the fruit. Some clones of the Guianas-Orinoco area produce larger, fleshy fruits of intermediate size. They are sometimes cultivated or tolerated in gardens. Such pineapples may have served as a basis for

Fig. 2.3 Variation for *Ananas comosus* var. *ananassoides* in French Guiana: a small dwarf type from the rain forest; a fleshy, relatively large fruit from a wild population growing on a rock savannah; and an intermediate, semi-domesticated type in a home garden (photographs courtesy Geo Coppens d'Eeckenbrugge)

Fig. 2.4 A *Ananas comosus* var. *ananassoides* in Mato Grosso (Brazil) and its typically arid habitat (photographs courtesy Geo Coppens d'Eeckenbrugge)

domestication. At the other end, some dwarf types have recently been cultivated as ornamentals for the cut flower market, at both national and international levels.

Vernacular names include *ananaí*, or *nanaí*, *ananas de ramosa* (Brazil, Pará), *curibijul*, *maya piñon*, *piñuela*, and *ananas do indio*. Since pre-Columbian times, the plant has been known for its digestive properties, as well as a vermifuge, antiamoebic, abortifacient, and emmenagog (Leal and Coppens d'Eeckenbrugge 1996). Its fruits were consumed in the Orinoco (Patiño 2002) and are still occasionally consumed in the Guianas.

A. comosus var. *erectifolius* is very similar to the preceding variety. Plants are medium sized, with abundant shoots, frequent crownlets at the base of the main crown, numerous erect, fibrous leaves, and a small, very fibrous, inedible fruit (Fig. 2.5). In some clones, the fruit appears to be rare. The essential difference with *A. comosus* var. *ananassoides* lies in the smooth leaves of *A. comosus* var. *erectifolius*, a trait which is under monogenic control (Collins 1960). *A. comosus* var. *erectifolius* is not known to occur in the wild. It was cultivated in the West Indies at the time of the contact with the European, and it is still cultivated by the natives in the Guianas, including the

Fig. 2.5 Fruit of *Ananas comosus* var. *erectifolius* (photograph courtesy Garth Sanewski)

Fig. 2.6 *Ananas comosus* var. *parguazensis* (photographs courtesy Geo Coppens d'Eeckenbrugge)

Orinoco basin, and in the north of the Amazon basin, for the strong and long fibers associated with its typical erect habit. Indeed, the dry fibers constitute 6% of the plant weight. They are used to make hammocks and fishing nets (Leal and Amaya 1991), but now suffer competition from synthetic fibers and nylon. Vernacular names include *curagua*, *curauá*, *curaná*, *kulaiwat*, and *pitte*. The typical absence of spines along the leaf margin, as well as its erect habit, is the likely result of artificial selection for high yield of easily extractable fibers among strains of *A. comosus* var. *ananassoides*. The reverse mutation to spiny or partly spiny leaves has been observed under cultivation and in germplasm collections. Genetic diversity studies (Duval et al. 2001b, 2003) indicate that the domestication process that produced *A. comosus* var. *erectifolius* from *A. comosus* var. *ananassoides* has taken place independently at different times and/or places. This variety has recently found a new economic use in the production of cut flowers.

The wild *A. comosus* var. *parguazensis* is also very similar to *A. comosus* var. *ananassoides*, from which it differs by wider leaves, slightly constricted at their base, and larger spines, some of them retrorse (Fig. 2.6). Its distribution mostly corresponds to the basins of the Orinoco and upper Rio Negro, with a few observations in eastern Colombia and in the northeastern Amazon (Coppens d'Eeckenbrugge et al. 1997). It grows in lowland forests, under canopies of variable densities, from clearings or river banks to dense forest. As compared to *A. comosus* var. *ananassoides*, it seems restricted to shadier environments, because of lower water use efficiency (Leal and Medina 1995).

In *A. comosus* var. *comosus*, the most widely cultivated pineapple and the basis of the world trade in fresh and processed fruit, the syncarp grows very significantly after anthesis, so the fruit are generally very large (up to several kilograms in certain cultivars), with many fruitlets ("eyes"); they are borne on a wide and strong, relatively short, peduncle. Seeds are rare in the fruits, because of reduced fertility, conjugated with stronger self-incompatibility and monoclonal cultivation. The plant has numerous wide leaves (40–80), with antrorse spines, generally smaller and denser than in other botanical varieties. They may be suppressed by dominant mutations, as the one leaving only a few spines near the leaf tip of cultivar "Smooth Cayenne" or the one governing the folding of the

lower leaf epidermis over the leaf margin ("piping" phenotype), suppressing all spines but the terminal one. The former mutation is more common in the Guianas, while the latter is mostly found in the western Amazon and in the Andes. These two regions also exhibit a wider overall cultivar diversity (Duval et al. 1997, 2003).

The clearest effects of domestication in *A. comosus* var. *comosus* consist in the reduced susceptibility to natural flowering induction, together with the formation of a larger number of wider, and generally shorter, leaves, a wider and longer stem allowing a larger starch storage capacity, a significant increase in the number of flowers, the enlargement of individual fruits, and reduced seed production through the combination of lower sexual fertility and stronger self-incompatibility. In the cultivars where the reduction of female fertility, i.e., the proportion of ovules producing a seed, is not very severe, it can be counterbalanced by the higher number of flowers. In any case, as vegetative reproduction is largely dominant in the genus, this reduced sexual potential affects the plant survival less than the changes in the vegetative organs and the plant vegetative cycle. Strictly speaking, the domestication syndrome in this botanical variety lies in its lack of adaptation to the natural conditions prevailing for the wild varieties. Pineapple plants from most cultivars can survive when their cultivation is abandoned, resisting competition in sufficiently open vegetation and even dry edaphic or climatic conditions; however, they do not propagate efficiently to form subspontaneous feral populations.

A. comosus var. *comosus* was planted throughout tropical America at the time of the Conquest. Its fruit was widely consumed and particularly appreciated in the form of fermented drinks (Patiño 2002). Other traditional uses are the same as for *A. comosus* var. *ananassoides*. Rotted pineapple was used on arrows and spear heads for poisoning (Leal and Coppens d'Eeckenbrugge 1996).

A. comosus var. *bracteatus* is particular as this variety is an assemblage of two cultivated forms that show the same geographic distribution as *A. macrodontes* and that are morphologically and genetically intermediate between the two *Ananas* species (Fig. 2.7). The most common one, corresponding to *A. bracteatus sensu* Smith & Downs, is a cultigen that was cultivated as a living hedge and harvested for fiber and fruit juice, or for traditional medicine, in southern

Fig. 2.7 The most common form of *Ananas comosus* var. *bracteatus* (photograph courtesy Garth Sanewski)

Brazil and Paraguay (Bertoni 1919). Indeed, its dense, long, and wide leaves are strongly armed by large antrorse spines, forming impenetrable barriers. It is very robust and still thrives in abandoned plantations, but it seems unable to colonize new habitats. The syncarp is of intermediate size (0.5–1 kg), borne by a strong scape, and covered by long and imbricate floral bracts, as in *A. macrodontes*. These bracts are bright pink to red at anthesis, giving the inflorescence a spectacular appearance. Indeed, a variegated mutant has been widely propagated as a tropical garden ornamental. Morphological and genetic variations appear very limited in this first form, being comparable to within-cultivar variations (Duval et al. 2001b, 2003) and suggesting a very narrow origin, possibly a single genotype. The second form, corresponding to *A. fritz-muelleri* Camargo, shares an additional trait with *A. macrodontes*, as it exhibits retrorse spines at the leaf base. According to Camargo (1943) and Smith and Downs (1979), it was also used in living fences. It is a very rare form, whose diversity has not been documented, only one clone being conserved in Brazil, by EMBRAPA and the botanical garden of Rio de

Janeiro. Nuclear and chloroplast DNA data confirm its closer proximity with *A. macrodontes*. The chromosome number is $2n = 2x = 50$ (Camargo 1943).

2.2.4 Capacity for Invasiveness

Although pineapples are hardy plants with good drought tolerance, they achieve this through a high stomatal resistance and hence slow growth rate. Generally, they have a weak root system and have not established as weeds of significance despite being grown commercially in many countries. Reproduction of most commercial cultigens is almost exclusively through vegetative propagules, which can only be distributed through human or animal intervention or extreme environmental events such as flooding. Wild varieties such as *A. comosus* var. *ananassoides* are known to be seedy. However, viability of seed and that of vegetative propagules is substantially reduced after 6 months. All these factors mean it is unlikely that pineapples would become weeds in nature.

2.3 Conservation Initiatives

Pineapple genetic diversity has long been underestimated. Most cultivars that formed the basis of its worldwide cultivation were collected in the Caribbean or near the northern shores of South America. "Smooth Cayenne" was collected in French Guiana and "Queen" in Barbados (although it might have been also brought from French Guiana, where it is a traditional cultivar of the natives). We do not know the origin of "Singapore Spanish", the third common cultivar in Asia. Commercial cultivation in tropical America has been dominated by a few regional cultivars, as "Red Spanish" in the Caribbean (now mostly limited to Venezuela), "Monte Lirio" in Central America, "Perolera" in the Andes of Colombia and Venezuela, and "Pérola" in Brazil. The many cultivars that were collected in the West Indies for glasshouse cultivation in Europe (Loudon 1822) have been lost. To our knowledge, only a handful of them have been recovered in the germplasm collections of Trinidad and Tobago and that of Cuba, and similar systematic collecting should be undertaken in other islands, as well as in Central America, particularly where the commercial planting of "Smooth Cayenne" and, more recently that of "MD-2", has not displaced home garden production.

In South America, the first effort for collecting and conserving pineapple germplasm was initiated by the exploration of southern Brazil and Paraguay, by Baker and Collins (1939). This area was chosen because it was supposed to be the region of origin and domestication, as stated by Bertoni (1919). Baker and Collins confirmed the existence of wild pineapples in this area, but never explored the Amazon basin north of Mato Grosso, which fed a circular reasoning about a southern origin of the pineapple, still biasing very recent literature. This view was seriously challenged in 1981, when Leal and Antoni (1981) showed that northern South America exhibited a larger number of botanical varieties (then considered as different species). This was confirmed by extensive collecting expeditions in Venezuela, Brazil, and French Guiana (Leal et al. 1986; Ferreira et al. 1992; Duval et al. 1997) and preliminary observations in Suriname (Suriname 1995). In addition, national collections were established in Colombia and Peru, gathering cultivars adapted to contrasting conditions, from the Amazon to steep slopes of the Andes (Bello and Julca 1993; Hernández and Montoya 1993).

As could be expected, pineapple genetic erosion has been particularly severe in southeastern Brazil and Paraguay, because of deforestation and agricultural intensification. The situation is very different for wild forms in less densely populated areas of the Amazon or the Orinoco, as they often thrive in open areas, on soils with limited water-holding capacity (sandy hills and shallow soils around rocks), or even in forest conditions, where they do not compete with agriculture. Preservation of cultivated forms depends on the proximity of markets. While Tikuna farmers of the Upper Amazon may cultivate more than a dozen landraces in small plots, for their own consumption or for small village markets, most *caboclos* around Manaus grow two or three more common cultivars in larger fields. Thus, the possibility of in situ conservation of most native germplasm diversity is limited to remote areas, where genetic erosion would be very difficult to monitor. On the other hand, Coppens d'Eeckenbrugge et al. (1997) proposed an effort of rigorous clonal selection in regional cultivars, to optimize their characteristics and enhance their attractiveness for growers

and consumers, in terms of adaptation, resistance, and market diversification. Similarly, the exploration and characterization of germplasm from the "deep" Amazon would certainly be rewarding, allowing the identification of competitive robust cultivars and an expansion of the genetic basis of commercial pineapple cultivation.

Field conservation has constituted the most serious option for pineapple germplasm conservation so far. The most important collections, in terms of numbers and diversity of forms and geographic origins, are those that were established directly from field collecting, i.e., those of EMBRAPA in Brazil, CIRAD in Martinique, INIA in Venezuela, and USDA in Hawaii. Other smaller but important collections are maintained in Côte d'Ivoire, Malaysia, Okinawa, Taiwan, and Australia. All field collections face problems about funding continuity. This may be particularly true for the two smaller collections of Colombia and Peru, which harbor unique materials with particular adaptations.

Concerning procedures for field conservation, Coppens d'Eeckenbrugge et al. (1997) have described the procedures followed at the CIRAD collection in Martinique. Biotechnology, on the other hand, provides alternative or complementary methods for ex situ conservation, through tissue culture and/or cryoconservation. Tissue culture techniques also offer the opportunity for rapid propagation while providing the convenience of medium- and long-term storage of germplasm and facilitating its safe distribution (Smith et al. 2005).

Low temperature (16–20°C) and low sugar (1.5% glucose) culture medium have been used to extend subculture times for up to 4 years (Sugimoto et al. 1991). Zee and Munekata (1992) observed that reducing the nutrient salts in the culture medium to one fourth was successful for medium-term (12 months), low-input maintenance of pineapple cultures. For long-term storage, cryopreservation has been utilized. González-Arnao et al. (1998) demonstrated that pineapple shoot apices could be preserved in liquid nitrogen following pre-treatment and the use of cryoprotectants. A problem with methods only based on tissue culture, however, is the need of regularly controlling the variation induced by mutation, a phenomenon that is particularly important in the pineapple (Collins 1960). Its monitoring is complicated and delayed because the resulting plantlets behave more like seedlings than field-multiplied material, so a supplementary cycle of traditional multiplication is required.

Pineapple seeds can maintain viability for 2 years or more in dry and cool conditions, opening the possibility of a pineapple seedbank, provided that proper methodology and procedures can be optimized. Seed cryoconservation should also be tested. Economic seed conservation techniques would be particularly interesting for wild germplasm and primitive landraces. For more advanced material, their interest is more limited, as the objective is the conservation of clones, presenting particular genetic combinations.

2.4 Role in Elucidation of Origin and Evolution of Pineapple

The use of molecular biology in recent decades has provided key elements on the origin and domestication of pineapple.

2.4.1 Phylogeography

The first studies were conducted using enzymatic systems and evidenced a high polymorphism in the genus (80–100%), with a strong heterozygosity on a sample including an important number of accessions collected in Venezuela (García 1988). Another study on the USDA collection (missing representatives of wild populations from the north of the Amazon) indicated that 86% of the total variation was found within botanical varieties (then considered distinct species), underlining a moderate genetic divergence (Aradhya et al. 1994). Both studies evidenced a clear separation of a group constituted by *A. comosus* var. *bracteatus sensu* Smith & Downs and *A. macrodontes*.

A first genetic study (Noyer 1991) showed a low cytoplasmic diversity, with only one polymorphic probe–endonuclease combination out of 56 tested on 75 accessions covering a wide *A. comosus* diversity. Polymorphism was then investigated at the nuclear rDNA level and six groups were identified. The largest group includes clones of the varieties *comosus* (all except one), *parguazensis*, and *ananassoides* from Venezuela. *A. comosus* var. *bracteatus* accessions

formed a second group. Other groups correspond to one or two accessions (Noyer et al. 1998).

Following joint French–Brazilian pineapple prospecting expeditions to explore genetic diversity in the genus, a nuclear DNA RFLP analysis was conducted on a sample of 301 accessions, most of these collected in South America with a large distribution range (Duval et al. 2001b). High levels of variation were found within *A. macrodontes* and the wild forms *A. comosus* var. *ananassoides* and *A. comosus* var. *parguazensis*. Genetic diversity varied within cultivated forms, ranging from very low (*A. bracteatus sensu* Smith & Downs), to very high (*A comosus* var. *erectifolius*). The structure of genetic diversity appeared loose. *A. macrodontes* separated well but shared 58.7% of the markers with *Ananas* and was very close to the diploid *A. fritzmuelleri* Camargo. Within *Ananas*, only *A. comosus* var. *parguazensis* accessions form a consistent cluster. The scattering of botanical varieties and the occurrence of intermediate forms indicates a very probable gene flow, which is consistent with the lack of reproductive barriers between them.

Chloroplast restriction site variation was then used to study a subsample of 97 accessions of *Ananas* chosen for their genetic diversity and 14 accessions from other genera of the Bromeliaceae for phylogenetic purposes (Duval et al. 2003). No sister group was evidenced among these bromeliads. *A. macrodontes* and *A. comosus* varieties were represented by 11 haplotypes and formed a monophyletic assemblage with three strongly supported groups. Two of these groups are consistent with the nuclear data analysis and with geographical data.

The first group includes the tetraploid *A. macrodontes*, represented by only one haplotype and the diploid *A. fritzmuelleri* Camargo, both from the south of the subcontinent and adapted to low light conditions. The contrast in *A. macrodontes* exhibiting high nuclear but low cytoplasmic diversity favors the hypothesis of a recent speciation process by autopolyploidization. The nature of its parental relationship with *A. fritzmuelleri* Camargo is difficult to evaluate because of the extreme rarity of the latter (no accession could be recovered during the 1990s prospecting expeditions).

The second group includes the majority of *A. comosus* var. *parguazensis* accessions, all from the Rio Negro region. The third and largest group includes cultivated forms, *A. comosus* var. *comosus* and *A. comosus* var. *erectifolius*, as well as wild forms, *A. comosus* var. *ananassoides*, and the remaining accessions of *A. comosus* var. *parguazensis*, from the whole *Ananas* distribution range.

The comparison of molecular data obtained using uniparentally and biparentally inherited markers indicate hybridization between these groups in the Rio Negro region, as well as the hybrid status of *A. bracteatus sensu* Smith & Downs from the south.

2.4.2 Domestication Processes

Pineapple was domesticated more than 3,500 years ago, as shown by archaeobotanical remains dated from 1200 to 800 BC (Pearsall 1992) and glottochronological data indicate that the crop has been highly significant to Mesoamerican people for more than 2,500 years (Brown 2010). A likely time frame for the divergence between wild and cultivated pineapple lies between 6,000 and 10,000 years BP. Yet, it has not resulted in such a clear morphological or genetic differentiation as to make it a different species.

Molecular studies and morphological observations have suggested a two-phase pathway for the domestication and differentiation of the cultivated pineapple. Indeed, two hot spots for cultivated *A. comosus* var. *comosus* diversity were found. The first one rests in the eastern Guiana Shield and hosts a wide nuclear and cytoplasmic diversity along with a number of intermediate forms between *A. comosus* var. *comosus* and the wild *A. comosus* var. *ananassoides* that is commonly observed in the forest. These intermediate forms are noticeable by their variation in fruit size. These data point out this region as a likely primary center of domestication for the fruit. The second hot spot lies in the upper Amazon. No wild or intermediate forms have been found in this region, which appears as an important center of diversification of agriculture (Schultes 1984; Clement 1989) and could be a center of diversification for the domesticated pineapple. The plant would have been brought there by humans, which allowed for completion of the domestication process while in the absence of counteracting gene flow from wild forms.

A. comosus var. *erectifolius* is cultivated for its fiber and is morphologically very similar to the variety

ananassoides, except for the smooth character of the leaf. Its very high genetic diversity, scattering in the phenetic and phylogenetic trees, and proximity with various *ananassoides* genotypes, generally from the same origins, indicate that the variety *erectifolius* evolved directly from the variety *ananassoides* following convergent domestication processes in various places.

The third cultivated pineapple, *A. bracteatus sensu* Smith & Downs, is limited to the southeast of the subcontinent where it is grown as a fence. This form is very homogenous, displays the most common cytoplasmic haplotype shared with other cultivated forms and the variety *ananassoides*, and shares nuclear markers specific to the southern group constituted by *A. macrodontes* and *A. fritzmuelleri* Camargo. These data point out this form as a hybrid between representatives of these two groups.

2.5 Role in Development of Cytogenetic Stocks and Their Utility

Some commercial cultivars of local importance such as the Puerto Rican "Cabezona" are triploid (Lin et al. 1987). Furthermore, some wild clones such as the *ananas dos indios* population from Aguas Emendadas, near Brasilia, have seedless fruit that appear larger than those of other wild clones in this region (Dujardin 1991). Indeed, the production of 0–6.5% of diploid gametes, in diploid plants, results in the spontaneous formation of triploids and, more rarely, tetraploids. The latter produce about 90% viable pollen, through regular meiosis, and when crossed with diploid plants, they produce a few viable seeds, giving vigorous, healthy tetraploid seedlings (Collins 1960). Despite this vigor, polyploidy in itself does not look promising for pineapple breeding. Autotetraploids of "Smooth Cayenne" have a vegetative growth period 5 weeks longer than diploids and give smaller fruits with fewer, but larger, fruitlets and lower sugar content; however self-incompatibility is not affected (Collins 1960).

Collins (1933) emphasized the possibility of producing triploids by exploiting chromosome non-reduction in the pistillate parent to retain all the characters of a good heterozygote, avoiding sexual recombination, and adding new genes and characters to an existing cultivar. However, spontaneous triploidization appears too infrequent and unpredictable for its exploitation.

When *A. comosus* is crossed with *A. macrodontes*, 5–10% of the seeds formed are viable and give hybrids that are tetraploid, vigorous, highly fertile, and self-fertile. A few rare triploids, which are smaller, sterile, and resemble more the tetraploid parent, are also produced (Collins 1960).

2.6 Role in Classical and Molecular Genetic Studies

2.6.1 Morphological and Agronomic Traits

As observed by Collins (1960), hybridizations between botanical varieties are comparable with crosses between cultivars of *A. comosus* var. *comosus*, so there is no limit in transferring traits from one form to the other. The most investigated one concerns the presence of spines along the leaf margin, a very important trait as far as crop management is concerned. Collins and Kerns (1946) have shown that it is mainly governed by two genes, *S* and *P*, and their results have been corroborated at CIRAD in Martinique and at EMBRAPA in Brazil (Cabral et al. 1997). The recessive *s* allele determines the common spiny phenotypes. The dominant *S* allele determines the "spiny tip" phenotype, with only partial spininess, often concentrated at the leaf tip, as in "Smooth Cayenne". The third allele of the series is *Se*, which is found in *A. comosus* var. *erectifolius*. It is dominant over both *S* and *s*. The *P* gene, only found in cultivars, controls the "piping" character, which consists in the folding of the lower epidermis over the leaf margin, resulting in a complete absence of spines. It is dominant, with an epistatic effect upon the *S* gene. According to Collins (1960), a third gene, named *B*, interferes with the *S* gene. Its dominant allele, present in *A. comosus* var. *bracteatus*, would have determined a 3:1 spiny/"spiny tip" phenotypic ratio and a similar ratio in one F_2 family. However, the same cross was repeated in Martinique, producing a 1:1 ratio, as expected from the segregation of the *S* gene only.

Another simple genetic trait concerns the presence of high anthocyanin density, giving the plant a dark red color. Its effect is so clear that it can be easily differentiated from the quantitative effects of minor genes for the red pigmentation. The dominant dark red allele can be found in *A. comosus* var. *erectifolius*, as well as in a few cultivars of *A. comosus* var. *comosus* (e.g., "Roxo de Tefé" and "Red Mundo Nuevo"). The major genes for spininess and anthocyanins are not linked (Cabral et al. 1997).

Acid and sugar content are higher in *A. comosus* var. *ananassoides* than in *A. comosus* var. *comosus*. Their hybrid progenies segregate widely for these traits, with values that are intermediate for acidity, but closer to the wild parent, particularly for sugar content. Their fruits have very pleasant flavors. When *A. comosus* var. *erectifolius* is used as a parent, fruits of the hybrid can be even sweeter, with refractometric indices commonly above 20°Brix. However, these fruits are smaller and highly fibrous. The gain in sugar content is generally lost in subsequent backcrosses onto *A. comosus* var. *comosus*, as fruit size increases to more normal levels. Collins and Hagan (1932) observed that the progeny from a cross between such a wild pineapple and cv. Smooth Cayenne retained its high tolerance to the root-knot nematode *Meloidogyne javanica*. On the other hand, comparable levels of tolerance are found in some cultivars. This and other examples of pest and disease resistance breeding are discussed in Sect. 2.7.

When *A. comosus* var. *bracteatus* is used as a parent, larger fruits are obtained, weighing from 0.56 to 3.20 kg, with a wide range of variation in flavor (Collins 1960; Geo Coppens d'Eeckenbrugge unpublished). In addition, these three varieties present interesting characters of rusticity, such as a strong root system, resistance to nematodes, wilt, heart rot, and root rot, which are transmitted to the hybrid progenies (Collins 1960).

Interspecific hybrids within *Ananas* present an intermediate morphology, with crowned fruits and shoots from the stem as well as from short stolons. Their leaf margins are wavy like those of their *A. macrodontes* parent, some with the same large spines. When the cultivated parent bears a mutation suppressing spines, these tetraploid hybrids segregate for this trait. They are self-fertile, as their *A. macrodontes* parent, and produce very seedy fruits. Backcrossing to *A. comosus* var. *comosus* reduces this high fertility and increases morphological variation, with some plants approaching the backcross parent (Collins 1960).

2.6.2 Molecular Genetics and Genome Mapping

Carlier et al. (2004) published the first pineapple genetic map and used the two-way pseudo-testcross approach to construct two individual maps of botanical varieties *comosus* and *bracteatus* using a segregating population of 46 F_1 individuals from fully fertile crosses between the two varieties. To construct the map, a combination of three different types of markers was used: random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), and intersimple sequence repeats (ISSRs). The *A. comosus* var. *comosus* map contained 157 markers (33 RAPDs, 115 AFLPs, eight ISSRs, and the piping locus) with 30 linkage groups, 18 of which assembled four markers or more (Carlier et al. 2004). A relatively large percentage (43%) of markers remained unlinked, a fact perhaps reflecting the small size of the mapping population. This map covered approximately 31% of the *A. comosus* var. *comosus* genome estimated as 4,146 cM with a calculated ratio of 127 kb/cM for the relationship between physical and genetic distance. In the case of *A. comosus* var. *bracteatus*, 50 linkage groups were established containing 335 markers (60 RAPDs, 264 AFLPs, and 11 ISSRs) with 26 linkage groups containing at least four markers. In this case, map coverage is approximately 57.2% of the genome calculated as 3,693 cM with a ratio of 120 kb/cM.

Since the publication of the first *A. comosus* linkage map, selfing of one of the F_1 plants at the CIRAD collection at Martinique has produced an F_2 population for further mapping. J. Leitão's group has greatly improved the quality and resolution of the genetic map and new versions have been published (Carlier et al. 2007; Botella and Smith 2008). The linkage groups shown in the latest map gather a total of 651 markers, with 505 AFLP, 124 RAPD, 20 simple sequence repeats (SSRs), 1 express sequence tag (EST), and 1 morphological trait (piping).

With respect to pineapple genes that have been isolated, cloned, and characterized, these include an

ACC synthase and an ACC oxidase (Cazzonelli et al. 1998); a NAD^+-dependent malate dehydrogenase (Cuevas and Podestá 2000); ananain (Carter et al. 2000); a Cu/Zn-superoxide dismutase (Lin et al. 2000); two distinct polyphenol oxidases (Stewart et al. 2001); and the cysteine protease inhibitor cystatin (Shyu et al. 2004). A retrotransposon-like sequence, repeatedly integrated in the genome in multiple variable sequences and still potentially capable of transposing (Thomson et al. 1998), and the genomic sequence coding for bromelain inhibitors (Sawano et al. 2002) have also been isolated and characterized. Moreover, recent studies on genes involved in root development (Neuteboom et al. 2002) and in fruit ripening and nematode–root interactions (Moyle et al. 2005a, b, 2006) have resulted in a very large number of sequenced ESTs.

2.7 Role in Crop Improvement Through Traditional and Advanced Tools

As there are no reproductive barriers among botanical varieties of *A. comosus*, wild and semi-domesticated pineapple germplasm may bring considerable variation for the benefit of pineapple breeding. Hybridization generates a wide variation in most traits, which opened prospects for new shape and colors for the fruit (Fig. 2.8), the introduction of resistance/tolerance traits into the main crop, and also the perspective of exploiting wild pineapple germplasm in the ornamental plant market.

2.7.1 Characters of Interest in Hybrid Breeding for the Fruit

Wild pineapple is highly efficient in its vegetative propagation, multiplying from stem suckers, slips, the crown, and even multiple crowns. Suckering is still important in *A. comosus* var. *bracteatus* and is particularly spectacular in *A. comosus* var. *erectifolius*. However, vegetative multiplication must be limited by the breeder in *A. comosus* var. *comosus* to obtain only a few basal stem suckers, allowing a further cultivation cycle or providing strong material for planting anew.

Tolerance to drought is remarkable in *A. comosus* var. *ananassoides*. However, an ample range of tolerance also exists among cultivars, and the use of a tolerant cultivar such as "Perola" would save the extra cost of several backcross generations.

The highest potential of hybrid breeding between botanical varieties lies in the transfer of resistance/tolerance to major pests and diseases. Thus, clones of *A. comosus* var. *ananassoides*, *A. comosus* var. *erectifolius*, and *A. macrodontes* are thought to exhibit levels of resistance to the nematodes *Rotylenchulus reniformis* and *Meloidogyne javanica*, allowing very low levels of nematode reproduction (Ayala 1961;

Fig. 2.8 Example of variation obtained through hybridization of the large-fruited *A. comosus* var. *comosus* (*upper right*) with small-fruited wild or primitive pineapples (photograph courtesy of Garth Sanewski)

Ayala et al. 1969; Sipes and Schmitt 1994). It is difficult, however, to test for relative differences in genetic resistance to nematodes because of the underlying genetic differences in plant growth parameters and responses to environment. Various methods of quantifying the effect of nematode challenge have been reported for pineapple including assessment of number and distribution of galls or lesions, as well as the effect on root and plant growth. The difficulty of assessment is probably partially responsible for conflicting assessments of genetic resistance and/or tolerance in pineapple.

In early work by Collins and Hagan (1932), an *A. comosus* var. *comosus* x *A. comosus* var. *ananassoides* hybrid progeny called "Lot 520" was considered as highly tolerant of the nematode, *Meloidogyne* sp. Although it was infected by the nematode, there were few galls and plant and root growth were little affected. Ayala et al. (1969) also reported that *A. comosus* var. *ananassoides* has good resistance to infection by *M. incognita* and *R. reniformis*. Sarah et al. (1997) tested *A. comosus* var. *ananassoides*, *A. comosus* var. *parguazensis, A. comosus* var. *bracteatus*, and many *A. comus* var. *comosus* clones for resistance to *Pratylenchus brachyurus* and found none were clearly resistant, whereas *A. comosus* var. *comosus* "Perola" was the least affected. Dinardo-Miranda et al. (1996) tested 13 varieties, mainly clones of *A. comosus* var. *comosus* for resistance to *M. incognita*, and found that only "Huitoto" could be considered as a poor host. Sipes and Schmitt (1994) reported on the most comprehensive study and found *A. comosus* var. *ananassoides* and "Lot 520" supported high levels of both *M. javanica* and *R. reniformis*. In that study, "Smooth Cayenne" was the most tolerant variety. Soler et al. (2009) found the *A. comosus* var. *comosus* hybrid "MD-2" displayed little impact on vegetative growth following infection by *R. reniformis*. One clone of *A. comosus* var. *ananassoides* was also found to tolerate infection very well and another moderately well. Williams and Fleisch (1993) reported that the *A. comosus* var. *comosus* hybrid clone "57-3" displays little growth suppression when infected with nematodes.

A review of the various studies indicates that there are conflicting reports regarding genetic resistance. Usually, the more comprehensive studies suggest that there are probably no genotypes that resist infection by any species of nematode to any extent, but some genotypes tolerate infection better than others. Often the varieties with the least vegetative growth depression will be those with a relatively smaller vegetative mass (Williams and Fleisch 1993). The domesticated *A. comosus* var. *comosus* clones of "Perola" and "Cayenne", as well as many hybrids, appear to offer some tolerance as do some clones of *A. comosus* var. *ananassoides*. The latter is suspected of exhibiting some resistance. Given the large diversity within *A. comosus* var. *ananassoides*, it would not be surprising to find more examples of genetic tolerance to nematode, but the usefulness of this for breeding is questionable given the primitive nature of theses clones. Resistant clones would, however, have significant use in molecular studies.

Resistances to the root pathogens *Phytophthora cinnamomi* and *Phytophthora nicotianae* var. *parasitica* are also reported for wild varieties of pineapple. This work was started by the Pineapple Research Institute (PRI) in Hawaii in 1936. Tests of various hybrids developed by the PRI indicated a level of resistance particularly in hybrids involving *A. comosus* var. *bracteatus* and *A. comosus* var. *ananassoides*. As a consequence, an expedition to South America was undertaken in 1937 to collect wild species and landraces that could be used for resistance breeding (Anderson and Collins 1949). From 1936 to the 1960s, many cultivars with resistance to *P. cinnamomi* and or *P. nicotianae* var. *parasitica* were developed by the PRI from the germplasm collected in South America. Of the germplasm collected, *A. macrodontes* was considered the most resistant, almost immune, but developing commercially acceptable varieties with it as a parent was very slow. No commercial varieties using *A. macrodontes* were ever developed despite a dominant resistance mechanism, mainly because of the poor fruit quality. *A. comosus* var. *bracteatus* was considered highly resistant and progress in breeding commercial types using it was relatively quick. *A. comosus* var. *ananassoides* was moderately to highly resistant and proved to be the most useful parent (Collins 1953). Most of the *P. cinnamomi* and *P. nicotianae* var. *parasitica* resistant varieties developed have approximately one-sixteenth *A. comosus* var. *ananassoides* in their parentage (Williams and Fleisch 1993). *A. comosus* var. *erectifolious* had a similar level of resistance as *A. comosus* var. *ananassoides*, but was a poor parent for other agronomic characteristics. No resistant, commercial varieties

with *A. comosus* var. *erectifolious* parentage were developed. The *A. comosus* var. *comosus* varieties "Red Spanish" and "Pernambuco" were also moderately resistant and contained many good agronomic traits (Smith 1966). Using these varieties, the PRI breeding program incorporated heart rot and root rot resistance into many varieties all with a reasonable complement of other desirable agronomic characters. This was achieved by backcrossing an F_1 progeny onto a commercial variety, usually one with a high proportion of "Smooth Cayenne" genes, over two to three generations (Collins 1953). While none of these varieties are grown commercially today, many would be worthwhile parents for use in breeding where *P. cinnamomi* or *P. nicotianae* var. *parasitica* resistance is a targeted trait. Some of these varieties are held by the University of Hawaii (Williams and Fleisch 1993). Resistant varieties include "PRI-10388" syn. "Spanish Jewel," "PRI-59-656," "PRI-52-323," and "PRI-61-2223" (Smith 1965; Rohrbach and Johnson 2003). Two of these, "PRI-59-656" and "PRI-52-323," were grown commercially on a small scale in Hawaii before improved chemical control methods and high yielding "Smooth Cayenne" clones became available (Williams and Fleisch 1993).

While most resistant varieties developed had *A. comosus* var. *ananassoides* in their parentage, some resistant varieties were derived from two apparently susceptible parents. Resistance was considered quantitative and additive (Smith 1966). Varieties could differ in their susceptibility to both *P. cinnamomi* and *P. nicotianae* var. *parasitica* (Johannessen and Kerns 1964). The variety "59-656" is claimed to possess good resistance to both the pathogens (Smith 1965).

2.7.2 Characters of Interest in Hybrid Breeding for Ornamental Plants

The family Bromeliaceae is well recognized for its extraordinary diversity and ornamental appeal. However, until very recently, *Ananas* has not been exploited significantly as an ornamental, as was the case of a great number of other genera in the family. Small but increasing quantities of *Ananas* plants and blooms are now being marketed in various countries for their ornamental appeal, usually *A. comosus* var. *bracteatus* "Tricolor" and *A. comosus* var. *erectifolious* "Selvagem 6" (Fig. 2.5). Both these varieties, while currently commercially exploited, have limitations and do not incorporate the breadth of ornamental potential within the *Ananas* gene pool. There remains exciting potential for further breeding. Breeding programs for ornamental pineapple are reported for Brazil, Australia, France, and Malaysia (Duval et al. 2001a; Chan 2006; Souza et al. 2006, 2009; Sanewski 2009).

Several markets exist for ornamental *Ananas* products, each with an emphasis on different plant characteristics. These markets include the cut-flower market for pre-petal syncarps, miniature fully formed fruit, and attractive cut foliage (F. Vidigal personal communication). The landscape or potted plant market will also take plants with ornamental fruit or foliage characteristics.

For attractive blooms, *A. comosus* var. *bracteatus* is good for imparting a bright red coloration to the syncarp and *A. macrodontes* will impart a pink color. *A. comosus* var. *erectifolious* "Selvagem 6" is a good parent for obtaining smooth reddish leaves, including those in the crown. An example of this hybrid is shown in Fig. 2.9.

For miniature fruit, *A. comosus* var. *ananassoides* is a good parent, as is *A. comosus* var. *erectifolious*. It is important that the small fruit has a strong attachment to a long (50 cm), thin stem and the crown is well formed with no side shoots. Large fruits and fruit on a short stem are less useful in flower arrangements.

Potted or landscape plants should have an attractive foliage, possibly variegated or reddish in color with smooth leaf margins. A dwarf, clumping habit is desirable for potted plants. An attractive syncarp and miniature fruit are also desirable. Again, *A. comosus* var. *ananassoides*, *A. comosus* var. *erectifolious*, and *A. comosus* var. *bracteatus* are excellent parents.

Of all the *Ananas*, *A. comosus* var. *ananassoides* displays considerable diversity in fruit and leaf color and appearance. The collection of *Ananas* held by EMBRAPA holds accessions highly suited as parental stock (Souza et al. 2006). Interspecific crosses also show ornamental interest (Fig. 2.9), and the potential for utilizing other genera might also exist. Most Bromeliaceae contain the same diploid number of 50 chromosomes as *Ananas* (Brown et al. 1997). Successful intergeneric hybrids with *Ananas* are reported for *Aechmea*, *Cryptanthus*, *Neoregelia* (Anonymous

Fig. 2.9 Two smooth-leaved hybrids obtained from crosses between *A. comosus* var. *bracteatus* and *A. comosus* var. *erectifolius* (*left*), or a "piping" leaved *A. comosus* var. *comosus* cultivar with *A. macrodontes* (*right*), both selected for the cut-flower market (photographs courtesy of Garth Sanewski)

2007), and *Tillandsia* (Valds et al. 1998). Many of the other genera of Bromeliacae exhibit greater diversity of foliage morphology and color than do *Ananas*, but none produce an attractive small fruit. The potential for combining the interesting decorative fruit form of *Ananas* and more striking foliage morphology and color might therefore exist.

2.7.3 Advanced Tools for Crop Improvement

Protoplast culture and somatic hybridization, as a tool for introgression of genes, have had no impact on pineapple improvement to our knowledge. There has been a successful attempt to isolate protoplasts of the cultivar "Perolera" (Guedes et al. 1996), but plant regeneration was not achieved.

Pineapple transformation, however, offers the possibility to make small targeted changes to the recipient plant's genome and is seen as an excellent strategy for genetic improvement. A review of pineapple transformation has recently been published (Ko et al. 2008) and methods involving the introduction of recombinant DNA to pineapple cells and tissues via *Agrobacterium tumefaciens*-mediated transformation and direct gene transfer through microprojectile bombardment are reported. Biolistics has been used to deliver genes conferring herbicide resistance (Sripaoraya et al. 2001) and blackheart resistance (Ko et al. 2006) into "Smooth Cayenne." Other groups focused on using *Agrobacterium* to introduce ACC synthase genes to control ripening (Firoozabady et al. 2006; Trusov and Botella 2006).

Despite these advances, consumer resistance to transgenic fresh fruit is limiting wider use of this technology. Incorporation of only native genes from wild relatives and with expression only in plant parts not intended for consumption is the approach worth considering. In addition, before businesses and institutions will have freedom to operate with transgenic lines, intellectual property ownership must be ascertained, and strategies put in place to ensure plants are free from encumbrance, which would otherwise restrict the sale of product.

2.8 Genomics Resources Developed

The amount of genomic data in databases is still scanty, despite the economic importance of pineapple, but has been increasing in the last few years. A search for pineapple genomic data through the National

Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) found about 60 microsatellite and other DNA marker loci from var. *bracteatus* and over 5,700 ESTs from var. *comosus*. About 140 SSR markers have also been published on EMBL database (http://srs.ebi.ac.uk), the main contributors being the Biotechnology Research Institute of Malaysian Sabah University for 76 SSRs (Kumar et al. unpublished) and CIRAD in France with 50 SSRs (Blanc et al. unpublished). Also, recently an entire collection of ESTs was generated during an investigation into fruit ripening and nematode–plant interactions during root invasion (Moyle et al. 2006) and has been made publicly available by an online pineapple bioinformatics resource named "PineappleDB" (http://www.pgel.com.au).

2.9 New Perspectives for Commercial Development

The pineapple yields many products in addition to the edible fruit. Crude extracts from the fruit, stem, and leaves yield several proteinases, mainly bromelain but also ananain (Rowan et al. 1988; Lee et al. 1997) and macrodontain's I and II (López et al. 2001). Bromelain has demonstrated broad bioactivity including antiedematous, anti-inflammatory, antithrombotic, fibrinolytic, immunomodulatic (Maurer 2001), and anthelminthic (Aye et al. 1996; Hordegen et al. 2006). Innovative studies where Chinese cabbage plants were transformed with a bromelain construct demonstrated enhanced resistance to a bacterial soft rot (Jung et al. 2008). Fiber extracted from the pineapple leaf is processed into paper, cloth, and composite plastics (Hepton and Hodgson 2003). The domesticated *A. comosus* var. *comosus* is the predominant source of these products primarily because it is cultivated on a large scale for fruit, making the extraction of additional compounds cost-effective. The primitive forms of pineapple have, however, been traditionally used in similar ways by indigenous people of South America. *A. comosus* var. *ananassoides* is the principal wild pineapple. A drink made from the fruit of the wild *ananassoides* is considered by some indigenous Amazonian tribes to have an abortive effect and this activity has been supported by clinical studies (Nakayama et al. 1993). *A. comosus* var. *ananassoides* is also commonly used in central Brazil for gastric pain. Recent studies (Silva et al. 2008) have demonstrated antiulcerogenic properties, which support this traditional use. *A. comosus* var. *erectifolious* could be considered as semi-domesticated and is now grown on a commercial basis for its leaf fiber (Leão et al. 2009). The "Curaua" leaf fiber has traditionally been used for twine, cloth, fishing line, nets, hammocks, etc. (Boom and Moestl 1990; Leal and Amaya 1991), but is now being tested in biocomposites with potential for the automotive industry (Zah et al. 2007).

2.10 Concluding Remarks

The indigenous people of South America have led the process of domestication and selection of the pineapple with so much success that only a few hybrid fruit cultivars have been produced through systematic breeding out of tropical America (Leal and Coppens d'Eeckenbrugge 1996). To adapt the crop to intensive cultivation and current standards, modern plant breeders attempt to remove the remaining undesirable "wild" traits such as natural flower initiation, small fruit size, excessive vegetativeness and long peduncles from parental stock. There appears limited scope, however, to revisit the use of most of the wild clones in breeding programs for fruit production as their phytomorph is highly unsuited to modern, efficient fruit production systems. *A. comosus* var. *ananassoides* in particular, while represented by a diverse range of clones, is too primitive in form to be of immediate use in fruit breeding. Its homozygotic tendencies for spiny leaves, vegetative growth characteristic and generally small, often unpalatable fruit are highly unsuited. Hybridization to improve on these characteristics would require many generations and almost all the desirable characteristics are already present in more commercially suited *A. comosus* var. *comosus* clones. Collins (1960) estimated that one generation and four backcrosses taking 20–25 years would be needed to produce commercial types from wild clones. This delay might be slightly shortened if clones with larger and fleshier fruits from northern South America are considered. *A. comosus* var. *bracteatus*, on the other hand, is less homozygotic for wild traits when used as a parent with *A. comosus* var. *comosus* clones and fewer generations are needed so

it might have greater potential for use in breeding. Finally, it must be kept in mind that several hybrids resulting from ancient programs of introgression of genes from wild varieties still exist in germplasm collections.

Many of the useful resistance traits are probably polygenic making gene acquisition difficult. While this might be the current picture, it is also possible that some useful resistance traits remain undiscovered as very few of the wild clones have ever been investigated. Most have only been collected in recent times. The increasing pressure toward more environment-friendly cultivation methods may soon give a much higher priority to the research on mechanisms and sources of resistances.

While the potential use of wild types for fresh fruit breeding awaits detailed characterization of collections, many are immediately useful for other purposes. As an example, many of the *A. comosus* var. *ananassoides*, *A. comosus* var. *bracteatus*, *A. comosus* var. *erectifolious*, and *A. macrodontes* are highly suited to the production of ornamental varieties. The other industrial and pharmaceutical uses are yet to be fully investigated.

There is no doubt that the *Ananas* collections held in various centers are an unexploited resource that needs further investigation to realize their full potential.

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Chapter 3
Citrus

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3.1 Botany of the Genus *Citrus*

Citrus is one of the world's most important fruit crops and has a total world production of 105.4 million tons (FAO 2006). It is also valuable for human nutrition and well-being. The genus *Citrus* belongs to the subtribe Citrinae, tribe Citreaea, subfamily Aurantioideae of the family Rutaceae. Based on leaf, flower, and fruit properties, the genus is further divided into two subgenera, namely, *Citrus* and *Papeda*. A number of studies have been carried out, using their morphological characteristics, on the relationships between genera and species, which have led to the formulation of numerous classification systems. The most commonly used citrus classifications are by Swingle who recognized only 16 species (Swingle and Reece 1967) and Tanaka who recognized 162 species (Tanaka 1977). Most *Citrus* species are diploids with nine pairs of chromosomes ($2n = 2x = 18$), though polyploids have also been reported. Cytogenetic studies have revealed that the citrus chromosomes are small but highly variable (Naithani and Raghuvanshi 1958; Raghuvanshi 1962; Guerra 1984, 1993). Karyotypes based on Geimsa C-banding (Guolu 1988) and staining with the intercalating fluorochromes chromomycin A3 (CMA) and 4′-6-diamidino-2-phenylindole (DAPI, Guerra 1993) show that many chromosome pairs must be heteromorphic. DAPI and CMA staining of citrus metaphase chromosomes showed that several chromosomes contain large blocks of terminal heterochromatin (Miranda et al. 1997). The genome size of citrus is approximately 367 Mb, which is nearly three times the size of *Arabidopsis* genome.

Citrus plants are small to medium sized, spreading, evergreen trees with thorny shoots, growing to about 2–15 m tall with most species being single-trunked with very hard wood but with a relatively thin (<5 mm) bark (Manner et al. 2006; Gmitter et al. 2007; Roose and Close 2008). The stem is green, with unifoliate, alternate leaves. Leaf shape varies from ovate to lanceolate; the size varies from 4 to 10 cm long and may possess more or less broadly winged petioles. Flowers are perfect or staminate due to abortion of the pistil (Roose and Close 2008). They are fragrant, borne solitary, or in short cymes in the axils of the leaves or in small lateral or terminal inflorescences. The sepals are four to five lobed. The petals are five, thick, linear, strap-shaped, and alternate with the sepals (Schneider 1968). The stamens are polyadelphous, cohering toward the bases in a few bundles and are usually four times as many as petals. The yellow, four-lobed anthers surround the pistil at or near the level of the stigma (Spiegel-Roy and Goldschmidt 1996). The ovary is superior and is composed of 6–14 carpels joined to each other and to a central axis (Soost and Roose 1996). The style is prominent but usually deciduous and contains as many tubes as there are cells in the ovary (Gmitter et al. 2007). The fruit is a hesperidium berry whose form and size vary from globose to oblong and oblate. They are highly fragrant and are divided into 10–14 segments consisting of juice vesicles, separated by thin septa, with seeds near the inner segment angle (Manner et al. 2006; Roose and Close 2008). The segments are surrounded by a white endocarp (albedo) and then the rind (flavedo), which has many oil glands and is usually colored orange or yellow at maturity. Seed content is variable among and within species.

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Citrus is grown in the tropical and subtropical regions around the world, primarily between the latitudes of 40°N and 40°S, from equatorial, hot–humid climates through warm–subtropical, and even cooler maritime climates (Spiegel-Roy and Goldschmidt 1996). It can be grown in a wide range of soil types from coarse sand to heavy clay, provided the soils are well drained (Gmitter et al. 2008). The long history of selection and vegetative propagation of citrus has, on the one hand, led to the perpetuation of the "elite" germplasm lines but, on the other hand, to the neglect and disappearance of the progenitor wild types (Krueger and Navarro 2007). This may have reduced the genetic diversity of citrus. While limited information is available on genetic resources/diversity of citrus, no information is available on the frequency of occurrence. With Southeast Asia being the center of origin, greatest amount of diversity of citrus resources may be expected to be found there (Reuther 1977; IBPGR 1982). Wild citrus may rarely be existing as scattered trees in remote areas rather than as pure stands. Even when natural populations are present, it is difficult to determine whether they represent wild ancestors or are derived from naturalized forms of introduced or selected varieties (Krueger and Navarro 2007).

3.2 Citrus Conservation Initiatives

To assess the genetic vulnerability, information on the extent and distribution of genetic diversity is a must. In this regard, limited information is available on citrus due to its wide cultivation. This has made it difficult to assess the magnitude of the actual risk of genetic erosion through the replacement of a large number of unselected citrus varieties by a small number of improved cultivars (Duran-Vila 1995). Citrus germplasm is traditionally conserved in clonal orchards belonging to botanical gardens or scientific institutions (Table 3.1). The accessions of living trees are the source of propagative materials for distribution as well as often being able to serve as resources for a limited amount of characterization and evaluation

Table 3.1 Summary of the number of accessions of citrus conserved in different countries

No. of accessions	Institution/University/Organization where maintained	Country
953	Concordia citrus collection	Argentina
374	EEAOC citrus germplasm collection	Argentina
1,858	Sylvio Moriera citrus collection	Brazil
602	National Research Center for Cassava and Tropical Fruit Crops	Brazil
390	Fruit Crop Research Center	Brazil
96	Universidad Catolica citrus collection	Chile
>1,000	National Citrus Germplasm Repository, Beibei, Chongqing, Sichuan province	China
280 in vivo and 110 in vitro	Huazhong Agricultural University, Huazhong	China
304	Instituto de Investigaciones de Citricos y otros Frutales	Cuba
>600	Garo Hills	India
>600	Eight sites belonging to Agricultural Institutes and Universities at Chetalli, Bangalore, Rahuri, Tirupati, Abohar, Bhatinda, Yercaud, and New Delhi	India
>500	–	Indonesia
>1,200	Fruit Tree Research Stations at Tsukuba, Okitsu, and Kuchinotsu	Japan
>100	Institute of Plant Breeding	Philippines
>500	Three sites including Phichit Horticultural Research Center and Nan Horticultural Research Center	Thailand
412	INIA Salto Grande collection	Uruguay
281	Facultad de agronomia Salto collection	Uruguay
99	Direccion de servicios agricolas collection	Uruguay
>250	Florida Citrus Arboretum	USA
>200	The Texas A&M University, Kingsville Citrus Center	USA
>100	Rio Farms Citrus Variety Collection	USA
865	Citrus Variety Collection	USA
–	National Institute of Agricultural Science and Technology and Phu Ho Fruit Research Center	Vietnam

(Krueger and Navarro 2007). In situ conservation of citrus has been attempted in most of the Southeast Asian countries, where citrus is supposed to have originated. These include China, India, Indonesia, Malaysia, Philippines, and Thailand. However, growing population and other development pressures in these countries have led to deforestation and loss of genetic diversity. This had made ex situ preservation of citrus genetic resources imperative. Ex situ collections also make genetic resources more readily available to users. Outside of the centers of origin/diversity, large ex situ collections are found in Argentina, Australia, Brazil, France, Morocco, New Zealand, South Africa, Spain, Turkey, and the USA (Krueger and Navarro 2007).

To coordinate the interactions between the various entities dealing with citrus germplasm, the Global Citrus Germplasm Network (GCGN) was established under the aegis of the Food and Agriculture Organization (FAO). GCGN is chaired by a General Coordinator and guided by Coordinating Board and will serve to link different initiatives in different parts of the world dealing with citrus genetic resources exploration, conservation, and utilization (Krueger and Navarro 2007). All citrus germplasm banks have a field collection. A duplicated protected collection is maintained by a few germplasm banks to maintain healthy genotypes and to diminish risks of losses of plants due to biotic or abiotic stresses. Usually, collections include two copies of each accession except for genotypes of low use for which only one plant is maintained.

High costs of traditional conservation system have led to the use of cryopreservation for long-term conservation of citrus genetic resources (Perez 2000). Various organs and tissues such as embryogenic callus lines, somatic embryos, shoot tips, ovules, pollen, embryo axes, and intact seeds have been cryopreserved (Mumford and Grout 1979; Bajaj 1984; Marin and Duran-Vila 1988; Kobayashi et al. 1990; Lambardi et al. 2004; Wang and Deng 2004; Malik and Chaudhury 2006) using techniques such as "slow-cooling", "one-step freezing", "encapsulation–dehydration", and "encapsulation–vitrification." Organs and tissues of various species such as *C. aurantifolia*, *C. aurantium*, *C. deliciosa*, *C. limon*, *C. sinensis*, *C. paradisi*, *C. sudachi*, *C. halimii*, *C. madurensis*, *C. latipes*, *C. macroptera*, and *C. hystrix* have been cryopreserved.

3.3 Origin and Evolution of *Citrus*

The lack of sufficient descriptions, specimens, and original habitats has made it difficult for the biogeographers to define precisely the centers of origin and ancestors of citrus. A multitude of natural interspecific hybrids and cultivated varieties, including spontaneous mutants, obscure further the history of citrus (Gmitter et al. 2008). It is considered to have originated from Southeast Asia, especially East India, North Burma, and Southwest China, possibly ranging from northeastern India eastward through the Malay Archipelago, north into China and Japan, and south to Australia (Tanaka 1954; Webber 1967; Scora 1975; Gmitter and Hu 1990; Soost and Roose 1996). The oldest known reference to citrus appears in Sanskrit literature that dates back to before 800 BC followed by descriptions in Chinese, Greek, and Roman literature (Webber 1967; Scora 1975).

Understanding taxonomy, phylogenetic relationships, and genetic variability in citrus is critical for determining the origin, evolution, and genetic relationships, characterizing germplasm, controlling genetic erosion, designing sampling strategies or core collections, establishing breeding programs, and registering new cultivars (Herrero et al. 1996). Barrett and Rhodes (1976) carried out a comprehensive phylogenetic study and evaluated 146 morphological and biochemical tree, leaf, flower, and fruit characteristics. They suggested that there were three true ancestral citrus species, namely, *C. maxima* (pummelos), *C. reticulata* (mandarins), and *C. medica* (citrons). Scora (1975) also viewed these three species to be the only true citrus and all other species as introgressions of these ancestral forms. More recently, the phylogeny and genetic origin of the important species of citrus has been investigated using molecular markers (Nicolosi et al. 2000; Moore 2001; Barkley et al. 2006). These studies have supported the hypotheses of Barrett and Rhodes (1976) and Scora (1975), though there may be other species that might also be considered as ancestral. *C. aurantifolia*, *C. micrantha*, and *C. halmii* are also included in the list of "true" citrus species by many researchers. Molecular markers, isozymes, chloroplast, and nuclear genome analysis were utilized to assess diversity, phylogenetic relationships, and parentage in lemon (*C. limon*) accessions (Gulsen and Roose 2001a, b). However, these studies have not

been able to clearly differentiate between all the species. Hence, there is a need for additional taxonomic studies to further clarify the taxonomic distinctions.

3.4 Role in Development of Cytogenetic Stocks and Their Utility

Chromosome addition, deletion, and substitution lines are extremely valuable tools for genome analysis. They allow direct localization of genes to specific chromosomes based on their expression or non-expression in lines carrying the respective added, deleted, or substituted chromosome or chromosome segment (Snowdon et al. 2002). As the chromosomes of citrus are small, it is difficult to study them in detail. This has limited the development of molecular cytogenetic tools in citrus. Another limiting factor is the availability of appropriate flower buds, for studying meiosis, only in the spring (Roose and Close 2008). Studies on chromosome banding in citrus have focused on distinguishing the chromosomes of different species and varieties (Guerra 1984, 1993; Yamamoto and Tominaga 2003). For the detection of cytogenetic variation in citrus chromosomes, fluorescence in situ hybridization (FISH) using repetitive sequence probes (rDNA) has been used (Matsuyama et al. 1996). A combination of chromosome banding and rDNA hybridization has revealed species-specific distinct patterns (Moraes et al. 2007). In situ hybridization of 18S–5.8S–25S rDNA probes labeled with biotin or rhodamine and 5S rDNA probes labeled with digoxigenin have been used to locate rDNA sites on root-tip metaphase chromosomes of Troyer citrange (*C. sinensis* × *Poncirus trifoliata*), Sacaton citrumelo (*C. paradisi* × *P. trifoliata*), and African shaddock × Rubidoux trifoliate (*C. maxima* × *P. trifoliata*). Counterstaining with the fluorochromes CMA3 and DAPI uniquely identified many but not all chromosomes. Counts of 18S–25S rDNA sites on metaphase chromosomes or interphase and prophase nuclei suggested that Troyer had four major and usually one minor site in all complete metaphases examined, Sacaton citrumelo had six sites, and a *C. maxima* × *P. trifoliata* hybrid had five sites in two complete metaphases (Roose et al. 1998). To date, no reports are available on localization of individual gene sequences or large insert clones such as bacterial artificial chromosomes (BACs) onto citrus chromosomes (Roose and Close 2008).

Euploidy in citrus is represented by monoploids, diploids, triploids, tetraploids, pentaploids, hexaploids, and octaploids. Haploids have been obtained by anther culture in *C. madurensis* (Chen et al. 1980) and other citrus species (Germana et al. 1991, 1994; Germana and Reforgiato 1997; Chiancone et al. 2006). Two haploid seedlings of *C. natsudaidai* Hayata were obtained from 3,000 seeds irradiated with gamma rays (Karasawa 1971). Only diploid plants were obtained by in vitro differentiation of microspores of *C. aurantium* (Hidaka and Omura 1989). Improvements in embryo rescue and somatic hybridization methodologies have greatly increased the range of possibilities for development of chromosome addition lines by interspecific and intergeneric hybridization. Tetraploid somatic hybrids have been obtained from intergeneric (compatible as well as incompatible) and interspecific somatic hybridization of citrus for scion and rootstock improvement (Grosser and Gmitter 2005). Attempts have also been made to combine citrus with over 30 sexually incompatible genera, including *Aegle*, *Atalantia*, *Citropsis*, *C. ichangensis*, *C. jambhiri*, *Eremocitrus*, *Fortunella*, *Microcitrus*, *Poncirus,* and *Severinia*, that exhibit desirable rootstock attributes (Mourao Fo and Grosser 1992; Grosser and Gmitter 2005).

3.5 Molecular Mapping in *Citrus*

To increase the efficiency of breeding programs, it is desired to have genetic linkage maps as they include important linkage relationships among molecular markers and genes that breeders wish to manipulate for cultivar improvement. Genetic maps of citrus may provide the basis for early screening procedures, permitting breeders to make initial selection among very young progeny based on the phenotype predicted by their genotype at molecular loci known to cosegregate with a particular phenotype (Durham et al. 1992) rather than relying on frequently difficult, time-consuming, and inefficient approaches based on phenotypic

Table 3.2 Citrus linkage maps

Mapped parent	Type of cross	Population size	Markers used	Total markers	Map length (cM)	Linkage groups	Reference
C. grandis cv. Acidless × *C. jambhiri* cv. Florida	*Citrus* F_1	35	Isozymes	2	–	2	Torres et al. (1985)
LB 1–21 (*C. reticulata* cv. Clementine × *C. paradisi* cv. Duncan) × *C. reticulata* cv. Clementine	*Citrus* BC_1	65	Isozymes, RFLP	35	314	8	Liou (1990)
C. grandis × *C. grandis*	*Citrus* self F_1	52	RAPD	34	600	7	Luro et al. (1996)
C. auratium	F_1	120	AFLP, RAPD, RFLP	247	1,000	20	de Simone et al. (1998)
C. latipes	F_1	120	AFLP, RAPD, RFLP	92	600	12	de Simone et al. (1998)
C. sunki	F_1	80	RAPD	63	732	10	Cristofani et al. (1999)
C. auratium	F_1	120	AFLP, RAPD, RFLP	247	1,021	10	Recupero et al. (2000)
C. latipes	F_1	120	AFLP, RAPD, RFLP	92	561	12	Recupero et al. (2000)
C. auratium	F_1	80	IRAP, RAPD, SSR	120	442	15	Ruiz and Asins (2003)
C. volkameriana	F_1	80	IRAP, Isozymes, RAPD, RFLP, SSR	97	460	10	Ruiz and Asins (2003)
C. sinensis	F_1	97	EST-SSRs	113	776	11	Chen et al. (2008)

AFLP amplified fragment length polymorphism, *EST-SSR* expressed sequence tag-simple sequence repeat, *IRAP* inter-retrotransposon amplified polymorphism, *RAPD* randomly amplified polymorphic DNA, *RFLP* restriction fragment length polymorphism, *SSR* simple sequence repeat

characterizations (Talon and Gmitter Jr 2008). Several linkage maps of citrus have been published (Table 3.2) since 1980s using isozymes as well as DNA markers such as randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), sequence characterized amplified region (SCAR), simple sequence repeat (SSR), and intersimple sequence repeat (ISSR) (Durham et al. 1992; Garcia et al. 1999; Roose et al. 2000; Sankar and Moore 2001; Chen et al. 2008). Several of these maps involved at least one parent that is a *Citrus* × *Poncirus* parent to ensure high heterozygosity, but the hybrids obtained have inedible fruit, making these mapping populations of limited use for fruit traits. These maps share few markers making their comparison difficult (Roose et al. 2000). They are also incomplete in that the number of major linkage groups often differs from the number of chromosomes. While such maps are still quite useful, more complete, high-density maps would be more useful for both quantitative trait loci (QTL) analysis and for anchoring BAC and genome sequence (Roose and Close 2008).

Gene tagging has been widely used in citrus. Citrus, being heterozygous, individuals differing for a trait are identified and crossed, and the trait in the progeny is measured and analyzed using bulk-segregant analysis with DNA markers to identify segregating markers

linked to the genes of interest. Several traits of horticultural importance including CTV resistance (Gmitter et al. 1996) from *P. trifoliata*, nematode resistance (Ling et al. 2000) from *P. trifoliata*, fruit acidity (Fang et al. 1997), dwarfing (Cheng and Roose 1995), and nucellar embryony (Garcia et al. 1999; Kepiro 2004; Nageswara Rao et al. 2008) from *C. latipes* × *C.aurantium* F_1 hybrids (Recupero et al. 2000) have been tagged. QTL analysis for tagging regions containing genes that influence salinity tolerance, *Alternaria* brown spot resistance, and several other traits have been identified, but fine mapping of QTLs has not been reported (Tozlu et al. 1999a, b; Dalkilic et al. 2005; Roose and Close 2008). F_1 progeny of *C. sunki* × *P. trifoliata* were evaluated for the detection of QTLs linked to citrus *Phythophthora* gummosis resistance (Siviero et al. 2006). Nineteen putative QTLs (8 in *C. volkameriana* and 11 in *P. trifoliata*) controlling the number of fruits per tree were detected in the *C. volkameriana* and *P. trifoliata* progeny (Garcia et al. 2000). Mapping of QTLs associated with freezing tolerance was accomplished using a *C. grandis* × *P. trifoliata* F_1 pseudo-testcross population (Weber et al. 2003). Molecular maps, particularly of those with very closely flanking or cosegregating DNA markers with the gene(s) of interest, may be very useful for further genomic manipulation, map-based cloning, and marker-assisted breeding programs (Recupero et al. 2000; Asins 2002; Gmitter et al. 2007).

The physical mapping of complex genomes is based on the construction of a genomic library and the determination of the overlaps between the inserts of the mapping clones in order to generate an ordered, cloned representation of nearly all the sequences present in the target genome (Talon and Gmitter Jr 2008). The construction of BAC libraries containing clones with large DNA fragments is essential for the generation of high-resolution physical maps. The relatively small genome size of citrus makes preparation and analysis of BAC libraries an attractive approach (Roose and Close 2008). Two BAC contigs with integrated fine genetic maps have been constructed from intergeneric citrus and *P. trifoliata* hybrid (Deng et al. 1997, 2001; Yang et al. 2001), resulting in full-length sequencing of the locus spanning several hundreds of kilobases and identification of the candidate genes (Yang et al. 2003).

3.6 Role in Crop Improvement Through Traditional and Advanced Tools

3.6.1 Traditional Breeding Efforts in Citrus

Citrus is vegetatively propagated. Due to its heterozygous nature, sexual hybridization to create new genotypes results in substantial variation of the characters in the progeny as they produce widely variant sexually derived progeny (Gmitter et al. 2009). It also has a long juvenile period of seedlings (5 or more years) making citrus breeding not only a difficult, but a costly and land-intensive proposition. Little is known about the genetic mechanisms controlling the inheritance of agriculturally important traits with only a few important traits showing single gene inheritance (Furr 1969; Gmitter et al. 1992). Nucellar embryony is also common in citrus. Conventionally, controlled crossing is carried out to combine desirable traits by cross-pollination. After the hybrid fruits have matured, the seeds are extracted from the fruits and planted in the greenhouse. Once the seedlings have attained sufficient size, they are either grafted onto rootstocks or directly planted in the field and evaluated for disease and pest resistance, stress tolerance, and overall growth characteristics during the juvenile period (Davies and Albrigo 1994), and subsequently for fruit characteristics of scions or rootstock traits of interest.

Though it takes only minutes to effect pollination, the difficult nature of citrus breeding lies in the elimination of undesirable hybrids and the evaluation of selections (Sykes 1987). The major objectives of rootstock breeding are aimed to control the tree size and to improve resistance and tolerance to biotic and environmental stresses such as citrus blight, CTV, *Phytophthora*, citrus exocortis viriod (CVC), nematodes, cold, drought, salinity, and flooding. Reduction of tree size without affecting yield or scion health is also desirable (Soost and Roose 1996). Most of these attributes are present in *P. trifoliata*, making it a valuable resource for the genetic improvement of citrus rootstocks (Gmitter et al. 2007). A number of breeding programs have focused on obtaining intergeneric hybrids between *C. sinensis* × *P. trifoliata*, *C. paradisi* × *P. trifoliata*, and *C. reticulata* × *P. trifoliata*

for use as rootstocks. Rapid growth and lack of branching are other desirable characters for convenient and economical nursery production of rootstock seedlings (Soost and Roose 1996). Mutation breeding programs have also been established for the genetic improvement of citrus. Mutations have been induced by gamma rays and chemicals and the mutants have been analyzed for the desired traits (Hensz 1977; Hearn 1984; Deng and Zhang 1988; Deng et al. 1993; Gulsen et al. 2007).

3.6.2 Ploidy Manipulation in Citrus

Anther culture may provide the breeders and geneticists with the ability to recover haploid plants that would facilitate the recovery of useful recessive alleles or mutations useful for citrus cultivar improvement and genetic studies (Gmitter et al. 1992). Citrus anthers have been cultured in attempts to produce haploid plants and colchicine has been used to obtain homozygous diploids (Chen et al. 1980; Germana et al. 1991, 1994; Germana and Reforgiato 1997; Chiancone et al. 2006). Anther culture of *C. madurensis* gave rise to haploids (Chen et al. 1980). Haploid seedlings of *C. natsudaidai* have also been obtained by irradiation (Karasawa 1971), after in situ parthenogenesis induced by irradiated pollen (Ollitrault et al. 1996) and through gynogenesis induced by in vitro pollination with pollen from a triploid plant (Germana and Chiancone 2001). The production of diploid plants by culturing anthers of tetraploid somatic hybrids may also provide breeders with greater access to these unique genetic combinations (Gmitter et al. 2009). Diploid regenerants have also been obtained from anthers of diploid plants of *C. aurantium*, with nuclear fusion observed during the routes of microspore development being the suggested cause of diploidy in these regenerants (Hidaka and Omura 1989).

Triploids have been regenerated by in vitro culturing of hybrid endosperms of *C. sinensis*, *C. paradisi*, and *C. grandis* (Gmitter et al. 1990). However, this method has not been adopted as a breeding strategy because it is species- and cultivar-dependent, and is far less efficient than creating triploid offspring by interploid hybridization (Gmitter et al. 2009). Intergeneric and interspecific somatic hybridization has been attempted to produce tetraploid somatic hybrids (Grosser and Gmitter 2005). These tetraploids are used either as rootstock candidates or as parents in breeding programs (Cameron and Soost 1969; Cameron and Burnett 1978; Grosser et al. 1998). Crosses of diploid and tetraploid parentage using monoembryonic seed parents have given rise to tetraploid progenies that might be due to non-disjunction leading to the doubling of chromosome number of one of the parents during cell division. *Aegle*, *Atalantia*, *Citropsis*, *C. ichangensis*, *C. jambhiri*, *Eremocitrus*, *Fortunella*, *Microcitrus*, *Poncirus*, and *Severinia* are some of the sexually incompatible genera, which exhibit desirable rootstock attributes, used for somatic hybridization with citrus (Mourao Fo and Grosser 1992; Grosser and Gmitter 2005). A seedless tetraploid "*Shiyueju*" tangerine was created by treating the shoot of diploid "*Shiyueju*" with colchicine (Chen and Ou 1985). When diploid *Fortunella japonica* was treated with colchicine, it gave rise to seedless tetraploid *F. japonica* (Li and Zhang 1988). Tetraploid plants have also been recovered by the incorporation of colchicine in standard tissue culture media (Gmitter and Ling 1991; Gmitter et al. 1991).

3.6.3 Tissue Culture Regeneration

Application of plant cell and tissue culture has the potential to unlock an entirely new round of genetic improvements for citrus crops, most of which cannot be achieved by any other means (Gmitter et al. 2008). Citrus is amenable to regeneration via organogenesis and somatic embryogenesis. Organogenesis has been induced in vitro from various explants such as shoot meristems of *C. aurantifolia* and *C. sinensis*, nodal explants of *C. junos*, *C. sinensis*, and *C. aurantifolia*, stem internodes of *C. sinensis*, leaf sections of *C. sinensis* and *C. aurantifolia*, root tissues of *C. medica* and *C. aurantifolia*, mature and immature embryos of *C. limon*, and thin sections of mature stem segments of *C. sinensis* (Maheshwari and Rangaswamy 1958; Chaturvedi and Mitra 1974; Sauton et al. 1982; Barlass and Skene 1986; Moore 1986; Duran-Vila et al. 1989; Omura and Hidaka 1992; Al-Khayri and Al-Bahrany 2001; Kim et al. 2001; Kotsias and Roussos 2001; Huang et al. 2002; Cavalcante-Alves et al. 2003; Kobayashi et al. 2003; Mukhtar et al. 2005; Soneji et al. 2007; Perez-Tornero and

Porras 2008). Somatic embryogenesis has been induced in cultured nucelli of *C. sinensis* and *C. limon*, undeveloped ovules of *C. limonimedica*, nucellar embryos of *C. limonia* and *C. unshiu*, endosperm-derived callus of *C. grandis*, *C. paradisi*, and *C. sinensis*, juice vesicles of *C. unshiu*, anthers of *C. madurensis*, *C. natsudaidai*, and *C. aurantium*, styles of *C. aurantium*, *C. deliciosa*, *C. paradisi*, and *C. sinensis*, and pistil thin cell layers of *C. deliciosa*, *C. limon*, *C. madurensis*, *C. medica*, *C. tardiva*, and *C. sinensis* (Rangan et al. 1969; Starrantino and Russo 1980; Chaturvedi and Sharma 1985; Gmitter and Moore 1986; Gmitter et al. 1990; Nito and Iwamasa 1990; Carimi et al. 1995, 1998, 1999; Calovic et al. 2003; Chiancone et al. 2006; Jajoo 2010). The identification of valuable somaclonal variants holds great promise for cultivar improvement especially for those citrus species that are difficult to manipulate by sexual hybridization (Gmitter et al. 1992) and is being exploited to identify clones with improved traits such as fruit quality improvements across an extended season of maturity and disease resistance (Nadel and Spiegel-Roy 1987; Grosser et al. 2003). Plant regeneration systems are also potentially useful for obtaining genetic change through cell transformation or mutagenesis.

3.6.4 Genetic Engineering

For the introduction of specific traits into citrus without altering the genetic background, genetic engineering may be an efficient alternative. Genetic engineering has been applied for a number of traits in citrus (Table 3.3). CTV-derived genes such as the coat-protein gene, *p23 ORF*, have been introduced into citrus cultivars such as *C. aurantifolia*, *C. aurantium*, and *C. paradisi* in efforts to produce resistance to CTV (Gutierrez et al. 1997; Dominguez et al. 2000; Ghorbel et al. 2001, Fagoaga et al. 2006; Ananthakrishnan et al. 2007). CTV R genes, derived from the CTV resistant *P. trifoliata* genome, have also been introduced into *C. sinensis* (Soneji et al. 2007). The cDNA of the *Xa21*, a *Xanthomonas* resistance gene, has been introduced into citrus in efforts to produce resistance to citrus canker (Omar and Grosser 2007). *Arabidopsis* genes such as *LEAFY* or *APETALA1* that alter the growth habit, reduce juvenility, and regulate vegetative and other behavior have been introduced into citrange, a hybrid of *C. sinensis* × *P. trifoliata* (Pena et al. 2001). Genetic transformation and regeneration of mature tissues of citrus, which could bypass the juvenile phase, has also been attempted (Cervera et al. 1998). For salinity tolerance, a gene from yeast has been introduced into citrus (Cervera et al. 2000). Genes involved in the metabolic pathway regulation such as the carotenoid biosynthetic genes have been introduced in *C. paradisi* (Costa et al. 2002). Ethylene biosynthesis genes have also been introduced in Carrizo citrange (*C. sinensis* × *P. trifoliata*), *C. sinensis*, and *P. trifoliata* (Wong et al. 2001). Attempts have also been made to introduce juice quality-related pectin methylesterase gene for enzyme associated with the "cloud separation" into citrus (Guo et al. 2005).

3.7 Genomics Resources Developed

Expressed sequence tags (ESTs) are useful in identifying gene transcripts, making them instrumental in gene discovery and sequence determination. The first set of ESTs was reported from citrus seeds and mature fruits in rapid cell development phase (Hisada et al. 1997). Since then several groups have contributed to the sequencing and development of ESTs from reproductive and vegetative organs and tissues at different developmental stages and challenged with biotic and abiotic agents, and elicitor and hormonal treatments (Bausher et al. 2003; Shimada et al. 2003; Talon and Gmitter Jr 2008). At present, around 494,484 ESTs developed from various citrus species have been deposited in the National Center for Biotechnology Information (NCBI) EST database (http://harvest.ucr.edu) (Table 3.4).

Microarrays have also been developed for citrus for transcript profiling. Construction of a cDNA microarray to monitor expression of mRNA from 2,213 genes during fruit development was the first transcript profiling data to be reported (Shimada et al. 2005). A spotted array of 6,875 clones obtained from 22,000-EST collection was used to investigate gene expression patterns during fruit ripening and other traits (Forment et al. 2005; Cercós et al. 2006). Many higher density citrus microarrays consisting of 12,000 unigenes or 24,000-element cDNA array containing 20,000 unigenes, based on nearly 90,000 high-quality sequences generated from 52 different cDNA libraries

Table 3.3 Summary of genetic transformation of *Citrus*

Genotype used	Explant used	Method used	Gene(s) introduced	Reference
Citrus sinensis cv. Trovita	Protoplasts	PEG-mediated	*npt*II	Kobayashi and Uchimiya (1989)
C. jambhiri	Protoplasts	PEG-mediated	*cat, npt*II	Vardi et al. (1990)
C. sinensis cvs. Washington Navel and Trovita	Cell suspensions	*At*-mediated	*hpt, npt*II	Hidaka et al. (1990)
C. reticulata cv. Ohta Ponkan	Protoplasts	Electroporation	*uidA*	Hidaka and Omura (1993)
C. sinensis cv. Hamlin	Protoplasts	Electroporation	*gfp*	Niedz et al. (1995)
C. sinensis cv. Pineapple	Greenhouse internodal stem segments	*At*-mediated	*npt*II, *uidA*	Pena et al. (1995)
C. reticulata × *C. paradisi*	Cell suspensions	Particle bombardment	*npt*II, *uidA*	Yao et al. (1996)
C. aurantifolia	In vitro internodal stem segments	*At*-mediated	CTV-*CP*	Gutierrez et al. (1997)
C. aurantium	In vitro internodal stem segments	*At*-mediated	CTV-*CP*	Gutierrez et al. (1997)
C. aurantifolia cv. Mexican	Greenhouse internodal stem segments	*At*-mediated	*npt*II, *uidA*	Pena et al. (1997)
C. sinensis cv. Washington navel	In vitro epicotyl segments	*At*-mediated	*npt*II, *uidA*	Bond and Roose (1998)
C. sinensis cv. Pineapple	Greenhouse internodal stem segments	*At*-mediated	*npt*II, *uidA*	Cervera et al. (1998)
C. sinensis cv. Tarocco	In vitro epicotyl segments	*At*-mediated	*npt*II, *rolA, rolB, rolC*	Gentile et al. (1998)
C. aurantifolia cv. Mexican	In vitro internodal stem segments	*At*-mediated	*npt*II, *uidA*	Perez-Molphe and Ochoa-Alejo (1998)
C. paradisi cv. Duncan	In vitro epicotyl segments	*At*-mediated	*npt*II, *uidA*	Luth and Moore (1999)
C. aurantifolia	In vitro epicotyl segments or greenhouse intermodal stem segments	*At*-mediated	*npt*II, *gfp*	Ghorbel et al. (1999)
C. aurantium	In vitro epicotyl segments or greenhouse intermodal stem segments	*At*-mediated	*npt*II, *gfp*	Ghorbel et al. (1999)
C. aurantifolia cv. Mexican	Greenhouse internodal stem segments	*At*-mediated	CTV-*CP*	Dominguez et al. (2000)
C. sinensis cv. Itaborai	Protoplasts	PEG-mediated	*gfp*	Fleming et al. (2000)
C. aurantium	Greenhouse internodal stem segments	*At*-mediated	CTV-*CP*	Ghorbel et al. (2000)
C. paradisi cv. Rio Red	In vitro epicotyl segments	*At*-mediated	*npt*II, *uidA*, unCTV-*CP*, *gna*	Yang et al. (2000)
C. sinensis cv. Pineapple	Greenhouse internodal stem segments	*At*-mediated	*P R-5*	Fagoaga et al. (2001)
C. aurantifolia cv. Mexican	Greenhouse internodal stem segments	*At*-mediated	CTV-*p23*	Ghorbel et al. (2001)
C. sinensis	In vitro epicotyl segments	*At*-mediated	CS-*ACS1*	Wong et al. (2001)
C. paradisi cv. Duncan	In vitro internodal stem segments	*At*-mediated	*CitPSY*, *CitPDS*, *CitLCY-B*	Costa et al. (2002)
C. aurantifolia cv. Mexican	Greenhouse internodal stem segments	*At*-mediated	CTV-*CP*, unCTV-*CP*	Dominguez et al. (2002a, b)
C. reticulata cv. Ponkan	Embryogenic callus	*At*-mediated	*bar*, *pAT29-barnase*	Li et al. (2002)
C. sinensis cv. Hamlin	In vitro epicotyl segments	*At*-mediated	*npt*II, *gfp*	Mendes et al. (2002)
C. sinensis		*At*-mediated	*npt*II, *uidA*	

(*continued*)

Table 3.3 (continued)

Genotype used	Explant used	Method used	Gene(s) introduced	Reference
	In vitro epicotyl segments or greenhouse intermodal stem segments			Almeida et al. (2003a, b)
C. limonia	In vitro epicotyl segments	*At*-mediated	*npt*II, *uidA*	Almeida et al. (2003a)
C. sinensis cvs. Valencia, Hamlin, Natal and Pera	In vitro epicotyl segments	*At*-mediated	*PMI*	Boscariol et al. (2003)
C. paradisi cv. Duncan	In vitro internodal stem segments	*At*-mediated	ntCTV-*CP*	Febres et al. (2003)
C. sinensis cv. Itaborai	Protoplasts	PEG-mediated	*gfp*, CTV-derived sequence	Olivares-Fuster et al. (2003)
C. sinensis cv. Valencia	Embryogenic callus	*At*-mediated	*bar*, *pAT29-barnase*	Li et al. (2003)
C. sinensis cv. Hamlin	Protoplasts	Electroporation	*egfp*	Niedz et al. (2003)
C. aurantifolia cv. Mexican	Greenhouse internodal stem segments	*At*-mediated	CTV-*p23*	Fagoaga et al. (2005)
C. aurantium	Greenhouse internodal stem segments	*At*-mediated	CTV-*p23*	Fagoaga et al. (2005)
C. sinensis cv. Valencia	Protoplasts	PEG-mediated	*gfp*, *T S P M E*	Guo et al. (2005)
C. paradisi cv. Duncan	In vitro internodal stem segments	*At*-mediated	*Ac*	Trainin et al. (2005)
C. macrophylla	In vitro epicotyl segments	*At*-mediated	ds(*p23* + *3′UTR*)	Batuman et al. (2006)
C. sinensis cv. Hamlin	In vitro epicotyl segments	*At*-mediated	*att A*	Boscariol et al. (2006)
C. sinensis cv. Pineapple	In vitro epicotyl segments or greenhouse internodal stem segments	*At*-mediated	*ipt*, *R*/*RS* recombinase system	Ballester et al. (2007)
C. sinensis cv. Bingtang	In vitro epicotyl segments	*At*-mediated	*gfp*	Duan et al. (2007)
C. paradisi cv. Duncan	In vitro epicotyl segments	*At*-mediated	*nptII*, *gfp*, CTV R genes	Chen et al. (2007)
C. sinensis cv. Hamlin	Protoplasts	PEG-mediated	*gfp*, *Xa21*	Omar and Grosser (2007)
C. sinensis cv. Midsweet	In vitro epicotyl segments	*At*-mediated	*nptII*, *gus*, *gfp*, CTV R genes	Soneji et al. (2007)
C. sinensis cv. Navelina	Greenhouse internodal stem segments	*At*-mediated	*npt*II	Rodriguez et al. (2008)
C. sinensis cv. Pineapple	Greenhouse internodal stem segments	*At*-mediated	CPsV-*CP*	Zanek et al. (2008)

or 22,000 oligoarray containing 21,495 independent ESTs, have also been developed (Talon and Gmitter Jr 2008). An Affymetrix citrus GeneChip containing 30,264 probe sets (22 probes each) for measurement of citrus transcripts and 5,023 probe sets (56 probes each) to serve as single nucleotide polymorphism (SNP) markers for 3,219 genes has also been designed from the HarvEST:Citrus database and is commercially available (Close et al. 2006). Transcript profiling has been carried out using genes from various species such as *P. trifoliata*, *C. latipes* × *C. aurantium*, *C. sunki* × *P. trifoliata*, *C. volkameriana*, and *C. grandis* × *P. trifoliata* for fruit growth and ripening involving traits such as ethylene production, anthocyanin and flavonoid biosynthesis, carbohydrate buildup, acid reduction, and chlorophyll degradation for responses to pathogenic infections such as leaf spot, mold, post-bloom fruit drop, root rot, canker, citrus variegated chlorosis, Huanglongbing or greening, CTV, and citrus leprosis virus, and for environmental stresses such as cold temperatures, drought, flooding, salinity, and high and low soil pH (Romero-Aranda

Table 3.4 Current *Citrus* EST entries in GenBank

Citrus species/hybrids	No. of ESTs
Citrus sinensis	208,909
Citrus clementina	118,365
Poncirus trifoliata	62,344
Citrus reticulata	55,980
Citrus unshiu	19,066
Citrus aurantium	14,584
Citrus × *limonia*	11,045
Citrus latifolia	8,756
Citrus aurantiifolia	8,219
Citrus limettioides	8,188
Citrus × *paradisi*	8,039
Citrus × *paradisi* × *Poncirus trifoliata*	7,954
Citrus reticulata × *Citrus temple*	5,823
Citrus reshni	5,768
Citrus sunki	5,216
Citrus macrophylla	1,929
Citrus sinensis × *Poncirus trifoliata*	1,837
Citrus clementina × *Citrus tangerina*	1,834
Citrus limon	1,505
Citrus medica	1,115
Citrus jambhiri	989
Citrus nobilis × *Citrus kinokuni*	645
Citrus tamurana	358
Citrus natsudaidai	202
Citrus hassaku	154
Total	558,898

Source: International Citrus Genome Consortium

et al. 1998; Moya et al. 2003; Lahey et al. 2004; Shimada et al. 2005; Cercós et al. 2006; Tsukuda et al. 2006; Fujii et al. 2007; Gandia et al. 2007).

3.8 Conclusion

Citrus is grown throughout the world in the tropics and subtropics. It is vegetatively propagated by grafting or budding. Limited information is available on the extent and distribution of genetic diversity of citrus, making it difficult to understand the actual risk of genetic erosion. Hence, concerted efforts are required for efficient conservation of citrus germplasm. Though citrus breeding is very challenging, different breeding programs throughout the world have made significant progress in the application of conventional and modern approaches for genetic improvement and cultivar development. Advances in plant cell and tissue culture also have major impacts on genetic improvement of citrus. Efforts have been made to produce haploids via anther culture as they are highly beneficial in breeding programs and in understanding citrus genetics. For the production of seedless cultivars, triploids have been generated by fusion of haploid with diploid protoplasts and/or endosperm culture. Use of somatic hybridization for the recovery of tetraploids has also expanded the range of germplasm available to citrus breeders.

Linkage mapping and tagging will aid in identification and cloning of candidate genes. These candidate genes can then be introduced into citrus via genetic engineering and exploited for the improvement of citrus without altering its integrity. In recent years, citrus genomics has progressed rapidly. The development of BAC libraries, an extensive EST collection, microarrays, and dense, sequence-based maps will be of immense importance in whole genome sequencing of citrus and will aid in elucidation of gene function, regulation, and expression. Advances in genomic science will have a great impact on citrus improvement and will continue to provide new information and gene targets for manipulation.

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Chapter 4
Mangifera

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4.1 Introduction

Mango (*Mangifera indica* L.) is one of the most important fruit crops having socio-economic significance in many countries. It is known as the king of fruits owing to delicious quality of its fruit rich in vitamins and minerals. Its long period of domestication is well evidenced from its mention in the ancient scripture. In India, ancients valued mango not merely for its sentiment or religious consideration, but they realized its importance in economic and cultural life of the society. Muslim Kings (Nawabs) promoted the practice of planting best varieties. Lakhi bagh (one lakh plants) planted by Akbar the Great, which is a testimony of this, is well known in the history. "Ain-I-Akbari," an encyclopedia written during the period 1590 AD amply gives the understanding of mango of that period. Mango is a rich source of vitamin A and C, flavones, carotenes, glucosides, sterol, termene, aromatic acids, essential oil, fatty acids, and phenolics. It is a powerful nutritive fruit, containing most of the essential substances needed by the body. It contains vitamins and minerals along with important chemicals that keep our body fit and fine.

Increasing importance has been given in the recent years to the wild germplasm, in particular wild relatives of crops and trees as a source of material that might enhance productivity, disease resistance, drought survivability, salinity, etc. There is a need for greater genetic diversity in many tropical species and it is very important in mangoes, as they are more suitable for arid and semi-arid regions. Incidentally, as the mango has increased importance as an export commodity, the commercial industry has come to rely on relatively few cultivars that conform to the current demands of the export market. The result has been a narrowing of the genetic diversity and a growing concern about the loss of wild crop relatives and the threat of devastating diseases or pests (Campbell 2007).

Conservation is very important, because many species are becoming extinct and many others are threatened and endangered. The diversity of some fruits is well collected, while for other fruits relatively little has been done (Arora 1994). Gaps in collections are found both between species and between regions. Kostermans and Bompard (1993) indicated that *Mangifera blommesteinii*, *M. leschenaultii*, *M. superba*, and *M. paludosa* are in real danger of extinction. It is to be mentioned here that collection and utilization of wild species is not an easy task, as they require specific climate and do not so easily get acclimatized to the ex situ conditions on introduction.

Biodiversity can be located either in the wild or in the backyard. With regard to mango the variability can be traced in wild, wherein many species grow naturally even today viz., the occurrence of *M. sylvatica* in the northeastern parts of India or *M. andamanica* in Andaman group of islands. In the wild, this diversity has come about mainly because of chance seedlings and seed propagation mainly by natural elements over a period due to the dispersal of seeds. In the backyard primarily the seedling populations have been the cause of diversity mainly by selection of desired types by man or unintentional (Fig. 4.1). Hence, there is a need to maintain the germplasm including the wild types for their possible incorporation into mango crop improvement programs.

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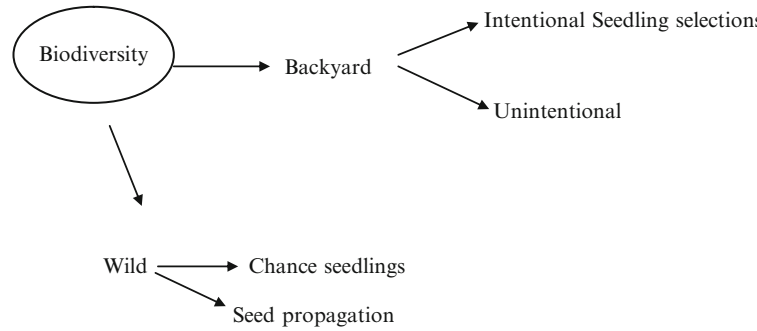

Fig. 4.1 Biodiversity of *Mangifera* species

4.2 Origin, Taxonomy and Distribution of *Mangifera* spp.

4.2.1 Origin

The genus *Mangifera* belongs to the family Anacardiaceae, which comprises 73 genera and about 830 species of mainly tropical plants. Kostermans and Bompard (1993) listed 58 species in the genus *Mangifera*, majority of which were distributed in tropical Asia. The origin of this genus and its center of diversity is mainly Southeast Asia. *M. indica* L. is the most important species in this genus and is now cultivated pan-tropically throughout the world. Other commercially valued species that produce edible fruits are *M. caesia* Jack, *M. foetida* Lout., *M. kemanga* Bl., *M. laurina* Bl., *M. odorata* Griff., *M. pajang* Kostermans, and *M. sylvatica* Roxb. (Tanaka 1976; Bompard 1993).

The mango (*M. indica*) originated in northeastern India, the Indo-Myanmar border region, and Bangladesh, where still it is found as a wild tree with very small fruits. It is also observed in the lower Himalayan tract, near Nepal, Bhutan, and Sikkim. Bompard (1993) has listed more than 60 species worldwide, the highest diversity being found in the heart of the distribution area of the genus *Mangifera*, i.e., the Malayan Peninsula, Borneo, and Sumatra. Mukherjee (1953) mentioned that mango has been under cultivation for at least 4,000 years with over 1,000 varieties in cultivation. Almost all these have arisen as selections made from open-pollinated seedlings.

Mango is grown in many countries. India's share in world production of mango is 56%. Mango production in India has been increasing ever since independence. Mango contributes for 39.5% of the total fruit production in India. In India about thousand varieties are known to exist, though all of them are not commercially important. This is mainly because of the long period of domestication, high genetic variability, and selection and future multiplication by vegetative means. In spite of the large variability, sufficient headway has not been made in the improvement of these varieties. Naik et al. (1958) opine that it is the large variability, too many cultivars and heavy regional preferences, which has hindered the production of the commercial varieties on a large scale. However, one has to admit that this large variability has not been exploited to the full potential.

4.2.2 Taxonomy

The genus *Mangifera* belongs to the order Sapindales in the family Anacardiaceae. The classification is given below:

Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Sapindales
Family: Anacardiaceae
Genus: *Mangifera*

Species of *Mangifera* is medium to large (10–40 m in height), evergreen with symmetrical, rounded canopy; bark is usually dark gray-brown to black, rather smooth, superficially cracked, or inconspicuously fissured, peeling off in irregular, rather thick pieces; long taproots (up to 6–8 m and more); leaves are simple, exstipulate, alternately arranged, 15–45 cm in length, variable in shapes like oval–lanceolate, lanceolate, oblong, linear–oblong, ovate, obovate–lanceolate, or roundish–oblong; upper surface is shining and dark green while the lower

is glabrous light green; petiole swollen at the base; terminal inflorescence, narrowly to broadly conical panicle up to 45 cm long, branching usually tertiary, rarely quaternary; panicle bear 500–6,000 flowers of which 1–70% are bisexual, remainder are male; calyx 4–5 partite, corolla consists of 4–5 pale yellow petals and stamens usually 1–5 but rarely 8, one or rarely two stamens are fertile and the rest are sterile; pollen grains are of variable size from 20 to 35 μm; ovary is sessile, one-celled, oblique and slightly compressed; fruit is a more or less compressed, fleshy drupe; and seed large and compressed (Mukherjee 1950; Naik and Gangolly 1950; Singh 1960; Hooker 1978).

4.2.3 Distribution

The highest species diversity of mango occurs in north-eastern states of India, Malaysia, particularly in peninsular Malaya, Borneo and Sumatra representing heart of the distributional range of the genus (Ara et al. 2005). Also, rich species diversity of *Mangifera* can now be found in the Island of Borneo, Malay Peninsula, Indonesian archipelago, Thailand, Indo-China, and Philippines, which includes seedling types growing in the forest area and have many primitive characters such as polyembryony and dwarf growth habit. Based on the morphological, phyto-geographical, cytological, anatomical, taxonomic, systematic, pollen studies, and other molecular evidences it is now clear that the genus probably evolved within a large area including Burma, Siam, Indo-china, and the Malay peninsula as the center of origin, and the diversity of the genus *Mangifera* is in Southeast Asia (Popenoe 1920; Mukherjee 1951; Iyer and Subramanian 1989). The highest concentration of *Mangifera* species is reported to be in the geographical areas of Malay Peninsula (19), followed by the Sunda Islands (16) and the eastern Peninsula (14) including *M. indica* and its allied species such as *M. sylvatica*, *M. pentandra*, *M. caloneura*, *M. caesia*, *M. foetida,* and *M. odorata* (Mukherjee 1949). Wild *M. indica* that is botanically related to *M. sylvatica*, *M. caloneura*, *M. zeylanica,* and *M. pentendra* and all other *Mangifera* species so far examined have $2n = 2x = 40$ (Mukherjee 1950; Roy and Visweswariya 1951; Sharma 1987; Mustaffa 1993; Mitra 2001).

Mango culture gradually spread to different parts of the world from the Indian subcontinent (Table 4.1).

Table 4.1 Distribution of *Mangifera* spp.

Country/Island	Number of species
India	5
Sri Lanka	2
Andaman Islands	3
Burma	6
Thailand	9
Indochina	10
Peninsular Malaysia	19
China	1
Sumatra	11
Java	9
Kalimantan, Sabah, Sarawak	10
Bali	2
Philippines	8
Celebes	4
Moluccas	5
Timor	2
Irian Java, Papua New Guinea	2

It is imperative to note that selection by man from seedlings of unknown parentage has played the most significant role in the development of new mango cultivars. Mukherjee (1948) has listed all of the valid species in *Mangifera* with their geographical distribution (Table 4.2). The introduction of mango into Florida was made in 1861 (Knight 1980). Further introductions were made systematically from various countries, resulting in diverse collection within a small area. Proximity of various genotypes from different geographical regions led to the production of many new seedlings by interpollination. Hence, Florida became a secondary center of mango diversity (Knight and Schnell 1993). In Sri Lanka, there is a large diversity for *M. zeylanica*. It is locally known as "Etamba." Mukherjee (1985) has listed the species under threat, these are as given below:

1.	Endangered	*M. cochinchinensis*
		M. flava
		M. lagenifera
		M. pentandra
		M. reba
		M. superba
2.	Vulnerable	*M. duperreana*
		M. inocarpoides
		M. monandra
		M. timorensis
		M. zeylanica
3.	Rare	*M. andamanica*
		M. camptosperma
		M. gedebe

Table 4.2 Valid species in *Mangifera* and geographical distribution in Tropical Asia

Valid species	Geographical areas of distribution
M. duperreana Pierre	Cochinchina, Siam
M. pentandra Hook. f.	Burma, Malaya, Indochina
M. cochinchinensis Engl.	Cochinchina
M. lanceolata Ridl.	Malaya
M. indica Linn.	Tropics of old world
M. longipes Griff.	Burma, Malaya, Sunda Archipelago, Philippines
M. caloneura Kz.	Burma, Siam
M. siamensis Warbg ex Craib	Siam
M. oblongifolia Hook. f.	Malacca, Siam, Indochina
M. minor Bl.	New Guinea, Celebes, Solomon Island
M. zeylanica Hook. f.	Ceylon
M. caesia Jack.	Malacca, Sumatra, Java
M. sylvatica Roxb.	India, Burma, Indochina
M. khasiana Pierre	Assam
M. gracilipes Hook. f.	Malacca
M. camptosperma Pierre	Burma, Siam, Cochinchina, Sumatra
M. gedebe Miq.	Java
M. microphylla Griff. ex Hook. f.	Malaya
M. griffithii Hook. f.	Malaya
M. sclerophylla Hook. f.	Malaya
M. merillii sp. nov.	Philippines
M. beccarii Ridl.	Sarawak
M. similes Bl.	Sumatra, Java
M. altissima Blanco	Philippines
M. rumphii Pierre	Banda Island
M. philippinensis sp. nov.	Philippines
M. havilandi Ridl.	Sarawak
M. rigida Bl.	Sumatra
M. maingayi Hook. f.	Malaya
M. longipetiolata King	Malaya
M. quadrifida Jack.	Malay, Sumatra, Borneo
M. spathulaefolia Bl.	Borneo
M. timorensis Bl.	Timor, Banda, Sumatra
M. monandra Merr.	Philippines
M. andamanica King	Andaman Island
M. lagenifera Griff.	Siam, Malaya, Sumatra
M. macrocarpa Bl.	Malaya, Sunda Archipelago, Anambas Island, Indochina
M. foetida Lour.	Malaya
M. odorata Griff.	Malaya, Philippines
M. kemanga Bl.	Malaya Peninsula and Archipelago
M. caesia Jack.	Malaya, Sunda Archipelago, Philippines
M. superba Hook. f.	Malaya

Although, there is awareness among the researchers with regard to the utility of the species, hardly any effort has been made to characterize and to generate basic information regarding their crossability with the cultivated species. Screening of the species needs to be urgently carried out for pest and disease resistance. However, one of the main problems encountered in the conservation of species, has been its precise climatic requirement. It is known fact that whenever a species was introduced from its original place of growing, it has failed to acclimatize in the place of introduction. Hence, conservation of the species needs to be carried out at the place of origin or in the place that resembles its place of origin.

4.3 Domestication of *Mangifera* spp.

Mangifera has 73 genera and 1,000 named mango species. The edible fruits are produced by 27 species of the genus. Wild mangoes are potentially valuable for the breeding programs. Some species of section Euantherae and *M. pentandra* have horticultural implications as they bear several desirable characteristic features. Asia has lot of diversity of these genera, however, modern agriculture, urbanization, and unfavorable moist climate has caused loss of many species and genera. The wild mangoes are vulnerable and in danger of extinction. However, in India due to large geographical area, diverse climatic conditions and alloploid nature of its origin, a great genetic diversity still exists. The fruits of wild species including *M. andamanica*, *M. capmptosperma*, *M. griffithii,* and *M. nicobarica* are eaten by the tribes of Andaman (Fig. 4.2).

Every part of the *Mangifera* plants is beneficial and has been utilized in folk medicine. The medicinal properties and purported uses attributed are antioxidant, antiviral, antiparasitic, antiseptic, antitussive, antiasthmatic, expectorant, cardiotonic, contraceptive, aphrodisiac, hypotensive, laxative, and stomachic. Mangiferin, found in the stem bark, has potent immunomodulatory properties and is believed to inhibit tumor growth in early and late stages (Kim et al. 2009). Young and unripe fruits are utilized for culinary purposes as well as for preparing pickles, *chutneys* and *amchoor* owing to their acidic taste. Ripened fruits are utilized for preparing squash, nectar, jam, cereal flakes, custard powder, baby food,

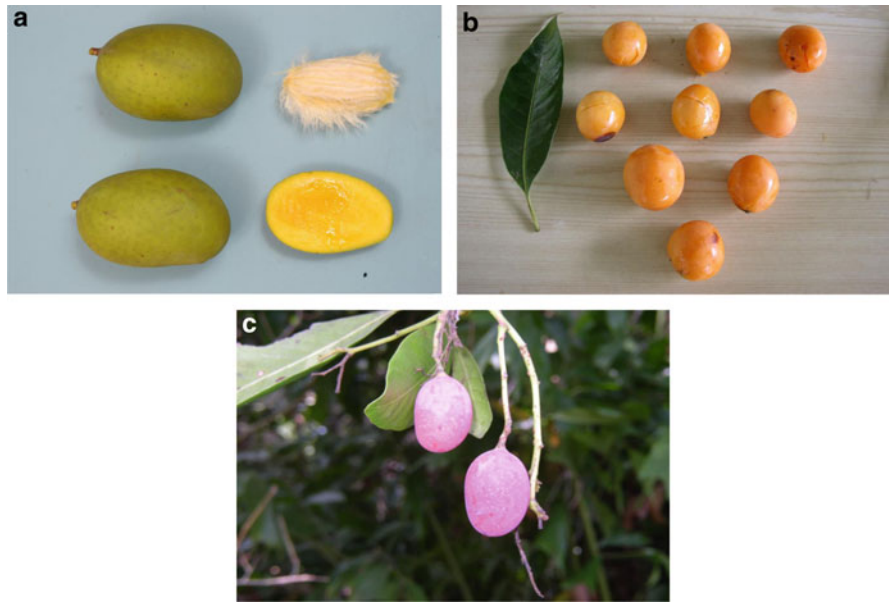

Fig. 4.2 Diversity in wild mango species. (**a**) *Mangifera odorata*, (**b**) *Mangifera andamanica* and (**c**) *Mangifera Griffith*

mango leather, and toffee. Tender leaves are used as vegetable in Java and Philippines, ash of burnt leaves used as a household remedy for burns and scalds, fumes of leaves burnt for relief from hiccups and throat infections and leaves are masticated to tone up the gums. The dried flowers are rich in bioactive tannin (15%), which is useful against diarrhea and chronic dysentery, and the bark yields mangiferine useful against diphtheria and rheumatism. Stem and trunk exude gum and the wood finds use in making furniture, flooring, packing boxes, splint, brush backs, boats, and oar blades (CSIR 1962; Purseglove 1968).

Members of the genus *Mangifera* are known for their strong aroma, intense peel coloration, delicious taste and are nutritionally rich source of vitamin A, C, β-carotene, and minerals (Singh 1960; Tharanathan et al. 2006). Also, several other bioactive constituents have been isolated, purified and identified, which includes mangiferen, mangiferic acid, mangiferol, alkylgallates, monoterpenes, amino acids, sugars, biflavones, and saponins (Khan and Khan 1992; Khan et al. 1994). The wild forms of *Mangifera* provide enormous economic potential. These plants not only sustain the recent crop improvement program but also can be utilized by future generations as new sources of genes against virulence of pathogen, pest, drought, frost, and many other unforeseen circumstances. *Mangifera koetijape* is known to have exploitation potential for horticultural traits.

The natural forest of West and Central Africa is rich in natural resources and has tremendous biodiversity (FAO 1983), particularly in trees that provide food, fuel, fiber, medicine, and various other products, including construction and building materials. *Irvingia gabonensis* Engl and *Irvingia wombolu* Vermoesen, the eating and the cooking types of bush mango, respectively, have been identified by the International Center for Research in Agroforestry as priority wild fruit tree species for domestication (Ladipo et al. 1995). They produce edible fruits and seeds (Adebayo-Tayo et al. 2006). These wild mango trees are observed in Cameroon, which are very tall and can reach 50 m high, and 2½ m in diameter. Tree bark is gray in color. *I. gabonensis* is "sweet" and the other has a bitter "skin" (*I. wombolu*). The germination rate of these two species is 80%. Local names of these are Ewondo Andok, Bangangté Bush mangolo, Bassa Ndoka, Mwiba, Boulou En'doé, ando'o, Mvae Ando, Maka Nouak, Douala njaka, Bibaya pygmies Pékié, Bayang Besay, maka pékié. *I. gabonensis* can be found in the humid forest zone of Cameroon, while *I. wombolu* is more localized in the southwest of the country. "Andok" the powder prepared from these species provides energy, macronutrients, calcium, and iron. The fruit of *I. gabonensis* weighs about 200 g, while the *I. wombolu* weighs about 85 g when harvested. Both varieties do not produce fruits during the same season. *I. gabonensis* produces fruits from

June to August, while *I. wombolu* produces during January to March.

The wild mango (*Irvingia* spp.), also called "Dika" tree, is classified in the Irvingiaceae family of plants and is a commercially and socially important fruit tree of the West and Central Africa. The tree has been identified as one of the most important fruit trees for domestication in the region, because of its relative importance to the food industry. Dika fruit is a drupe with a thin epicarp, a soft fleshy thick mesocarp, and a hard stony endocarp encasing a soft dicotyledonous kernel. The kernel, with about 62.8% lipids, 19.7% carbohydrates, 8.9% protein, 5.3% dietary fiber, and 3.2% ash has been incorporated in human nutrition for controlling dietary lipids and weight gain. Dika kernels are widely marketed locally, nationally and between countries in West Africa, especially for their food thickening properties. The economic importance of the kernel is further strengthened by its use as a pharmaceutical binder and a base material in the manufacture of soap, cosmetics, confectionary, and edible fats (Ogunsina et al. 2008).

In conclusion, the wild species of the genus, *Mangifera* can be considered as sources of supplemental food, nutritionally balanced diets, timber, fuel wood, bioactive compounds, household income, and national revenues. Moreover, many related species of *M. indica* could be important for breeding purposes, for use as rootstocks, for processing, and for consumption. Moreover, the possibilities should be explored, and such promising species need to be domesticated and commercialized as unexploited sources of revenue and also as good sources of improved nutrition.

4.4 Reproductive Mechanism

Mango, like some of the other perennial crops viz. citrus has varied reproductive mechanism, i.e., both sexual and asexual. The various botanical features of mango are discussed below.

4.4.1 Polyembryony

Depending on the mode of reproduction from seeds, wild mango species can be classified into two groups viz., monoembryonic and polyembryonic. Monoembryonic seeds contain one zygotic embryo probably of hybrid origin and only one seedling per seed is produced. Polyembryonic seeds contain more than one embryo, of which one is zygotic; the other embryos originate from the nucellus tissue, surrounding the embryo sac. The seedlings from the nucellar tissues are true to type and hence can be used where uniformity of seedlings are desired. Maheshwari and Rangaswamy (1958) suggested that the nucellar cells destined to form adventitious embryos are recognizable by their dense cytoplasm and starchy contents. They gradually push into the embryo sac cavity where they divide and differentiate into embryos. However, the incidence of polyembryony is genetically determined. Leroy (1947) considered that adventive embryonic reflects the effect of one or more recessive genes. Sturrock (1968) crossed mono- and polyembryonic varieties and found that polyembryony is recessive and is controlled by a single pair of genes.

4.4.2 Floral Biology

Inflorescence of the genus *Mangifera* is basically terminal, although axillary and multiple panicles may also arise quite frequently from axillary buds. The hermaphrodite and male flowers occur on the same panicle. The total number of flowers in a panicle may vary from 1,000 to 6,000 depending on the variety (Mukherjee 1953). Initial fruit-set depends on the percentage of perfect flowers and the final set need not depend on this (Iyer et al. 1989). When the proportion of perfect flowers drop to 1% then only it becomes critical for optimum fruit-set.

Flowers in the genus *Mangifera* open early in the morning and anthesis is completed by noon under sunny conditions. Maximum number of flowers opens between 9 and 10 a.m. The stigmatic receptivity is maximum during the first 6 h, although it continues up to 72 h after anthesis. Singh (1960) has reported that stigma becomes receptive even before anthesis. The minimum time required for pollen grains to germinate is 1.5 h (Sen et al. 1946: Singh 1954: Spencer and Kennard 1955). *Mangifera* pollens do not rapidly lose viability even after prolonged storage. Singh and Singh (1952) observed 98% viability after 11 months in storage at 7°C and 25% RH and 65.7% viability

after 24 months of storage at 0°C and 25% RH by acetocarmine stainability test.

4.4.3 Pollination

Mangifera is cross-pollinated. Pollination is carried out by insects such as housefly, honey bees, and thrips. Pollination by other agents like wind and gravity has been suggested to some extent (Popenoe 1917; Maheshwari 1934; Malik 1951). In nature more than 50% of the flowers do not receive any pollen. Dijkman and Soule (1951) suggested that self-pollination can also occur quite frequently.

4.4.4 Incompatibility

The prevalence of self-incompatibility in genus *Mangifera* was confirmed by Singh et al. (1962). However, the existence of self-sterility was suspected earlier (Dijkman and Soule 1951). The studies carried out by Mukherjee et al. (1968) and Sharma and Singh (1970) revealed the presence of self-incompatibility. Embryological studies evidenced that in spite of normal fertilization after self-pollination, degeneration of endosperm occurs 15 days after pollination (Mukherjee et al. 1968). The self-incompatibility system operating in mango is sporophytic type. Ram et al. (1976) have reported the presence of cross-incompatibility in certain mango genotypes.

4.4.5 Cytology

The chromosome number in *M. indica, M. sylvatica* Roxb, *M. caloneura* Kung, *M. zeylanica* Hools, and *M. odorata* Ariff is reported to be $2n = 40$ (Mukherjee 1950). The same chromosome number has also been reported by Roy and Visweswariya (1951). However, the polyembryonic variety *M. indica* cv. Vellaikolumban was found to have $2n = 80$ (Roy and Visweswariya 1951), although subsequent studies showed that it is a diploid (Majumder and Sharma 1990). Eleven types of chromosomes are there in *M. indica* and *M. sylvatica* and these two species differ from one another mainly in the assortment of these 11 chromosome types (Mukherjee 1950). He also suggested that primitive type or types, which gave rise to the mango varieties originated through allopolyploidy. Mukherjee (1958) reported that mango is an allopolyploid owing to high chromosome number, high number of nuclear chromosomes, secondary association of bivalents, regular pairing and absence of multivalent formation and good pollen fertility. However, it is not conclusive. Arumuganathan and Earle (1991) reported that nuclear DNA content of *M. indica* is 0.45 pg, which is equivalent to 441 Mbp.

4.5 Mango Improvement Through Breeding

Special emphasis is given to the breeding potential of several noteworthy species, which are of particular interest for horticulture and may open new possibilities for mango cultivation. A significant fraction of this rich gene pool, including wild and semi-cultivated species, is currently on the verge of disappearance. Adequate practical measures must rapidly be implemented to ensure the long-term survival of mango genetic resources, by both ex situ and in situ conservation. Representative samples of the diversity of selected species other than *M. indica* need to be established in living collections.

In order to exploit fully the potential of wild mango species, a cooperative effort involving all those concerned with mango cultivation and conservation must be made before it is too late (Bompard 1993). There are over 60 wild *Mangifera* species currently recognized in Southeast Asia, with many species locally rare and/or included in the IUCN (International Union for the Conservation of Nature and Natural Resources) Red List of Threatened Species; while some species are vulnerable (*M. pajang, M. zeylanica*), data deficient (*M. lalijiwa, M. odorata*) and extinct in the wild (*M. casturi*). These species are not well represented in gene banks either within or outside of Southeast Asia. A project has been developed recently, which aims at the identification, collection and propagation of *M. casturi, M. griffithii, M. lalijiwa, M. laurina, M. odorata, M. pentandra, M. pajang, M. zeylanica, M. foetida,* and *M. caesia* for long-term maintenance and use in a living gene bank. However, establishment of *M. caesia, M. foetida,* and *M. pajang* using current grafting techniques on *M.*

indica rootstocks has been unsuccessful. All other species have been established and are currently being molecularly characterized to find their suitability for breeding with *M. indica*. *M. casturi, M. griffithii, M. lalijiwa, M. laurina, M. odorata*; and *M. zeylanica* have shown the most horticultural potential for use with *M. indica* (Campbell 2007).

The cultivated germplasm comprises *M. indica*, *M. odourata, M. foetida, M. caesia,* and *M. pajang,* cultivated in Southeast Asia. For future genetic improvement there is a need to conserve in situ and ex situ and also needs cataloging. The gene pool of mango consisting of wild mangoes should bear regular cropping with good yield under favorable conditions. They should have tolerance to saline conditions, resistance to pests, diseases and precocious bearing (Farzana and Fanwar 2005).

4.5.1 Objectives in Mango Breeding

The objectives in mango breeding have two different sets of aims. One is from the point of view of scion breeding and another from the point of view of rootstock breeding. However, it is to be mentioned here that the breeding objectives in mango vary from region to region. This is specially so in India. With the export market increasing for attractive skin color for the hybrids skin color is one of the foremost features. This is true particularly for Bangladesh where the fruits of majority of the varieties have green color. Development of dwarf mango hybrids has also been one of the objectives in India, as there is potential to increase productivity per unit area. As no polyembryonic rootstock has shown consistent dwarfing effect on the scion and since from monoembryonic seeds true to type rootstocks cannot be raised, transfer of characters from wild mango species has become a necessity.

In other countries, such as Israel, rootstock breeding is aimed at developing rootstocks resistant/tolerant to problematic soils and also breeding varieties with better appearance and yield with longer harvest periods (Lavi et al. 1993). In Australia, the main objective has been to improve the cv. "Kensington," which bears regularly but is susceptible to anthracnose and bacterial black spot and has poor shelf-life. Hence, Floridian and Indian wild mango cultivars are used as parents. The Brazilian mango breeding aims at developing varieties with improved fruit yield and quality, regular bearing and small tree size (Pinto and Byrne 1993). Hence, the objectives in mango breeding vary with the region.

In general, mango-breeding projects can have the following main objectives:

1. Developing dwarf plant types
2. Irregular bearing
3. Better fruit quality
4. Better shipping quality and
5. Resistance to diseases and pests (Iyer 1991)

With regard to rootstock breeding, the main desirable features are:

1. Polyembryony
2. Dwarfing
3. Tolerance to adverse soil conditions (high pH, calcareous soil, etc.) and
4. Good scion-compatibility

However, combining all desirable traits in a single variety is difficult because of high heterozygosity and long juvenile period (Singh 1977). Hence, breeding objectives have to be defined for specific purposes (Sharma 1987). This can be achieved by including wild species having economic traits in the mango breeding programs.

4.5.2 Pre-selection Indices in Mango Breeding

Several breeders have reported the existence of correlation of certain economic traits with the characteristics of the plant. Pre-selection indices have been utilized to identify both wild and cultivated species having important traits for mango improvement. Mango is perennial in nature and because of its long juvenile phase evaluation of progenies takes long time and requires more area. Pre-selection indices help in overcoming these problems to a great extent. Leaf flavor was reported to be directly correlated with fruit flavor (Majumder et al. 1972; Whiley et al. 1993). The emergence of new growth flushes simultaneously with fruiting or immediately after harvest is indicative of regular bearing (Sharma et al. 1972). A higher phloem:xylem ratio was found to be associated with dwarfness (Kurian and Iyer 1992). If the ratio exceeds 1.0, trees tend to be least vigorous; if it is from 0.6 to 1.0 they have

medium vigor; trees with less than 0.6 are most vigorous. Higher phenolics in the apical bud have also been shown to be associated with dwarfing (Iyer 1991). Majumder et al. (1981) had earlier reported that lower stomata density is an indication of dwarfness. However, such as correlation could not be confirmed by other workers (Iyer 1991).

4.5.3 Interspecific Hybridization

Wild species of mango can be of great value for breeding. Iyer (1991) suggested that these species could be useful in crop improvement in two ways (1) species having edible fruits with other desirable characters and (2) species that could act as gene donors for specific traits like resistance to pests and diseases. Bompard (1993) has enumerated the potential use of wild species in breeding. *M. laurina*, which has subglabrous and laxly flowered panicles, is well adapted to areas with perpetual wet climates and this could be of some use in incorporating resistance to anthracnose. *Mangifera orophila*, a newly described species from Malaysia and *M. dongnaiensis* from Vietnam, which are restricted to mountain forests l,000–l,700 m above sea level may make growing mango in Mediterranean a distinct possibility.

Fruits of some wild species such as *M. magnifica* are completely free from fibers. *M. rufocostata* and *M. swintonioides* have off-season bearing habit. *Mangifera paiang* and *M. foetida* have good quality fruits. The variety "Wani," belonging to the species *M. caesia* from 'Bali and Borneo has a distinct taste. *M. casturi*, a newly described species occurring in South Kalimanatan, is a prolific bearer with small, black and sweet fruits. All these species have good potential in breeding (Bompard 1993; Kostermans and Bompard 1993). Angeles (1991) reported that *M. altissima* is not affected by serious pests including hoppers, tip borers and seed borers. Yadav and Dinesh (1999) also reported varied forms of *M. sylvatica*, which were polyembryonic in nature, having good fruit quality in the northeastern Indian States. Fairchild (1948) reported that, hybrids having pollinating quality can be produced by crossing five-stamened mango with the Indian mango having only one fertile stamen. Bhujanga Rao et al. (1963) reported on obtaining 32 interspecific hybrids of *M. odorata* and *M. zeylanica*.

Very little work has been done on the evaluation of wild *Mangifera* species. Hence, there is an urgent need to conserve, screen and use them in the breeding program. Mukherjee (1963) felt that different *Mangifera* species could be intercrossed easily, based on the success obtained from the crossing of *M. zeylanica* and *M. odorata*.

4.6 Genomic and Molecular Studies

Ravishankar et al. (2004) observed that the grouping of cultivars based on their embryo types indicated monoembryonic and polyembryonic types of Indian mango cultivars to have a different genetic basis. Lopez-Valenzuela et al. (1997) were able to differentiate mango cultivars by embryo type and geographical origin using random amplified polymorphic DNA (RAPD) markers. These techniques can be successfully used to identify the characteristics of other mango species so that it can be successfully incorporated in mango improvement program. Litz (2004) opines that the primary components in mango genetic engineering are the efficient somatic embryogenesis and plant recovery from elite material, induction of random mutations in embryogenic cultures and challenging for resistance to a specific selective agent and transformation with gene that mediates a horticultural trait. Mango cultivars are identified on the basis of morphological characters, which are not conclusive, as they are influenced by environmental conditions. Of late, reliable genetic markers have been developed and introduced for the identification of mango germplasm. These include isozymes, RAPDs and variable number tandem repeats (VNTRS).

There are few studies using intersimple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), and intertranslation space of ribosomal DNA (ITS) markers that attempted to determine genetic relationship among *Mangifera* species. Yonemori et al. (2002) studied 14 species of Thailand using ITS markers. Parsimony and neighbor joining (NJ) analyses revealed that the common mango was closely related to *M. laurina, M. sylvatica,* and *M. oblongifolica. M. foetida* and *M. odorata* were related to *M. indica* in both parsimonious and NJ trees, although these two species are classified into a different subgenus (subgenus *Limus*) from the

subgenus *Mangifera* to which *M. indica* belongs. ITS sequence analysis revealed several species to have nuclear additivity and polymorphism among *M. indica* cultivars. Therefore, hybrid origin of many of the *Mangifera* species was proposed.

DNA characterization through AFLP analysis has been attempted to explore the genetic relationship and diversity between and within four *Mangifera* species. A total of 35 accessions comprising eight cultivars and three landraces of *M. indica*, 11 landraces of *M. odorata* Griff., seven landraces of *M. foetida* Lour., and six landraces of *M. caesia* Jack were studied. Eight primer combinations produced a total of 518 bands, 499 (96.3%) of which were polymorphic among the 35 accessions. Clustering analysis showed that all 35 accessions were basically classified into four groups corresponding to the four *Mangifera* species. The results indicate that the genetic relationship of these four *Mangifera* species based on AFLP analysis is in good agreement with their classification by classic methods (Yamanaka et al. 2006). In another study, Weerarathne et al. (2005) used RAPD analysis for assessing the genetic diversity of *M. zeylanica*. They observed that intraspecific variation was found in the population even within the same climatic zone. Further studies have to be carried out using molecular approach to identify important economic values of the wild species for inclusion into mango breeding program.

4.7 Genomics Resources Developed

A few genomic studies have been conducted in *Mangifera*. Majority of the nucleotide sequences deposited in NCBI database are from *M. indica* (Table 4.3). Other species of the *Mangifera* are rarely investigated for any genomic sequences. A few expressed sequence tag (EST) sequences are available for these species and are part of interspecies variation studies. Nearly, 68 sequences have been deposited from Indian Institute of Horticultural Research (IIHR), Bangalore alone from both studies on ripening and internal breakdown during ripening (Vasanthaiah et al. 2006) in *M. indica*. Recently various laboratories around the world working on genetic markers in mango have developed microsatellite markers for mango. These markers have been standardized for cultivated mango. They have not been studied for cross-species amplification. At IIHR a study was carried out to amplify 36 microsatellite primers for six species. Majority of the primers amplified other species indicating a good inter species amplification. The following tables with Tables 4.4 and 4.5 showcase the nucleotide sequences deposited by various institutions. These ESTs can be successfully utilized in various breeding programs as they are source for mango crop improvement.

4.8 Strategies for Future Line of Work

The strategies for increasing mango productivity should comprise developing new varieties or commercial cultivation of hitherto uncultivated varieties after

Table 4.3 Nucleotide sequences for *Mangifera* species

Total gene sequences	320
M. indica	296
M. cochinchinensis	3
M. mangicola	3
M. sylvatica	2
M. caloneura	1
Other related taxa	15

Table 4.4 Microsatellite DNA sequences of *Mangifera* species deposited by various laboratories

MiIIHR	88	Division of Biotechnology, Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bangalore, Karnataka 560089, India
MiSHR	48	USDA-ARS, SHRS, 13601 Old Cutler Rd., Miami, FL 33158, USA
MiCIR	36	Duval M., Flhor, CIRAD, Boulevard de la, Lironde – TA50/PS4 – Montpellier, 34398, Montpellier Cedex 5, France
LMMA	16	Fruticultura Subtropical y Fitopatologia, E.E. La Mayora-Csic, E.E. La Mayora, Algarrobo-Costa, Malaga 29750, Spain
MIAC	11	Chitose Honsho, University of Miyazaki, Faculty of Agriculture; 1–1 GakuenKibanadaiNishi, Miyazaki 880-2192, Japan

Table 4.5 Nucleotide sequences available for *Mangifera* species

S. No	Accession No	Locus type	Source	Tissue type
1	FJ645928	Caffeic acid O methyl transferase (COMT)	*M. indica*	Fruit
2	FJ529207	MAP1 (MAP1) mRNA	*M. indica*	–
3	EU805508	Polygalacturonase mRNA	*M. indica*	Fruit
4	EU057145	L-4-DRM desaturase-like gene	*M. indica*	–
5	EU057144	L-5-DFW desaturase-like gene	*M. indica*	–
6	EU057143	L-4-DRM desaturase-like gene	*M. indica*	–
7	EU057142	L-4-DFM desaturase-like gene	*M. indica*	–
8	EU057141	K-4-DRM desaturase-like gene	*M. indica*	–
9	EU057140	K-4-DFM desaturase-like gene	*M. indica*	–
10	EU057139	K_5_DRW desaturase-like gene	*M. indica*	–
11	EU057138	K-5-DFW desaturase-like gene	*M. indica*	–
12	EU057137	L-3-TFC thioesterase-like gene	*M. indica*	–
13	EU057136	L-1-TRM thioesterase-like gene	*M. indica*	–
14	EU057135	L-1-TFM thioesterase-like gene	*M. indica*	–
15	EU057134	K-3-TRC thioesterase-like gene	*M. indica*	–
16	EU057133	K-3-TFC thioesterase-like gene	*M. indica*	–
17	EU057132	K-1-TRM thioesterase-like gene	*M. indica*	–
18	EU057131	K-1-TFM thioesterase-like gene	*M. indica*	–
19	AY987389	Ripening-related pectate lyase mRNA	*M. indica*	Fruit
20	DQ631803	6-phosphogluconolactonase mRNA	*M. indica*	–
21	DQ538513	Succinate dehydrogenase mRNA	*M. indica*	–
22	DQ497004	LEAFY (LFY) gene	*M. persiciforma*	–
23	DQ366708	Beta-1,3-glucanase mRNA	*M. indica*	–
24	AY300808	Auxin response factor-like protein (ARF2) mRNA	*M. indica*	–
25	AY255705	Auxin response factor-like protein (ARF1) mRNA	*M. indica*	–
26	DQ270547	Profilin 1 (Man i 3.01) mRNA	*M. indica*	–
27	DQ400579	Profilin 2 mRNA	*M. indica*	–
28	AM040280	Beta-galactosidase (pman11 gene)	*M. indica*	–
29	AM040279	Beta-galactosidase (pman19 gene)	*M. indica*	–
30	Z71276	Small GTPase mRNA	*M. indica*	pericarp
31	X75329	THMF5 mRNA for 3-ketoacyl-coA thiolase B mRNA	*M. indica*	–
32	AJ505612	Aminocyclopropane carboxylic acid synthase (acs gene) mRNA	*M. indica*	–
33	AJ505609	Endo-1,4-beta-glucanase (cel gene)	*M. indica*	–
34	AJ505583	Poly(A) binding protein (pabp gene) mRNA	*M. indica*	–
35	AJ297435	1-aminocyclopropane-1-carboxylic acid oxidase (aco1 gene) mRNA	*M. indica*	–
36	AJ890472	ITS1, 5.8S rRNA gene and ITS2	*M. indica*	Leaf
37	AJ890470	ITS1, 5.8S rRNA gene and ITS2	*M. sylvatica*	Leaf
38	AJ890469	ITS1, 5.8S rRNA gene and ITS2	*M. zeylanica*	Leaf
39	AJ890468	ITS1, 5.8S rRNA gene and ITS2	*M. camptosperma*	Leaf
40	AY651064	S-adenosylmethionine decarboxylase (SAMDC) mRNA	*M. indica*	Fruit
41	AB071689	ITS1, 5.8S rRNA gene and ITS2	*M. sylvatica*	Leaf
42	AB071688	ITS1, 5.8S rRNA gene and ITS2	*M. macrocarpa*	–
43	AB071687	ITS1, 5.8S rRNA gene and ITS2	*M. laurina*	–
44	AB071686	ITS1, 5.8S rRNA gene and ITS2	*M. gracilipes*	–
45	AB071685	ITS1, 5.8S rRNA gene and ITS2	*M. griffithii*	–
46	AB071684	ITS1, 5.8S rRNA gene and ITS2	*M. pentandra*	–
47	AB071683	ITS1, 5.8S rRNA gene and ITS2	*M. odorata*	–
48	AB071682	ITS1, 5.8S rRNA gene and ITS2	*M. oblongifolia*	–
49	AB071681	ITS1, 5.8S rRNA gene and ITS2	*M. gedebe*	–
50	AB071680	ITS1, 5.8S rRNA gene and ITS2	*M. foetida*	–
51	AB071679	ITS1, 5.8S rRNA gene and ITS2	*M. flava*	–
52	AB071678	ITS1 , 5.8S rRNA gene and ITS2	*M. caloneura*	–

(*continued*)

Table 4.5 (continued)

S. No	Accession No	Locus type	Source	Tissue type
53	AB071677	ITS1 , 5.8S rRNA gene and ITS2	*M. cochinchinensis*	–
Chloroplast genes deposited:				
1	U39269	Ribulose 1,5-bisphosphate carboxylase (rbcL) gene	*M. indica*	–
2	EF205595	Inverted repeat region	*M. indica*	–

germplasm evaluation. Some of the strategies that need to be adopted in future are listed below:

1. Surveying and use of GIS tools for developing maps on the genetic diversity in *Mangifera* species
2. Germplasm and species evaluation by morphological and molecular means
3. Systematic evaluation of germplasm and species for agronomical traits
4. Development of molecular data: SSR, EST, cDNA, and single nucleotide polymorphisms (SNPs) in mango
5. Use of embryo rescue to increase the progeny population size of the hybrids
6. Increasing the productivity/unit space by growing dwarf varieties, high-density orchards, pruning and chemical regulation of tree vigor
7. Enhancing fruit quality and composition, self life and organoleptic properties

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Chapter 5
Morus

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5.1 Introduction

Mulberry (*Morus*; Moraceae) is a tree crop being cultivated widely in Asian countries for leaf to feed the monophagous silkworm, *Bombyx mori* L. Mulberry leaf is the only natural feed available for *B. mori*; thus, mulberry is one of the vital components of sericulture industry that provides employment to a large number of people in countries including China, India, Bangladesh, Pakistan, and several other Asian countries. Furthermore, being a perennial tree crop with a crop cycle of over 50 years, mulberry offers additional benefits such as conservation of soil and water, enhancement of biodiversity by providing shelter to shade loving plants, and food to birds and small animals. Considering these economic potentials, a good amount of research has been devoted to developing mulberry varieties with better leaf yield and adaptability. China and India being the top silk-producing countries have developed a number of mulberry varieties suitable for a wide range of agroclimatic conditions (Datta 2000; Pan 2000, 2003; Tables 5.1 and 5.2). Most of these mulberry varieties were developed from a few species, such as *M. alba*, *M. atropupurea*, *M. bombycis, M. indica*, *M. latifolia*, and *M. multicaulis*, though more than 68 species have been reported from the genus *Morus* (Datta 2000). The major reasons for this lack of utilization of other species are low leaf yield and poor silkworm feeding quality due to coarseness of the leaf, low moisture content and retention in the harvested leaves, low protein content, etc. (Das 1984; Zhao et al. 2005a). Thus, the genetic resources of these wild species remained mostly unutilized. Fortunately, there is a growing awareness that new varieties developed from the limited gene pool of the cultivated species are genetically more homogenous and are, thus, more vulnerable to pathogens and adverse environmental conditions (Asins and Carbonell 1989), highlighting the importance of wild genetic resources for enriching the already depleted genetic pool of the cultivated species. Consequently, efforts have been made in several sericulturally important countries including China, India, Japan, and Korea, to explore, collect, characterize, conserve, and utilize the precious wild genetic resources of mulberry. Considering this current emphasis on exploration and utilization of the wild genetic resources of this very important tree crop, an overview of origin, distribution, cultivation practices, availability of both domestic and wild genetic resources, and their conservation strategies, along with efforts on genetic improvements through traditional breeding and modern biotechnological methods has been given in this chapter.

5.1.1 Origin and Distribution

Mulberry (*Morus*) is believed to have originated in the northern hemisphere, particularly in the Himalayan foothills, and spread to the tropics of southern hemisphere (Benavides et al. 1994; Hou 1994). While reviewing the centers of origin of crop plants, Vavilov (1951) placed *Morus* L. in China–Japan center of plant origin. Excavations of early Tertiary Moraceae fossils

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Table 5.1 High-yielding mulberry varieties developed in China

Sl.	Variety	Selection and breeding method	Year	Suitable zone
1	Siansang 305	Mutation breeding	2001	The Huanghe River valley
2	Beisang 1	Selected from local seedling mulberry	1995	The Changjiang River valley, The middle and lower reaches of the Huanghe River
3	Nongsang 8	Hybridization breeding	2000	The Changjiang River valley
4	Huangluxuan	Selection from local variety	1998	The Huanghe River valley
5	Jihu 4	Hybridization breeding	1989	Northeast zone
6	Dazhonghua	Polyploidy breeding	1996	The Changjiang River valley
7	Xinyiyuan	Mutation breeding	1995	The Changjiang River valley, The middle and lower reaches of the Huanghe River
8	Nongsang 14	Hybridization breeding	2000	The Changjiang River valley
9	Yu 237	Hybridization breeding	1989	The Changjiang River valley
10	Xuanqiu 1	Selected from local seedling mulberry	1989	Northeast zone
11	7307	Selected from local seedling mulberry	1989	The Changjiang River valley
12	Husang 32	Selection from local variety		The Changjiang River valley, The middle and lower reaches of the Huanghe River
13	Xiang 7920	Hybridization breeding	1995	The Changjiang River valley
14	Canzhuan 4	Selected from local seedling mulberry	2001	The Changjiang River valley
15	Huamingsang	Selected from local seedling mulberry	1994	Chizhou, Xuanzhou, Anqing in Anhui province and Linyi in Shandong province
16	7946	Hybridization breeding	1998	The Huanghe River valley
17	Yu 2	Hybridization breeding	1989	The Changjiang River valley
18	Shigu 11-6	Mutation breeding	1995	The Changjiang River valley, The middle and lower reaches of the Huanghe River
19	Xuan 792	Selection breeding	1989	The Huanghe River valley
20	Yu 711	Hybridization breeding	1995	The Changjiang River valley, The middle and lower reaches of the Huanghe River
21	Yu 151	Hybridization breeding	1989	The Changjiang River valley
22	Hongxin 5	Hybridization breeding	1995	The Changjiang River valley, The middle and lower reaches of the Huanghe River
23	Lunjiao 40	Selection from local variety	1989	The Zhujiang River valley
24	Wan 7707	Selected from local seedling mulberry	1994	Chizhou, Xuanzhou, Anqing in Anhui province and Linyi in Shandong province
25	Huangsang 14	Selected from local seedling mulberry	1989	The Changjiang River valley
26	Lunjiao 40	Selection from local variety	1989	The Zhujiang River valley
27	Shi 11	Selection from local variety	1989	The Zhujiang River valley
28	Xinyizhilan	Introduced variety	1995	The Changjiang River valley; The Huanghe River valley
29	Jialing 16	Polyploidy breeding	1998	The Changjiang River valley
30	Tang10×Lun 109	Hybrid mulberry seed	1989	The Zhujiang River valley
31	Nongsang 12	Hybridization breeding	2000	The Changjiang River valley

further supported this northern hemisphere origin with subsequent migration into the southern hemisphere (Collinson 1989). Most of the contemporary molecular studies also revealed an early diversification of Moraceae in Eurasia and subsequent migration into the southern hemisphere (Zerega et al. 2005). The temperate origin of mulberry is further evident from the nature of the growth as at the end of each growing season the apical meristems of the long shoots abort and are abscised. Today, the genus *Morus* is present in all regions between 50°N Lat. and 10°S Lat., and from sea level to altitudes as high as 4,000 m (Tutin 1996; Machii et al. 1999), which include Asia, Europe, North and South America, and Africa (Table 5.3; Fig. 5.1). Continental America has four species viz., *M. insignis, M. celtidifolia, M. corylifolia*, and *M. mexicana* (Table 5.3). In India, there are many species, of which *Morus alba* and *M. indica* are fully domesticated while *M. serrata* and *M. laevigata* grow wild in the Himalayas. China has 24 species but only four species viz., *M. alba*, *M. multicaulis*, *M. atropurpurea*, and *M. mizuho* are largely cultivated for sericulture

Table 5.2 High-yielding mulberry (*Morus alba*) varieties developed in India

Variety	Region	Developed at	Origin
Kanva-2	South India (irrigated)	CSRTI, Mysore	Selection from natural variability
S-36	South India (irrigated)	CSRTI, Mysore	Developed through EMS treatment of Berhampore Local
S-54	South India (irrigated)	CSRTI, Mysore	Developed through EMS treatment of Berhampore Local
Victory-1	South India (irrigated)	CSRTI, Mysore	Hybrid from S30 × C776
DD	South India (irrigated)	KSSRDI, Thalaghattapura	Clonal selection
S-13	South India (rainfed)	CSRTI, Mysore	Selection from polycross (mixed pollen) progeny
S-34	South India (rainfed)	CSRTI, Mysore	Selection from polycross (mixed pollen) progeny
MR-2	South India (rainfed)	CSRTI, Mysore	Selection from open pollinated hybrids
S-1	Eastern and NE India (irrigated)	CSRTI, Berhampore	Introduction from (Mandalaya, Myanmar)
S-799	Eastern and NE India (irrigated)	CSRTI, Berhampore	Selection from open pollinated hybrids
S-1635	Eastern and NE India (irrigated)	CSRTI, Berhampore	Triploid selection
C776	Saline soils	CSRTI, Berhampor	Hybrid from English balck and *C. multiculis*
S-146	N. India and hills of J and K (irrigated)	CSRTI, Berhampore	Selection from open pollinated hybrids
Tr-10	Hills of eastern India	CSRTI, Berhampore	Triploid developed from "S1"
BC-259	Hills of eastern India	CSRTI, Berhampore	Back crossing of hybrid of Matigare local × Kosen with Kosen twice
Goshoerami	Temperate	CSRTI, Pampore	Introduction from Japan
Chak Majra	Subtemperate	RSRS, Jammu	Selection from natural variability
China White	Temperate	CSRTI, Pampore	Clonal selection

Adopted from Datta (2000)

Table 5.3 The species distribution in different countries

Sl. No.	Country	Endemic species	Total species
1	China	17	24
2	Japan	14	19
3	S. Korea	1	6
4	India	2	4
5	Indonesia	2	3
6	Taiwan	1	4
7	Thailand	2	2
8	Argentina	1	1
9	Columbia	1	3
10	Mexico	2	3
11	Peru	1	1
12	USA	9	14
13	Bulgaria	1	6

purposes and the remaining are considered wild species. In Japan, out of the 19 species only *M. alba*, *M. bombycis*, and *M. latifoila* are mostly cultivated. In Africa, *M. mesozygia* has been reported to occur in humid, subhumid, and semi-arid areas. It grows well from sea level to an altitude up to 1,000 m (Le Houerou 1980). The vernacular names attached with some of these species also indicates either their origin or their morphological distinctiveness. For instance, *M. alba* is called "white mulberry" because of the fruit and bark color. According to Sharma et al. (2000) white mulberry is native to China but has spread into several other countries as shown in Fig. 5.2. Similarly, *M. nigra* is called "black mulberry" due to the dark red fruit it bears. Black mulberry, a native of Iran, is cultivated for its fruits in South Europe, Southwest Asia and is the most important species in the Mediterranean countries (Tutin 1996). The black mulberry (Turkish name "Kara Dut") is widely grown in Turkey for its delicious edible fruits (Yaltirik 1982). Owing to the mediterranean conditions, the northeastern part of Turkey, in particular Coruh valley, has notable populations of black mulberry. The *M. rubra* is called "red mulberry" due to stem and fruit color. Red mulberry is native to North America, and it has been cultivated in America since colonial times and its fruit is made into wine and also the fruit is considered a valuable agricultural and wildlife feed. Likewise, based on the place of origin *M. tartarica* is called as "Russian mulberry," *M. serrata* as "Himalayan mulberry," *M. mesozygia* as "African mulberry," *M. celtidifolia* as "Mexican mulberry," *M. microphylla* as "Texas mulberry" and *M. australis* as "Chinese mulberry."

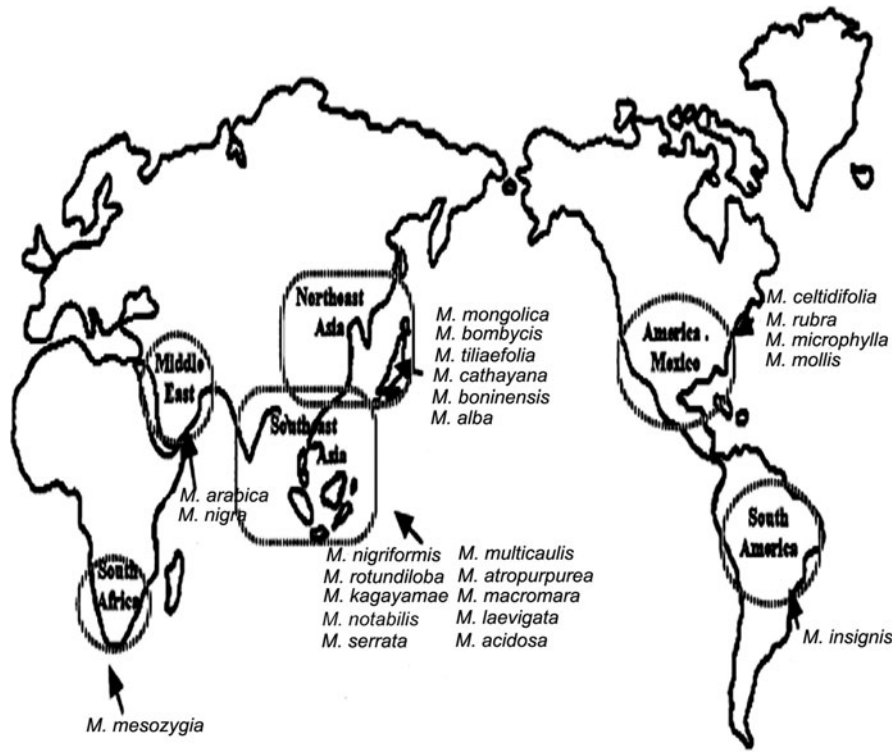

Fig. 5.1 Geographical distribution of *Morus* sp. showing their great diversity in north and South Asia (Reproduced from Sharma et al. 2000)

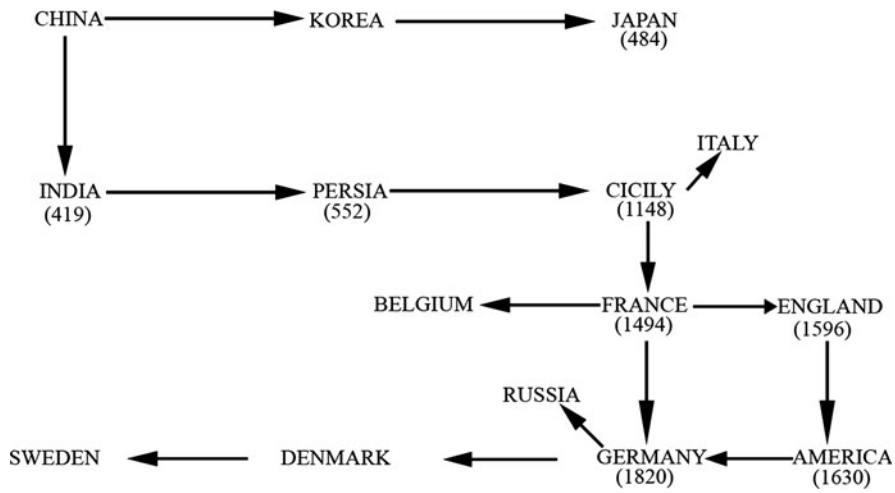

Fig. 5.2 A *line diagram* showing the domestication of the most widely grown mulberry species, *M. alba*, a native of China, to areas as far apart as India, Europe, and America (Reproduced from Sharma et al. 2000)

5.1.2 Taxonomic Position

Taxonomy of the genus *Morus* was started by Linneaus (1753) by recognizing seven species. Later, the taxonomic position of the genus and the number of species within it has undergone many modifications as the system of classification evolved from the sexual systems (eighteenth century) to the modern molecular phylogenetic system (twenty-first century). Hooker (1885), following the natural system of classification, placed the genus, *Morus* L, in the tribe Moreae of the family Moraceae under the order Urticales. Takhatajan (1980) based on phylogenetic relationships deduced from morphological characters placed *Morus* under the family Moraceae of the order Urticales, which is considered an advanced order among the woody flowering plants. Recently, the angiosperm phylogenetic group (AGP

Table 5.4 Mulberry species recognized by Koidzumi (1917)

Sl. No.	Species	Sl. No.	Species
1	*M. bombycis* Koidz.	13	*M. latifolia* Poir.
2	*M. alba* L.	14	*M. acidosa* Griff.
3	*M. indica* L.	15	*M. rotunbiloba* Koidz.
4	*M. kagayamae* Koidz.	16	*M. notabilis* C. K. Schn.
5	*M. boninensis* Koidz.	17	*M. nigriformis* Koidz.
6	*M. atropurpurea* Roxb.	18	*M. serrata* Roxb.
7	*M. laevigata* Wall.	19	*M. nigra* L.
8	*M. formosensis* Hotta	20	*M. rubra* L.
9	*M. mesozygia* Stapf.	21	*M. celtidifolia* Kunth
10	*M. cathayana* Hemsl.	22	*M. tiliaefolia* Makino
11	*M. microphylla* Bickl.	23	*M. macroura* Miq.
12	*M. rabica* Koidz.	24	*M. multicaulis* Perr.

II 2003), based on evidences from molecular phylogenies, placed the family Moraceae in the order Rosales. In the same way, the genus *Morus* have also been classified into many species based on morphological and phenological characters (Koidzumi 1917; Engler and Prantl 1924; Bounocore 1941; Leroy 1949; Ledebour 1951; Hotta 1954; Katsumata 1972). Among the taxonomists, who classified the genus *Morus*, Koidzumi (1917) and Hotta (1954) deserve special mention. Koidzumi (1917) divided the genus *Morus* into two sections, the *Dolichostylae* (long style) and the *Macromorus* (short style) and under each section two groups, viz., Papillosae and Pubescentae, were recognized based on the nature of stigmatic hairs. He classified the groups into 24 species and one subspecies, using morphological characters of leaf, inflorescence, and sorosis (Table 5.4). Although, Leroy (1949) subdivided the genus into three subgenera, viz., *Eumorus* (all species except for the neotropic species *M. insignis*), *Gomphomorus* (the neotropical species *M. insignis* and its synonym *M. trianae*), and *Afromorus* (the African species *M. mesozygia*), this division has not received wide acceptance. Later, Hotta (1954) divided *Morus* into two sections, viz., the *Dolychocystolithiae* and the *Brachycystolithiae*, according to the shape and position of cystolith cells in the leaf and he recognized 35 species. So far, more than 150 species of mulberry have been cited in the Index Kewensis, but majorities of them have been treated either as synonyms or as varieties (mostly of *M. alba*) rather than species, and some have been transferred even to allied genera. For instance, *M. tinctoria* and *M. zanthoxylon* have been transferred to the genus *Maclura* Nuttal. It is remarkable that *Morus* is the only genus of the Moraceae that has not been revised yet (Berg 2001). Nonetheless, 68 species of mulberry have now been widely recognized and majority of them are found in Asia, especially in China, Japan, Korea, and India (Datta 2000). However, it is important to note that most of these species undergo natural cross hybridization and produce fertile hybrids (Das and Krishnaswami 1965; Dwivedi et al. 1989; Tikader and Dandin 2007). This high success rate of cross-species reproduction suggests that these species have relatively closer genetic relationships and thus, their "species" status needs to be investigated further (Wang and Tanksley 1989). Another reason for this paucity of information on the general taxonomy of *Morus* is that most of the recent investigations were confined only to the sericulturally important species. Therefore, information on other species and their relationships with domesticated species remains very scanty (Zhao et al. 2006).

5.1.3 Morphological Characteristics

Morphologically, mulberry is a fast growing deciduous woody perennial tree with deep root system. Salient morphological characters of a few important species, viz., *M. alba, M. rubra*, *M. nigra*, *M. serrata*, and *M. laevigata* are given in Table 5.5.

5.1.3.1 Leaf

Wide variations in leaf morphology are observed among different species and accessions within species (Fig. 5.3). Leaves of white mulberry are simple, alternate, stipulate, petiolate, entire, or lobed. The number of lobes varies from one to five. Leaves of the red mulberry are larger, thicker, blunt toothed, and often lobed. They are rough on their upper surfaces and pubescent underneath. The smaller black mulberry leaves are similar to those of the red mulberry morphologically, but with sturdier twigs and fatter buds. The shape of the leaf may vary according to the age of the plant, growth, positions in the branches, period of growth, etc. Leaves of wild mulberry species such as *M. laevigata*, *M. serrata*, and *M. tiliaefolia* are considered too rough, leathery and thick to be used for silkworm rearing.

Table 5.5 Morphological characters of domesticated (*M. alba*) and wild (*M. laevigata*, *M. serrata*, *M. nigra*, and *M. rubra*) species of mulberry

Morphological characters	*M. alba*	*M. laevigata*	*M. serrata*	*M. nigra*	*M. rubra*
Bud color	Brown	Brown	Dark brown	Dark red	Black
Bud size (mm^2)	16.50–39.90	24.10–79.55	19.40–79.20	18.00–75.10	15.50–38.00
Branch color	Gray or grayish yellow	Gray or grayish yellow	Gray colored	Dark colored	Dark colored
Branching	Erect	Semi-erect	Semi-erect	Semi-erect	Erect
Leaf lobation	Lobed to unlobed	Lobed to unlobed	Lobed to unlobed	Lobed to unlobed	Lobed to unlobed
Leaf color	Pale green	Dark green	Dark green	Dark green	Dark green
Leaf surface	Smooth	Rough	Rough	Smooth	Rough
Leaf margin	Larger round serration	Shallow lobed	Large round serration	Large round serration	Smaller more pointed serration
Leaf length (cm)	10–15	28–32	20–25	10–15	7–10
Inflorescence length (cm)	3–4	5–12	2–5	3–5	2–4
Fruit color	White-red	White-red	Red	Dark black	Red
Petiole groove	Present	Present	Absent	Present	Present
Lenticel size (mm^2)	0.41–2.00	0.70–2.25	1.44–8.00	1.40–7.50	0.38–1.95

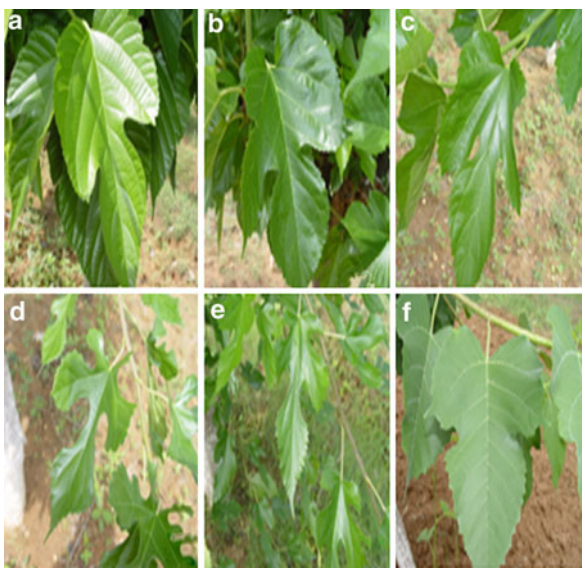

Fig. 5.3 Variations in the leaf morphology of mulberry. (**a**) *M. laevigata*, (**b**) *M. laevigata*, (**c**) *M. alba,* (**d**) *M. indica*, (**e**) *M. indica*, (**f**) *M. serrata*

5.1.3.2 Flower

Although mulberry trees are predominantly dioecious, monecious plants are not very rare. It is also reported that mulberry changes its sex depending on environmental conditions or other factors such as physical injury such as pruning (Das and Mukherjee 1986; Tikader et al. 1995). It is presumed that mulberry possesses both male- and female-determining genes, but their expression is determined by external stimuli such as climate or internal stimuli such as physiological factors (Minamizawa 1963; Tiku et al. 1988). On the basis of the observations on some cultivars, Mukherjee (1965) opined that dioecism evolved from monoecism in mulberry. Sexual reversal using hormones, chemicals, and growth regulators was also reported by several authors (Jaiswal and Kumar 1980, 1981a, b; Ogure et al. 1980a, b; Kumar et al. 1985; Das and Mukherjee 1986; Rabindran et al. 1987; Sikdar et al. 1988). Similarly, Tikader et al. (1995) reported sexual reversal in a male plant due to physical injury.

The inflorescence in mulberry is a catkin with pendent or drooping peduncle bearing unisexual flowers. Male catkins are usually longer than the female catkins. Inflorescence of *M. laevigata* is the longest among the inflorescence of mulberry species, with ca. 5–12 cm long in males and ca. 5–6 cm long in females. Male flowers are loosely arranged and after shedding the pollen, the inflorescence dries and falls off. There are four persistent perianth lobes and four stamens, filaments incurved in bud. Female inflorescence is usually short and the flowers are arranged

compactly. There are four persistent perianth lobes. The ovary is single celled and the stigma is bifid. The ovules are pendulous. Pollination is anemophilous.

5.1.3.3 Fruit

The fruit is a sorosis that is composed of a collection of individual fruits (Achens). Once the female flowers are pollinated, the white stigma turns into brownish colored and finally dries off. Subsequently, the fleshy bases of the perianth begin to swell and become completely altered in texture and color. The ripened fruit is succulent, fat, and full of juice. Because the color of the fruit varies greatly from white to black with different color shades upon ripening (Fig. 5.4), the color of the fruit is not a reliable character to identify mulberry species. White mulberries, for example, can produce white, lavender, or even black fruits depending, to certain extent, on the timing of harvest. If the harvesting of fruits is delayed, the over ripened fruits of white mulberry turn into somewhat black. Ercisli and Orhan (2007) reported that the coloring compounds tend to concentrate in the outer drupelet cells in *M. alba*, whereas in the fruits of *M. nigra* and *M. rubra*, these substances concentrate in all the cells of drupelets. White mulberry fruits are generally very sweet. Red mulberry fruits are sweet and usually deep red or almost black. Black mulberry fruits are attractive, large, and juicy, with a good balance of sweetness and tartness that makes them the best-flavored fruits in mulberry. The ripened fruits of all mulberry species are very perishable, hence, it should be handled carefully while harvesting and subsequent processing like transportation and marketing.

Fig. 5.4 Variation in the color, size, and shape of fruits in mulberry

5.1.3.4 Seed

The seed is light yellow or brown in color, oval shaped with a nearly flat surface at the micropylar region. The seed coat contains two layers, the outer hard and brittle layer called the testa and the inner thin papery and slightly brownish layer called the tegmen. Inside the seed coat, there is the kernel, which contains outer endosperm and inner embryo. Embryo consists of a primary axis (plumule and radicle) and two cotyledons. On germination, the plumule gives rise to the shoot system and the radicle to the root system. The size and weight of the seed vary from variety to variety. Generally the seeds retain viability only for few weeks under room temperature, but if stored under controlled temperature and humidity, the viability can be prolonged for 3–6 months. The optimum temperature for germination is 28–30°C. Mulberry seed contains 25–35% of yellow dying oil.

5.1.4 Cytology and Karyotypes

In nature, mulberry exists in different ploidy levels, though a great majority such as *M. alba*, *M. indica*, and *M. rotundiloba* are diploids with 28 chromosomes ($2x$, $2n = 28$). However, higher ploidies such as triploids with 42 chromosomes ($3x$, $3n = 42$; *M. bombysis*), tetraploids with 56 chromosomes ($4x$, $4n = 56$; *M. laevigata, M. cathayana*, and *M. boninensis*), hexaploids with 84 chromosomes ($6x$, $6n = 84$; *M. serrata* and *M. tiliaefolia*), and octaploids with 112 chromosomes ($8x$, $8n = 112$; *M. cathayana*) are also not very rare in mulberry (Basavaiah et al. 1989). The highest ploidy among phanerogams was reported in mulberry, viz., docosaploidy with 308 ($22x$, $22n = 308$; *M. nigra*). A haploid, *M. notabilis* with 14 chromosomes in the somatic cells, was also found in the nature (Maode et al. 1996). Although, different ploidy levels are available, due to poor leaf yield, slow growth rate, and poor feeding performance, genotypes with higher ploidy levels are not used for silkworm rearing; hence they were totally neglected and considered as wild species (Hamada 1963; Das et al. 1970; Tojyo 1985).

5.1.5 Current Agricultural Status

Diploid and triploid forms of a few species such as *M. alba*, *M. indica*, *M. bombycis*, *M. latifolia*, and *M. multicaulis* are cultivated for leaves, and *M. nigra* is cultivated for fruits. The local people sometimes use the wild mulberry species for fruits, fuels, constructing fencing, timbers, and as fodders for live stocks, etc. Fruits of wild species like *M. laevigata* are big and sweet with no harmful effect, which is used by the native tribal folks in the Himalayan regions. The potential of mulberry trees for landscaping has also been explored in Asia, Europe, and America (Tipton 1994). *M. pendula* is used for ornamental purposes. Owing to these various usages, mulberry is considered as "Kalpa Vruksha" (the wishing tree) in India.

5.2 Genetic Resources and Their Conservation

The genetic resources of crop plants comprise the genetic material of all species both wild and domesticated, which include traditional varieties, landraces, elite lines, and special varieties developed by breeders and other researchers, and their wild relatives. As these precious genetic resources are vanishing rapidly due to destruction of habitats, over exploitation for human benefits, and increased vulnerability to the drastically changing environmental conditions, appropriate measures have to be taken urgently to conserve these genetic resources for the future needs.

In mulberry, due to the high economic importance attached with the domesticated species, most of the efforts on crop improvement were confined largely to them and their triploid forms. Thus, the wild species and wild relatives of the domesticated species were totally neglected. These restricted research and farming trend, in turn, resulted in the loss of considerable genetic diversity (Tikader and Dandin 2007). Further, due to the increased urbanization and industrialization, large areas of the natural habitat of mulberry have been destroyed and many populations have been reduced below the size needed for their continued survival without management. Nonetheless, because of the recent realization that many populations of the wild mulberry species such as *M. serrate*, *M. leavigata, M. tiliaefolia*, and *M. tartarica* have a number of agronomically important traits including resistance to abiotic and biotic stresses and faster growth (Tikader and Thangavelu 2002) and their conservation and utilization would be beneficial and essential, attempts have now been initiated to conserve these genetic resources (Tikader and Dandin 2007). Likewise, red mulberry (*M. rubra*) is also considered as the most endangered tree species in Canada. Consequently, much emphasis has now been placed for its proper conservation in USA and Canada.

5.2.1 Conservation Practices

The conservation of genetic resources entails several activities ranging from establishment of protected areas to building of DNA libraries, many of which may greatly benefit from knowledge generated through diversity assessment of the plant species and the structure of ecosystems. Therefore, extensive investigations on the genetic diversity of populations in the wilderness need to be carried out through applying various methods such as molecular marker systems, before planning the conservative strategies. There are a number of conservation strategies but some of the most important and widely adopted such as (1) in situ, (2) ex situ, (3) field gene bank, (4) on-farm participatory, and (5) cryopreservation are described below.

5.2.1.1 In Situ Conservation

In situ conservation is defined as the conservation of plants in their original habitats. The planned area serves as the home land of the plant species. Thus, it should be protected from all destructive and disturbing activities. The area may be well protected by raising boundary walls or fencings to prevent grassing, cutting of trees, etc. The major advantage of in situ conservation is that it permits continued activities evolutionary forces including natural selection, mutation and population structuring, etc., thereby promoting free evolution of the species. Considering the importance of mulberry as potential resources for employment in the rural areas, the national committee on environmental planning and coordination (NCEPL) and man and biosphere (UNESCO) of India identified 14 biosphere

reserves in the country. Among them, provinces such as Uttarkhand, Nandadevi, Namdapha, Kaziranga, Manas, Nokrek, North Andaman, and Great Nicobar (Rao 2002) for in situ conservation of mulberry. Accordingly, efforts have now been made to collect information on the location of availability of mulberry genetic resources with details on the "declared protected area network of India" including biosphere reserves, national parks and botanical gardens, wild life sanctuaries, etc. (Rao 2002). In a similar effort in Canada, red mulberry has been declared as the most endangered tree species by COSEWIC, the Committee on Status of Endangered Wildlife in Canada (G5 S2). Red mulberry is also listed as "threatened" or "rare" in three northern US states. Efforts to conserve this species have received strong support from land managers and naturalists and a recovery plan has recently been developed for the species *M. rubra* in Hamilton's Royal Botanical Gardens, Ball's Falls Conservation Area, Niagara Glen, Rondeau Provincial Park, Point Pelee National Park, Fish Point Provincial Nature Reserve, Pelee Island, Middle Island, and East Sister Island.

5.2.1.2 Ex Situ Conservation

When the genetic resources are conserved outside of their original habitats, it is called ex situ conservation. Conservation of plants in botanical gardens, experimental stations, research institutes, on-farm conservation by farmers with traditional agricultural systems, nursery or home garden, or field gene banks is all come under the category of ex situ conservation. Historically, botanical gardens and arboreta play significant roles in collection and conservation of wild species (Frankel and Soule 1886). Although, conservation of seeds at low temperature is the most preferred method of germplasm conservation, clonally propagated crops like mulberry with long juvenile period and high genetic heterozygosity, conservation of the germplasm accessions through preservation of seeds is not practiced as seeds do not duplicate the true genetic constitution of the plant. Hence, mulberry genetic resources are conserved mainly through maintenance of the whole plant either in the field or preserving vegetative parts in a viable form or using both ways. Ex situ field gene banks are developed through planting of stem cuttings/saplings or by grafting the buds on appropriate rootstocks. The ideal genetic resource conservation programs generally have an active collection of germplasm that is used for evaluation of accessions for economic traits and distribution of genetic resources for breeders and other research groups, and a base collection used exclusively for the purpose of long-term preservation. In order to have more security on the genetic resources, duplicates of base collections are usually maintained in geographically different locations.

Collection, Characterization, and Evaluation

The sampling of plant materials for conservation of genetic resources can be generally categorized into four groups: wild relatives, weedy relatives, landraces, and elite lines. In order to collect appropriate samples from populations and species, extensive survey on distribution, extent of genetic diversity within and between populations, occurrence of new and rare alleles, etc. are to be investigated prior to make the decision on collection. Recently, attempts have been made to study the genetic diversity in populations of wild species and landraces through molecular markers to aid collection of samplings (Vijayan and Chatterjee 2003; Chatterjee et al. 2004; Vijayan et al. 2004a, b; Zhao et al. 2006). Furthermore, most of the mulberry species are hard to collect in the form of seeds, and therefore they have to be collected in the form of vegetative propagules (stem cuttings). Because the vegetative propagules may not remain viable for a long period, utmost care has to be taken while collecting and transporting the samples. Modern biotechnological tools like tissue culture, cryopreservation, etc. have recently been adopted to resolve this problem to a great extent (Withers 1995). Once samples are collected, they should be properly recorded with specific accession numbers with such details as name of the locations from which they are collected, prevailing environment conditions of the place, geographic features, race name, variety name, species name, and other important morphological features. Subsequently, the samples are brought to the germplasm and subjected to preliminary evaluations. Evaluation of the sample is essential to understand the genetic potentiality of mulberry accessions for their effective

conservation and utilization. Using their preliminary evaluation results and other relevant important information collected, a systematic documentation (passport data) is prepared, which is essential for the ultimate use of these resources. According to Tzenov (2002) documentation is the most critical activity to make the germplasm utilization feasible through providing the descriptive information to the breeders and other scientists. Recently, many countries have also worked out descriptors, data standard, and technical code for evaluating mulberry germplasm resources, for instance, in China and India.

Mulberry Genetic Resources in China

China, being the major silk-producing country in the world, has made extensive exploration to collect maximum extent of genetic resources from wild species, local races, and elite genotypes. Four different types of collection methods were under taken in China. (1) Collection of local indigenous varieties, i.e., most of them are selected or bred through natural or artificial selections giving emphasis to their suitability to local condition such as weather, soil, and high resistance to pest and disease. The percentage of the local varieties accounts for about 65% of total resources in China. (2) Introduced varieties, viz., mainly from Japan, the former USSR, Korea, India, Thailand, Vietnam, etc. (3) Evolved varieties, which constitutes those developed through line selection, traditional breeding, mutation breeding, and biotechnology. These varieties have a wider acceptability, and thus they occupy more than 30% of the mulberry genetic resources in China currently. (4) The wild types have less economic value but have genes of many precious characteristics such as resistance to pest, diseases, and abiotic stresses. Owing to these concerted efforts, currently more than 1,860 numbers of germplasm accessions comprising 15 species are being maintained in various provinces like Zhejiang, Jiangsu, Guangdong, Guangxi, Shandong, Sichun, Anhui, Hubei, Hunan, Hebei, Shanxi, Shuanxi, Xinjiang, etc. (Table 5.6). The national mulberry gene bank of the Sericultural Research Institute, Chinese Academy of Agricultural Sciences (CAAS), Zhenjiang, Jiangsu province, China, ranks No. 1 in the world on mulberry germplasm conservation (Pan 2000).

Mulberry Genetic Resources in Japan

Japan also undertook extensive exploration and collection of mulberry genetic resources. The National Institute of Sericultural and Entomological Science (NISES) is currently maintaining more than 1,375 germplasm accessions (Table 5.6; Machii et al. 1999). Similarly, the National Institute of Agrobiological Sciences (NIAS), which is now responsible for maintaining and conserving the mulberry genetic resources, presently has 1,502 genotypes in the gene bank (Kazutoshi et al. 2004). These genotypes are preserved on the campus field and greenhouse and are being used to dissect the morphological and agricultural traits to improve mulberry cultivars for beneficial uses. These genetic resources are classified into five groups based on their origin such as wild type, domestic type, bred type, and the source of unknown type. These genetic resources are also classified into

Table 5.6 Species wise distribution of mulberry germplasm accessions available in Japan, China, India, and Korea

Species	Japan	China	India	Korea
M. bombycis Koidz.	583	22	15	97
M. latifolia Poir.	349	750	19	128
M. alba L.	259	762	93	105
M. acidosa Griff.	44	–	–	1
M. wittorium Hand-Mazz.	–	8	–	–
M. indica L.	30	–	350	5
M. mizuho Hotta	–	17		–
M. rotundiloba Koidz.	24	4	2	–
M. kagayamae Koidz.	23	–	–	1
M. australis Poir.	–	37	2	–
M. notabilis C.K.Schn.	14	–	–	–
M. mongolica Schneider		55	–	–
M. boninensis Koidz.	11	–	–	–
M. nigriformis Koidz.	3	–	–	–
M. atropurpurea Roxb.	3	120	–	–
M. serrata Roxb.	3	–	18	–
M. laevigata wall.	3	19	32	1
M. nigra L.	2	1	2	3
M. formosensis Hotta.	2	–	–	–
M. rubra L.	1	–	1	–
M. mesozygia Stapf.	1	–	–	–
M. celtifolia Kunth.	1	–	–	–
M. cathayana Hemsl.	1	65	1	–
M. tiliaefolia Makino	1	–	1	14
M. microphylla Bickl.	1	–	–	–
M. macroura Miq.	1	–	–	–
M. multicaulis s Perr.	–	–	15	–
Morus spp. (unknown)	15	–	106	259

working collection, base collection, and active collection. Working collection is utilized for field examinations and investigations, while the base collections are preserved for long-term investigations. Genetic resources that are judged to distribute out of base collection are grouped as active collection, which are mostly domestic varieties. At present, 430 genotypes belong to the working collection and 945 genotypes including 780 genotypes of active collection are base collection.

Mulberry Genetic Resources in India

Wild relatives of genus *Morus* were reported in India from the tropical and subtropical Himalayan belts from Indus to Arunachal Pradesh. Hooker (1885) and Brandis (1906) reported four species, viz. *M. alba, M. indica, M. laevigata*, and *M. serrata* from this region. Parkinson (1923) reported wild populations of *M. laevigata* from Andaman and Nicobar islands. Kanjilal et al. (1940) discovered wild populations of *M. serrata*, *M. laevigata*, and *M. acidosa* in Assam and northeastern India. Gamble and Fischer (1957) recorded two species, viz., *M. alba* and *M. indica*. Nair (1977) documented the occurrence of *M. serrata* in the sub-Himalayan belt up to an altitude of 3,300 m. Portuguese, British, French, Dutch, and Mohammedan rulers in the process of colonization introduced many exotic genotypes into the Indian mulberry gene pool (Rao 2002). Presently, rich *Morus* biodiversity exists both under natural and managed habitats in India.

In India, currently the ex situ mulberry germplasm holding being maintained at Central Sericultural Germplasm Resources Centre (CSGRC), Hosur, Tamil Nadu, possesses more than 654 mulberry accessions (Table 5.6; Tikader and Vijayan 2010). Additionally, several small-scale germplasm collections are also available at different Central Sericultural Research and Training Institutes located in Mysore (Karnataka), Berhampore (West Bengal), and Pampore (Jammu and Kashmir).

Mulberry Genetic Resources in Bulgaria

In Bulgaria, naturally growing mulberry has been reported since the ancient times and now *M. alba*, *M. bombycis*, *M. multicaulis*, *M. kagayamae*, *M. rubra*, and *M. nigra* are growing in different parts of the country. The Sericulture Experiment Station (SES) in Vratza collects, characterizes and evaluates both indigenous and exotic mulberry varieties. Currently more than 140 mulberry accessions are maintained in the germplasm at SES-Vratza (Tzenov 2002).

Mulberry Genetic Resources in Korea

In Korea, the Department of Sericulture and Entomology has collected more than 614 accessions of mulberry comprising 205 indigenous, 150 exotic, and 259 unclassified strains (Table 5.6). These mulberry accessions are being evaluated for various morphological and agronomical characters. The exotic accessions are from nearly all countries practicing sericulture from the temperate and subtropical belt including Japan, Iran, China, Uzbekistan, Pakistan, India, Canada, Taiwan, America, Philippines, and Lebanon.

Mulberry Genetic Resources in Turkey

Domestication of mulberry in Turkey started approximately 400 years ago (Ercisli and Orhan 2007). Different regions of Turkey have their own local mulberry genotypes with different names. Many of these genotypes are growing as wild populations, and thus, efforts are now under way to conserve these precious genetic resources. As a result, germplasm collections have been set up in Adana, Tokat, and Malatya regions (Gunes and Cekic 2004; Koyuncu 2004). Currently, the Malatya Fruit Research Institute has a vast number of mulberry accessions, which were mainly collected from different parts of Turkey. Today, due to introduction and naturalization, all the three major types of mulberry, viz., *M. alba, M. nigra*, and *M. rubra* can be seen in Turkey (Gokmen 1973; Yaltirik 1982).

5.2.1.3 On-Farm Participatory Conservation

Another form of conservation is the on-farm conservation that is generally carried out with the farmers' participatory breeding (FPB). Herein, special emphasis is given for sustaining and utilizing on-farm biodiversity by the farmers (Eyzaguirre and Iwanaga 1996).

In India, rich *Morus* diversity exists under managed habitats, i.e., in the backyards, kitchen gardens, farmhouses, horticultural gardens, agricultural lands, and roadside plantations. These are the first-hand selections of the farmers and rural folks for varied utilizations. Therefore, conservation of these potentially useful genetic resources is being promoted in most of the sericulturally important countries. In mulberry, the wild species like *M. laevigata*, *M. serrata, M. tartarica, M. cathayana*, and many others do not get much attention in the formal sector for cultivation for sericulture purposes. However, these wild species have been used for other non-sericultural purposes such as horticulture and agro-forestry. Farmers and aboriginals largely use fruits and timbers of these species as a livelihood. Thus, the biodiversity of these species are conserved through the on-farm participation of aboriginals and farmers.

5.2.1.4 Cryopreservation

Conservation of genetic resources of clonally propagated species like mulberry is mostly done by maintaining the germplasm in the field. Maintenance of plants in the field is simple and technically less demanding, and also is easy to utilize the material for breeding and related studies. However, the risk of destruction by natural calamities, pests, and diseases cannot be ruled out. For this reason, safety duplicates of the living collections are established using alternate strategies of conservation, and it is in this area that biotechnology contributed significantly by providing complementary in vitro conservation options through tissue culture techniques and cryopreservation. In vitro conservation also offers other distinct advantages. For example, the material can be maintained in a pathogen-tested state, thereby facilitating safer distribution. Further, the cultures are not subjected to environmental disturbances (Withers and Engelmann 1997). For short period of conservation, in vitro techniques are adopted, but for longer durations, cryopreservation is used. Cryopreservation has been found potentially suitable for long-term preservation of clonally propagated plants such as mulberry, because it requires less space, labor, and is cost-effective. The techniques for cryopreservation that are currently in use varied greatly and include the older classical techniques based on freeze-induced dehydration of cells as well as newer techniques based on vitrification (Engelmann 2000). Classical cryopreservation techniques involve slow cooling down at a controlled rate (usually 0.1–4°C/min) down to about −40°C, followed by rapid immersion of samples in liquid nitrogen. They are generally operationally complex, as they require the use of sophisticated and expensive programmable freezers. In the new vitrification-based procedures, cell dehydration is performed prior to freezing by physical or osmotic dehydration of explants. This is followed by ultra-rapid freezing, which results in vitrification of intracellular solutes, i.e., formation of an amorphous glassy structure without occurrence of ice crystals, which are detrimental to cellular structural integrity. These techniques are less complex and do not require a programmable freezer, hence are suited for use in any laboratory with basic facilities for tissue culture. Cryopreservation involves storage of plant material at ultra-low temperatures in liquid nitrogen (−196°C). At this temperature, cell division and metabolic activities remain suspended and the material can be stored without changes for long periods. Thus, cryopreservation method ensures genetic stability of the mulberry germplasm and requires limited space, protects material from contamination, involves very little maintenance, and is considered a cost-effective method for conservation of mulberry germplasm. In fact, cryopreservation is the only available method for long-term conservation of clonally propagated plant-like mulberry. In mulberry the most appropriate material for cryopreservation was found to be winter buds, though embryonic axes, pollen, and synthetic seeds have also been used (Niino and Sakai 1992; Niino et al. 1992a, b, 1993; Niino 1995). Keeping this in view, many laboratories across the world have established cryopreservation laboratories. For instances, the cryopreservation facilities at the Central Sericultural Germplasm Resources Centre (CSGRC), Hosur, is actively involved in preservation of its 908 mulberry germplasm accessions (Rao et al. 2007). Success has been achieved in the cryopreservation of several accessions belonging to *M. indica*, *M. alba*, *M. latfolia*, *M. cathayana*, *M. laevigata*, *M. nigra*, *M. australis, M. bombycis*, *M. sinensis*, *M multicaulis*, and *M. rotundioba*. Likewise, with the epoch making research on cryopreservation by Sakai (1960), Japan has undertaken cryopreservation of mulberry in a large scale. About 450 germplasm accessions within several species have been cryopreserved in liquid

nitrogen tanks in mulberry gene bank now (Kazutoshi et al. 2004). Shoot tips of pre-frozen winter buds of *M. bombycis* Koidz. were able to withstand long storage in liquid nitrogen. The general procedure for cryopreservation of shoot tips is that the shoot segments were first pre-frozen at −3°C for 10 days, −5°C for 3 days, −10°C for 1 day, and −20°C for 1 day before immersion in liquid nitrogen. Buds were cultured on Murashige and Skoog's (MS) medium after thawing in air at 0–20°C. Survival rate was 55–90%. Prior to pre-freezing at −20°C partial dehydration of the bud up to 38.5% has found improving the recovery rates. The survival rates of the winter buds stored in liquid nitrogen up to 3–5 years did not change significantly. Encapsulation of winter hardened shoot tips of many mulberry species with calcium alginate coating was also tested successfully. In addition, Yakua and Oka (1988) conducted experiments on cryopreservation of intact vegetative buds of mulberry (*M. bombycis*) attached to shoot segments by pre-freezing and storing in liquid nitrogen. The buds were later thawed, and the meristems were excised for culture on MS medium supplemented with 1 mg I^{-1} BA to regenerate plants. Either pre-freezing at −10°C or −20°C along with rapid thawing at 37°C or pre-freezing at −20°C or −30°C along with slow thawing at 0°C was a suitable condition for high percentages of survival and shoot regeneration.

5.3 Role in Elucidation of Origin and Evolution of Allied Crop Plants

The intergeneric relationship of Moraceae was investigated recently by Zerega et al. (2005), who used both nuclear and chloroplast DNA sequences to elucidate the infra-familial relationship of Moraceae. The study revealed that *Morus* is closer to *Trophis* within the family. Other than this single study no other recent report is available on the intergeneric relationships in the family Moraceae. Some other economically important plants in the family are breadfruit (*Artocarpus altilis*), *Maclura pomifera* (Osage orange), jackfruit (*A. heterophyllus*), fig (*Ficus* spp.), che (*Cudrania tricuspidata*), African breadfruit (*Treculia rabica*), and paper mulberry *(Broussonetia papyrifera)*. Regarding sericultural use, it was reported that leaves of *Cudrania triloba* could be partly substituted for mulberry leaves, though the larval period is prolonged and cocoons become much smaller. Rearing of silkworms on *Cudrania javanensis* (*Vanieria cochinehinensis* Lour. var. *gerontogea*) leaf was also reported. However, the cocoons were found much smaller than those obtained with *Cudrania triloba* leaves. *Maclura pomifera* (Osage orange) is another alternative host for silkworms. Recently, an artificial diet containing dried leaf powder of *Maclura pomifera* was shown to be almost as good as a diet including mulberry leaf powder (Yusa 1975). Osage orange is a hardy tree native to the South-Central United States. The plants are cultivated widely, often as a hedge plant because of its spiny, impenetrable branches, and it is a source of a flexible as also durable wood and of a yellow–orange dye, from the root bark, that is similar to the more widely used fustic (*Maclura tinctoria*). The heartwood of fustic yields a yellowish or olive dye, also called fustic, which has been used chiefly for dyeing woolens, and it has largely been replaced by synthetic aniline dyes. In its native habitat of Central and South America, the fustic is also a timber tree. Fiber plants of the mulberry family include the paper mulberry (*Broussonetia papyrifera*) and the upas tree (*Antiaris toxicara*) of the East Asian tropics, where the bast fiber is utilized for rough fabrics and for paper, often after a crude retting process.

5.4 Classical and Molecular Genetic Studies

Although genetic studies in heterozygous perennial tree like mulberry with long juvenile period is not an easy task, considering the great economic importance of mulberry, efforts have recently been started with both classical and modern biological tools to understand the genetics and cytogenetics of mulberry.

5.4.1 Classical Genetic Investigations

The dioecious nature coupled with long juvenile period and high heterozygosity acts as the great impediment for developing inbred lines in mulberry. Therefore, the genetic basis of most of the agronomically important traits remains almost obscure. Barring a few incidents of attempts to understand the crossing

ability of a few elite lines of the domesticated species with their wild counter parts (Dandin et al. 1987; Dwivedi et al. 1989; Tikader and Dandin 2001), not much progress has been made in understanding the breeding behavior of mulberry. These interspecific hybridizations among *M. alba*, *M. australis*, *M. cathayana*, *M. latifolia*, *M. nigra*, and *M. sinensis* revealed varied results as in some crosses fertility was very high, whereas in some others it was very poor. For instance, seed fertility in crosses between *M. alba* × *M. indica*, *M. alba* × *M. cathayana* was high, whereas the same among domesticated species like *M. alba* and wild species like *M. laevigata* and *M. serrata* was poor (Das and Krishnaswami 1965; Katagiri et al. 1982). However, recently, Tikader and Dandin (2007) from an extensive pre-breeding programs using the domesticated species *M. indica* (*M. alba*) and wild species *M. laevigata* and *M. serrata* under ideal conditions found 95% and 90% seed settings in crosses between *M. indica* × *M. laevigata* and *M. indica* × *M. serrata*, respectively. The F_1 hybrids expressed heterosis for single leaf weight, leaf area, leaf yield, and rooting of the stem cuttings. The leaf texture and morphology of the F_1 hybrids were intermediate between that of the parents and the leaf was found soft and palatable to the silkworm, *B. mori* L. Tikader and Dandin (2008) opined that introgression of useful traits from wild species to the domesticated ones is quite possible even in the F_1 generation. In short, much has to be done in mulberry to understand the genetic basis of most of the agronomic traits using its wild allied species.

5.4.2 Molecular Markers for Characterization of Genetic Resources

In the past two decades, research on mulberry has enormously benefited from molecular markers. Significant progress has been made in molecular characterization of mulberry genetic resources. Molecular markers have also been used for elucidating the relationship between domesticated and wild species. Application of DNA fingerprinting in mulberry was initiated in Japan by Katagiri et al. (1984) and Machii (1989). Random amplified polymorphic DNA (RAPD) was the first DNA-based marker used in mulberry. Using RAPD the genetic diversity among a few cultivars was initially tested (Xiang et al. 1995; Lichuan et al. 1996; Lou et al. 1998; Zhang et al. 1998). Later, Bhattacharya and Ranade (2001), Chatterjee et al. (2004), Srivastava et al. (2004), and Zhao and Pan (2004) used RAPD markers for assessing the inter- and the intraspecific relationships in mulberry. Considering the inconsistency in the result from RAPD markers, even in the same laboratory, attempts were made to explore other DNA markers in mulberry. Consequently, Vijayan and Chatterjee (2003) tested the suitability of intersimple sequence repeat (ISSR) markers in mulberry and found that (AG), (TG), (AC), (ACC), (ATG), (AGC), (GAA), (GATA), (CCCT), (GGAGA), and (GGGGT) produced excellent results. Subsequently, ISSR markers were used to estimate the genetic diversity among indigenous cultivars of *M. alba* and *M. indica* (Awasthi et al. 2004; Vijayan et al. 2004a, c, 2005, 2006a, b) and among different ecotypes present in China (Zhao et al. 2006). Relationships between temperate and tropical mulberry species (Vijayan 2004) and interspecific variability (Vijayan et al. 2004c) were also investigated in detail using ISSR and RAPD markers. Sharma et al. (2000) used amplified fragment length polymorphism (AFLP) markers for the first time to elucidate the interrelationships of different *Morus* species. In the mean time, efforts were made to develop microsatellite primers for mulberry. Accordingly, Agarwal and Udaykumar (2004) and Zhao et al. (2005a) developed several simple sequence repeat (SSR) primers for mulberry. Using these SSR primers, Zhao et al. (2005a) were able to discriminate the wild genetic resources from the domesticated ones. Wild species such as *M. laevigata*, *M. cathayana*, *M. nigra*, *M. mongolica*, and *M. wittiorum* were grouped together into a cluster separated from the clusters formed by the domesticated species *M. alba* and its close relatives.

In order to conserve the wild mulberry genetic resources efficiently and economically, Vijayan et al. (2004b) evaluated genetic diversity present in different populations of the wild mulberry *M. serrata* present in different parts of northern India. They found significant genetic diversity among geographically isolated populations. On the basis of the results, they identified populations to be conserved. In a similar study, Chatterjee et al. (2004) investigated the intraspecific diversity among populations of *M. laevigata* collected from different locations in India. This study revealed stronger genetic relationships between *M. laevigata*

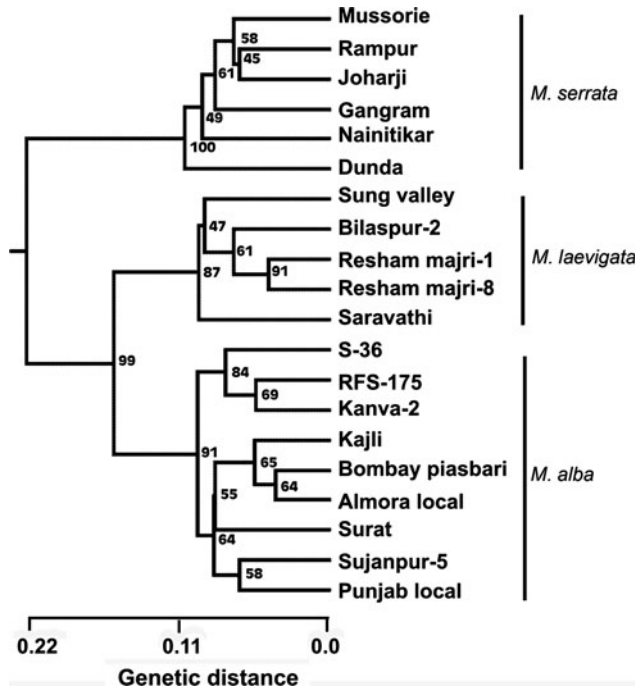

Fig. 5.5 Genetic relationships among three species *M. alba* (domesticated), *M. laevigata* (wild), and *M. serrata* (wild) as revealed by ISSR markers

populations in Andaman Islands with those in Himalayan foot hills of West Bengal, India. Additionally, the genetic relationships between cultivated and wild mulberry species were also investigated using molecular markers (Vijayan et al. 2006b), and it was found that *M. laevigata* is closer to *M. indica* and *M. alba* than to *M. serrata*. In a related investigation, the internal transcribed spacers (ITS) of nuclear ribosomal RNA (nr*ITS*) and the cpDNA gene (*trnL-F*) were used to elucidate the phylogenetic relationships among different species of *Morus* (Zhao et al. 2005b). This study, further, confirmed the closer relationship of *M. laevigata* with *M. alba*. These studies have generated significant information essential for undertaking breeding programs to broaden the genetic base of the domesticated mulberry species (Fig. 5.5).

5.5 Crop Improvement Through Traditional and Advanced Tools

During the past two decades, substantial progress has been made in applying both traditional and modern biotechnology in mulberry genetic improvement. Applications of biotechnology have assisted mulberry breeding programs in several ways from micropropagation of hard to root species to developing transgenic mulberry with enhanced tolerance to abiotic stresses.

In vitro culturing of axillary buds and shoot tips was used for screening and selecting salt-tolerant genotypes from germplasm accessions. This procedure has been found effective in early identification of salt-tolerant hybrids and genotypes. Hossain et al. (1991) and Vijayan et al. (2003) used this technique to isolate five salt-tolerant genotypes from 63 germplasm accessions, which included both domesticated and wild species. Among the wild genotypes that showed comparatively higher salt tolerance include *M. rotundiloba* and *M. nigra*; these genotypes could sustain growth up to 14.1 dS m^{-1} salinity level. Further testing of these selected genotypes under ex vitro conditions confirmed not only the efficacy of in vitro screening and selection but also the salt tolerance of these selected species. Seed germination under in vitro saline condition was also tested as a criterion for identification of salt-tolerant maternal parent with promising results (Vijayan et al. 2004d). Using these techniques, screening of germplasm accessions is underway in many research institutes in India to identify potential parents from both domesticated and wild species. In this context it is important to note that recently Tikader and Kamble (2008) reported

occurrence of diploid forms of *M. laevigata* in saline regions in Andaman Nicobar Island, India. Introgressive breeding with these species can increase salinity tolerance level of the domesticated species.

5.6 Domestication and Commercialization

Domestication of mulberry for sericultural purposes is believed to have taken place in China sometime before 2200 BC (FAO 1990). Later, when the silkworm rearing has spread to other countries, domestication of mulberry has also been expanded to other countries and currently mulberry is grown in several Asian countries. However, still only a small fraction of the *Morus* species is used for commercial purposes and great majority of the species are still growing in the wilderness.

5.6.1 Commercial Importance of Leaves

As mentioned, varieties of only few mulberry species like *M. alba*, *M. bombycis*, *M. indica*, and *M. latifolia* are used for silkworm rearing because of the hairy, coarseness, and thickness of the leaves of the wild species, which render them unattractive to the silkworm *B. mori*.

5.6.2 Commercial Importance of Fruits

Mulberry fruits are rich in anthocyanins, and hence hold great potential for using for health benefits and as natural food colorants. Anthocyanins are water-soluble and yield attractive colors such as orange, red, purple, black, and blue. Anthocyanins also possess antioxidant properties and are being investigated for antineoplastic, radiation-protective, vasotonic, vasoprotective, anti-inflammatory, chemopreventive, and hepato-protective properties. Recently, new techniques have been developed to isolate anthocyanins from mulberry fruit juice. It is estimated that from 1-l mulberry fruit juice 148–2,725 mg of anthocyanins can be obtained. Mulberry fruits are also used for making spirit beverage like "Mouro," which is distilled from fermented fruits of *M. nigra* (Soufleros et al. 2004). Therefore, *M. nigra*, a wild species as far as sericulture is concerned, is being cultivated extensively for fruits in Turkey (Gokmen 1973; Yaltirik 1982). Mulberry fruits have also been used medicinally as a worming agent, as a remedy for dysentery, and as a laxative, hypoglycaemic, expectorant, anthelmintic, odontalgic, and emetic (Baytop 1996). Recently, it has gained an important position in the local soft drink market, although its biological and pharmacological effects are still poorly defined (Bae and Suh 2007). Fruits of *M. laevigata* are very long and sweet and are suitable for all the above-mentioned purposes.

5.6.3 Commercial Importance of Wood

Mulberry wood has been used for manufacturing sports article and turnery items, as the wood peels well on a rotary lathe and does not require antiseptic treatment. It is compared with teak as regards shock resistance ability, strength, hardiness, etc. The hard wood from wild species like *M. laevigata* and *M. serrata* is being used for manufacturing tennis racket, and cricket bats for fine grain and polishing. Mulberry wood is also suitable for making house buildings, agricultural implements, furniture, spokes, poles, shafts, and bent parts of carriage and carts. The wood is also found suitable for low-grade plywood and for paneling, carving and turnery, tea boxes, and toys. The wood of *M. laevigata* is reported as termite resistant and used as pole in house building in Andaman and Nicobar islands.

5.6.4 Medicinal Values of Leaves and Fruits

As mentioned earlier, mulberry fruit juice has many medicinal properties such as antiphlogistic, antivinous, astringent, bactericide, diaphoretic, ditiretic, emollient, escharotic, expectorant, fungicide, laxative, nervine, purgative, refrigerant, restorative, sedative, tonic, and vermifuge (Duke and Wain 1981). Additionally, barks

of roots and stem have purgative, anthelmintic, and astringent properties. Leaves of mulberry are considered disphoretic and emollient. A decoction of mulberry leaf can also be used as a medicine for inflammation of throat (Reed 1976). Mulberry leaves have long been used in Chinese medicine for the prevention and treatment of diabetes; they contain some compound, which suppress high blood sugar levels. Scientists in Japan have pinpointed a number of biologically active compounds in extracts of the leaves of the white mulberry. They serve as antioxidants. The root bark contains calcium malate; the bark of the branches contains tannins, phlobaphens, a sugar and phytosterol, ceryl alcohol, fatty acids, and phosphoric acid. An alkaloid, deoxyjirimycin (DNJ), has been extracted from the root bark of the wild mulberry *M. nigra* that interferes with the synthesis of sugar chains and is supposed to be the magical drug for diabetes.

5.6.5 Miscellaneous Usage of Mulberry

The fast growth of wild mulberry species like *M. laevigata* makes it suitable for paper industry as well. Mulberry stem and stem powder are good media for mushroom production. Incorporation of shade-dried mulberry leaves in poultry feed showed an increase in egg production with desirable yolk color without any adverse effect on body weight and egg quality (Narayana and Setty 1977). Another characteristic of the mulberry stem is the resilience, flexibility, and fast growth, which makes it a good raw material for the preparation of baskets. In Sujanpur, Pathankote, and Dhar villages of Punjab, Haridwar in Uttaranchal, and Jammu and Kashmir, mulberry twigs are used for making baskets and several other agricultural implements. The farmers usually plant mulberry in their backyard, roadside, bund areas, and other fallow lands to get sufficient twigs of mulberry and derive additional income as well as part time job for family members. The basket is used to carry various household purposes, transportation of manures, vegetables, cereals, etc.

5.7 Invasive and Weedy Natures

Although mulberry has great economic potential, it is not devoid of any deleterious effects on the ecology as the fast growing white mulberry (*M. alba*). When introduced into non-native areas it may quickly disrupt the native habitat by becoming a highly invasive species and upset the natural ecosystem. This has proven true in many places, including parts of Latin America, the United States, and South Asia. A conspicuous example of such invasiveness was observed in the vicinity of the city of Islamabad where it was introduced for its scenic value, but is now replacing the native flora at an alarming rate. White mulberry was introduced to the United States during colonial times, in an effort to establish a silkworm industry, it now occurs throughout the country with the exception of Arizona and Nevada. The ecological threats posed by white mulberry include its hybridization with and replacement of the native wild red mulberry (*M. rubra*) and its ability to invade natural areas including fields, forest edges, and roadsides (Ambrose and Kirk 2004).

5.8 Future Prospects

Considering the great potential of mulberry sericulture in the economic developments of rural areas in the Asian countries, it is expected that in the coming years considerable amount of research will be carried out for understanding the genetic architecture of mulberry and also to widen the genetic diversity of the present day cultivated species by incorporating genes from wild relatives. In the light of the rapid genetic erosion of genetic diversity of the cultivating species and the great disturbance occurring throughout the wild habitats of mulberry, it is important to conserve the precious wild genetic resources urgently. The economic boom that is sweeping across the Asian countries has already destroyed a great chunk of the natural habitats of wild mulberry species. Thus, measures are to be taken urgently to conserve the precious genetic resources of this valuable plant species to make it available for the future generations to use. It is also equally important to encourage exchange of genetic materials among nations as breeding of improved varieties largely dependent on the access to suitable parental germplasm. Since wild mulberry species have a number of desirable traits including resistance to disease and pests, abiotic stresses like drought and salinity, exchange of genetic materials

among countries has to be undertaken based on the international treaty on plant genetic resources for food and agriculture (ITPGRFA) to facilitate access to the genetic resources in harmony with the Convention on Biodiversity (CBD) through an efficient mutually agreeable multilateral system of benefit sharing. Strategies for undertaking joint exploration of wild mulberry germplasm resources by major sericultural countries including China, India, Japan, etc. under the aegis of International Sericulture Commission or FAO need to be formulated. International cooperation on fund raising and monitoring of such attempts must be fostered by FAO, UNDP, and WWF for effective conservation and management of the wild mulberry genetic resources. Further, mulberry breeders have to be exceptionally careful to ensure that they have the right to use germplasm in their breeding programs and also to ensure that they use the material legally.

A taxonomical revision of genus *Morus* at global level is urgently required to remove the current confusion on the identity of species and genotypes. Supplementary data from molecular phylogeny with both nuclear and cpDNA sequences will be of much use for delineating the inter and intraspecific relationships.

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Chapter 6
Musa

Rodomiro Ortiz

6.1 Basic Botany of *Musa*: A Perennial Tropical Herb

Although native to the tropics of the Asia and Oceania, banana and plantain are now found throughout the tropics and subtropics. As perennial crops banana and plantain grow quickly and can be harvested all year round in the tropics. Plantain and banana are important food crops in the humid, lowland tropics worldwide, and in mid-altitude agroecologies.

6.1.1 Distribution

In 2006, bananas were cultivated in 4.2 million ha, whereas 5.5 million ha of plantains were grown (FAO 2007). The global average yields in 2006 were 17 tons ha^{-1} for bananas and 6 tons ha^{-1} for plantains. The world production in 2006 was estimated at 71 million tons for bananas and 34 million tons for plantains. India, Brazil, China, Philippines, and Ecuador are the top five dessert banana producers (Office of the Gene Technology Regulator 2007). However, such outputs are an approximation because the bulk of world banana and plantains production (about 85%) comes from relatively small plots and kitchen or backyard gardens, where statistics are lacking (FAO 2003). World consumption stands on average in excess of 15 kg per caput: 13 kg for the developed world and 21 kg for the developing world that almost produced domestically the entire crop. The main exporters are Ecuador, Philippines, Costa Rica, Colombia, and Guatemala, whereas the European Union, USA, Japan, China, and Canada are among the main importers of this fruit crop.

6.1.2 Geographical Locations of Genetic Diversity

Wild relatives of plantains and bananas are found primarily in the tropicals, namely from India to Polynesia. Their center of diversity seems to be either Malaysia or Indonesia (Daniells et al. 2001). Unfortunately, there is little information about the precise geographic dispersal of each *Musa* section (Pollefeys et al. 2004). Champion (1967) gave the first limits for each *Musa* section. This work was further updated by Hotta (1967) who added Australimusa in Borneo and Liu et al. (2002) by including Callimusa in China.

Plantain and bananas evolved by natural hybridization between the two species *M. acuminata* (contributing genome A) and *M. balbisiana* (contributing genome B). *Musa* species originated in Southeast Asia, but a great diversity exists in sub-Saharan Africa where each of the different types is grown in distinct agro-eco-zones. Plantains are popular in the humid lowlands of West and Central Africa, while cooking and beer bananas as well as recent introductions of Asian cooking bananas are cultivated in the East and Central African mid-altitudes (Vuylsteke et al. 1993a). West and Central Africa are the secondary center of plantain diversification, whereas East and Central Africa are considered a secondary center of diversity for bananas of the *Musa* AAA group. These secondary centers of diversification result from accumulation of somatic mutations and human selection during the long history of the crop's cultivation.

In Memorian: Dirk R. Vuylsteke (1958–2000).

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6.1.3 Morphology

Plantain and banana (*Musa* spp.) are giant perennial herbs that produce succeeding generations of ratoon crops (Swennen and Ortiz 1997). The first cycle after planting is called the plant crop. The ratoon is the sucker succeeding the harvested plant. The second cycle is called the first ratoon crop. The third cycle is the second ratoon crop, and so on. The growth cycle of *Musa* consists of two phases: vegetative and reproductive. The vegetative phase (or "shooting") begins with the production of leaves by the planted sucker and ends when the inflorescence appears at the top of the plant. The reproductive phase begins with the transition of the vegetative meristem into a floral shoot. The division of phases is arbitrary, and it takes several weeks before the inflorescence emerges at the top of the plant. The fruit filling period, or the time between flowering and harvest, completes the reproductive phase and the growth cycle.

During the growth cycle, plants develop essentially three major components: an underground corm producing suckers and roots, a pseudostem consisting of encircling leaf sheaths and carrying the leaves, and an inflorescence containing female flowers that develop into fruits. Swennen and Ortiz (1997) provide further details on the morphology and growth of *Musa*, while Fortescue and Turner (2005) give more information on the growth and development of ovules of banana, plantain, and enset (*Enset* spp.), and Graven et al. (1996) report on the structure and macromolecular composition of the seed coat of the Musaceae, which show unique components of aromatic phenols.

6.1.4 Cytology and Karyotype

All plantains and almost all important bananas are triploid ($2n = 3x = 33$ chromosomes), but some diploid ($2n = 2x = 22$) banana cultivars are also grown by farmers, especially in Asia and the Pacific. *Musa* chromosomes are relatively small (Ortiz 2000), and flow cytometry data suggest that each will be about 50 Mbp of DNA. Mitotic metaphase chromosomes of both "Calcutta 4" (*M. accuminata*) and "Butohan 2" (*M. balbisiana*) ranged from 1.4 to 3.6 μ m in length (Osuji et al. 2006). There are ten metacentric, six submetacentric, four subacrocentric, and two acrocentric chromosomes, the two largest submetacentric chromosomes having secondary constrictions. Each chromosome set shows a major 45S rDNA site (Osuji et al. 1998), whereas 5S rDNA sites vary between two and six (Doleželova et al. 1998; Osuji et al. 1998; Bartoš et al. 2005). D'Hont et al. (2000) indicated that rDNA sites are often associated with satellites, which can be separated from the chromosomes, thereby being a potential error source for chromosome countings.

Osuji et al. (1997a) showed that the repetitive DNA components of the two genomes in $3x$ plantains (AAB) were substantially different since the two or three chromosome sets in the hybrids were labeled differentially. Their results suggested that there were large arrays of repetitive sequence at the centromeres of the chromosomes, since these regions were both stained brightly as chromocenters using the DNA stain DAPI. Furthermore, the major genomic repeats represented in the labeled genomic DNA were from the centromeres as shown by in situ hybridization.

The occurrence of $2n$ gametes (both $2n$ eggs and $2n$ pollen) in *Musa* suggests that the unilateral polyploidization ($2n \times n$) can account for $3x$ cultivars (Ortiz 1997a). Further allele introgression from $2x$ to polyploids can occur through unilateral or bilateral polyploidization ($2n \times 2n$) (Ortiz 1997b).

6.1.5 Genome Size

Flow cytometry on cell nuclei of wild bananas was useful for determining the size of nuclear genome of *Musa*, which appears to be smaller than many other angiosperms (Doležel et al. 1994). The nuclear genome was relatively small, e.g., 1C ~610 Mbp for *M. acuminata* (Kamaté et al. 2000; Hřibová et al. 2007). The nuclear DNA content of *M. balbisiana* was significantly lower (537 Mbp as average genome size) than that of *M. acuminata* subspecies and cultivars (Lysák et al 1999; Kamaté et al. 2000). The genome of diploid *Musa* is thus threefold that of *Arabidopsis thaliana*. Larger variation in genome size (8.8%) was found among $3x$ *Musa* cultivars (ranging from 559 to 613 Mbp). The genome sizes in $3x$ cultivars may vary with 2C DNA values ranging from 1.61 to 2.23 pg. Likewise, the genome sizes (2C) of

4*x* cultivars range from 1.94 to 2.37 pg. The genomic base composition of main *Musa* taxa had a median value of 40.8 ± 0.4% GC (Kamaté et al. 2000). In excess of 50% of the banana genome may include repetitive and non-coding DNA sequences.

6.1.6 Taxonomic Position

Banana and plantain are monocotyledonous plants, belonging to the section Eumusa within the genus *Musa* of the family Musaceae in the order Scitamineae (Zingiberales), one of four sister orders in the monophyletic grouping Commelinids, along with the Poales (grasses), Commelinales, and Aracales. *Musa* can therefore benefit from comparative genetics, since it is a sister group to the well-researched grasses, e.g., rice.

Paterson et al. (2004) showed that rice duplication is more recent than its divergence from *Musa* but more ancient than its divergence from *Sorghum* and *Hordeum*. They dated on about 142 million years ago (Mya) the divergence time between *Musa* and rice following the KS statistics of 1.73 of *Musa* in comparison to rice genes. However, the comparison of the genomic sequence of two *Musa* species with orthologous regions in the rice genome showed microsynteny regions that have persisted since the divergence of the Commelinid orders Poales and Zingiberales at least 117 million years ago (Mya) (Lescot et al. 2008). This research was also able to verify the previously hypothesized large-scale duplication event in the common ancestor of major cereal lineages within the Poaceae. Likewise, their study of time distributions for *Musa–Zingiber* (Zingiberaceae and Zingiberales) orthologues and paralogues provided strong evidence for a large-scale duplication event in the *Musa* lineage after its divergence from the Zingiberaceae approximately 61 Mya. Moreover, comparisons of genomic regions from *M. acuminata* and *M. balbisiana* indicated a highly conserved genome structure and revealed that these genomes diverged circa 4.6 Mya.

The Musaceae family consists of large perennial herbs with pseudostems composed of leaf sheaths. The leaves are spirally arranged with a large lamina and strong midrib. The diploid banana species are *M. acuminata* and *M. balbisiana*. The triploid types are AAA dessert and highland beer and cooking bananas, AAB plantains and dessert bananas, and ABB cooking bananas. In the East African highlands, beer and cooking bananas are the staple food and the region records the highest consumption figures in the world. Plantain and banana are high-yielding crops and particularly suited to the lowland, humid, farming systems in the tropics. Several criteria are used to distinguish the different types of plantain and banana further (Swennen et al. 1995). They include form of consumption, inflorescence type, height of pseudostem, and genome composition.

6.2 Conservation Initiatives on *Musa* Genetic Resources

A worldwide network of *Musa* field genebanks in producing countries conserves and makes available most of the *Musa* gene pool. This informal network plans to follow a strategic and rationalized approach for preserving *Musa* genetic resources, through sharing roles and a common responsibility for conservation, making a wider range of germplasm available to more users (IPGRI 2006). As a means for promoting the use of collections and diversity they should provide quality information on their genetic resources holdings. A Taxonomic Advisory Group for *Musa* (2006) was recently launched as an open discussion forum to help addressing issues and constraints concerning *Musa* taxonomy and global conservation.

6.2.1 Evaluation of Genetic Erosion

Edibility of mature *Musa* fruits was the result of female sterility and parthenocarpy, and such edible types were, without doubt, selected and maintained by humans, initially in Southeast Asia and the Pacific islands (Simmonds 1962). The B genome could contribute to the increased fertility in some 3*x* cultivars by correctly positioning the embryo sacs (Fortescue and Turner 2005). In most of Southeast Asia the edible, seedless types (mostly 3*x* cultivars), which are vigorous and have large fruits, replaced the original 2*x* ancestors (Daniells et al. 2001). In Papua New Guinea, 2*x* cultivars are, however, agriculturally significant and a wide range of diversity remain in farmers' fields.

Colonization–extinction processes also influenced the evolution of wild *Musa* populations, which show a meta-population structure and are connected by migrating individuals. For example, Ge et al. (2005) showed that gene flow via pollen in wild *M. balbisiana* (using microsatellites) was 3.65 times greater than gene flow via seed in China, which was estimated from chloroplast-DNA polymorphism. Pollination of *Musa* is mainly by honeybees and bats (Start and Marshall 1976). In China, due to human overexploitation of broadleaf forests, long-tongued fruit bats have, however, decreased dramatically and only honeybees act as the active pollinators for wild banana (Liu et al. 2004). Ortiz and Crouch (1997) have shown how effective are natural pollinators vis-à-vis artificial hand-pollination in *Musa*.

6.2.2 Musa *Germplasm Information Systems*

The *Musa* Germplasm Information System (MGIS) was established as a system for the exchange of germplasm data. MGIS is a database containing detailed and standardized information on the accessions (including wild species) stored in different *Musa* genebanks around the world (http://mgis.grinfo.net/). Analysis of data in MGIS can allow duplicate or unique materials in ex situ conservation to be identified and can help to highlight classification problems and gaps in data. Likewise, MGIS provides an inventory of ex situ *Musa* collections and related research results: taxonomic descriptions, agronomic performances of cultivars in various environments, molecular characterization, host plant resistance, health status, origin, and environment in collecting sites. MGIS can be used in association with geographic information systems (GIS) to produce maps illustrating the distribution of *Musa* diversity. Genebank curators have the primary responsibility for entering data in this MGIS. Data in MGIS remain the property of the respective genebank curators involved in this network. Inventorying the diversity of cultivars, their uses and names will assist on studying the history of the *Musa* crop, as was shown by Rossel (1998) through her taxonomic–linguistic study of plantain in Africa.

6.2.3 Musa *Genebanks: Local, National and International*

There are 43 banana collections in the world, in 33 different countries (Silva et al. 2001), which may be holding a total of 1,500–3,000 accessions (including wild species) that represent the wide range of morphological variation and genome constitutions in *Musa*. The Fundación Hondureña de Investigación Agrícola (FHIA, San Pedro de Sula, Honduras) inherited from the United Fruit Company one of the largest collections of *Musa* genetic resources, which was initially set up for the use of their plant breeders and geneticists. This field genebank included once the world's largest collection of bananas, nearly 800 accessions of wild species and cultivars (including about 200, 2*x* accessions), from locations as far apart as the Solomon Islands and India and from the northern most part of Luzon (Philippines) to Bali (Indonesia). Some of the materials brought back from Asia and held in this genebank were successfully used by the *Musa* breeding program in Honduras. FHIA was among the first banana breeding programs to produce improved banana cultivars grown by smallholder farmers in the tropics due to this wealth of genetic material it has at its disposal (Rosales et al. 1999).

The largest international in vitro *Musa* genebank was established by the then International Network for the Improvement of Banana and Plantain (INIBAP) at Katholieke Universiteit Leuven in 1984. This INIBAP Transit Center (ITC) obtained in October 2003 an international status by the signing of an international agreement between Belgium and the then International Plant Genetic Resources Institute (IPGRI and now known as Bioversity International, Rome, Italy). In 1994, the collection was placed under the auspices of the Food and Agriculture Organization (FAO) of the United Nations to hold in trust *Musa* genetic resources. The aim of this in vitro genebank (also known as the International *Musa* Germplasm Collection) is to conserve all available banana, plantain, and wild species genetic resources and to supply plant materials to any bona fide users. This collection holds 1,168 accessions among which 15% are wild relatives and 75% landraces covering most of the genetic diversity within genus *Musa* (http://www.agr.kuleuven.ac.be/DTP/TRO/_data/itc.htm). The ITC also preserves advanced cultivars (10%) from banana improvement programs worldwide and supplies daily

five accessions of the *Musa* genetic resources it holds on trust. About 28% of the accessions have been sent to Africa, 24% to Latin America and the Caribbean, 21% to Asia, and 27% to other users.

The in vitro plantlets at ITC are checked for the presence of pathogens, such as bacteria, fungi, and viruses. Only those that are pest-free (currently 2/3) are available for distribution. Ploidy levels of most of this International *Musa* Germplasm Collection held at ITC were determined through flow cytometry by the Laboratory of Molecular Cytogenetics and Cytometry at the Institute of Experimental Botany (IEB, Czech Republic). Accessions of this international collection are tested for *Musa* viruses in virus indexing centers at the Queensland Department of Primary Industries and Fisheries (QDPI, Australia), Centre de coopération internationale en recherche agronomique pour le développement (CIRAD, France), and the Plant Protection Research Institute (PPRI, South Africa).

The ITC accessions are distributed internationally following the FAO/IPGRI Technical Guidelines for the Safe Movement of *Musa* Germplasm. Shoot-tip culture and third country quarantine facilitated the introduction of new *Musa* germplasm worldwide (Vuylsteke et al. 1990). ITC accessions are supplied to users under the terms and conditions of a Standard Material Transfer Agreement (SMTA) of the Multilateral System of Access and Benefit Sharing of the International Treaty on Plant Genetic Resources for Food and Agriculture. Additional Conditions to the SMTA may apply for the distribution of materials under development, i.e., breeding materials acquired by Bioversity International from other *Musa* improvement programs worldwide. For a wide range of accessions, lyophilized leaf tissues are also available in order to respond to requests for DNA. Delivery time depends on the type of plant material requested and ranges between 2 and 4 months for in vitro cultures and about 2 weeks for lyophilized leaf tissues. The material will be accompanied by a health statement, phytosanitary certificate, and a copy of the SMTA.

6.2.4 Modes of Preservation and Maintenance

Most banana and plantain cultivars (especially 3*x*) do not produce seeds, and the seeds produced by 2*x* wild *Musa* species are difficult to conserve. Their genetic diversity is, therefore, conserved through vegetative propagation or in test tubes under slow-growth conditions. For example, the *Musa* cultivars, wild species, and improved cultivars of the International *Musa* Germplasm Collection held in trust at the ITC are preserved as in vitro plantlets in medium-term storage and cryopreserved for long-term conservation. Field genebanks used suckers for propagating their holdings. A few *Musa* genebanks also keep in vitro backups, especially of their native accessions and cultivars.

In the ITC, each accession uses 20 shoot cultures that are kept on nutrient medium in continuous light conditions at 16°C and recultured once a year on average. They replace every 10 years the tissue culture material with fresh material grown out in the field to control for the risks of somaclonal variation. The ITC accessions are cryopreserved on liquid nitrogen at −196°C. For safety purposes, the entire International *Musa* Germplasm Collection held at ITC will be eventually cryopreserved – following a "black box" approach, at Montpellier by the Institut de Recherche pour le développement (IRD, France).

6.3 Role of Wild Musa Relatives in Elucidation Origin and Evolution of *Musa* Crop Plants

The edible cultivars (2*x*- and 3*x*-derived *M. acuminata*) were brought by humans to India, Myanmar, Thailand, and Philippines, where *M. balbisiana* was a native species. Natural hybridizations led to offspring involving both A and B genomes, especially in South Asia. *M. balbisiana* appears to be more suitable for drought-prone environments and pest-resistant than *M. acuminata*; as such characteristics are often found in cultivars with the B genome. Following the initial hybridization, a wide range of edible types arose, survived, and were brought under domestication. As a result, a diverse selection of *Musa* cultivars became available in Southeast Asia at the time of the earliest developments of agriculture many thousands of years ago (Price 1995).

Subsequent eastward movements of *Musa* germplasm, which were associated with human migration, brought new types of edible cooking cultivars (3*x* Maia Maoli/Popoulu group) to the Pacific islands.

Perhaps the same proto-Polynesians that carried the edible cultivars east to the Pacific Islands also carried west some edible cooking cultivars that ended in Africa. They took advantage of the sucker propagation, since such propagules survived for several months during the long sea voyages. More recently phytoliths dating to the first millennium BC in Cameroon has ignited debate about the timing of the introduction of the *Musa* crop to Africa (Mbida et al. 2000). Further phytolith evidence at a sediment core from a swamp at Munsa, Uganda (Lejju et al. 2006), suggested the presence of bananas in this site during the fourth millennium BC.

6.3.1 List of Plant Species Related to Cultivated Musa Species

The genus *Musa* includes five sections (*Eumusa*, *Rhodochlamys*, *Callimusa*, *Australimusa*, and *Ingentimusa*) as per their chromosome numbers and morphological characters. Two sections show a chromosome number of $2n = 20$ (*Callimusa* and *Australimusa*) and other two sections (*Eumusa* and *Rhodochlamys*) have a basic chromosome number of 11 ($2n = 22$). The species in the sections *Callimusa* and *Rhodochlamys* are of ornamental interest only, as the characteristic of fruit parthenocarpy is absent; i.e., they do not produce edible fruit. The section *Australimusa* includes *M. textilis* (or abaca – the source of Manila hemp). Within this section the edible Fe'i bananas, which are found mainly in the Pacific islands, have evolved. Sharrock (2001) provides an assessment of the diversity of this section, especially for the origin, distribution, and use of Fe'is cultivars. The fifth section includes *M. ingens* ($2n = 14$), whereas a section yet to assign for *M. lasiocarpa* and *M. boman* (Horry et al. 1997). There are four known genomes in *Musa*: A, B, S, and T that belong to *M. acuminata*, *M. balbisiana*, *M. schizocarpa*, and *Australimusa* species, respectively (D'Hont et al. 2000). Genomic in situ hybridization (GISH) provided a means for determining the genome structure of interspecific cultivated clones. GISH results were consistent with the putative genomes as suggested by phenotypic descriptors with some exceptions, e.g., the Asian cooking banana "Pelipita", which shows 8 A and 25 B instead of the expected 11 A and 22 B chromosomes.

Bartoš et al. (2005) indicated that in *Eumusa*, 2C DNA content ranged from 1.13 to 1.377 pg, and *M. balbisiana* having the lowest DNA content of all sections. *M. beccarii* ($x = 9$) from *Callimusa* had the highest 2C nuclear DNA content (1.561 pg). Species belonging to *Rhodochlamys* and *Australimusa* had 2C DNA contents ranging from 1.191 to 1.299 pg, and from 1.435 to 1.547 pg, respectively. Their research also showed that 5S rDNA loci in *Musa* varied from 4 to 8 per diploid cell. While different numbers of 5S rDNA loci were observed within *Eumusa* and *Rhodochlamys*, four 5S rDNA loci were observed in all accessions of *Australimusa*. *M. beccarii* contained 5S rRNA gene clusters on five chromosomes. These authors also reported that the number of 45S rDNA loci was conserved within individual sections.

Research shows that both demographic history and dispersal mechanisms also accounts for *Musa* genetic diversity among populations and across geographical regions. Pollefeys et al. (2004) provide a preliminary analysis of the distribution of wild *Musa* species using available information from the available literature, MGIS, and DIVA-GIS software. The freely available geographic information system DIVA-GIS (http://www.diva-gis.org/) can be used for many different purposes but particularly for mapping and analyzing biodiversity data, such as the distribution of species.

The family Musaceae contains *Musa* and *Ensete*, which are clearly distinguishable by their distinct morphology and through research with genetic markers. A third genus, *Musella*, with a single species was recently added to this family. In *Musa*, the bracts and flowers are inserted independently on the inflorescence axis, the bracts are usually deciduous, and the basal flowers are generally female, whereas in *Ensete*, the bracts and flowers, which are integral with each other and with the axis, are persistent and the basal flowers are often hermaphrodite (Sharrock 2001).

Ensete gilletii ($2n = 18$) has a 2C DNA content of 1.210 pg and 5S rRNA in six chromosomes (Bartoš et al. 2005). Population differentiation in wild enset appears to be relatively low vis-à-vis other outbreeding, perennial species (Birmeta et al. 2004). Further analysis showed that enset cultivars (*E. ventricosum*) clustered distinctly from wild enset samples, suggesting that today's cultivars evolved from a few wild progenitors. The propagation system and harvesting time precludes gene flow between wild enset and

cultigen pools because the cultivars are harvested prior to flowering, and propagated by suckers. Research of distribution patterns of enset in southern Ethiopia showed that a substantial fraction of the landraces had a restricted distribution range and low abundance, while a number were moderately common with the remaining few being cosmopolitan (Tesfaye and Lüdders 2003). Regional distribution was positively correlated with local abundance; i.e., highly widespread landraces were also typically more abundant. The authors of this research also indicated that landrace diversity was not evenly distributed throughout the region with mountains recording the highest diversity. The extensive farmers' exchanges of planting materials result in a similarity coefficient among sites. In this regard, Birmeta et al. (2002) indicated that genetic distances, as well as amount of within-site diversity, may instead be connected with the pattern of distribution for various ethnic groups in the enset-based agricultural system, and their dependency on enset as a staple food.

6.3.2 Application of Morphotaxonomy, Chemotaxonomy, Biochemical Markers, and Molecular Markers

Taxonomic and phylogenetic groups within the genus *Musa* were initially established through a numerical morphology-based scoring system (Simmonds and Shepherd 1955). Numerical taxonomy was further used for taxonomy of the wild *Musa* species (Simmonds and Weatherup 1990). Using the above research results, a *Musa* descriptor list became available (IPGRI-INIBAP/CIRAD 1996). Such descriptors are very useful for cataloging *Musa* genetic resources (Daniells et al. 2001).

Qualitative and quantitative variation in vegetative, inflorescence, and fruit traits was also used for grouping banana and plantain cultivars held at the genebank of the International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria) (Ortiz 1997c). Groupings that resulted following principal component analysis supported conventional taxonomic groupings of 3*x* cultivars. Pseudostem height and number of fruits in combination with the number of hermaphrodite flowers and persistence of the male bud sufficed to group plantain cultivars (Swennen et al. 1995). Multivariate analysis also revealed that starchy bananas (ABB) appear to be separated into two subgroups with one being close to the African plantains and the other being close to the Asian (ABB) cooking bananas (Osuji et al. 1997b). Similarly, principal component analysis, using fruit traits due to their high heritability, separated cooking from beer banana cultivars of the AAA subgroup Lujugira–Mutika (Nsabimana and van Staden 2005).

A phenotypic distance index, using quantitative descriptors, was useful for classifying African plantains (3*x*, AAA), dessert (3*x*, AAA, AAB), and cooking (3*x*, ABB) bananas (Ortiz et al. 1998a). The phenotypic distance matrix was developed by calculating the average difference between each pair of accessions for all quantitative descriptors. The between-cluster variance was larger (0.001779) than the within-cluster variance (0.001380). Wright's ϕ_{FS}, which measures the overall diversity, was 0.5663. This value suggested little gene flow among triploid taxonomic clusters via pollen, which explains the higher population differentiation exhibited by this vegetatively propagated crop with very low male fertility. The results also suggested that variation observed within each *Musa* taxonomic cluster arose from mutations accumulated throughout the history of cultivation of this crop.

Chemotaxonomy using biochemical markers such as flavonoids and isozymes were subsequently used for identifying *Musa* commercial cultivars, species, and subspecies (Bonner et al. 1974; Table 6.1). The more recent use of nucleic acid technologies in phylogenetic research needed the development and application of efficient DNA extraction procedures from *Musa* plants. A modified CTAB DNA extraction procedure for *Musa* (Gawel and Jarret 1991c) has been the most widely used. Results so far have shown that molecular markers are essential for characterization and classification of *Musa* germplasm collections (Table 6.1). Chloroplast and nuclear DNA provided different insights into variation patterns of *Musa* populations and together can assist for developing sound conservation practices.

The recent advances brought by molecular taxonomy still need to deal with both complementary and contrasting data along with a judicious filling up of data gaps (Heslop-Harrsion and Schwarzacher 2007), which will be required to resolve the relationships and phylogeny in the *Musa* genus. Moreover, cluster

Table 6.1 Genetic marker-facilitated *Musa* taxonomy, cultivar true-to-type assessment and population genetics

Genetic markers	References
Isozymes	Jarret and Litz (1986a, b), Horry (1989), Bhat et al. (1992a, b), Lebot et al. (1993), Mandal et al. (2001), Mendioro et al. (2007)
Anthocyanins	Horry and Jay (1988)
Flavonoids	Horry (1989)
Chloroplast DNA (cpDNA), restriction fragment length polymorphisms (RFLP)	Gawel and Jarret (1991a, b), Bhat et al. (1994, 1995b), Carreel et al. (2002), Ge et al. (2005)
Polymerase chain reaction (PCR)-RFLP of organelle DNA sequences	Nwakanma et al. (2003a), Ning et al. (2007), Boonruangrod et al. (2008)
Ribosomal gene spacer (rDNA) length (IGS)	Lanaud et al. (1992)
RFLP	Gawel et al. (1992), Jarret et al. (1992), Bhat et al. (1994, 1995b), Carreel et al. (1994), Raboin et al. (2005)
DNA oligonucleotide and amplification fingerprinting	Kaemmer et al. (1993)
Random amplified polymorphic DNA (RAPD)	Howell et al. (1994), Bhat and Jarret (1995), Bhat et al. (1995a), Crouch et al. (2000), Newbury et al. (2000), Vidal and De García (2000), Pillay et al. (2001), Ude et al. (2003), Ferreira et al. (2004), Gübbük et al. (2004), Onguso et al. (2004), Muhammad and Othman (2005), Martin et al. (2006) , Ray et al. (2006), Uma et al. (2006), El-Dougdoug et al. (2007), Jain et al. (2007), Nsabimana and van Staden (2007), Lakshmanan et al. (2007), Agoreyo et al. (2008)
Interretrotransposon amplified polymorphism (IRAP)	Muhammad and Othman (2005), Nair et al. (2004), Teo et al. (2005), Häkkinen et al. (2007)
High annealing temperature-random amplified polymorphic DNA (HAT-RAPD)	Ruangsuttapha et al. (2007)
Primer sequences derived from a 520 bp highly repetitive DNA from *M. acuminata* ssp. *malaccensis*	Jarret et al. (1993)
Oligodeoxyribonucleotide probes specific for simple repeat motifs	Bhat et al. (1995b)
PCR assay for copy number of repetitive elements	Baurens et al. (1996)
A sequence family of species-specific repetitive element (Brep 1)	Baurens et al. (1997a), Noyer and Lagoda (2000)
Sequence-tagged microsatellite site (STMS)	Kaemmer et al. (1997), Grapin et al. (1998)
Cleaved amplified polymorphic site (CAPS)	Kaemmer et al. (1997)
Simple sequence repeats (SSR) or microsatellites	Ortiz et al. (1998b), Creste et al. (2003, 2004), Ge et al. (2005), Noyer et al. (2005), Oriero et al. (2006), Ning et al. 2007
Amplified fragment length polymorphisms (AFLP)	Loh et al. (2000), Wong et al. (2001, 2002), Ude et al. (2002a, b, 2003), Bhat et al. (2004), Noyer et al. (2005)
Sequence characterized amplified regions (SCAR) rDNA	Ramaje et al. (2004)
Nuclear DNA content and genomic distribution of 5S and 45S	Bartoš et al. (2005)
P450-based analogues (PBA)	Wan et al. (2005)
Methylation-sensitive amplification polymorphisms (MSAP)	Noyer et al. (2005)
Intersimple sequence repeats (ISSR)	Ray et al. (2006), Lakshmanan et al. (2007), Racharak and Eiadthong (2007)

analyses ensuing from DNA marker data needs to link with research on morphological traits to unravel relationships among *Musa* species and cultivars, which are complicated by interspecific hybridization, heterozygosity, and polyploidy, which are common in this genus. In this regard, De Langhe et al. (2005) indicated that numerical taxonomy can be useful for comparing morphological and molecular data. They suggested using large morphological and molecular datasets because an incomplete match of morphological and molecular data may imply that some traits are being influenced by the environment or that DNA markers did not sample critical genome regions. Furthermore, the use of DNA markers and sequences need to be supplemented with classical and molecular cytogenetic research.

6.4 Role in Development of *Musa* Cytogenetic Stocks and Their Utility

Musa species are one among the best examples of organisms whose complex cytogenetic structure ensued through extensive chromosomal rearrangements As a result non-homologous chromosomes involved in a reciprocal translocation may associate during the first meiotic prophase to form multivalents (vis-à-vis normal bivalents), which will behave as a single recombination unit or linkage group. Hence, genes that should segregate independently will show varying degrees of genetic linkage in the offspring of such structural hybrids. There will likely be reduced recombination of genes located near the break points at the origin of the translocations, thereby reducing the genetic distances between those loci. An unbalanced distribution of interchanged chromosomal segments – as a result of disjunction of multiple chromosome associations at meiotic metaphase usually will lead to some unviable gametes, thereby losing their gene combinations. This differential gametic viability accounts for a distortion of the expected gene segregation of loci on rearranged chromosomes.

Aneuploids arise after $3x \times 2x$ crosses in *Musa* and along with euploid ($2x$, $3x$, and $4x$) and highly ploid ($5x$ onwards) hybrids (Osuji et al. 1997c). Their chromosome number ($2n$) can range from about 22 to 32. Flow cytometry provides a rapid method for detecting aneuploids in a *Musa* population segregating for ploidy levels (Roux et al. 2003). Seedlings with 23 or 24 chromosomes could often be recognized by their general aspect, "*but the converse was also true that often they could not*" (Shepherd 1999). As expected, it will depend on the individual chromosome that was surplus to the balanced $2x$. Some trisomics ($2n + 1$) may be both vigorous and female-fertile. Such aneuploid stocks can assist for identifying individual *Musa* chromosomes and their further use in genetic research.

Endogamy may lead to inbreeding depression in *Musa*, though some bananas can tolerate occasionally a generation of inbreeding. Selfing may not succeed for producing inbred lines in this highly heterozygous species. Haploids are, therefore, being sought through anther culture of $2x$ sources. Autopolyploidy can result from chromosome doubling using in vitro methods or colchicine treatments. Such methods allow for manipulating ploidy (scaling up and down the chromosome numbers) and for devising new genetic enhancement methods for the crop. *Musa* double haploids can be useful genetic resources for gene mapping and other genetic research, but large numbers are needed from a single heterozygous recombining pollen source.

6.5 Role in Classical and Molecular Research in Understanding *Musa* Genetics

6.5.1 Use in Classical Genetic Studies

The wild $2x$ banana "Calcutta 4" (*M. acuminata* ssp. *burmannicoides*) is widely available in most *Musa* genebanks and extensively used in breeding programs for its resistance to black Sigatoka (*Mycosphaerella fijiensis*), yellow Sigatoka (*Mycophaerella musicola*), Panama disease (*Fusarium oxysporun* f. *cubense*), banana weevil (*Cosmopolites sordidus*), and burrowing nematodes (*Radopholus similis*). Offspring ensuing from plantain × "Calcutta 4" crosses or intermating diploid *Musa* accessions were broadly used for genetic research (Ortiz 2000 and references therein). For example, fruit parthenocarpy inheritance in diploid banana (Simmonds 1952) was the most known genetic research on an important descriptor until the 1990s. Albinism – complete lack of chlorophyll – in any plant tissue, in diploid plantain–banana hybrid seedlings (Ortiz and Vuylsteke 1994a) was among the first research results of the 1990s, which provided a means to reinvestigate the almost unapproachable *Musa* genome. Further genetic research in plantain–banana offspring led to the discovery of alleles inherited in a Mendelian fashion – in either monogenic or olegogenic epistatic systems – controlling $2n$ pollen (Ortiz 1997a), dwarfism (Ortiz and Vuylsteke 1995a), apical dominance (Ortiz and Vuylsteke 1994b), persistency of male bracts and hermaphrodite flowers (Ortiz 1996a), bunch orientation (Ortiz and Vuylsteke 1998a), and pseudostem waxiness (Ortiz et al. 1995c). Furthermore, the underpinnings of host plant resistance to black Sigatoka (Ortiz and Vuylsteke 1994c), banana weevil (Ortiz et al. 1995b), banana streak virus (Ortiz 1996b), and burrowing nematodes (Dochez et al. 2009) were elucidated and added to the previous

knowledge on genetics for resistance to yellow Sigatoka (Vakili 1968), Moko (Vakili 1965a), and Panama (Vakili 1965b) diseases. Accessions of *M. acuminata*, used by banana breeding programs (especially in Central America and the Caribbean), were also important genetic resources for elucidating inheritance of important *Musa* traits (Ortiz 1995 and references therein).

6.5.2 Use as Parents in Interspecific or Intergeneric Crosses

More recently, *M. acuminata* along with *M. balbisiana* are being used for developing mapping populations or as genetic resources for genomics (Table 6.2). The first mapping population (Fauré et al. 1993) ensued from a *M. acuminata* ssp. *banksii* × "SF 265" – a 2*x*, AA cultivar. The second (and saturated) map was developed through selfing M53 – a diploid AA cooking cultivar (Noyer et al. 1997). Ongoing mapping research involves a cross between "Borneo" (*M. acuminata* ssp. *microcarpa*) and "Pisang lilin" (*M. acuminata* ssp. *malaccensis*) (Roux et al. 2008). Due to the nature of the crop, association genetics and linkage disequilibrium mapping may, however, be better tools for unraveling important genes and valuable alleles in banana and plantain.

6.5.3 Mapping of Genes and Polygenic Clusters

Table 6.2 lists the available DNA marker systems for *Musa* genetic enhancement. Some of them result from research on both *M. acuminata* and *M. balbisiana* accessions and cultivars. This polymorphism provides DNA markers that will be extremely useful for genetic mapping, molecular-assisted selection, germplasm characterization, and evolutionary research in *Musa*. Furthermore, molecular cytogenetics can be useful for developing physical maps in this crop. For example, genomic in situ hybridization (GISH) enabled to distinguish the four genomes of banana cultivars and allowed defining the genome constitution of several cultivars (D'Hont 2005). Fluorescent in situ hybridization (FISH) was also used to analyze the distribution of repeated sequences along the *Musa* genome.

6.5.4 Assessment of Gene Actions, Physiological Pathways, and Host–Parasite Interactions

The dominant gene for fruit parthenocarpy (P_1), the recessive gene for black Sigatoka resistance (bs_1), and ploidy significantly affected the quantitative variation observed in plantain–banana 4*x*, 3*x*, and 2*x* hybrids (Vandenhout et al. 1995; Ortiz and Vuylsteke 1995b; Craenen and Ortiz 1996, 1997). Such results showed that in multigenic systems and much of the observed quantitative trait variation can be accounted by alleles with large phenotypic effects in a few loci. The results also confirm the influence of black Sigatoka in yield of plantain–banana hybrids. For example, the bunch weight of 4*x* banana hybrids appears to be significantly correlated with the disease development time (Craenen and Ortiz 1998).

The Asian cooking bananas (3*x*, ABB) are regarded among the most promising cultivars for transient dry environments, whereas plantains (3*x*, AAB) and some of their hybrids could be very sensitive to short dry spells (Ekanayake et al. 1994). It seems that the B genome (from *M. balbisiana*) provide traits for better adaptation to drought-prone environments. In this regard, Ekanayake et al. (1998) showed that ABB cooking banana had higher leaf stomata conductance than other *Musa* groups: AA, AAA, AAB, and (AAB × AA) hybrids.

The DNA of banana streak badnavirus (BSV) hybridized to the genomic DNA of the plantain Obino l'Ewai suggested integration of viral sequences (Harper et al. 1999). This discovery of the first para-retrovirus integration in the *Musa* genome makes it very unique. This integrated sequence, possibly in the nuclear genome of the *M. balbisiana* ancestor (Geering et al. 2005), leads to a plant para-retrovirus episomal infection. Sequencing of clones of Obino l'Ewai genomic DNA revealed an interface between BSV and *Musa* sequences, and a complex BSV integrant. In situ hybridization showed two BSV sequence locations in Obino l'Ewai chromosomes, and a complex arrangement of BSV and *Musa* sequences through probing stretched DNA fibers.

Table 6.2 DNA marker maps, marker-aided breeding, genetic tools, and gene cloning in *Musa* genomics research

Resource	Reference
Map with restriction fragment length polymorphisms (RFLP), random amplified DNA polymorphisms (RAPD) and isozyme markers	Fauré et al. (1993)
Microsatellites or simple sequence repeats (SSR)	Jarret et al. (1994)
RFLP, SSR and amplified fragment length polymorphism (AFLP) map	Noyer et al. (1997)
Chloroplast (cpDNA) and mitochondrial (mtDNA) RFLP	Fauré et al. (1994)
Derived cleaved amplified polymorphic sequence (dCAPS)	Umali and Nakamura (2003)
Sequence-tagged site (STS) cpDNA	Baurens et al. (1997b)
Copia-like repetitive element	Baurens et al. (1997c)
Genomic in situ DNA–DNA hybridization	Osuji et al. (1997a)
Fluorescent in situ hybridization (FISH) as an aid for physical mapping	Osuji et al. (1998)
Inter*Alu* PCR-like genomic profiling	Baurens et al. (1998)
SSR analysis of segregation of 2*n* eggs and heterozygosity	Crouch et al. (1998a)
Sequence-tagged microsatellite site (STMS)	Lagoda et al. (1998)
Fluorescent in situ hybridization (FISH) of the 18S–25S and 5S ribosomal DNA sites	Doleželova et al. (1998)
SSR for predicting hybrid performance	Tenkouano et al. (1998)
RFLP, SSR and AFLP linkage map and quantitative trait loci analysis	Carreel et al. (1999)
Variable number of tandem repeats (VNTR)	Crouch et al. (1999a)
AFLP, RAPD and VNTR analyses of full-sib 3*x* hybrid population	Crouch et al. (1999b)
SSR and marker-aided breeding	Tenkouano et al. (1999a, b)
Fluorescent AFLP analysis to study dwarfism	Engelborghs et al. (2000)
RAPD for A and B genomes	Pillay et al. (2000)
PCR-RFLP of the ribosomal DNA internal transcribed spacers (ITS)	Nwakanma et al. (2003b)
RAPD and flow cytometry	Oselebe et al. (2006b)
Gipsy-like long terminal repeats (LTR)	Balint-Kurti et al. (2000)
Methylation-sensitive amplification polymorphism (MSAP) for detecting DNA methylation changes after micropropagation	Peraza-Echeverria et al. (2001)
Repetitive DNA sequences	Valárik et al. (2002)
Internal region of the reverse transcriptase (RT) gene of *Ty*1-*copia*-like retrotransposons	Teo et al. (2002), Tan et al. (2002)
Secondary digest (SD)-AFLP and MASP for assessing CCGG methylation	Baurens et al. (2003)
AFLP markers linked to banana streak disease expression	Lheureux et al. (2003)
Improved anchored PCR technique for analysis of T-DNA insertions in transgenic banana	Pérez-Hernández et al. (2006)
Cloning and characterization of banana fruit polyphenol oxidase (PPO)	Gooding et al. (2001)
Cloning of malate synthase gene during fruit ripening	Pua et al. (2003)
Phylogenetic and molecular analysis of the ribulose-1,5-bisphosphate carboxylase (*rbcS*) small subunit gene family	Thomas-Hall et al. (2007)
Structural and phylogenetic analysis of Pto-type disease resistance gene candidates	Peraza-Echeverria et al. (2007)
Super-serial analysis of gene expression (SuperSAGE) for global gene expression pattern	Coemans et al. (2005)
Bacterial artificial chromosome (BAC) library	Vilarinhos et al. (2003), Aert et al. (2004), Šafář et al. (2004)
Plant transformation-competent binary bacterial artificial chromosome (BIBAC) library	Ortiz-Vázquez et al. (2005)
SSR for B genome	Buhariwalla et al. (2005)
Genomic library to isolate and characterize microsatellite loci	Creste et al. (2006)
Selective amplification of microsatellite polymorphic loci (SAMPL)	Giménez et al. (2005)
Expressed sequence tags (EST)	Santos et al. (2005)
Highly repeated fraction	Hřibová et al. (2007)
Resistance gene analogs (RGA)	Pei et al. (2007)
BAC-end sequences	Cheung and Town (2007)
RAPD markers for *Fusarium* wilt	Javed et al. (2004), Zambrano et al. (2007)
Host plant resistance (R)-genes encoding proteins with nucleotide-binding site (NBS) and C-terminal leucine-rich repeat (LRR) domains	Miller et al. (2008)
Syntenic relationships with rice	Lescot et al. (2008)
High-resolution BAC-fluorescent in situ hybridization (FISH)	De Capdeville et al. (2009)

6.5.5 Detection of Precise Ploidy Level and DNA Base Composition

Pollen size and stomata traits also were often used for assessing ploidy in *Musa* breeding populations (Vandenhout et al. 1995; Dumpe and Ortiz 1996; Tenkouano et al. 1998b). For example, chloroplast number in guard cells appears to increase proportionally to cell size as per ploidy level. More recently, flow cytometric analysis has been used for determining ploidy in other *Musa* genetic or breeding resources (Asif et al. 2001; Nsabimana and van Staden 2006; Oselebe et al. 2006a, b; Pillay et al. 2006).

6.5.6 Deciphering of Favorable Alleles

Epistasis can further enhance fruit sizes of high-yielding plantain–banana hybrids. Both additive × additive and additive × intralocus interactions of bs_1 and P_1 loci may also increase fruit mass (Ortiz et al. 1997b). This type of gene action was further corroborated by comparing half-sib hybrid offspring derived from French plantains and the wild banana "Calcutta 4" (Ortiz 1997d). Hybrid offspring from "Obino l'Ewai" showed on an average higher bunch weight than "Bobby Tannap"-derived hybrid offspring, whereas "Bobby Tannap" had higher bunch weight per se than "Obino l'Ewai." Such result can be accounted for by non-additive gene action (heterozygosity and epistasis) controlling bunch weight in plantain. Tenkouano et al. (1998a) indicated that the potential bunch weight cannot, therefore, be predicted based on parental performance but instead by using specific combining ability tests.

6.6 *Musa* Improvement Through Traditional and Advanced Tools

Wild *M. acuminata* and AA cultivars are the main 2*x* parental sources for plantain and banana breeding. The wild "Calcutta 4" and the 2*x* cultivar "Pisang lilin", a translocation heterozygote, have been extensively used in interploidy and interspecific crosses by *Musa* breeding programs for introgressing their host plant resistance to fungi into the cultigen pool (Ortiz et al. 1995a). Likewise, the 2*x* "Pisang jari buaya" remains as the main source of host plant resistance to plant parasitic nematodes, but new sources are becoming available (Stoffelen et al. 2000; Dochez et al. 2005, 2006; Quénéhervé et al. 2008a, b). Cultivars with host plant resistance to burrowing nematode showed significantly higher levels of catecholamine dopamine and lignin in the vascular bundle as well as cell-wall-bound ferulic acid esters in the cortex (Wuyts et al. 2007). The highest host plant resistance to banana weevil was observed in "Calcutta 4" and some of its 2*x* hybrids, e.g., "TMB2x 8075-7" (Kiggundu et al. 2003a). The 2*x* *Musa* gene pool also shows significant genetic variation for plant height, sucker vigor, fruit number, and size, which are also important traits for banana and plantain genetic enhancement. Recurrent selection at the 2*x* level remains as the main breeding approach for developing parental sources for further use in unilateral or bilateral sexual polyploidization aiming the genetic enhancement of *Musa* cultigens. Diploid hybrids can also arise from 3*x* × 2*x* crosses and can be of further use in recurrent selection schemes (Vuylsteke et al. 1997). In recent decades, some 2*x* breeding stocks (with genes from wild *Musa* species) became available for further use in banana and plantain improvement (Rowe 1984; Vuylsteke and Ortiz 1995; Tenkouano et al. 2003).

The search for both female fertility and 2*n* eggs in 3*x* cultigens remains an important activity for *Musa* breeding (Ortiz and Vuylsteke 1995c; Ssebuliba et al. 2006). This systematic screening paved the way for plantain improvement and the successful breeding of plantains with host plant resistance to black Sigatoka (Vuylsteke et al. 1993b). *Musa* breeders assess male fertility (Mukasa and Rubaihayo 1993; Dumpe and Ortiz 1996; Ssebuliba et al. 2008), especially at the 2*x* level to identify new pollen sources for further crossing schemes. Quick and reliable pollen quality assessments are therefore very important for *Musa* breeding (Fortescue and Turner 2004). Banana nectar could be used as a medium for testing pollen viability and germination in *Musa* (Nyine and Pillay 2007). Male fertility may be affected by seasonal variation (Ortiz et al. 1997c), and one needs to identify the best timing for successful crossing. Likewise, pollen production and viability may vary along the rachis (Krishnakumar et al. 1992).

Genetic bottlenecks could happen during the evolution of vegetatively propagated crops such as banana and plantain because the breeders of these crops (farmers in the early days or nowadays mostly trained professionals) may select a few sports (or mutants) with the desired characteristics, which could replace old cultivars in a large-scale area. Triploid plantains provide an interesting example in which most of the variation observed in approximately 120 known worldwide landraces resulted from mutations accumulated throughout the history of cultivation of this crop and farmer selection of a few strains (Ortiz 1997b). Diploid banana species and 3*x* plantain producing 2*n* eggs were the tools for broadening the genetic base in this important tropical fruit. Promising 4*x* hybrids may be obtained by hybridizing 2*n* eggs from plantains with *n* pollen from 2*x* accessions (Vuylsteke et al. 1993c). Plantain-derived 2*x* result also from such crosses and such germplasm has provided a means for both genetic research and further enhancement of the plantain genome at the 2*x* level (Vuylsteke and Ortiz 1995), avoiding the complex inheritance patterns of polyploid species.

In plantains, heterozygous 3*x* clones – which are still very old farmers' selections – are the sources of allelic diversity that are released to the 4*x* hybrids through 2*n* eggs and further broadened by the alleles provided by the 2*x* bananas or wild *Musa* species (Ortiz 2004). Advanced ploidy manipulations may lead to secondary 3*x* hybrids resulting from crosses between selected 4*x* and elite 2*x* stocks, both producing *n* gametes (Ortiz 2003). Triploid *Musa* hybrids may also occur due to unilateral sexual polyploidization among selected 2*x* stocks because one of the parents produces 2*n* gametes. Such breeding methods for plantain should be regarded as part of an evolutionary improvement approach because conventional breeding will be enhanced by innovative knowledge-led methods, as described above, for introducing additional genetic variation.

Biotechnology coupled with the use of genetic resources (including wild species) could help *Musa* breeders to genetically enhance the crop and meet this century's demand for banana and plantain (Crouch et al. 1998a; Vuylsteke et al. 1998). Available techniques for the transfer of genes could significantly shorten the breeding process and overcome some of the agronomic and environmental problems (Rout et al. 2000), which may not be possible through conventional methods. In the first decade of this millennium, new protocols for optimizing large-scale banana micropropagation were published (Arinaitwe et al. 2000; Nauyen and Kozai 2001; Madhulatha et al. 2004; Roels et al. 2005; Gübbük and Pekmezci 2004; Kalimuthu et al. 2007). Rapid mass propagation through in vitro methods coupled with field decapitation assisted the deployment of new *Musa* cultivars among small-scale farmers in northwestern Tanzania (Gallez et al. 2004). About 340,000 suckers were distributed by this undertaking and additional 680,000 suckers from farmer to farmer, amounting to 1,020,000 suckers, which led farmers to benefit from the new cultivars because they outyielded the local landraces by an average of 40%.

6.6.1 Traditional and Molecular Breeding Efforts

The first 4*x* hybrid ensued from a "Gros Michel" (3*x*, AAA) × *Musa acuminata* ssp. *malaccensis* (2*x*) cross. This 4*x* hybrid offspring arose from 2*n* eggs of "Gros Michel" and *n* pollen of the 2*x* banana. Other *Musa* breeding schemes include 3*x* hybrids from unilateral sexual polyploidization (one 2*x* parent producing 2*n* gametes) or 4*x* × 2*x* crosses (Ortiz 1997b), and 4*x* hybrids from 4*x* × 4*x* crosses or from bilateral sexual polyploidization at the 2*x* level.

Polyploidy can be induced in *Musa* by immersing newly germinated diploid seedlings in 0.5% aqueous colchicine solution (Vakili 1967). *M. balbisiana* 4*x*-derived plants were taller and more robust but had slower growth rate, droopier leaves, fewer suckers, and scantier root systems than their 2*x* counterparts, whereas the 4*x* level affected fruit size and shape in both *M. balbisiana* and *M acuminata*. Tetraploidy did not affect bunch size in *M. acuminata* spp. *banksii* but significantly reduced the bunch size of *M. acuminata* ssp. *microcarpa* cv. "Zebrina." Doubling of the anthocyanin concentration in the leaves of the pigmented banana plants was also brought upon by tetraploidy. Tetraploidy by chromosome doubling through colchicines of an ancestral 2*x* or an improved 2*x* hybrid has been advocated for developing 4*x* parents for further use in a 4*x* × 2*x* breeding approach for the genetic betterment of banana (Tomepke et al. 2004; Bakry et al. 2007).

Nowadays, some in vitro standard protocols allow commercially viable propagation of banana and plantain cultivars in the tropics. However, in vitro propagated *Musa* plants may not manifest consistently superior horticultural performance compared to conventional propagules under severe pest epidemics (Vuylsteke and Ortiz 1996). Nonetheless, the advantages of tissue culture-derived plants as improved planting material would be most relevant for establishing field nurseries for further clean, conventional propagation of newly bred or selected genotypes.

Cellular and molecular biology tools as well as induced mutations are also available for *Musa* breeding (Jain and Swennen 2004). Molecular marker-assisted breeding has the potential to dramatically enhance the pace and efficiency of genetic improvement in *Musa* (Crouch et al. 1999b). In this regard, microsatellites were shown to be powerful and reliable DNA markers for genetic analysis, hybrid fingerprinting, and marker-assisted breeding in *Musa* (Crouch et al. 1997). There was clearly a high cost associated with the generation of the necessary sequence data for such markers. However, once microsatellite primers were designed and tested the technology then they were easily transferred and relatively simple to use.

Advances in functional genomics (Roux et al. 2008) started offering new research tools for geneticists, who are cooperating in the *Musa* Genomics Consortium. Old and new knowledge of structural and functional genomics and genes, reproductive physiology, cytogenetics, and comparative genomics with rice, *Arabidopsis*, and other model species will enhance our understanding of *Musa* and its diversity enormously (Heslop-Harrsion and Schwarzacher 2007). Such wealth of information will also assist in developing novel approaches for the genetic betterment of the crop in this twenty-first century.

6.6.2 Tissue Culture, Somatic Hybridization, and Genetic Transformation

Micropropagation offers many advantages for *Musa*, it may, however, be adversely affected by somaclonal variation, i.e., genetic variation among plants regenerated from tissue culture. Somaclonal variation is ubiquitous in *Musa*, and off-types range between 0 and 70% depending on the genotype (Vuylsteke et al. 1988, 1991; Vuylsteke 1998). It appears that at least one particularly labile portion of the *Musa* genome may be susceptible to the stress imposed during in vitro culture, which could lead to higher rearrangement and mutation rates than other portions of the genome (Oh et al. 2007). Somaclonal variation may be detected by visual screening and cytology (Sahijram et al. 2003). DNA fingerprinting and flow cytometry techniques are now being used for assessing somaclonal variation in *Musa* clones (Damasco et al. 1996; Sahijram et al. 2003; Srangsam and Kanchanapoom 2007). Breeding new cultivars showing host plant resistance to *Fusarium* wilt appears to be suitable through somaclonal variation for some Cavendish bananas (Hwang and Ko 2004). However, Vuylsteke et al. (1996) indicated that somaclonal variants of plantains showing changes in leaf or inflorescence were inferior to the original landrace from which they were derived because of their low bunch weight and small fruits. Nonetheless, one somaclonal variant of "Agbagba" ("AO 2B2-2") expressed lower host plant susceptibility to the black Sigatoka and had a higher bunch weight, more fruits per bunch with higher average weight, greater average length, and greater average girth that the original source (Nwauzoma et al. 2002). Furthermore, Vuylsteke et al. (1995) bred "PITA-9" – a plantain hybrid, from the almost sterile False Horn plantain set through cross-breeding of a female-fertile somaclonal variant.

Embryogenic cell suspension-derived banana plants offer another option for mass propagation of *Musa*. A somatic embryogenesis technique may reduce significantly costs due to a high multiplication rate. Banana plants with similar field performance to those produced by the conventional in vitro budding method could be regenerated from embryogenic cell suspension (Côte et al. 2000). None of the cell suspension-derived plants exhibited off-type traits in the field. Kulkarni et al. (2004) suggested that cell suspension cultures coupled with mutagenesis (e.g., though γ-irradiation) could provide another option for *Musa* improvement. Table 6.3 provides a summary of recent advances on tissue culture and other methods for *Musa* genetic resources enhancement and preservation.

Protoplast fusion has been advocated as useful complementary tool for producing somatic hybrid $4x$ parents to further use them in interploid crosses with other $2x$ genetic resources or for direct release of $3x$

Table 6.3 Most important advances since the mid-1990s on tissue culture and other methods for *Musa* genetic resources enhancement and preservation

Method	Reference
In vitro induced 4*x* through culturing shoot tips in liquid medium supplemented both with 5 mM colchicine for 48 h or 30 μM oryzalin (3,5-dinitro-*N*4,*N*-dipropylsulphate) for 7 days, both in combination with 2% (v/v) DMSO	Duren et al. (1996)
In vitro mutation breeding	Bhagwat and Duncan (1998)
Micropropagation to dissociate cytochimeras	Roux et al. (2001)
In vitro 4*x* plants through proliferating culture in liquid medium with 1.25 mM of colchicine for 48 h	Bakry et al. (2007)
Haploidy through anther culture	Assani et al. (2003)
Somaclonal variation by induction of adventitious shoots from excised sucker shoot tips grown in Murashige and Skoog medium containing 15 mg l^{-1} of N^6-benzyladenine	Giménez et al. (2001)
Plant regeneration via somatic embryogenesis	Daniels et al. (2002)
Plant regeneration from protoplasts	Assani et al. (2001, 2002)
Microcallus production and whole plant regeneration from recalcitrant protoplasts	Assani et al. (2006)
Direct regeneration of male inflorescence-derived plants	Harirah and Khalid (2006)
Direct shoot and cormlet regeneration from leaf explants	Venkatachalam et al. (2006)
Plants regeneration via somatic embryogenesis in a bioreactor	Gomez Kosky et al. (2002, 2006)
Plant regeneration from protoplasts via somatic embryogenesis	Xiao et al. (2007)
Embryogenic cell suspensions from shoot meristematic tissue	Strosse et al. (2006)
Cryopreservation of banana meristem cultures	Panis et al. (1996)
Cryopreservation for eliminating cucumber mosaic or banana streak viruses	Helliot et al. (2002, 2003)
Cryopreservation protocol using droplet vitrification of apical meristems	Panis et al. (2005)
Cryopreservation protocol for long-term conservation	Agrawal et al. (2008)

somatic hybrids by $x + 2x$ protoplast fusion (Assani et al. 2003). Optimizing protoplast fusion parameters should, therefore, be a prerequisite for the establishment of somatic fusion technology for *Musa* breeding. In this regard, Matsumoto et al. (2002) attempted somatic hybridization between 3*x* and 2*x* bananas through protoplast electrofusion and nurse culture techniques. Protoplasts from embryogenic cell suspensions of "Maçã" (3*x*, AAB) were fused with protoplasts from non-embryogenic calli of "Lidi" (2*x*, AA). Direct somatic embryogenesis was achieved after culturing fusion-treated protoplasts with rice nurse cells. Likewise, Assani et al. (2005) found that protoplast fusion with the fusogen polyethylene glycol was best, whereas electric fusion was better, as measured by mitotic activities, somatic embryogenesis, and plantlet regeneration rate.

Plant regeneration in cell suspension cultures of the cooking banana (3*x*, ABB) "Bluggoe" (Dheda et al. 1991) provided the means for establishing most of the *Musa* genetic transformation protocols. More recently, generation of cell suspensions of East African highland bananas (3*x*, AAA) was achieved using scalps (Sakik et al. 2007). Cell suspension structure and cell growth rate depended on the East African highland banana cultivar regardless of the hormone treatment in the induction medium.

Transgenic breeding provides an additional or complementary tool to *Musa* breeders who will be able to introduce value-added traits into banana and plantain cultivars (Table 6.4). Transgenic approaches show potential for the genetic improvement *Musa* with a wide set of transgenes currently available that may confer resistance to nematodes, fungi, bacteria, and viruses (Tripathi 2007). One of the first reports of a transgenic banana showing host plant resistance against a major pest was on a Cavendish banana, which was transformed using *Agrobacterium tumefaciens* to express a protein engineered rice cystatin (OcIΔD86) that provides host plant resistance to the parasitic burrowing nematodes (Atkinson et al. 2004). Transgenic bananas expressing human lysozyme (*HL*) gene also showed resistance to race 4 (FocR4) of *Fusarium* wilt (Pei et al. 2005). The transgenic clones H-67 and H-144 remained healthy after field testing and were able to fruit. Both transgenic clones had the highest level of *HL* expression, as revealed by Northern blotting analysis, and that their expression of *HL*

Table 6.4 Transgenic *Musa* protocols and outputs

Protocols	Germplasm	Outputs	Reference
Agrobacterium-mediated plant transformation system	Cavendish banana Grand "Nain" (3*x*, AAA)	This system allowed for the recovery of putative transformants within 4 weeks after cocultivation of tissue samples with *Agrobacterium*. Two or more cycles of meristem rooting and micropropagation allowed for the selection of plants from this putative transformant population which demonstrated chromosomal integration of foreign DNA by Southern analysis with no indication of chimeric tissues	May et al. (1995)
Particle bombardment of embryogenic suspension cells	Cooking banana (3*x*, ABB) and plantain (3*x*, AAB) cultivars	Bombardment parameters were optimized for a modified particle gun resulting in high levels of transient expression of the β-glucuronidase gene in both banana and plantain cells. Bombarded banana cells were selected with hygromycin and regenerated into plants. There was a stable integration of the transferred genes into the banana genome	Sági et al. (1995a)
Electroporation and particle bombardment of regenerable protoplasts isolated from embryogenic cell suspensions	Cooking banana "Bluggoe" (3*x*, ABB)	Several chimeric *uidA* gene constructs were used for their introduction by electroporation. By using polyethylene glycol and heat shock, the frequency of transiently expressing protoplasts reached 1.8% as shown by an in situ β-glucuronidase assay. A duplicated 35S promoter with an alfalfa mosaic virus leader sequence (pBI-426) induced the highest expression rate among the constructs tested. Embryogenic cell suspensions were also bombarded with accelerated particles coated with a high expression *uidA* gene construct (pEmuGN) using a biolistic gun. After a partial optimization of the procedure, transient GUS assays reproducibly showed the presence of 400 blue foci in 30 µl of settled cell volume	Sági et al. (1995b)
Microprojectile bombardment of embryogenic cell suspensions from immature male flowers as the explant	"Grand Nain"	Cells were cobombarded with the neomycin phosphotransferase (*nptll*) selectable marker gene under the control of a banana bunchy top virus (BBTV) promoter or the CaMV 35S promoter, and either the β-glucuronidase (*uidA*) reporter gene or BBTV genes under the control of the maize polyubiquitin promoter. Plants were regenerated, under selection with kanamycin, and were cotransformed with *nptll* and either the *uidA* or BBTV genes. Transgenes were stably integrated in the banana genome	Becker et al. (2000)
Microprojectile bombardment of apical meristems	Banana accessions (2*x* AA, BB) and cultivars (3*x* AAA), plantain (3*x* AAB) cultivars and hybrids (4*x*, AAAA, AAAB)	An efficient regeneration protocol, which appeared to be independent of ploidy level and genomic background was developed by using apical meristems. Transient expression of the β-glucuronidase (*gus* A) gene was observed in 60–70% of explants 48 h after microprojectile bombardment. Organogenesis from apical shoot meristems may offer a simple efficient method for easier transformation of a broad range of *Musa* species than the use of embryogenic cell suspensions	Tripathi et al. (2003)

(*continued*)

Table 6.4 (continued)

Protocols	Germplasm	Outputs	Reference
Agrobacterium-mediated transformation of embryogenic cell suspensions	Banana cultivar "Rasthali" (3*x*, AAB)	Two hundred putative transformants were recovered, of which a set of 16 was tested by histochemical analysis for GUS expression and by Southern blot analysis with a probe for the *gusA* gene. The plants were positive for GUS expression and integration of the *gusA* gene. Two of the transformants were grown to maturity in a greenhouse. Bananas were harvested to test GUS expression by histochemical analysis. The fruit from both transgenic plants were positive for GUS expression	Ganapathi et al. (2001)
Centrifugation Assisted *Agrobacterium tumefaciens*-mediated transformation (CAAT) of embryogenic cell suspensions	Banana cultivars "Grand Nain" and "Lady Finger" (3*x*, AAB)	This protocol resulted in 25–65 plants per 50 mg of settled cell volume of embryogenic suspension cells, depending upon the *Agrobacterium* strain used, and gave rise to hundreds of morphologically normal, transgenic plants. One factor that led to a significant enhancement in transformation frequency was the introduction of a centrifugation step during cocultivation. Marker gene expression and molecular analysis demonstrated that transgenes integrated stably into the banana genome	Khanna et al. (2004)
Agrobacterium-mediated transformation of apical shoots	False Horn plantain "Agbagba" (3*x*, AAB)	Transient expression of the β-*glucoronidase* (*uid* A) gene was achieved in transformed apical shoot tips. The hygromycin-resistant shoots were regenerated 4 to 5 weeks after cocultivation of explants with *Agrobacterium*. The two step selection procedure allowed the regeneration of shoots which were uniformly transformed. The integration of the *uid* A gene was confirmed by PCR and Southern blot analysis	Tripathi et al. (2005)
Agrobacterium-mediated transformation of scalps by vacuum infiltration	"Grand Nain"	The binary vector pC2301 was used for the initial transformation and the histochemical detection of GUS. Polymerase chain reaction (PCR) assays demonstrated that the transformation protocol was successful	Acereto-Escoffié et al. (2005)
Agrobacterium-mediated transformation of male-flower-derived embryogenic cell suspensions	"Mas" (2*x*, AA)	Depending upon conditions and duration of cocultivation in liquid medium, up to 490 transgenic plants per 0.5 ml packed cell volume of embryogenic cell suspensions were obtained. Histochemical GUS assay and molecular analysis in several tissues of the transgenic plants demonstrated that foreign genes were stably integrated into the banana genome	Huang et al. (2007)

was well correlated with their FocR4 resistance. Likewise, MSI-99 (a synthetic substitution analogue of magainin that inhibited the growth and spore germination of *F. oxysporum* f. sp. *cubense* at 16 lg ml^{-1}) was used for producing transgenic bananas showing host plant resistance to both *Fusarium* wilt and *Mycosphaerella musicola* (Chakrabarti et al. 2003).

The Queensland University of Technology submitted recently an application for the limited and controlled release of transgenic bananas to the Australia's Office of the Gene Regulator (http://www.ogtr.gov.au/ir/dir079.htm). This release may take place in Cassowary Coast, Queensland, on a total area of up to 1.4 ha between 2008 and 2010. The transgenic bananas contain the *ced-9* gene from the nematode *Caenorhabditis elegans*, which is expected to protect the transgenic plants from pathogenic microorganisms. The *ced-9* gene encodes a

protein that prevents plant cells from undergoing programmed cell death (apoptosis) in response to pathogen attack. The gene may also affect growth and development of the transgenic plants and will confer enhanced tolerance to a range of abiotic stresses.

The recent outbreak of *Xanthosoma* wilt in eastern and central Africa prompted the use of genetic engineering as a strategy for incorporating host plant resistance to this banana bacterial wilt (Tripathi et al. 2004; Biruma et al. 2007). Banana cultivars with transgenes encoding for plant ferredoxin-like protein (pflp) and hypersensitive response assisting protein (hrap) isolated from sweet pepper are being sought. Kiggundu et al. (2003b) also suggested host plant resistance to banana weevil as another target for transgenic *Musa*. The authors indicated that exogenous cysteine proteinase inhibitors may control banana weevil. Cysteine proteinases are not part of the human digestive system, thereby allowing humans to consume cysteine proteinase inhibitors safely.

The release of a transgenic *Musa* crop needs, however, to follow an ecologically and socially acceptable process (Baurens et al. 1999), e.g., avoiding controversial vectors, selectable markers, or gene constructs. Hence, combining ecofriendly transgenic approaches with *Musa* cross-breeding offers an environmentally friendly option for improving bananas and plantains. A recent ex ante impact assessment (Smale and Tush emereirwe 2007) shows the potentially pro-poor application of transgenic cooking bananas in East Africa and the likely social consequences of the choice of host cultivar for trait insertion. Their research suggest that the demand for planting material of transgenic cooking banana may vary according to household and physical farm characteristics, markets, and the attributes of the new cultivars, e.g., host plant resistance to pests.

6.7 Genomics Resources Developed for *Musa* Improvement

Several thousand expressed sequence tags (ESTs) and gene sequences analyzed by isolation of mRNA, which are important for examination of gene expression, responses, and differentiation of the plants, and examination of diversity are becoming available (Heslop-Harrsion and Schwarzacher 2007). EST comparisons will be a very valuable resource for identification of genes that are differentially expressed under stress conditions.

The bacterial artificial chromosome (BAC) library from the wild 2*x* banana "Calcutta 4" has become a valuable tool for many of the goals of the Global *Musa* Genomics Consortium (Table 6.2). For example, a physical map by BAC-FISH will allow the characterization of translocation breakpoints. The BAC library from the wild *M. balbisiana* provides another important genomics resource, especially for studying the structure and evolution of *Musa*. It has been also used in map-based cloning of a genetic factor that is involved in the activation of integrated para-retroviral sequences of BSV. The third library derived from the black Sigatoka-resistant source *M. acuminata* spp. *errans* cv. "Tuu Gia" (2*x*, AA) and was cloned in a transformation-competent binary BAC (BIBAC), amenable for transgenic research. Another BAC library was also developed from "Calcutta 4" (A James et al. CICY, Mexico, unpublished) and complements the previous one due to the use of a *Bam*HI cloning site. The last library was from "Grand Nain" (3*x*, AAA) and became the first one from a commercial dessert banana cultivar (P Piffanelli et al. CIRAD, France, unpublished results). De Capdeville et al. (2009) showed the potential of BAC-FISH for banana breeding when using this technique on the wild banana "Calcutta 4" and *M. velutina*. Their research suggest that this technique could validate colinearity between potential crossing parents and how the method be also helpful in *Musa* mapping initiatives, e.g., allowing the identification of chromosomal rearrangements between related *Musa* species and cultivars. Other available genomics resources are given in Table 6.2.

6.8 Scope for Domestication and Commercialization of *Musa* Genetic Resources

Banana and plantain are not considered to be invasive species (Office of the Gene Technology Regulator 2007) and may be found in mixed cropping systems in the tropics, where they prefer well-drained soils. The main uses are as indicated before as staple food but also as fodder and fiber (Nelson et al. 2006). They can be used as crop shade, mulch, and living fence.

Alcohol, beer, vinegar, and wine can be produced from banana and plantain fruit. The small male flowers (inside the "bud") of certain cooking banana cultivars are cooked and eaten in the Philippines and the entire bud can be cooked as a vegetable. Likewise, leaf buds can be eaten as vegetable.

Pro-vitamin A carotenoids (e.g., β-carotene, the most important one) are important for protecting against vitamin A deficiency and anemia – because of the involvement of vitamin A in iron metabolism (Englberger et al. 2003a). For example, some Fe's bananas showed up to 4,960 μg of β-carotene in 100 g of the edible portion vis-à-vis 21 μg per 100 g in Cavendish export bananas (Englberger et al. 2003b). The cultivar "Utin Iap" had up to 6,360 μg β-carotene per 100 g of edible fruit (Englberger et al. 2003c; Englberger and Lorens 2004). It seems that carotenoid levels may correspond to the color intensity with high levels in those with deep orange flesh, which can be used as a guide (Daniells 2004). In Cameroon, the daily vitamin A supply by banana and other plant foods could be significant: reaching 13% on average per day (max. ~20%) for both children and mothers (Honfo et al. 2007). However, β-carotene (as well as vitamin C) content significantly decreased by frying plantain chips at high temperatures, especially for thin slices (Demasse et al. 2007). The optimum frying was with plantain slices of 1 mm thickness and at 150°C for 5–7 min. Similarly, the processing technique may affect starch bioavailability of East African cooking (matooke) bananas (Murange et al. 2007). Although solubilized matooke starches may adequately meet the energy requirement of a growing animal, extrusion cooking confers a marginal advantage over the cooked flour because the extruded flour's lower peak viscosity. Such an advantage would be enhanced in humans if the rations are taken as porridges.

Banana flowers, fruits, and roots are still used medicinally in some Pacific islands' cultures, whereas ashes produced from burning banana leaves and pseudostems are used in curries and as a salt substitute in India. Some *Musa* species and hybrids with colorful floral bracts and flowers are utilized in ornamental landscapes and tropical flower arrangements, e.g., *M. aurantiaca*, *M. laterita*, *M. mannii*, *M. ornata*, *M. rosea, M. rubra*, *M. sanguinea*, *M. siamensis*, and *M. velutina* (Häkkinen 2007). Constantine (2006) provides an overview on the available methods for propagating ornamental Musaceae through seeds, suckers, and in vitro.

In the Pacific, banana trunks are still used to line underground ovens (to provide steam), together with banana leaves placed over the food to keep it dirt-free. Furthermore, banana fruits and stems are made into silage and used as cattle feed and the underground parts are also used for pig and cattle feed. Banana stalks were used as canoe rollers in ancient times in Hawai'i and are still today in use at some of the remote Micronesian and Polynesian islands. Banana leaf and plant fibers are used to make thread and cloth, or to wrap some food before steam cooking, e.g., tamales in Peru. Abaca's leaf fibers are used to make string, thread, cordage, and rope. Moreover, *Musa* leaves are used as packing material, parcels to hold things, leis and garlands, or for house roofs and wall linings. Banana sap has been used in many cultures as dye. Leaves are also used as plates and tablecloths.

6.9 Some "Dark Sides" and Their Addressing: Minimizing Risks of Transgenic *Musa* to wild species

Although transgenic crops were originally developed for temperate climates and industrialized agriculture, this technology has the potential to address some of the most challenging biotic and abiotic constraints faced by farmers in non-industrialized agriculture (Ortiz and Smale 2007). Genetic transformation should also add an economically valuable trait while maintaining other desirable characteristics of the host cultivar, e.g., enhanced product quality or micronutrients can be added to a well-adapted cultivar that already yields well under local conditions. Likewise, the use of transgenic crops for delivering vaccines has been advocated (Mason and Arntzen 1995). A plant-based production of an oral hepatitis B virus (HBV) vaccine has potential economic and other advantages over the available vaccine delivery by injection. Banana emerged as one of the best candidates for the delivery of a bite-sized vaccine for HBV, especially to millions of people in the developing world. Arntzen (2005) indicated that the use of crop-based vaccines has been delayed by regulatory concerns regarding the entry of transgenic crops into the food supply.

As indicated before, most *Musa* cultivars are 3*x* ($2n = 33$) and are seedless due to human selection for this trait during the species domestication process. Hence, edible cultivars are normally vegetatively disseminated by plantlets developed from the buds that are attached to its underground stem or rhizome or through tissue culture. Furthermore, most 3*x* cultivars show unviable pollen in contrast to wild *Musa* species. Sterility also arises by triploidy of the germinative tissue, irregular or late growth of pollinic tubes in the styles of the female flower, absence of fertilization even with the development of the tube by unknown causes, and necrosis of the female flower nectary at blooming (Silva et al. 2001). Likewise, many parthenocarpic 2*x* show often sterility in both sexes as a result of meiotic abnormalities due to chromosome anomalies. Hence, sterility and both propagation methods in the cultigens minimize any risks for potential "super-weeds" arising from gene flow from a transgenic 3*x* *Musa*, the main ploidy target for this endeavor, to wild species. Wild banana seeds in ripe fruit that fall into the ground do not show a high survival rate or viability (Office of the Gene Technology Regulator 2007). Wild banana seeds may remain dormant for up to 1 year, germinate, and die.

6.10 Recommendations for Future Actions: Learning for Introducing Exotic and Bred-*Musa* Germplasm into the African Farming Systems

African plantains in the lowlands and East African highland bananas (for both cooking and beer) are examples of African farmers' ingenuity, tenacity, and organizational and inventive capacity in adapting this imported *Musa* crop species from Asia to respective environments (Ortiz and Hartmann 2003). Asia – where AA, AAA, AAB, and AAA bananas are regarded today mostly as dessert fruit or as ingredient for snacks, cakes, or medicines – shows a wide range of genome composition among the relatively small number of local cultivars therein, whereas there are many local cultivars among the AAB plantains of West Africa and the AAA East African bananas – where both *Musa* crops are staple food for their growing population (Kaori et al. 2006). Such local cultivar diversity could arise from the interactions between farmers, traders, and consumers. Moreover, Shigeta (2002) suggested that diversity, perenniality, and mass-of-harvest are the essential elements of African vegeculture centered on *Musa* and enset, which could shape the worldview of plantain and banana farmers in sub-Saharan Africa.

Although their asexual propagation may limit crop evolution, today West, Central, and East Africa are acknowledged as secondary centers of variation for plantains and bananas, because farmers' selected sports (mutants) arising in their fields, which account for most of the caloric intake from fruit crops in the African diet. Some African populations built also a close relationship with the crop over hundreds of years, e.g., Haya banana growers in Northwest Tanza nia (Maruo 2002) or among the Nyakyusa in southern Tanzania, who are known as the "banana-eaters" (Maruo 2007). Furthermore, their farmers developed crop husbandry skills, tools, vocabulary, and cultivar diversity in relation to the plants. People also created symbolic meanings for the plants that are related to prosperity, the idea of the sacred, and gender values, which show the key role of plantain in the development of their rural community. African farmers may grow plantain and banana in agroforestry or multilayer systems that maximize land use, e.g., under coffee trees and within vegetables around the Kilimanjaro (Hemp 2006). Such an ancient small landholder system, which evolved since introducing the crop in Africa, has not changed significantly in recent decades and today sustains a high biodiversity in some agroecosystems of sub-Saharan Africa.

Population pressure, poor soil fertility, and pests are, however, affecting banana and plantain production in Africa (Craenen et al. 2000 and articles therein). In the 1980s, some African governments encouraged IITA to launch a plantain and banana improvement program, which was spurred by the threat brought to the continent by black Sigatoka, which causes significant yield loss of the crop (Mobambo et al. 1993). An interim measure adopted by IITA in the late 1980s was the introduction from Asia of black Sigatoka-resistant cooking bananas (Ferris et al. 1997), while the long-term strategy was to develop black Sigatoka-resistant plantains. After their introduction to Nigeria, cooking banana plantlets were produced in two tissue culture laboratories located at IITA High Rainfall Station (Onne, near

Port Harcourt) and the Agricultural Development Program at Owerri (Imo State). With the collaboration of 24 institutions, vegetatively propagated planting materials (suckers) were distributed to 29,585 farmers in 710 villages.

An impact assessment study examined the adoption and diffusion of cooking banana in Nigeria. The highest adoption levels were obtained for those utilization methods similar to local and traditional methods of plantain consumption and lowest for non-traditional uses. Cooking banana gained a high level of acceptance and spread among the people and thus established itself within the farming system in the region (Tshiunza et al. 2002). The crop has been adopted by 55% of farmers, occupying about 26% of total fields, while its cultivation has increased by more than 930% since the introduction, with a multiplication rate of 600% across farmers. Bearing in mind that cooking banana was neither a traditional crop nor an improved cultivar from an existing one, the level and rate of adoption and diffusion are quite high and encouraging. At the end of the 1990s, about 80% of farmers, who adopted this new crop, were selling 10–90% of their total cooking banana production, while the other 20% produced entirely for household consumption. About 58% farmers sold at least 50% of their cooking banana. At the end of the 1990s, the average selling price of cooking bananas was N6.5 per kg compared to N13.3 per kg for plantains (about US$ 1 = N111). However, the cooking bananas may have an increased overall value because of their significantly higher bunch weight than plantains. Furthermore, the consumption patterns of plantain and cooking banana are very similar, which greatly contributed to the rapid integration of cooking banana within the existing plantain consumption and cropping systems (Tshiunza et al. 2001a). As indicated by Lemchi et al. (2005b), the major factors, which have strongly influenced the adoption process, were the level of educational attainment, social status, primary occupation, intensity of training received, availability of commercially produced products in the market or target area, trialability, and the number of desirable attributes of the utilization methods.

The introduction of cooking bananas and their subsequent adoption and diffusion made a positive impact in the region: on farmers' farm enterprises, farm resource use and allocation, income and food base of the people, as well as employment generation Hence, the potential of cooking banana in contributing to bridging the hunger gap and uplifting the income level of farmers in the region is quite high (Tshiunza et al. 2001b). As such, it is no longer appropriate to regard cooking banana as a stopgap measure, rather a suitable supplement (or even substitute) to plantain for some farmers and consumers in Nigeria.

Researchers at IITA were able in the early 1990s to rapidly (about 5 years) develop improved plantain–banana hybrid germplasm using a range of conventional and innovative approaches: interspecific hybridization, ploidy manipulation, embryo culture, rapid in vitro multiplication, field testing, and selection (Vuylsteke 2000). This result is a noteworthy achievement, considering that programs elsewhere required decades of breeding before *Musa* hybrids became available. The potential impact of using black Sigatoka-resistant plantains shows a cost–benefit impact of 10:1 over fungicides during periods of adequate production in rural southeastern Nigeria, while this advantage may reduce to 5.5:1 during periods of scarcity in plantain production – which dramatically influences the prices of plantain fruit (Ortiz et al. 1997a). "PITA 14" (or "TMPx 7152-2" – ensuing from a cross between a plantain landrace × Calcutta 4) appears to be one of the most promising IITA plantain hybrids because of its early fruiting, high bunch weight, and big fruits (Ortiz and Vuylsteke 1998c). "PITA 14" also responds to sucker removal with above 20% yield gain and about 15% reduction in length of growth cycle (Tenkouano et al. 2007). It is noteworthy that several farmers in southeastern Nigeria established sucker multiplication plots of "PITA-14" and are selling its suckers to other farmers (Lemchi et al. 2005a). An impact study shows that each farmer obtained about US$8.62 from "PITA14" and US$4.33 for their local landrace. The combination of host plant resistance and increased yield accounts for "PITA-14" high adoption potential. Owing to this early success, IITA and research-for-development partners started in 2002 large-scale introduction (on-farm) of hybrids with black Sigatoka resistance to the farming community in 11 Nigerian States of the plantain belt, Ghana, Tanzania, and Uganda (Tenkouano and Swennen 2004). Average adoption level was 40.33% (ranging from 36 to 47%) in the targets project areas of Nigeria. The adoption process was strongly influenced by household size, educational attainment, farming experience, frequency of

extension visit, overall experience from innovation, market access, and access to credit and profit as a result of the technology (Faturoti et al. 2006). Outside Africa, IITA-bred hybrids have been reaching farmers in Latin America; e.g., some hybrids were distributed (Nicaragua) to farmers in the departments of Leon and Chinandega with possible spillover into neighboring locations and across borders, and Asia, e.g., in the Philippines (de la Cruz Jr et al. 2007). Furthermore, the hybrid "BITA-3" (Ortiz and Vuylsteke 1998b) is being produced for chips in Kerala (India), where it compares favorably with the local cultivars.

Clearly, the level of adoption of the technology appears to be associated with an induced model of adoption where farmers are able to assess the yield before embarking on the cultivation of the new *Musa* cultivars and are thereafter supported with educational materials and incentives for adopting the new technology. However, as pointed out by Faturoti et al. (2007), governmental intervention may be sometimes needed in the areas of policy initiatives and should go beyond the ad hoc response that are often triggered by natural disaster such was the case with black Sigatoka outbreak in the mid-1980s.

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Chapter 7
Passiflora

Roxana Yockteng, Geo Coppens d'Eeckenbrugge, and Tatiana T. Souza-Chies

7.1 Introduction

The species of the genus *Passiflora* L., commonly known as passionflowers, are recognized for their tasty fruits, pharmaceutical properties and ornamental flowers. Although *Passiflora* species are numerous, few have significant commercial value. The vast majority are wild and poorly known. However, they constitute an important source of unexploited genetic diversity for the improvement of cultivated *Passiflora* species and the introduction of new crops. In addition, wild *Passiflora* species have potential useful characters, such as the resistance to particular pathogens, which can be incorporated by traditional breeding or molecular methods in crop species. The knowledge of the relatedness among *Passiflora* species is essential for the identification of wild crop relatives for future breeding programs. In this chapter, we review the advances in genetics, breeding and conservation of *Passiflora* species.

7.2 Botany

The pantropical family Passifloraceae comprises more than 600 species (Vanderplanck 2000) accommodated in 18 genera. Of this, *Passiflora* is the largest with ca. 520 species (MacDougal and Feuillet 2004). The first report of a *Passiflora* species was made in 1553 by Pedro Cieza de León during the Spanish colonial period in South America (Linnaeus 1753). In 1574, the Spanish botanist Nicolás Monardes suggested that flowers of *Passiflora* symbolized the Passion of the Christ. Linnaeus (1753) named the genus *Passiflora*, from the latin *flos passionis* that means "suffering flower". Since Linnaeus' publications, in which he described 22 and 24 *Passiflora* species (Linnaeus 1745, 1753), more than 500 new species have been described (MacDougal and Feuillet 2004) and their number continues to increase (Coppens d'Eeckenbrugge et al. 2001a; MacDougal 2001, 2003; Cervi 2002, 2006; Feuillet 2002, 2004; MacDougal and Hansen 2003; Jørgensen 2004; Jørgensen and Weigend 2004; MacDougal 2004, 2006; Vitta and Bernacci 2004; Krosnick 2005; Porter-Utley 2007; Vásquez et al. 2007; Azevedo 2008).

The center of diversity of *Passiflora* is located in the neotropics, with South America accounting for 95% of all the species (Fig. 7.1). A few species are also found in subtropical, and even temperate, regions of North and South America and 24 species are native from Southeast Asia, Australia and the Pacific Islands (Krosnick and Freudenstein 2005). Overall, passionflowers are adapted to a wide range of climates (0–40° latitude), altitudes (0–4,500 m) and ecosystems (from tropical humid forest to arid regions).

Passiflora flowers are mostly pollinated by bees, hummingbirds and bats (MacDougal 1983, 1994; Endress 1994; Büchert 1998; Kay 2001), but other types of pollinators such as wasps, butterflies and moths have also been recorded (MacDougal 1983). Many species, such as *P. edulis* f. *flavicarpa* O. Deg. and *P. incarnata* L., are self-incompatible and their fertilization is done through cross-pollination. The coevolution between plants of *Passiflora* and larvae of butterflies of tribe Heliconiini has been the subject

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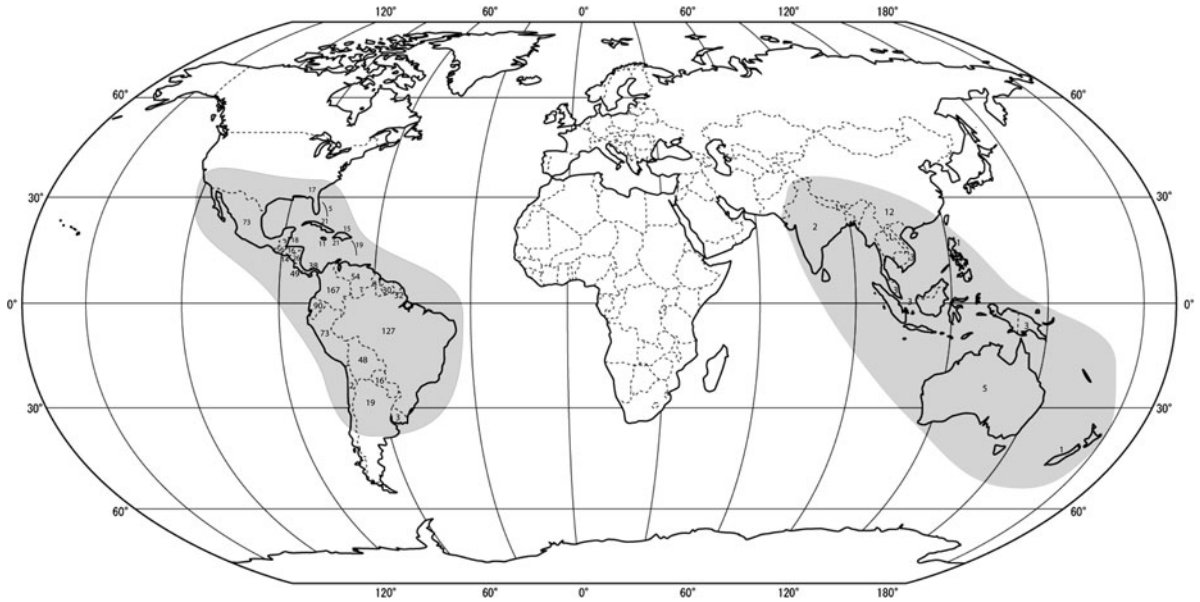

Fig. 7.1 Distribution of *Passiflora* richness according to Ocampo et al. (2007) and Shawn Krosnick (personal communication)

of several evolutionary studies (Gilbert 1971; Benson et al. 1975; Benson 1978; Gilbert 1980, 1982, 1983; Mitter and Brooks 1983; Spencer 1988). The caterpillars of these butterflies feed exclusively on *Passiflora* leaves.

7.2.1 Systematics

Based on previous classifications (Linnaeus 1753; Jussieu 1805a, b; De Candolle 1822; Roemer 1846; Harms 1923, 1925), Killip (1938) wrote the largest monograph on the genus including descriptions for 335 American species. His classification was based on floral characters and subdivided the genus into 22 subgenera. The Old World species were accommodated in the section *Disemma* of the subgenus *Decaloba* (Harms 1925). Escobar (1989) added the subgenus *Porphyropathantus*. The subgeneric classification of the genus has been recently challenged on morphological grounds by Feuillet and MacDougal (2003), who recognize only four subgenera which are subdivided into supersections, sections and series.

Cultivated species are essentially regrouped into four supersections of the largest subgenus *Passiflora* (see Sect. 7.4). At the subgenus level, the Feuillet and MacDougal classification is partly supported by recent molecular phylogenetic studies, as they clearly distinguish three major clades within *Passiflora*, corresponding to the three major subgenera (Muschner et al. 2003; Yockteng and Nadot 2004a; Hansen et al. 2006). The first problem is that this classification does not consider hundreds of species, such as the Old World species *Passiflora tetrandra* Banks ex DC. The second problem in the classification is the small subgenus *Deidamioides*, which regroups 13 species with dissimilar morphology. In fact, these species appear to be dispersed in phylogenetic reconstructions (Yockteng and Nadot 2004a; Krosnick and Freudenstein 2005). At lower levels, some supersections are supported by phylogenetic studies. For instance, the phylogeny based on nuclear sequences of glutamine synthase (ncpGS) supports mainly all supersections of subgenus *Decaloba* and the supersections *Coccinea, Passiflora* and *Tacsonia* of subgenus *Passiflora* (Yockteng and Nadot 2004a). However, the supersections *Stipulata* and *Laurifolia* of subgenus *Passiflora* do not form a clade in phylogenies. Members of the supersection *Stipulata* are dispersed in two non-related groups, in which section *Dysosmia* appears at the base of subgenus *Passiflora* and sections *Tacsonioides* and *Granadillastrum* are regrouped in one clade. Supersection *Laurifolia* is also divided into two well-supported but non-related clades. On one hand, the species of series *Tiliifolia* form a well supported clade closely related to species of supersection *Stipulata* except for the species in

section *Dysosmia*. On the other hand, the species of series *Laurifoliae* and *Quadrangulares* form a clade closely related to supersection *Passiflora*. The results of this phylogenetic study point out the necessary revision of the classification by MacDougal and Feuillet. Molecular reconstructions are essential in this task; however, their robustness and resolution have not been sufficient for appropriate inferences on the divisions under the supersection level. Moreover, the interpretation of molecular data is highly complicated by the level of reticulation and the complexity of genome transmission in Passifloraceae. Interspecific hybridizations seem common in the genus, and chloroplast inheritance is often paternal or even biparental (see Sect. 7.5.3). Thus, phylogenetic inferences based on cpDNA sequences face the most severe problems anticipated by Harris and Ingram (1991) for classical phylogenetic methods, i.e., high intraspecific and even intraindividual variation and consequently lineage sorting, and also reticulate evolution of the chloroplast genome through hybridization and introgression. Studies based on nuclear ribosomal genes may suffer similar limitations. Many *Passiflora* species have originated from recent radiation, involving recent isolation and hybridization events, giving an incomplete concerted evolution of ribosomal gene copies in the genome (Lorenz-Lemke et al. 2005). Understanding the evolution of *Passiflora* is likely to be a long task that will impose many different molecular and morphological approaches as well as original methods for the interpretation of their data.

Although the classification of MacDougal and Feuillet (2004) needs to be revised and completed with the inclusion of many species, we have used it in the present text because it is the most recent to date.

1. Subgenus *Astrophea* (DC.) Mast. – 57 species
1.1. Supersection *Astrophea* Feuillet & MacDougal
1.1.1. Section *Astrophea* Feuillet & MacDougal
1.1.2. Section *Capreolata* MacDougal & Feuillet
1.1.3. Section *Leptopoda* Killip ex Feuillet & Cremers
1.2. Supersection *Pseudoastrophea* (Harms) Feuillet & MacDougal
1.2.1. Section *Pseudoastrophea* (Harms) Feuillet & MacDougal
1.2.2. Section *Botryastrophea* (Harms) Killip
1.2.2.1. Series *Botryastrophea* (Harms) MacDougal & Feuillet
1.2.2.2. Series *Carneae* Feuillet

2. Subgenus *Deidamioides* (Harms) Killip – 13 species
2.1. Section *Deidamioides* (Harms) Feuillet & MacDougal
2.2. Section *Polyanthea* DC
2.3. Section *Tetrastylis* (Bard. Rodr.) Harms
2.4. Section *Mayapanthathus* MacDougal & Feuillet
2.5. Section *Tryphostemmatoides* Harms

3. Subgenus Decaloba (DC.) Rchb. – 214 species
3.1. Supersection *Pterosperma* Gilbert & MacDougal
3.2. Supersection *Hahniopathanthus* (Harms) MacDougal & Feuillet
3.3. Supersection *Disemma* (Labill.) MacDougal & Feuillet
3.3.1. Section *Octandranthus* Harms
3.3.2. Section *Disemma* (Labill.) MacDougal & Feuillet
3.3.3. Section *Hollrungiella* Harms
3.4. Supersection *Multiflora* (Small) MacDougal & Feuillet
3.5. Supersection *Auriculata* MacDougal & Feuillet
3.6. Supersection *Cieca* (Medic.) MacDougal & Feuillet
3.7. Supersection *Bryonioides* (Harms) MacDougal & Feuillet
3.8. Supersection *Decaloba* (DC.) MacDougal & Feuillet
3.8.1. Section *Decaloba* DC.
3.8.2. Section *Xerogona* (Raf) Killip

4. Subgenus *Passiflora* Feuillet & MacDougal – 236 species
4.1. Supersection *Passiflora* Feuillet & MacDougal
4.1.2. Series *Passiflora* Feuillet & MacDougal
4.1.3. Series *Palmatisectae* Feuillet & MacDougal
4.1.4. Series *Pedatae* Killip ex Cervi
4.1.5. Series *Setaceae* Killip ex Cervi
4.2. Supersection *Stipulata* Feuillet & MacDougal
4.2.1. Section *Granadillastrum* Tr. & Planch
4.2.2. Section *Calopathanthus* Harms
4.2.3. Section *Tacsonioides* DC.
4.2.4. Section *Kermesinae* (Cervi) Feuillet & MacDougal
4.2.5. Section *Dysosmia* DC.
4.3. Supersection *Laurifolia* (Cervi) Feuillet & MacDougal
4.3.1. Series *Laurifoliae* Killip ex Cervi
4.3.2. Series *Quadrangulares* Feuillet & MacDougal
4.3.3. Series *Tiliafolia* Feuillet & MacDougal
4.3.4. Series *Marginatae* Killip ex Cervi

4.4. Supersection *Coccinea* Feuillet & MacDougal
4.5. Supersection *Distephana* (DC.) Feuillet & MacDougal
4.6. Supersection *Tacsonia* (Juss.) Feuillet & MacDougal
4.6.1. Section *Rathea* (Karst.) Harms
4.6.2. Section *Insignes* Feuillet & MacDougal
4.6.3. Section *Colombiana* Esc.
4.6.3.1 Series *Leptomischae* Esc.
4.6.3.2. Series *Colombianae* Esc.
4.6.3.3. Series *Quindiensae* Esc.
4.6.4. Section *Parritana* Esc.
4.6.5. Section *Fimbriatistipula* Esc.
4.6.6. Section *Tacsoniopsis* Tr. & Planch
4.6.7. Section *Elkea* Feuillet & MacDougal
4.6.8. Section *Tacsonia* (Juss.) Feuillet & MacDougal
4.6.9. Section *Boliviana* (Harms) Feuillet & MacDougal
4.6.10. Section *Trifoliata* Feuillet & MacDougal
4.6.11. Section *Manicata* (Harms) Feuillet & MacDougal

7.2.2 Morphology

Passiflora species exhibit a highly diversified morphology. Passionflowers are in general lianas, vines or herbs, mostly climbing with woody or herbaceous stems and axillary tendrils. Some species are arborescent and are regrouped in subgenus *Astrophea*. The stem can be cylindrical, angular or even winged. All species present stipules, sometimes deciduous, differing widely in size and shape. Leaves are alternate, simple, entire, lobed or palmate; rarely compound, such as in *P. cirrhiflora* Juss., *P. deidamioides* Harms, *P. pedata* L. and *P. trifoliata* Cav. The margins of the leaves range from serrate to entire. The extreme diversity of leaf morphology in *Passiflora* has been attributed to the pressure exerted in their coevolution with their main herbivores, the larvae of the Heliconiine butterflies (Gilbert 1982). The genus is also characterized by the presence of extrafloral nectaries that vary in form, position and number. They are common on the leaf petiole and along leaf margins, and can be also found on sepals or bract margins. In subgenus *Decaloba*, they are most often found in the lamina. These extrafloral nectaries (EFN) attract and reward ants that often have a protective role against herbivores, especially butterfly caterpillars, and nectar robbers (Smiley 1986; Apple and Feener 2001; Leal et al. 2006). Most passionflowers have three bracts, rarely less. Bracts can be foliar, elliptic, oblong or oval, persistent or deciduous with entire or dentate margin. The flowers are axillary, regular and hermaphroditic. Only *P. tetrandra* Banks ex DC. is dioecious. The calyx can be patelliform, campanulate or tubular. The perianth is composed of five sepals and five petals often similar in color and shape, and its color varies from flat green to showy violet or red. The most striking floral feature is the corona that is constituted of filaments in one or several series. These can be long, short or fused into a tube, such as in *P. murucuja* L. This character plays an essential role in the attraction and selection of pollinators (Endress 1994; Kay 2003; Yockteng 2003). A circular flower nectary is at the base of the corona. The reproductive organs are borne by an androgynophore holding the five stamens and three carpels, except in some Asiatic species (Krosnick et al. 2006). The ovary has one locule with multiple ovules disposed in three parietal placentas. The fruits are small, purple or blackish indehiscent berries with one or few seeds, commonly in subgenus *Decaloba,* or yellow or orange to red capsules with many seeds, mostly edible and measuring up to 30 cm long, commonly in subgenus *Passiflora*.

7.2.3 Cytology

Cytological data are essential to study the relationship between wild and cultivated species and to plan out interspecific hybridizations for future breeding programs.

Until now, chromosome counts have been reported for 94 *Passiflora* species (Table 7.1). The most common chromosome numbers are $2n = 12$, $2n = 18$ and $2n = 24$, which correspond roughly to the three main subgenera. The most common chromosome numbers of subgenera *Passiflora* and *Decaloba* are $2n = 18$ and $2n = 12$, respectively. Few species of subgenus *Astrophea* have been examined but the results indicate that the predominant number in the group is $2n = 24$. Divergent hypotheses about the base chromosome number of the genus have been proposed. A recent study based on a phylogenetic analysis proposed $x = 12$ as the base number (Hansen et al. 2006). Karyotype analyses have shown that several characters (number and position of satellites, number and length of chromosomes and the position of the

Table 7.1 Chromosome numbers of *Passiflora* species[a]

Taxa	Number of chromosomes
Supersection *Astrophea*	
P. lindeniana Triana & Planchón; *P. pentagona* (Cervi) Killip, 5S = 2 45S = 4	$n = 12$
P. pittieri Mast.; *P. haematostigma* Mart. Ex Mast.- *P. pentagona* (Harms) Killip	$2n = 24$
Supersection *Decaloba*	
P. adenopoda DC.; *P. biflora* Lam.; *P. aurantia* var. *aurantia* G. Forst.; *P. bryonioides* Kunth ex Spreng.; *P. candollei* Triana & Planch.; *P. capsularis* L. 5S = 2; 45S = 2; *P. capsularis* var. *acutiflora* Mast.; *P. cinnabarina* Lindley; *P. citrina* MacDougal; *P. cobanensis* Killip; *P. conzattiana* Killip; *P. coriacea* Juss.; *P. costaricensis* Killip; *P. cubensis* Urban; *P. dioscoreaefolia* Killip; *P. escobariana* MacDougal; *P. gilbertiana* MacDougal; *P. gracilis* J. Jacq. Ex Link.; *P. herbertiana* Lindley; *P. juliana* MacDougal; *P. karwinskii* Mast.; P. misera Kunth, 5S = 2. 45S = 4; *P. morifolia* Mast., 5S = 2 45S = 2; *P. nubicola* MacDougal; *P. oaxacensis* MacDougal; *P. obtusifolia* Sessé & Mociño; *P. penduliflora* Bertero; *P. perfoliata* L.; *P. porphyretica* Mast. var. *porphyretica*; *P. pterocarpa* MacDougal; *P. pulchella* Kunth; *P. quinquangularis* Calderón; *P. rovirosae* Killip; *P. rubra* L., 5S = 2 45S = 2; *P. sanguinolenta* Mast. & Linden; P. sexflora Jusssubsp. *itzensis* MacDougal ; *P. standleyi* Killip; *P. suberosa* L.; *P. tricuspis* Mast., 5S = 2 45S = 4; *P. warmingii* Mast.; *P. xiikzodz; P. xiikzodz* subsp. *xiikzodz* MacDougal	$n = 6$; $2n = 12$
P. holosericea L.; P. lobata (Killip) Hutch. Ex. MacDougal	$2n = 14$
P. gracilis J. Jacq. Ex Link.; *P. microstipula* Gilbert & MacDougal	$2n = 18$
P. gracilis J. Jacq. Ex Link	$2n = 20$
P. exsudans Zucc.; *P. lutea* L.; *P. punctata* L.; *P. suberosa* L., 5S = 4, 45S = 10, 1C = 1.85; *P. tenuiloba* Engelm.	$n = 12$; $2n = 24$
P. misera Kunth, 5S = 6, 45S = 12; *P. suberosa* L.	$n = 18$; $2n = 36$
P. lutea L.	$2n = 84$
Supersection *Passiflora*	
P. actinia Hook, 5S = 2 45S = 6; *P. alata* Curtis, 5S = 2 45S = 4; *P. amethystina* Mikan, 5S = 2 45S = 6; *P. antioquiensis* Karst.1C = 1.5 4, 2C = 3.0 4; *P. caerulea* L., 1C = 1.45 2C = 2.9; *P. cincinnata* Mast., 5S = 2 45S = 4; *P. coccinea* Aubl.; *P. edmundoi* Sacco, 2C = 3.43, 5S = 2 45S = 6; *P. edulis* Sims; *P. edulis* fo. *flavicarpa* Sims. Deg., 2C = 3.19, 2C = 3.2, 5S = 2 45S = 4; *P. edulis* Sims; *P. edulis* Sims fo. *edulis*, 2C = 3.16; *P. elegans* Mast.; *P. foetida* L.; *P. galbana* Mast.2C = 3.52 6, 5S = 2 45S = 6; *P. gibertii* Brown, 2C = 3.92; *P. glandulosa* Cav., 5S = 2 45S = 6; *P. incarnata* L.; *P. jilekii* Wawra; *P. kermesina* Link & Otto; *P. laurifolia* L., 2C = 3.88, 5S = 2 45S = 4; *P. ligularis* Juss.; *P. magnifica* L. K. Escobar; *P. malacophylla* Mast.; *P. maliformis* L., 2C = 3.78; *P. manicata* (Juss.) Pers.; *P. mixta* L. f.; *P. mucronata* Lam. 2C = 3.4, 5S = 2 45S = 6; *P. nitida* Kunth 2C = 4.82; *P. quadrangularis* L., 2C = 5.36; *P. racemosa* Brot.; *P. riparia* Mart. ex Mast.; *P. seemanii* Griseb.; *P. serratodigitata* L.; *P. serrulata* Jacq.; *P. setacea* DC.; *P. subpeltata* Ortega; *P. tarminiana* Coppens & Barney; *P. tripartita* (Juss.) Poir.; *P. tripartita* var. *mollissima* (Kunth) Holm-Niels. & Jørg.; *P. trisulca* Mast.; *P. umbilicata* (Griseb.) Harms; *P. vitifolia* Kunth	$n = 9$; $2n = 18$
P. incarnata L.	$n = 18$; $2n = 36$
P. foetida L. 5S = 4 45S = 6; *P. foetida* var. *gossypifolia* (Desv.) Mast.	$n = 10$; $2n = 20$
P. foetida L.	$2n = 22$
P. subpeltata	$2n = 72$

[a]Chromosome numbers (Heitz 1926; Nakajima 1931; Simonet and Miedzyrzecki 1932; Nishiyama and Kondo 1942; Baldwin 1949; Bowden 1940; 1945; Darlington and Janaki Ammal 1945; Storey 1950; La Cour 1951; Simmonds 1954; Beckett 1960; Heiser 1963; Lloyd 1963; Harvey 1966; Beal 1969a, 1969b, 1971, 1973; Diers 1961; MacDougal 1983, 1994; Gill et al. 1984; Guerra 1986; Berry 1987; Lepper and Duharte Gongora 1988; Turner and Zhao 1992; Snow and Macdougal 1993; Oliveira and Coleman 1996; Barbosa and Vieira 1997; Soares-Scott 1998; Gilbert and MacDougal 2000; Melo et al. 2001; Olaya Arias et al. 2002; Melo and Guerra 2003; Souza et al. 2003a, 2003b; Yockteng 2003). Nuclear content (2C or 1C) (Souza et al. 2004; Zonneveld et al. 2005; Souza et al. 2008) and number of sites 45S and 5S of rDNA (Melo and Guerra 2003) are also indicated

centromere) are specific to each subgenus (Snow and Macdougal 1993). The majority of species studied so far are diploid, but some species display various ploidy levels such as *P. misera* Kunth. with $2n = 12$, 36, *P suberosa* L. with $2n = 12$, 24 and 36, *P. subpeltata* Ortega with $2n = 18$, 72 and *P. incarnata* with

$2n = 18, 36$. Tetraploid hybrids ($2n = 36$) have also been obtained from somatic hybridization of diploid species ($2n = 18$) (Soares-Scott et al. 2005). However, the main commercial species and horticultural hybrids, obtained through sexual hybridization, are in general diploids (Vieira and Carneiro 2004).

Genome size values (2C) obtained for 10 diploid species of subgenus *Passiflora* range from 3.16 to 5.36 pg while the genome size of a tetraploid species of subgenus *Decaloba* is 1.83. Data of genome size can be useful in the assessment of somatic and sexual compatibility in *Passiflora* species. Further studies are, therefore, needed to increase data of genome size in *Passiflora*.

Examining the sites of 5S and 45S of rDNA, Melo and Guerra (2003) observed that species with $x = 6$ present only two 5S sites and two or four 45S sites whereas other species with $x = 9$ or 10 display more than two sites for both 45S and 5S (Table 7.1). These data suggest a diploid origin for the species with $x = 6$ and a polyploid origin for genomes with $x = 9$ or 10. In addition, the numbers of 45S and 5S sites should correspond to the ploidy level of the individual analyzed. In fact, they allow differentiating the two cytotypes of *P. misera* in which the number of 5S and 45S is three times larger in the hexaploid cytotype than in the diploid cytotype. In addition, interspecific hybrids derived from species of supersection *Passiflora* can be differentiated by their number of 45S rDNA sites (Melo and Guerra 2003).

Wild and cultivated *Passiflora* species generally present meiotic stability (Barbosa and Vieira 1997; Melo et al. 2001; Souza et al. 2003b). In contrast, hybrids exhibit meiotic instability, but sexual hybrids seem more stable than somatic ones (obtained by protoplast fusion) (Soares-Scott et al. 2003). Pollen viability in cultivated species is 80%, whilst in wild species it varies from 78.2% to 99.5% (Barbosa and Vieira 1997; Souza 2002).

7.2.4 Agricultural Status

Since prehistoric times, people have consumed fruits of *Passiflora* species. Passionfruits are rich in minerals, alkaloids, flavonoids, caretonoids and vitamins A and C, indicating their nutritional quality. More than 80 *Passiflora* species produce edible fruits (Coppens d'Eeckenbrugge 2003). About half of these species belong to subgenus *Decaloba* and their fruits are generally small, rarely more than 1.5 cm in diameter, so they are only collected on wild plants in rural areas. Although fruits produced by subgenus *Decaloba* are rich in qualitative terms, their potential for the development of economic fruit crops is very limited. Species with larger fruits belong to subgenus *Passiflora*. More precisely, species of economic potential for fruit production belong to supersections *Passiflora*, *Laurifolia* and *Tacsonia* (Fig. 7.2). Table 7.2 provides a list of 48 species of which more than 20 have been cultivated as fruit crops at very different scales.

Although passionfruits were widely known and grown by Native Americans (Patiño 1963), their development into commercial crops is fairly recent. *Passiflora edulis* f. *flavicarpa* (Fig. 7.3) was long overshadowed by the purple maracuja, published as a distinct taxon only in 1932 in the Flora of Hawaii, as an introduction from Australia (Degener 1932). For this country, Winks et al. (1988) mention that *P. edulis* was introduced in 1861; however, commercial development started only 60–70 years later, the yellow form and its hybrids becoming important only in the late 1950s. In 1951, Hawaiian plantings of this form amounted to less than five acres, from which a few vines were selected. By 1958, 1,200 acres were devoted to this crop as a basis of a well-established Hawaiian passionfruit juice industry. It was introduced in Venezuela in 1954, and trials took place in the Cauca Valley (Colombia) in 1963 with materials from Hawaii, Brazil and Venezuela (Torres and Giacometti 1963; Morton 1967). Among Andean passionfruits, Castañeda (1956) only mentions sweet granadilla (*P. ligularis* Juss.) as a highly demanded

Fig. 7.2 Diversity of edible fruits of subgenus *Passiflora*. Photo from Geo Coppens d'Eeckenbrugge

Table 7.2 Cultivated species of *Passiflora*[a]

Species	Cultivation	Uses	Origin	Common vernacular names	Close relatives with potential
Supersection *Passiflora*					
Series *Passiflora*					
P. bahiensis	Possibly tolerated	Fresh fruits, drinks	NE Brazil		
P. cincinnata	Garden ornamental with edible fruit	Fresh fruit, drinks	Bolivia, S Brazil, Paraguay	Pachio del monte	
P. edulis f. flavicarpa	Pantropical fruit crop	Fruit juice, drinks, medicinal	Brazil, Paraguay, N Argentina	Yellow passionfruit, maracuja	
P. edulis f. edulis	Tropical highland fruit crop	Fresh fruit, drinks	S Brazil, Paraguay, N Argentina	Purple passionfruit, purple maracuja	
P. incarnata	Ancient native crop of N America; ornamental	Desserts, drinks, vegetable, garden ornamental	E North America (USA and N Mexico)	Maypop, may-apple	
Supersection *Laurifolia*					
Series *Laurifoliae*					*P. acuminata, P. odontophylla, P. capparidifolia, P. crenata, P. cerasina, P. rufostipulata, P. pergrandis, P. tolimana, P. chaparensis*
P. ambigua	Sporadically in native countries	Fresh fruit	From S Mexico to Ecuador and Brazil	Granadilla de monte	
P. laurifolia	Home gardens – West Indies	Fresh fruit, drinks	Tropical South America	Golden apple, water lemon	
P. nigradenia	Occasionally in native countries	Fresh fruit	Bolivia, Peru	Pachio amarillo	
P. nitida	Small commercial plots dispersed in Panama and South America	Fresh fruit	Panama, Colombia, Venezuela, French Guiana, Brazil and E Peru	Bell apple, maracujá de cheiro	
P. popenovii	Small commercial plots in S Ecuador and S Colombia	Fresh fruit	Ecuador	Curubejo, granadilla de Quijos	
P. riparia	Occasionally in native countries	Mature fruits eaten raw	Amazon	Chinchorcon	
Series *Quadrangulares*					*P. trialata*
P. alata	Cultivated commercially in southern Brazil, sporadically in other South American countries	Fresh fruit, desserts, drinks	Amazon and riverine forests of Central Brazil	Winged-stem passionflower, sweet maracuja	
P. quadrangularis	Minor commercial crop in South and Central America; tropical home gardens	Fresh fruit, desserts, drinks, candied mesocarp	Uncertain, probably N South America	Barbadine, giant granadilla	

(*continued*)

Table 7.2 (continued)

Species	Cultivation	Uses	Origin	Common vernacular names	Close relatives with potential
Series *Tillifoliae*					
P. ligularis	Tropical highlands of Central and South America	Fresh fruit	Mexico to Bolivia	Sweet granadilla	*P. danielli, P. fieldiana, P. magnifica, P. seemannii , P. palenquensis,*
P. maliformis	Small commercial cultivation in Colombia, home gardens in other countries	Fruit drinks	N Andes	Stone granadilla, couch apple, sweet calabash, water lemon	*P. triloba, P. serrulata, P. multiformis, P. platyloba*
P. tiliifolia	Rarely cultivated, in home gardens	Fruits eaten raw, drinks	Colombia, Ecuador, Peru	Machimbi, granadilla tripona	
Supersection *Stipulata*					
P. caerulea	Ornamental, under subtropical and Mediterranean climates	Bright red edible pulp, scarce and insipid	S Brazil, Argentina, Paraguay	Blue passionflower	
Supersection *Coccinea*					
P. coccinea	Rarely cultivated, in Guyana and Guadeloupe, as ornamental plant	Edible fruit	Amazonia	Scarlet passionflower	*P. quadriglandulosa, P. speciosa*
P. glandulosa	Rarely cultivated, in Guyana and Guadeloupe, as ornamental plant	Edible fruit	Amazonia	Maracuja cabeza de gado, markoesa	
P. vitifolia	Rarely cultivated, in Guyana and Guadeloupe, as ornamental plant	Edible fruit	From Honduras to NW South America	Cuchubao, curuvito	
Supersection *Tacsonia*					*P. ampullacea, P. mandonii*
P. antioquiensis	Becoming rare in home gardens in Colombia	Fresh fruit, medicinal	Colombia	Red banana passion-flower, curuba antioqueña	
P. cumbalensis	Cultivated around Bogotá, Colombia	Juice, drinks, sherbets	Andes (2,000–3,000 m) from N Colombia to C Peru	Rosy passionfruit, tacso, curuba roja	
P. mixta	Rarely cultivated, in home gardens	Juice, drinks, sherbets	Andes (2,500–3,600 m)	Curuba de indio	

P. pinnatistipula	Home gardens in the Andes	Fresh fruit	Andes (2,500–3,800 m) from Colombia to N Chile	Mountain granadilla, coldland granadilla, gulupa
P. tarminiana	Commercial and home gardens in native countries	Juice, drinks, sherbets	In high altitude in NW South America	Banana passionfruit, banana poka
P. tripartita var. azuayensis	Rarely, in home gardens of Ecuador	Juice, drinks, sherbets	Andes of Ecuador (2,600–3,300 m)	Tacso
P. tripartita var. mollissima	Commercial and home gardens in native countries	Juice, drinks, sherbets	Andes (2,000–3,200 m) from Venezuela to Bolivia	Yellow banana passionfruit, yellow curuba
P. tripartita var. tripartita	Rarely, in home gardens of Ecuador	Juice, drinks, sherbets	Andes of Ecuador (2,600–3,300 m)	Tacso

[a]This list was produced following the classification of Feuillet and MacDougal (2003). Species presenting the same potential are also listed in relation to the affinity to cultivated species

Fig. 7.3 *Passiflora edulis* Sims. f. *flavicarpa* Deg. Photo from Geo Coppens d'Eeckenbrugge

Fig. 7.4 *Passiflora edulis* f. *edulis* Sims. Photo from Geo Coppens d'Eeckenbrugge

Fig. 7.5 *Passiflora ligularis* Juss. Photo from Geo Coppens d'Eeckenbrugge

fruit while the banana passionfruit (*P. tripartita* var. *mollissima* (Kunth) Holm-Nielsen and Jørgensen) is only mentioned for its organoleptic qualities and its absence from two of the three Colombian cordilleras. The first experiment for the commercial cultivation of this species was reported by Jaramillo (1957).

Nowadays, 15 species are cultivated and a dozen are commercialized. The maracuja, *P. edulis* Sims, is the most important one. Its yellow form is cultivated in most tropical lowlands, particularly in tropical South America. Brazil is by far the main producer and consumer, with about 70% (half a million tons) of the world production. Ecuador is the main provider for the international market of passionfruit concentrate (Linden 2007). Colombia, the third main producer, has an appreciable home market and its contribution to international trade is variable. The purple form of *P. edulis*, *P. edulis* f. *edulis* (Fig. 7.4), originating from higher latitudes in southern South America, is confined to cooler climates. It has shown adaptation to tropical highlands, particularly in the Andes and in eastern Africa, as well as to subtropical climates, in Australia. The purple maracuja is present in small quantities on the international market, mostly provided by East African countries. While the yellow maracuja appears to be a cultigen, the purple form still exists in the wild in its birth place. Interestingly, the closest relative of *P. edulis* is *P. incarnata*, which was a common fruit crop of North America in Pre-Columbian times. The sweet granadilla, *P. ligularis* (Fig. 7.5), is cultivated for the fresh fruit market in the northeastern Andes, at moderate altitudes, and in Central America. Colombia is the main producer and consumer, and regularly provides small quantities at high price for the international market. The banana passionfruits, or curubas, are also widely cultivated in the Andes, above 2,000 m, and consumed in juices, sherbets and pastries. They mostly correspond to two cultigens, *P. tripartita* var. *mollisssima* (Fig. 7.6) and *P. tarminiana* Coppens and Barney. *Passiflora cumbalensis* (H. Karst.) Harms is commercialized at a very small scale around the city of Bogota in Colombia (Fig. 7.7). The ancient cultivation in home gardens of *P. antioquiensis* Karst. has been abandoned. Similarly, *P. pinnatistipula* (Harms) Killip is losing ground in High-Andean home gardens. The giant granadilla, *P. quadrangularis* L. (Fig. 7.8), is a cultigen present on the national markets in all tropical America. A close

Fig. 7.6 *Passiflora tripartita var. mollissima* (Kunth) Holm-Niels. & Jørg. Photo from Geo Coppens d'Eeckenbrugge

Fig. 7.7 *Passiflora cumbalensis* (H. Karst.) Harms. Photo from Geo Coppens d'Eeckenbrugge

relative, *P. alata* Curtis, has been recently selected and developed in Brazil, for the fresh fruit market (Kavati et al. 1998). Among the section *Laurifoliae* of subgenus *Passiflora*, *P. popenovii* Killip (Colombia, Ecuador) (Fig. 7.9), *P. nitida* Kunth (northern South America) and *P. riparia* Mart. ex Mast. (Amazon) are sold in local markets. *Passiflora nitida* is often confused with *P. laurifolia* L., which occurs wild in

Fig. 7.8 *Passiflora quadrangularis* L. Photo from Geo Coppens d'Eeckenbrugge

Fig. 7.9 *Passiflora popenovii* Killip. Photo from Geo Coppens d'Eeckenbrugge

northeastern South America, and is still, albeit rarely, cultivated in the Antilles.

The peculiar beauty of their flowers has made passionflowers particularly interesting for the industry of ornamental plants. The development of the ornamental potential of passionflowers is still confined to circles of amateurs, who have developed a number of interspecific hybrids. The earliest was obtained by Thomas Milne in 1819. Since then, more than 400 hybrids have been generated to obtain more showy flowers (Peixoto 2005). Breeders have also used tetraploidization to obtain larger flowers (Fischer 2004). This spectacular diversity of shapes and colors has not been translated into commercial success, mostly because potential market is essentially concentrated at high latitudes. Indeed, as a garden ornamental, the most successful species is *P. caerulea* L. Its winter hardiness has allowed it to take a relative importance under mild temperate climates. Thus, much remains to be done to develop passionflowers for the tropical garden (Abreu et al. 2009).

Table 7.3 Theraupetic properties of *Passiflora* species[a]

Species	Theraupetic properties
P. alata	Analgesic (M), anthelmintic, antiashmatic, antibacterial, anticancer, antidiarrheal, antifungal, antioxidant , antiviral, anxiety (R), burn, cough, diabetes, diuretic, emetic, emmenagogues, fever, hemorrhoids, hypertension, hysteria, insomnia, internal inflammation (M), maintenance of natural killer (NK) cell activity, morphine deaddiction, sedative (M), seizure, skin inflammation (Iv), venom antidote
P. actinia	Anxiety (R), sedative (M)
P. caerulea	Anthelmintic, antibacterial, diuretic, sedative, seizure
P. clathrata Mast.	Sedative
P. capsularis	Emmenagogues
P. contrayerva Sm.	Venom antidote
P. coriacea	Antioxidant (Iv)
P. edulis	Analgesic, anthelmintics, antibacterial (Iv), anticancer (Iv), antidiarrheal, antifungal (Iv), antiviral (Iv), anxiety (R), diuretic, hypertension, internal inflammation (M), sedative, skin inflammation (M)
P. edulis var. flavicarpa	Anticancer (Iv), internal inflammation (M)
P. foetida	Analgesic, anthelmintics, antibacterial (Iv), anticancer (Iv), anxiety (H), cough, emetic, emmenagogues, fever, hysteria, insomnia, skin inflammation
P. incarnata	Anthelmintics, antiashmatic, anxiety, burn, diarrhea, dysmenorrheal, hemorrhoids, hysteria, insomnia, maintenance of natural killer cell activity (R), morphine deaddiction, neuralgia, sedative, seizure (M)
P. laurifolia	Venom antidote (Iv)
P. maliformis	Analgesic, fever, hypertension
P. mexicana Juss.	Sedative
P. mixta (and *P. tripartita*)	Antiseptic for ulcers, diuretic, febrifuge, hepatic and bilious affections, "intestinal fevers"
P. mucronata	Anthelmintic
P. nitida	Antibacterial (Iv), antioxidant (Iv)
P. palmeri Rose	Antibacterial (Iv), antioxidant (Iv)
P. pedunculata Hort. ex Mast.	Venom antidote
P. pentagona	Anthelmintic
P. quadrangularis	Anxiety (R), diabetes, hypertension
P. salvadorensis (Donn. Sm.) MacDougal	Diuretic
P. sexflora	Venom antidote
P. suberosa	Skin inflammation
P. tenuifila	Antioxidant (Iv)
P. trifoliata	Antiseptic, sedative
P. vitifolia	Venom antidote

[a]Properties according to Uphof (1968), Girault (1984), Braga and Junqueira (2000), Dhawan et al. (2004), and others cited in Sect. 7.7.3. Some properties have been tested in mice (M), in rats (R), in humans (H) or In vitro (Iv).

Passionflowers are also known for their medicinal properties (Table 7.3). In particular, *P. incarnata* is exploited in the pharmaceutical industry for its sedative, antispasmodic and analgesic properties (McGuire 1999; Dhawan et al. 2004).

Several common species of *Passiflora* have become weeds, as is the case of *P. capsularis* L., in the coffee growing zone of Colombia. Some have extended their range to other continents early after the great voyages of the XVI and XVII centuries, and some are even considered invasive. *Passiflora foetida* L. and *P. suberosa* have become pantropical weeds, for upland rice and other field crops. Nevertheless, the competitive ability of *P. foetida* has been used to control erosion and the grass *Imperata cylindrica* in coconut fields in Philippines and in sweet potato fields in Papua New

Guinea, while their young leaves are used in Surinam and Java as a vegetable (Waterhouse 1994). Several species of supersection *Tacsonia* have naturalized in New Zealand (Heenan and Sykes 2003). The most surprising case is that of *P. tarminiana,* a cultigen showing limited potential of escaping cultivation in the northern Andes, but invading protected areas in Hawaii, New Zealand and Australia. The considerable impact of *P. tarminiana* on native floras generated prevention against the introduction of passionflowers in many tropical countries. The problem of invasive passionflowers is treated more thoroughly in Sect. 7.9.

7.3 Conservation Initiatives

7.3.1 Evaluation of Genetic Erosion

Passiflora species are essential components of natural and semi-natural habitats and are critical to maintain ecosystem health. Their conservation and sustainable use are vital for improving their agricultural production and preserving the environment. Furthermore, the disappearance of *Passiflora* species from the ecosystem would entail the loss of other organisms depending on them, such as the butterflies *Heliconius* and many nectar feeding insects and birds.

Characterization and evaluation of the genetic diversity of wild and cultivated *Passiflora* species are necessary to identify and prevent genetic erosion. The genetic diversity of different *Passiflora* species has been studied using various molecular tools as isoenzymes (Segura et al. 1998, 2003b, 2005; Fore and Spira 2002; Tague and Fore 2005), random amplified polymorphic DNA (RAPD; Fajardo et al. 1998, Aukar et al. 2002; Crochemore et al. 2003; Viana et al. 2003; Bellon et al. 2007; Junqueira et al. 2007), cpDNA (Sanchez et al. 1999), amplified fragment length polymorphisms (AFLPs; Segura et al. 2002; Ocampo et al. 2007) and sequences of chloroplast and nuclear regions (Lorenz-Lemke et al. 2005; Koehler-Santos et al. 2006a, b).

These genetic studies show that wild and cultivated *Passiflora* species have, in general, high intraspecific and interspecific genetic variability. Only one species, *P. elegans* Mast., has been reported to have low genetic variability (Lorenz-Lemke et al. 2005). The genetic diversity of some cultivated and wild species has been compared (Segura et al. 1998, 2002, 2003b, 2005; Bellon et al. 2007). In the group of banana passionfruits, cultigens (*P. tripartita* var. *mollissima* and *P. tarminiana*) show very limited variation, contrasting with the high diversity of wild species (*P. mixta* L. f.) (Segura et al. 1998, 2002, 2003b, 2005). In contrast, Bellon et al. (2007) report no differences in genetic diversity among wild and commercial accessions of *P. edulis*. Ocampo et al. (2007) emphasize a surprisingly high diversity in the same species. However, the susceptibility of cultivated species to many pathogens is a probable indication of genetic erosion (Vieira and Carneiro 2004). Furthermore, human activities can facilitate new encounters between plants and pathogens, causing damage in wild populations (Webster et al. 2007).

There are in situ and ex situ approaches to conserve biodiversity and different ways of using genetic resources. We review the different attempts to conserve germplasm of *Passiflora*.

7.3.2 In Situ Conservation

In situ approaches for the conservation of *Passiflora* species have been mainly limited to protected areas. These measures may be appropriate for particular species, such as those located in sparsely populated rain forests, but they may be insufficient when human pressure is high. In Colombia, Ocampo et al. (2007) showed that most of the genus diversity was particularly concentrated on the hillsides of the coffee growing zone (81% of the total 167 species), i.e., in a highly populated and perturbated region, out of protected areas. Several common species thrive in disturbed habitats, so their distribution even benefits from the human pressure. However, most of the 58 Colombian endemic species are found in these increasingly deforested hillside areas, which puts them at a high risk. Furthermore, the *Passiflora* diversity hotspots correspond strikingly well to particular ecotopes, hence the necessity of integrating biodiversity conservation measures in the general management of agricultural activities, recreational areas and water resources (Ocampo et al. 2007). Applying IUCN criteria, Ocampo et al. (2007) consider that 71% of the Colombian Passifloraceae are under some degree of threat, 10% being critically endangered, 6.1% vulnerable and

only 16% of the species are placed in the two "least concern" and "near threatened" categories, indicating the necessity of establishing conservation measures, at least in this country.

A proper documentation of *Passiflora* genetic diversity in relation with the geographic distribution of species is essential. Jørgensen et al. (2009), from the Missouri Botanical Garden (USA), have conducted a study to establish the geographical distribution of *Passiflora* species based on herbarium data and subsequently estimated their threat status. Preliminary results focused on subgenus *Decaloba*, Jørgensen (2009) found that 40% of these species are vulnerable or endangered, while another 40% are not currently threatened.

For a same level of habitat fragmentation or perturbation, the erosion risk varies according to several factors, such as the situation of the species in the ecological succession, its reproductive biology and its dependence on particular pollinators and dispersors. For instance, high genetic diversity at the local level with little differentiation among regions in *P. incarnata*, an early successional species, suggests that this genetic structure is related to founder effects and long-distance pollen-mediated gene flow (Fore and Spira 2002; Tague and Fore 2005). This situation contrasts with that of *P. mixta*, a similar weed that is affected by the fragmentation of the habitat of its pollinator, the hummingbird *Ensifera ensifera* (Lindberg and Olesen 2001).

7.3.3 Ex Situ Conservation

Conservation of *Passiflora* genetic resources out of their natural habitat is mainly done in botanical gardens and germplasm collections, maintained by national institutes. More than 50 *Passiflora* germplasm collections are maintained around the world (Ferreira 2005) and the *Passiflora* accessions in collections have increased in recent years, from 524 accessions in 1994 to 1,235 in 2004 (Ferreira 2005).

Figure 7.10 illustrates the information available of *Passiflora* germplasm obtained from several databases. A total of 2,203 *Passiflora* accessions are currently reported for a total of 159 species; 19% of these accessions correspond to the widely cultivated *P. edulis* and 24.7% are unclassified. When compared to other collections of crop genetic resources, the proportion of wild species is much more important (28.3%). The global effort may appear important; however, it must be related to the number of species, and also to their potential usefulness for breeding. Thus, Fig. 7.10 should be taken with caution. Many accessions are usually exchanged among collections, so duplication may be relatively important. While this is positive in terms of safety, the genetic diversity may be less important than suggested by the numbers.

The living plant collections of botanical gardens represent a significant source of conserved genetic resources. For instance, the Royal Botanic Gardens of Kew (UK) holds in their greenhouses 113 living passionflowers representing 72 different species and 21 hybrids or ornamental varieties. The Missouri Botanical Garden conserves 24 individuals of 15 species and 2 hybrids. In vivo plant collections with a scientific research purpose are also maintained by academic institutions, such as the Lawrence Gilbert's laboratory at University of Texas in Austin (USA), which maintains 101 species and 30 hybrids (Gilbert 1998). Unexpected sources of ex situ genetic resources of *Passiflora* are the living collections maintained in greenhouses by plant societies and amateurs (Passiflora Society International 2008). Private societies regrouping amateurs are created for the sole purpose of studying, collecting and preserving *Passiflora* seeds and plants. Many members of these societies have large in vivo collections with more than a hundred species collected around the world. These societies have also seed banks available for their members. In France, the collection maintained by C. Houel, including 359 accessions from 241 species, has received the status of national collection (Houel 2009).

An appropriate documentation of germplasm is indispensable to make this stored diversity useful to everyone. The germplasm collection should be systematically classified and evaluated. For instance, Crochemore et al. (2003) used molecular markers to evaluate the samples of *P. edulis* obtained from the germplasm collection of the Instituto Agronômico do Paraná (IAPAR) in Brazil, facilitating by this way the identification of misclassified samples and the classification of unidentified ones. In addition, it is necessary to continuously increase the genetic variability of germplasm collection. This initiative requires identification of the most suitable populations. For instance, genetic studies in banana passionfruits showed that it would be preferable to collect samples of *P. tripartita*

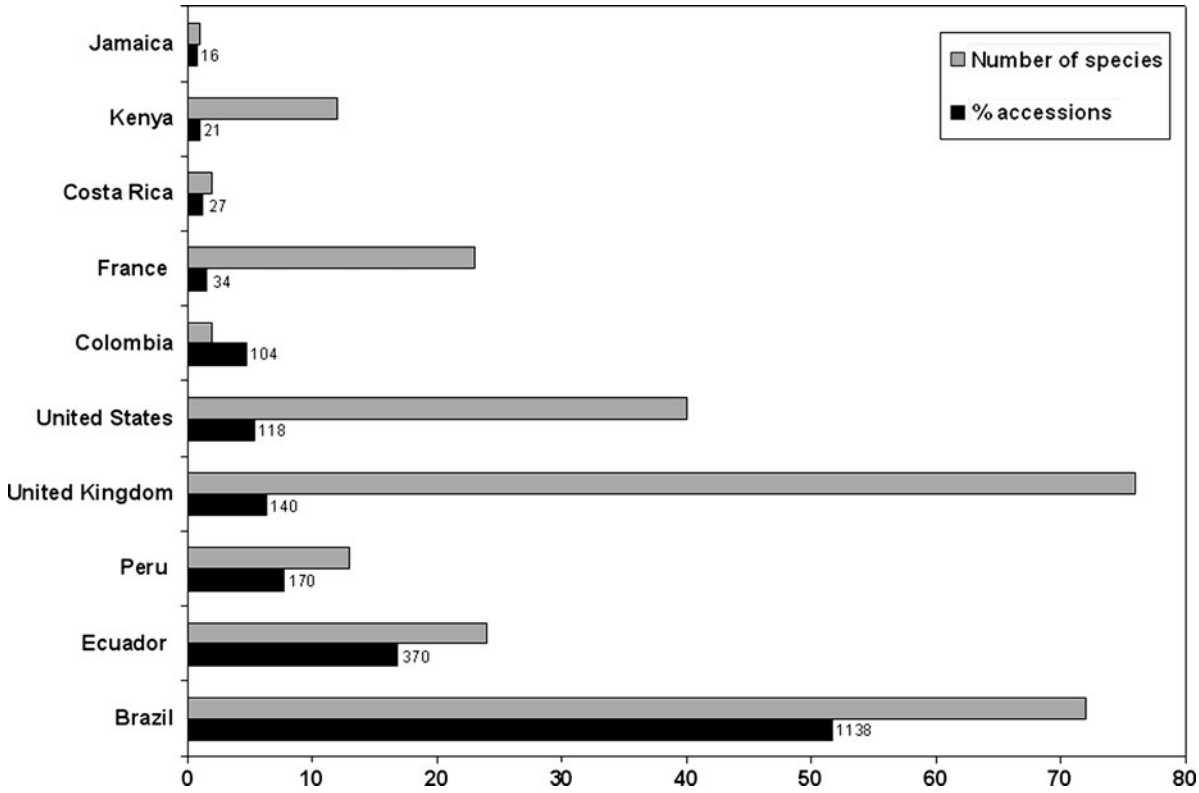

Fig. 7.10 Percentage of accessions and number of species represented in germplasm collections in each country. The number of accessions per country is also indicated. The information was obtained from the Germplasm Resources Information Network of the USDA-ARS (GRIN) (USDA 2010), the New World Fruit Database of the Biodiversity International that save information of many ex situ germplasm collections worldwide (Biodiversity International 2009), the Kew Botanical Garden and the Missouri Botanical Garden. The information about Brazil collections have been obtained from Ferreira (2005). Only data from the first ten countries with more abundant collections are illustrated in this chart

var. mollissima in southern populations (Ecuador and Peru) with high genetic variability, rather than in northern populations (Colombia and Venezuela), in order to enlarge the genetic diversity of these species in germplasm banks (Segura et al. 1998, 2002, 2003b, 2005).

7.4 Role in Elucidation of Origin and Evolution of Allied Crop Plants

The evolution of cultivated passionflowers has not been extensively investigated. However, despite the difficulties and inconsistencies in the taxonomic treatment of *Passiflora*, most of them show a close morphological relationship with several wild species, suggesting strong evolutionary affinities. The best approach is to identify the likely crop gene pools around these cultivated species. The closest relatives would be the taxa in the primary gene pool; slightly more distant relatives would constitute the secondary gene pool and so on.

The situation is different for ornamental plants, where vegetative propagation is the normal rule. Many crosses have been attempted according to the amateur creativity and potential genitors in the collection, with some surprising successes between what seem distantly related species. However, most of these hybrids set no fruits or empty fruits.

The present section is essentially devoted to the relatives of established fruit crops, all from subgenus *Passiflora* (Table 7.2). In each case, species exhibiting strong morphological affinities are considered. Elements from molecular studies are added at the end of each section, when available.

7.4.1 *Series* Passiflora *(Supersection* Passiflora*)*

P. edulis f. *flavicarpa* (yellow maracuja), *P. edulis* f. *edulis* (purple maracuja) and *P. incarnata* (maypop) can only be distinguished by a very limited number of morphological traits. They are much more clearly differentiated in their ecology. The first one is a vigorous tropical cultigen producing large yellow fruits. Although its origin is most probably Amazonian or periamazonian, the precise origin of yellow maracuja remains mysterious since it was developed out of South America. According to Vanderplanck (1991), the first documented cultivation was based on the seeds of a few fruits bought in a London market and sent to Argentina. Their descent would have been sent to the United States Department of Agriculture, and redistributed to Australia, New Zealand and later to Hawaii. This very narrow genetic base was somewhat widened through crosses with *P. edulis* f. *edulis*. The yellow maracuja shows strong self-incompatibility, even if a few pseudo-self-compatible clonal cultivars were selected in the 1950s. When reintroduced into South America, these selections were newly in contact with their natural pollinators and propagated again through seeds; so this trait was lost. Many cultivated populations still show the segregation for fruit color testifying that the yellow form was once crossed with the purple form. In contrast with our ignorance for the yellow maracuja, the origin of *P. edulis* f. *edulis* is clearly in southern South America, where it is still commonly found growing spontaneously, under subtropical conditions. Its adaptation to cool conditions allowed it to naturalize in other subtropical areas as well as in tropical highlands. The purple maracuja produces a fruit smaller than the yellow one, and appears at least partially self-fertile. The cross between the two forms of *P. edulis* is only fertile when the yellow form is used as the male genitor. Hybrids are intermediate and vigorous (Nakasone et al. 1967) with a regular meiosis, but have a lower chiasma frequency (Beal 1975).

The maypop was a common North American fruit crop in Pre-Columbian times. However, it is no more cultivated commercially, except for ornamental or pharmaceutical purposes. Crosses with *P. edulis* have shown limited success. Several authors report the obtention of fertile hybrids (Beal 1972; Anderson 1976; Winks et al. 1988), with more fertile F_2 and backcross progenies (Kajewski 1941) while others had to double their hybrid genome to restore fertility (Knight 1991). Thus, independent of the strong affinity between the two species, *P. incarnata* must be considered to be part of the secondary or tertiary gene pool of *P. edulis*. Similarly, *P. edulis* has been crossed with *P. cincinnata* Mast., with variable success (Howell 1989). The hybrid of Howell did not flower, while those obtained by Ruberté-Torres and Martin (1974) were intermediate and produced an edible fruit. Afterwards, species of different supersections, such as *P. caerulea* and *P. manicata* (Juss.) Pers., have also been crossed successfully with *P. incarnata* or *P. edulis* (Escobar 1985; Vanderplanck 1991) showing that morphological and genetic affinity are poor predictors of crossability in *Passiflora*.

7.4.2 *Series* Tiliifolia *(Supersection* Laurifolia*)*

This series includes two groups of species showing particularly large morphological and genetic similarity. The first one involves the cultivated *P. ligularis*, as well as the wild *P. tiliifolia* L. (Fig. 7.11) that can be often observed in close vicinity, in the same highland conditions. Despite its striking resemblance with *P. ligularis*, *P. tiliifolia* is rarely cultivated. Other very similar species are *P. palenquensis* Holm-Niels. & Lawesson, and *P. fieldiana*, which grow under very different ecological conditions (low to mid elevations). A close relative (*P.* af. *palenquensis*) has been observed under cultivation in the Pacific lowlands of

Fig. 7.11 *Passiflora tiliifolia* L. Photo from John Ocampo

Fig. 7.12 *Passiflora maliformis* L. Photo from John Ocampo

western Colombia. All these species produce fruits that are round to ovoid, tapering at apex, with a thin, smooth and brittle epicarp and sweet grayish arils, easy to consume in hand. Hybrids have not been reported between these species.

The second group involves the stone granadilla, *P. maliformis* (Fig. 7.12), and its close relatives, *P. platyloba*, *P. serrulata* Jacq. and *P. multiformis* Jacq. All of them exhibit yellow stipules, a same fruit shape and similar habit and habitat. In fact, intraspecific variation in these four species is so wide, as compared to presumed interspecific variation, that species delimitation should be re-examined objectively, on both morphological and genetic grounds. The only morphological trait that discriminates them is leaf lobation, but it may be highly variable within many species. The two groups are slightly differentiated in the molecular study of Yockteng and Nadot (2004a). Amplified fragment length polymorphism (AFLP) markers show a close affinity between *P. ligularis* and *P. tiliifolia*, but no particular affinity with *P. maliformis* (Segura et al. 2002; Ocampo et al. 2007).

7.4.3 *Series* Laurifoliae *(Supersection* Laurifolia*)*

The morphological similarity prevailing within this series made the identification and classification of species particularly difficult (Killip 1938). Several species may coexist in the same region such as *P. nitida*, *P. laurifolia*, *P. crenata* Feuillet & Cremers and *P. cerasina* Annonay & Feuillet in the lowland forests of French Guiana or *P. nitida*, *P. nigradenia* Rusby, *P. riparia*, *P. fernandezii* Escobar, *P. chaparensis* Vásquez and *P. venusta* Vásquez & Delanoy in Bolivia (Vásquez et al. 2007). The origin of *P. laurifolia* populations cultivated in the Antilles has to be clarified, and their relationship to wild conspecific materials from South America. In the northern South America, the most commonly cultivated *Laurifoliae* species is *P. nitida*, while *P. laurifolia* seems to occur only in the wild in the Guianas. No wild representative of *P. popenovii* has been reported, and the species is so difficult to propagate through seeds that vegetative propagation is often preferred. Its vernacular name "granadilla de Quijos" refers to an ancient Native American culture, and also a province of Ecuador, where it may have its origin (Patiño 1963). The situation is different for *P. nigradenia*, which is still found in both wild and cultivated conditions in Bolivia (Vásquez 1998).

The species of series *Laurifoliae* present a particular high potential. Indeed, they not only produce a highly aromatic fruit pulp, but also they exhibit at the same time a high genetic diversity and a high uniformity in most essential traits, so they could allow the breeder to create original and adapted cultivars without facing the problems of an excessive genetic segregation. However, their taxonomy should be first clarified, delineating species on an objective basis and establishing their affinities. The exceptional capacity to grow on flooded soils and their resistance to soil parasites is of outmost interest for developing rootstocks or transferring the corresponding genes to other passionfruit species. However, these species have several traits reflecting their lack of domestication. Their seeds tend to germinate very irregularly, often with marked latency, and they often allocate too many resources to vegetative growth. Thus, they cannot be accommodated on artificial supports, because they could invade other fruits trees in the orchard. In addition, their harvest season is short and impredictable. These problems should be addressed in order to fully develop their economic potential.

7.4.4 *Series* Quadrangulares *(Supersection* Laurifolia*)*

In contrast with the situation prevailing in other series of supersection *Laurifolia*, the high morphological

similarity of *P. alata* and *P. quadrangularis* reflects their close genetic affinity and fertile hybrids are easily obtained (JC de Oliveira personal communication). Their progeny segregates for resistance traits (Oliveira et al. 1996). Both species have been used successfully in crosses with *P. caerulea* (Vanderplanck 1991).

7.4.5 *Supersection* Tacsonia

The supersection *Tacsonia* comprises 61 species, 11 of which are cultivated and known with the name of banana passionfruits. The fruits of *P. tripartita* var. *mollissima* and *P. tarminiana* are present all the year on the Andean markets and a small quantity is even exported to Europe. Their thin leathery pericarp and generous orange, succulent arils ensure the highest pulp yields among passionfruits (around 60% for the two cultigens). *Tacsonia* species are differentiated by their large flowers presenting a long floral tube mostly pollinated by hummingbirds. In most of them, the hypanthium length varies between 7 and 13 cm, restricting the normal access to nectar to only one species, the sword-billed hummingbird, *Ensifera ensifera* (Snow and Snow 1980; Lindberg and Olesen 2001). As for this bird, their native distribution is limited to the Andean mountains between 1,400 and 4,000 m, from Venezuela to Chile.

Although this has not been established systematically, interspecific barriers are much more labile in this supersection as compared to supersection *Passiflora*. Spontaneous hybrids have been relatively frequently reported, involving the cultivated *P. arminiana*, *P. tripartita* var. *mollissima*, *P. pinnatistipula* as well as the wild *P. tripartita*, *P. mixta*, *P. cumbalensis* and *P. antioquiensis* (Killip 1938; Escobar 1981; Geo Coppens d'Eeckenbrugge personal observations). The most distinct species, *P. manicata*, has also been involved in artificial crosses, giving fertile hybrids (Escobar 1985). However, Schoeniger (1986) in an attempt to exploit the intercompatibility of *P. tripartita* var. *mollissima* with *P. mixta* and *P. cumbalensis* for the genetic improvement of the former, observed phenomena of unilateral incompatibility, loss of fertility, seed germination and general viability in advanced hybrid and backcross generations.

Segura et al. (2002, 2003a) have studied the relationships between cultivated species and some of their wild relatives with isozyme and AFLP markers. The four most common species, *P. tarminiana*, *P. cumbalensis*, *P. tripartita* and *P. mixta* were differentiated from the others. In the isozyme study, the accessions of the two latter species showed particular affinity, as they clustered together, following a more geographic than taxonomic pattern. A wider study (Segura et al. 2005) confirmed the existence of a gene flow between *P. mixta* and *P. tripartita*, as well as a higher genetic diversity in Ecuador, as compared to Colombia and Venezuela, two countries where the genetic diversity of the two cultigens appeared very low. The closer genetic affinity between *P. tripartita* and *P. mixta* seems consistent with their closer morphological affinity (Villacis et al. 1998); however, *P. tripartita* var. *mollissima* hybridizes easily with both *P. mixta* and *P. tarminiana*. The observation of meiosis in a few hybrids indicates a better chromosome pairing between the two cultigens than with the wild *P. mixta* (Olaya 2002). However, the two parental phenotypes of the cultigens are recovered in less than four generations in the progenies of their hybrids, suggesting very limited exchanges at the genome level. The nature of interspecific barriers among species of supersection *Tacsonia* is not clear yet.

7.5 Role in Classical and Molecular Genetic Studies

The genetics of species *Passiflora* is mainly known from cultivated species. Therefore, the genetics of wild species is not known except for a few population genetic studies. Data from gene actions, physiological pathways and genetic mapping are only available for the cultivated species *P. edulis*. However, the studies based on crop species constitute a reference for future research in wild species. They can even provide new tools, as could be the case of transferable microsatellite markers.

7.5.1 Population Genetic Studies

Genetics studies conducted in wild and crop *Passiflora* species reveal their genetic diversity, the geographic structure of populations and the relationship among

species. Passionflowers generally present high interspecific and intraspecific genetic variability except for *P*. elegans (Fajardo et al. 1998; Segura et al. 1998, 2002, 2003b, 2005; Sanchez et al. 1999; Aukar et al. 2002; Fore and Spira 2002; Crochemore et al. 2003; Viana et al. 2003; Lorenz-Lemke et al. 2005; Tague and Fore 2005; Bellon et al. 2007; Junqueira et al. 2007).

Species belonging to the same supersections are in general closely related. For instance, *P. elegans* and *P. actinia* of supersection *Stipulata* are close relatives (Lorenz-Lemke et al. 2005), as well as *P. tarminiana*, *P. tripartita* var. *mollissima* and *P. mixta* of supersection *Tacsonia* (Segura et al. 1998, 2003b). The genetic proximity among species has facilitated interspecific hybridization events. Genetic studies also permit to know how historical geological, climatic and ecological conditions have affected the distribution of species. For example, the high intraspecific variability of *P. actinia* is structured along a north–south gradient in Brazil, where northern populations are more diversified than the southern. This study gives evidence of migration of northern populations of *P. actinia* to the south probably caused by a change in temperature and humidity in southern region at the beginning of the Holocene (11,000–10,000 years BP). Populations of *P. elegans* show a low genetic variability not structured geographically, probably as a consequence of severe bottleneck events during Pleistocene glacial stage (Lorenz-Lemke et al. 2005). The genetic variation of banana passionfruit species, *P. tarminiana*, *P. tripartita* var. *mollissima* and *P. mixta,* is structured along a south–north gradient giving evidence that their center of diversity is probably in the southern region of their range (Segura et al. 2005). *Passiflora nitida* also has a high genetic variability with a geographical structure and a higher variation among populations than within populations (Junqueira et al. 2007). The opposite is found in *P. alata* in which the greater variation is found within populations while a geographical structure is absent, possibly due to high levels of gene flow between populations (Koehler-Santos et al. 2006a). Genetic variability among populations of *P. incarnata* is lower than within populations. The strong gene flow between geographically distant populations, enhanced by the combined action of self-incompatibility and long distance pollen transfer by pollinators, explain the low differentiation among *P. incarnata* populations (Tague and Fore 2005).

The study of geographical structure of genetic diversity is essential to prioritize the conservation of genetically diversified areas.

7.5.2 Genetics of Self-Incompatibility

The genetic control of self-incompatibility in *Passiflora* has been studied since 1959 only in cultivated species (Bruckner et al. 2005). In the yellow passionfruit species (*P. edulis* f. *flavicarpa*), self-compatibility is controlled by the combination of a gametophytic and a sporophytic systems (Rego et al. 1999, 2000; Suassuna et al. 2003; Souza et al. 2006). The gametophytic system is regulated by the *S* gene that has multiple alleles. A single allele is expressed in the haploid pollen grain that germinates only in a diploid pistil, which does not express the same allele. The sporophytic system is regulated by a cluster of three genes encoding for proteins with a role in the reception and recognition of pollen (Hiscock and Tabah 2003). Pollen will not germinate on the diploid stigma of a flower that contains either of the two alleles of the sporophyte parent that produced the pollen.

Self-incompatibility of *Passiflora* species facilitates the outcrossing of individuals and promotes the creation of new genotypes. Therefore, it must be taken into account in breeding programs to improve crop species, ensuring sufficient diversity of *S* haplotypes to provide fertilization and good fruit production.

7.5.3 Organellar Inheritance

Besides, mitochondria and chloroplast in *Passiflora* are inherited by different mechanisms. Studies present evidence that mtDNA is maternally inherited in passionflowers, while the inheritance of cpDNA can be maternal, paternal or biparental (Muschner et al. 2006; Hansen et al. 2007). The biparental plastid transmission, suspected by Corriveau and Coleman (1988) on the basis of observations in epifluorescence microscopy, was confirmed by Do et al. (1992) in crosses involving the two forms of *P. edulis*. Only the chloroplasts of *P. edulis* f. *flavicarpa* were found in the

reciprocal hybrids. In a cross with *P. coccinea* Aubl. as female parent, *P. edulis* f. *flavicarpa* also transmitted its chlorotype to the progeny. Mrácek (2005) observed that the plastids of *P. menispermifolia* Kunth. and *P. oerstedii* Mast. are biparentally transmitted. The heteroplasmy of hybrids also permitted to evidence that the progeny's plastome–genome was incompatible with the parental genome of *P. menispermifolia* (Mrácek 2005). The heteroplasmy could cause many problems in phylogenetic inferences based on chloroplast gene sequences. In species of subgenus *Passiflora*, the cpDNA is reported to be inherited paternally or biparentally whilst it appeared maternally inherited in two species of subgenus *Decaloba* studied so far (Muschner et al. 2006; Hansen et al. 2007). Although the differential inheritance of plastids could bring new evidence to support the subdivision of these two subgenera, it underlines that interpretation of phylogenies based on cpDNA sequences has to be very cautious. Moreover, the phenomenon of heteroplasmy could explain the incongruences between phylogenetic studies based on chloroplast sequences (Muschner et al. 2003; Hansen et al. 2006). In addition, it is not possible to infer the paternal origin of the pollen and seeds of hybrid individuals (Hansen et al. 2007).

7.5.4 Construction of Genetic Linkage Maps

Linkage maps are useful for identification of important plant genes controlling simply and quantitatively inherited traits. They could facilitate the identification of genomic regions that might affect the variation of important *Passiflora* traits involved in fruit production, fruit quality and disease resistance.

In self-incompatible species, like many *Passiflora* species, linkage maps are constructed using a strategy known as two-way pseudo-testcross, based on monoparental dominant markers that segregate in a 1:1 proportion (Grattapaglia and Sederoff 1994). The final result is the generation of two individual maps, one for each parental genotype. This method was used to generate genetic linkage maps of *P. edulis* f. *flavicarpa,* based on random amplified polymorphic DNA (RAPD) and AFLP markers in three different studies (Carneiro et al. 2002; Moraes 2005; Lopes et al. 2006). The linkage maps were obtained from the same F_1 population derived from a single cross between two clones of *P. edulis* f. *flavicarpa*, "IAPAR 123" (female parent) and "IAPAR 06" (male parent) (Carneiro et al. 2002; Moraes 2005; Moraes et al. 2005; Lopes et al. 2006).

Moreover, the development of specific microsatellites markers to yellow passionfruit (Oliveira et al. 2005) and the use of a maximum likelihood approach (Wu et al. 2002) permitted to generate a first integrative linkage genetic map using the same F_1 population. In this study, individual parental maps were integrated into one map based on the segregation of codominant markers (microsatellites) and dominant markers (AFLP) in one or both the parents (Oliveira et al. 2008). The integrated map is more saturated in markers and the linkage groups are longer than those in individual maps, which will probably facilitate the mapping of crucial traits for the crop species.

7.5.5 Mapping Genes and Polygenic Clusters

Once the genetic linkage map has been developed, the next step is to identify or map the genes controlling the traits of interest by quantitative trait loci (QTL) mapping.

Fruit phenotype is, in general, the result of the interaction of multiple genes as well as environmental factors. Therefore, QTL mapping is necessary to determine the genetic architecture underlying the fruit phenotype. Several fruit characters in yellow passionfruit (*P. edulis* f. *flavicarpa*) show a wide genetic variability and also a high coefficient of heritability (52.6–83%) (Moraes 2005; Moraes et al. 2005). Using an AFLP linkage map, Moraes (2005) mapped genomic regions associated with eight fruit-related traits (fruit yield, number of fruits, average fruit weight, average fruit length, average fruit width, percentage of pulp, soluble solids content and average of fruit size). A total of 41 QTLs were mapped with four to seven QTLs by trait (Moraes 2005). Most (90%) of the QTLs have small or medium effects on fruit traits explaining 15% of the trait's phenotypic variance. These values are higher than those found in some recent studies conducted in other plant species such as cucumber (Yuan et al. 2008) and melon (Obando et al. 2008). The expression of some traits such as fruit yield and the number of fruits are correlated and

probably controlled by the same genes or proximal genes. QTLs for important fruit quality traits in passionfruit were, therefore, located on the linkage map (Moraes et al. 2005). The key markers most closely linked to these QTLs can be further developed to provide tools for breeding and selection in *Passiflora*. A future QTL analysis in wild species would permit to locate genes coding desirable fruit traits that can be incorporated into cultivated species.

QTL mapping in *Passiflora* has also been used to identify genomic regions related to the resistance to one of the common pathogen *Xanthomonas campestris* pv. *passiflorae* (Lopes et al. 2006). This bacterial pathogen causes a disease in *Passiflora* that leads to a premature death of the plant. A unique quantitative resistance loci (QRL) was found in genetic linkage map explaining only 15.8% of the total phenotypic variance in the segregating population (Lopes et al. 2006). The results in *Passiflora* are not sufficient (Lopes et al. 2006) compared to the studies in other plant species that detected several QRLs explaining a large percentage of phenotypic variance for resistance to *X. campestris* (Studer et al. 2006; Soengas et al. 2007). However, it is necessary to continue this first effort in order to progress in the detection of genes controlling the resistance against pathogens in particular in wild resistant species.

7.5.6 Assessment of Gene Actions and Physiological Pathways

Some of the pathways studied in *P. edulis* are the ethylene pathway and its role in growth and development of the plant (Arjona and Matta 1991; Shiomi et al. 1996) and the jasmonate pathway and its role in defense response (Siqueira et al. 2008). The inositol phosphate biosynthesis pathway has also been studied. This pathway produces signal molecules having vital roles encompassing regulation of many processes indispensable to organism homeostasis (Abreu and Aragao 2007). One enzyme implicated in this pathway has been detected not only in *P. edulis* but also in *P. eichleriana* Mast., *P. caerulea, P. nitida* and *P. coccinea* Aubl.

Pelegrini et al. (2006) also described a new defense peptide *Pe*-AFP1 (*Passiflora edulis* antifungal peptide-1) that is a pathogen inhibitor. In vitro, this defense peptide inhibits the development of the filamentous fungi *Trichoderma harzianum*, *Fusarium oxysporum* and *Aspergillus fumigatus* but not of *Rhyzoctonia solani*, *Paracoccidioides brasiliensis* and *Candida albicans* (Pelegrini et al. 2006). The discovery of *Pe*-AFP1 can lead to the development of antifungal drugs against human and plant diseases and of transgenic plants resistant to fungal pathogens. Besides, these results are promising to conduct studies in the detection of new plant defense peptides in other *Passiflora* species, particularly in species already described as resistant to pathogens.

7.5.7 Host–Parasite Interactions

Species of *Passiflora*, especially crop species, suffer different diseases caused by virus, bacteria and fungi. In Table 7.4, the most common pathogens are listed. New descriptions of pathogens affecting *Passiflora* species appear frequently (Parry et al. 2004; Baker and Jones 2007; Tang et al. 2008; Villalobos et al. 2009). Some of these diseases produce considerable losses in passionfruit production. The harmful diseases are the fruit woodiness caused by the virus *Passiflora* woodiness virus (PWV) and cowpea aphid-borne mosaic virus (CABMV), the anthracnose, scab and septoriose caused, respectively, by the three fungi, *Colletotrichum gloeosporioides*, *Cladosporium* spp. and *Septoria passiflorae* and bacteriosis caused by the bacteria *Xanthomonas axonopodis* pv. *passiflorae*.

Larger transportation linked to human activity allows pathogens to encounter potential new hosts and can thus promote the emergence of new epidemics when pathogens could adapt to these novel hosts. Rarely these pathogens are specific to one species and thus they have the capacity to shift from a primary host to a new host species. For example, in Australia the *Passiflora* woodiness virus (PWV) described firstly in the indigenous species *P. aurantia* G. Forst. is now also infecting introduced species such as *P. edulis, P. caerulea* and *P. foetida* (Webster et al. 2007). Other examples are the infection by the *Passiflora* latent virus of the non-native species *P. tarminiana* in New Zealand (Tang et al. 2008) and the infection by fungus *Phytoplasma* spp. of introduced species *P. edulis* in Costa Rica (Tang et al. 2008; Villalobos et al. 2009). Pathogen species described

Table 7.4 Diseases affecting *Passiflora* species[a]

Pathogen	Species susceptible	Known resistant species
Bacteria		
Xanthomonas axonopodis pv. passiflorae	*P. alata, P. amethystina, P. cincinnata, P. coccinea, P. edulis* f. *edulis, P. edulis* f. *flavicarpa, P. nitida, P. setacea*	*P. actinia, P. amethystina, P. caerulea, P. edulis* f. *edulis, P. edulis f. flavicarpa, P. foetida, P. gibertii, P. laurifolia, P. maliformis, P. morifolia, P. mucronata, P. nitida, P. odontophylla, P. serrato-digitata, P. setacea, P. Tenuifila*
Fungi		
Alternaria spp.	*P. edulis* f. *flavicarpa*	*P. manicata*
Cladosporium cladosporioides	*P. edulis* f. *flavicarpa*	
Cladosporium herbarum	*P. nitida, P. cincinnata*	
Colletotrichum gloeosporioides	*P. alata, P. caerulea, P. cincinnata, , P. cirrhiflora, P. coccinea, P. edulis f. flavicarpa, P. garckei, P. gibertii, P. serratodigitata, P. setacea, P. tenuifila, P. tripartita* var. *mollissima*	*P. amethystina, P. caerulea, P. candida, P. coccinea, P. fuchsiiflora, P. gibertii, P. mucronata, P. nitida, P. serrato-digitata, P. odontophylla, P. setacea, P. Tenuifila*
Fusarium oxysporum f. sp. passiflorae	*P. edulis* f. *edulis, P. edulis* f. *flavicarpa, P. capsularis, P. cincinnata, P. foetida, P. laurifolia, P. Ligularis, P. morifolia, P. tripartita* var. *mollissima*	*P. alata, P. caerulea, P. edulis* f. *edulis* (wild), *P. gibertii, P. ligularis, P. macrocarpa, P. nitida, P. quadrangularis, P. Setacea*
Fusarium solani (*Nectria haematococca*)	*P. edulis* f. *edulis, P. edulis f. flavicarpa, P. caerulea, P. cincinnata, P. maliformis, P. pohli, P. setacea, P. sidaefolia, P. suberosa*	*P. actinia, P. alata, P. amethystina, P. caerulea, P. coccinea, P. edulis* f. *edulis, P. gibertii, P. mucronata, P. nitida, P. odontophylla, P. serrato-digitata, P. setacea, P. Tenuifila*
Glomerella cingulata	*P. edulis*	
Oidium neolycopersici, Oidium passiflorae	*P. caerulea, P. edulis, P. foetida*	
Phytophthora nicotianae var. nicotianae.	*P. edulis* f. *edulis, P. setacea, P. sidaefolia*	*P. caerulea* (tolerant)
Phytophthora parasitica	*P. edulis*	
Phytoplasma spp.	*P. edulis*	
Pseudocercospora spp.	*P. foetida, P. setacea*	
Virus		
Bean yellow mosaic virus (BYMV)	*P. caerulea*	
Chrysanthemum B carlavirus (CVB)	*P. caerulea*	
Citrus tristeza closterovirus (CTV)	*P. gracilis*	
Cowpea aphid-borne mosaic virus (CABMV)	*P. edulis* f. *flavicarpa*	*P. coccinea, P. incarnata, P. macrocarpa, P. Suberosa*
Cucumber Mosaic virus, cucumovirus (CMV)	*P. edulis, P. caerulea*	
East Asian *Passiflora* virus (EAPV)	*P. edulis*	
Maracuja mosaic tobamovirus (MarMV)	*P. edulis*	
Okra mosaic tymovirus (OkMV)	*P. edulis*	
Passiflora chlorosis potyvirus	*P. incence*	
Passiflora latent carlavirus (PLV)	*P. edulis, P. caerulea, P. foetida , P. suberosa, P. subpeltata, P. tarminiana*	
Passiflora virus Y (PaVY)	*P. edulis, P foetida*	
Passionfruit crinkle virus (PCV)	*P. edulis* f. *edulis, P. edulis* f. *flavicarpa, P. suberosa*	
Passionfruit mottle virus (PFMoV)	*P. edulis*	
Passionfruit rhabdovirus (PRV).	*P. edulis*	

(*continued*)

Table 7.4 (continued)

Pathogen	Species susceptible	Known resistant species
Passionfruit ringspot virus (PFRSV)	*P. edulis, P. foetida, P. quadrangularis*	
Passionfruit Sri Lankan mottle potyvirus (SLPMV)	*P. edulis* f. *flavicarpa, P. foetida, P. tripartita var. mollissima*	*P. suberosa*
Passionfruit vein-clearing rhabdovirus	*P. edulis*	
Passionfruit woodiness potyvirus (PWV)	*P. alata, P. amethystina, P. aurantia, P. edulis* f. *edulis, P. edulis* f. *flavicarpa, P. foetida, P. gibertii, P. mucronata, P. nitida, P. odontophylla, P. serrato-digitata, P. suberosa, P; subpeltata, P. tenuifila*	*P. actinia, P. coccinea, P setacea*
Passionfruit yellow mosaic tymovirus [PFYMV (PaYMV)]	*P. edulis f. flavicarpa*	
Purple granadilla mosaic virus	*P. edulis, P. alata, P. serrato-digitata, P. caerulea, P. maliformis, P. gibertii, P. edulis* f. *flavicarpa.*	
Nematodes		
Meloidogyne incognita		*P. caerulea, P. edulis, P. cincinnata, P. Quadrangularis*

[a]The diseases are listed according the following references: Delanoë (1991), Ploetz (1991), Chang (1992), Cole et al. (1992), Chang et al. (1994), Brunt et al. (1996), Pares et al. (1997), Gonzalez et al. (2000), Wolcan and Larran (2000), Davis et al. (2002), Gioria et al. (2002), Morales et al. (2002), Parrella and Castellano (2002), Pegg et al. (2002), Parry et al. (2004), Fischer et al. (2005), Junqueira et al. (2005), Iwai et al. (2006b), Liberato (2006), Nascimento et al. (2006), Ploetz (2006), Baker and Jones (2007), Dianese et al. (2008), Jankovics et al. (2008), Santos et al. (2008), Tang et al. (2008), Villalobos et al. (2009). Resistant *Passiflora* species of a specific disease are also listed: Delanoë (1991, 1992), Junqueira et al. (2005), Meletti et al. (2005)

initially in a phylogenetically distant host plant can also be found in species of *Passiflora*. For instance, the bean yellow mosaic virus infects now plants of *P. caerulea* in Italy (Parrella and Castellano 2002) and the worldwide *Citrus tristeza* closterovirus (CTV) infects the wild species *P. gracilis* J. Jacq. ex Link (Brunt et al. 1996). Closely related virus can develop the same disease symptoms in *Passiflora* plants. The fruit woodiness has been described to be caused primarily by only the PWV virus. However, Nascimento et al. (2006) found that the disease is also caused by the related CABMV potyvirus. While Brazilian plants are primarely infected by the CABMV, the Australian and Asian plants are mostly infected by the PWV.

Strategies of selection could be performed to choose the more resistant genotypes of cultivated species in order to reduce the susceptibility to diseases. Among the yellow passionfruit, Martins et al. (2008) found some genotypes moderately resistant to *Colletotrichum gloeosporioides*. Moreover, the resistance to the scab caused by the fungus *Cladosporium cladosporioides* has been enhanced by the selection of more resistant individuals of yellow passionfruit (Santos et al. 2008). However, the variability of resistance to different diseases of cultivated species appears in general to be very low (Junqueira et al. 2003).

Therefore, the identification of resistant species is essential to increase the variability of resistance in susceptible cultivated species by breeding strategies. The numerous wild species of *Passiflora* are a potential source of genotypes resistant to particular pathogens. Several wild species are resistant to the most common pathogens such as *P. actinia* that is non-susceptible to the PWV, the CABMV and the fungus *Colletotrichum gloeosporioides* (Junqueira et al. 2005) (Table 7.4). The feasibility to produce hybrids from interspecific crosses enhances the probability of obtaining resistant varieties of crop species (see Sect. 7.6.4).

7.6 Crop Improvement Through Traditional and Advanced Tools

7.6.1 On-Farm Selection and Genetic Resource Management

Passionfruits' genetic improvement owes much to the efforts of the native growers in South America. For

most species, even those of economic importance, many farmers still select a few good-looking fruits from higher yielding vines to establish the next orchard. In banana passionfruit, some growers select those seeds in the median part of the fruit. The intensity of selection is relatively weak particularly if the crop cycle is long (about 10-year-old banana passionfruit orchards). Growers may detect interspecific hybrids and favor them in their orchard. In Colombia, in a valley where *P. tripartita* var. *mollissima* cultivation has recently been established, the growers favor hybrids with *P. mixta* in an attempt to reduce the impact of anthracnose on fruit appearance. Other farmers bring spontaneous *P. mixta* × *P. tripartita* var. *mollissima* from the wild to grow them in their own home garden. Conversely, conservative farmers often discard those seedlings exhibiting particularly thin leaf lobes, indicative of hybridization with *P. mixta*, because hybrids tend to bear less fruits. Similar situations may exist in other cases where cultivated and wild species look very similar; some farmers tolerate wild vines of *P. tiliifolia* on the borders of their *P. ligularis* plots. Partial sympatry of similar cultivated and wild species also occurs in the series *Laurifoliae* in the lowlands.

As passionfruits have become more important commodities and even local markets have offered higher prices for more standardized products, plot size and planting densities have increased, favoring, unfortunately, the development of a wide cohort of pests and diseases. Although the shortening of crop cycles benefits the higher yield and better sanitary conditions of young vines, it increases both the costs of establishment of the crop and the need to get a higher and faster economic return. Such intensification has necessarily run parallel to the development of formal breeding in the passionfruit crops of wider importance. Local strains have been developed through mass selection for the sweet granadilla and banana passionfruit, while the development of yellow maracuja cultivars has been shared by the public and private sectors.

7.6.2 Development of Clonal Cultivars

Yellow maracuja breeding was initiated in Hawaii to improve yield and quality of the crop for the juice industry. Yields were multiplied fourfold, and juice yield improved from 25% to 35% (Morton 1967). To face the increasing phytopathological constraints of the crop, breeders attempted to widen its depleted genetic basis through hybridization with the purple form (Nakasone et al. 1967). Selection was most often carried out on an individual basis and the selected elite material was propagated clonally, which led to prefer pseudo-self-compatible genotypes (Fouque and Fouque 1980). When the elite material was strongly self-incompatible, cross-pollination had to be organized by interplanting distinct clones in the orchard (Knight 1972; Ito 1978).

In the context of clonal propagation of planting materials, another solution was attempted in Australia by grafting the plants on rootstock resistant or tolerant to the most severe soil pathogens. This practice imposed on the development of two parallel programs – a breeding program for scions and a rootstock program – without solving the problem of viral transmission via the planting materials (Coppens d'Eeckenbrugge et al. 2001b). Distinct species have been tested as rootstocks for *P. edulis*. *Passiflora incarnata* and *P. caerulea* were appreciated for their general resistance to soil pathogens as well as cool temperature at subtropical latitudes, but the latter was discarded because of its abundant suckering. Hybridization was attempted between both species and *P. edulis* f. *flavicarpa* for obtaining of better rootstock. Interspecific crosses involving *P. incarnata* were used as well for the scion, in order to improve the cold and virus resistance of commercial fruit types (Winks et al. 1988). However, their outcome is not clear, as the use of clones and pure *P. edulis* f. *flavicarpa* rootstock is still recommended in Australia (Department of Employment Economic Development & Innovation 2005).

7.6.3 Synthetic Population and Hybrid Breeding

When the Hawaiian selections of yellow maracuja came back to their home continent, they returned to sexual propagation, which had two major consequences. First, seed propagation reduced the impact of viral diseases as this planting material was not contaminated. Second, a higher level of agromorphological variation appeared in the crop, notably the segregation for fruit colors, still observed in many

South American populations, and also in all important agronomical traits. Considerable genetic gains for yield and fruit size and quality were made possible, first through mass selection, then through the development of synthetic varieties (see Meletti et al. 2005 for a review), initially composed by selected clones (Maluf et al. 1989) and then by half-sib or full-sib progenies (Meletti 1998). Early producing cultivars were developed for the fresh fruit market and for the juice industry, showing yields up to 50 tons/ha, pulp yields close to 50% and soluble solids around 15–16°Brix (Meletti et al. 2000). However, no similar success was obtained in terms of disease resistances (Vieira and Carneiro 2004), which have a considerable impact on production, mostly because no sources of appreciable resistance/tolerance had been identified within *P. edulis*. A similar partial success allowed the development of *P. alata* from a home garden or from the wild to a commercial crop for the fresh fruit market, through selection and standardization of fruit shape (Kavati et al. 1998). As limitations in yield and quality had been overcome, the focus of breeding efforts shifted towards genetic resistances, through the characterization of other species (see Sect. 7.5.7) and introgression programs (Sect. 7.6.4).

In Brazil also, the yellow and purple forms of *P. edulis* have been crossed to generate varieties with better fruit qualities. Although these varieties are, in general, highly susceptible to diseases (Vieira and Carneiro 2004), they permitted the selection of plants with good fruit yield and also some degree of resistance to bacteriose and anthracnose (Junqueira et al. 2005).

7.6.4 Introgression of Traits Through Interspecific Hybridization

The search of resistance traits is the most frequent reason for attempting many different crosses and the subsequent introgression at the interspecific level. A few projects have been justified on other grounds, such as adapting the crop to temperate latitudes and/or to smaller pollinators through a reduction in flower size, or introducing self-compatibility to improve pollination efficiency (Knight 1991).

Some species seem to be amenable to hybridization more easily than others, as in the case for *P. caerulea*. Self-incompatibility of many cultivated species, such as *P. edulis* and *P. incarnata,* could be a functional component of interspecific incompatibility, explaining cases of unilateral incompatibility as in the cross between the two forms of *P. edulis* (Coppens d'Eeckenbrugge et al. 2001b). According to the difficulty of particular crosses, the production of interspecific seeds has involved controlled crosses or more sophisticated techniques, such as hormones, to retard floral abscission or/and intra- and interspecific double pollination (Payan and Martin 1975). The frequent sterility of the resulting F_1 hybrids poses a further problem. Knight (1991) doubled the chromosome number of hybrids between *P. incarnata* and the two forms of *P. edulis* to restore their fertility. The subsequent tetraploid progenies were self-incompatible, which is consistent with the sporophytic system, remaining functional in polyploids. Their fruits gave a pleasant juice, despite a relatively clear color, with characteristics closer to maracujas than to maypops (Senter et al. 1993). Knight concluded that they had promise as a new fruit crop for warm–temperate zones. However, the only documented outcome today is the ornamental cultivar "Byron Beauty" derived from this hybrid (Knight et al. 1995).

Junqueira et al. (2005) attempted to transfer the resistance of *P. setacea* DC. against the anthracnose and PWV to *P. edulis* f. *flavicarpa* by introgression. The initial cross is fertile in both directions. The F_1 hybrids appear vigorous, closer to the *P. setacea* parent in many respects, and resistant. However, they face pollination problems related to abnormal floral morphology. Four backcross generations were obtained using the recurrent genitor as pollen-donor, allowing the recovery of a *P. edulis* phenotype. However, genetic resistance to the PWV seems to be inherited in a quantitative manner, diminishing with successive generations. The BC_3 still showed resistance to anthracnose; however, fruit yield was very low in comparison to susceptible cultivars, probably in relation to their very long androgynophore. The same authors report very similar results using *P. coccinea* instead of *P. setacea*, with hybrid vigor, general resistance transfer, abnormal floral proportions and very low fruit set in the F_1 and BC_1 generations. When exploring the potential of the cross with *P. caerulea*, they obtained sterile hybrids with good resistance to

bacteriosis and anthracnose, but susceptible to the PWV and attractive to caterpillars of *Dione* butterflies. Fertility was partly restored in the BC_2 generation. Many more such crosses within subgenus *Passiflora* have been attempted by the same group, including triple hybridizations. The compatibility of species has also been evaluated and some appear totally compatible, producing fertile seeds (Junqueira et al. 2005). For instance, the widely cultivated *P. edulis* f. *flavicarpa* seems totally compatible with *P. glandulosa* and very compatible with *P. coccinea* (79.2%) and *P. setacea* (85.7%) but it is totally incompatible to *P. actinia* and very incompatible with *P. caerulea* (8.6%) (Junqueira et al. 2005). Recently, 17 interspecific F_1 hybrids were generated from the crosses *P. laurifolia* × *P. nitida, P. edulis* f. *flavicarpa* × *P. coccinea*, *P. caerulea* × *P. amethystina* J.C. Mikan, *P. glandulosa* Cav. × *P. galbana* Mast., *P. coccinea* × *P. actinia, P. glandulosa* × *P. edulis* f. *flavicarpa, P. sidaefolia* M. Roemer × *P. actinia, P. galbana* × *P. actinia,* F_1 (*P. coccinea* × *P. setaceax P. coccinea*), F_1 (*P. coccinea* × *P. setacea*) × *P. mucronata* Lam., *P. eichleriana* × *P. gibertii* N.E. Br., *P. galbana* × *P. edulis* f. *flavicarpa, P. glandulosa* × *P. edulis edulis, P. glandulosa* × *P. sidaefolia, P. coccinea* × *P. setacea*. Their success was confirmed using RAPD markers (Junqueira et al. 2008).

Schoeniger (1986) conducted a long series of experiments of interspecific crosses in the supersection *Tacsonia*, with the aim of introgressing genetic resistance to oidium and anthracnose from wild *P. mixta* and *P. cumbalensis* into *P. tripartita* var. *mollissima*. In this case, F_1 hybrids also showed remarkable vigor, with larger leaves, stipules, bracts and flowers. According to Escobar (1981), pollen viability is equal to or higher in the hybrids, as compared to parental species. However, fertility strongly decreases in F_2 and BC_1 generations, following a lack of flowering and low fruit set, aggravated by poor seed germination and high mortality. Considerable variation appeared for all organs, including fruits (production, size, shape, succulence and flavor of arils), with cases of transgressions in the segregation and abnormal leaf and flower shapes. Selfing F_2 and BC_1 plants produced similar results, and some plants exhibited traits that were unknown in the parent species (Schoeniger 1986). Later, Coppens d'Eeckenbrugge crossed *P. tripartita* var. *mollissima* with *P. tarminiana* and *P. mixta* and characterized the resulting F_1 hybrids; crosses were fully fertile. The hybrids were morphologically intermediate, with a tendency to be more similar to their maternal parent. They showed high vegetative vigor, with large leaves and larger, but somewhat rarer, fruits, of intermediate characteristics. Concerning resistance to anthracnose, which produces black dots depreciating the fruits, the hybrids between the susceptible *P. tripartita* var. *mollissima* and the resistant *P. tarminiana* expressed the symptoms at an intermediate level, suggesting a quantitative inheritance of the resistance and presaging difficulties for its effective introgression (Primot et al. 2005). These examples show that the introgression of resistance traits into cultivated passionfruits through interspecific crosses will be a long process. Sexual compatibility is not a synonym to genome compatibility and disease resistances are complex traits whose inheritance often seems to depend on a dosage effect. Lack of resistant germplasm in *P. edulis* f. *flavicarpa* leaves few other solutions than pursuing the effort. The situation may be different in species, such as *P. tripartita*, for which resistant conspecific materials may be explored, particularly in wild botanical varieties. *P. mixta*, a wild species, which shows spontaneous genetic exchanges with the cultigen, is also worth exploring.

The success of introgression at the interspecific level will depend not only on the number of crosses and generations obtained, but also probably more on the development of specific tools to understand and follow the transfer of targeted traits. A particular investment is necessary in cytogenetics, studying the chromosomal structure of the parents and recombination in the hybrids, as well as molecular tools, such as genetic maps and trait markers. Furthermore, cytogenetics could provide some keys for a better understanding of interspecific incompatibility in *Passiflora*.

7.6.5 Somatic Hybridization

Somatic hybrids of *Passiflora* species have been generated from protoplasts isolated from leaf tissues or callus derived from suspension cultures. These somatic hybrids are artificial polyploids and present, in general, chromosomal instability. For example, despite their high pollen viability, meiotic

irregularities have been found in somatic allotetraploid hybrids ($4n = 36$) obtained from the cross between the diploid parents *P. edulis* f. *flavicarpa* ($2n = 18$) and *P. amethystina* ($2n = 18$) (Vieira and Carneiro 2004). In contrast, the somatic allotetraploid hybrids generated from protoplasts of *P. edulis* f. *flavicarpa* and *P. cincinnata* do not present aneuploidy. They have stable meiotic behavior and normal pollen viability suitable for the introgression of genes such as the resistance against *Xanthomonas campestris* pv. *passiflorae* present in *P. cincinnata* (Vieira and Carneiro 2004). Other desirable traits such as cold tolerance have been transmitted via somatic hybridization from *P. incarnata* to *P. edulis* f. *flavicarpa* (Otoni et al. 1995).

7.6.6 Genetic Transformation

To counteract the negative effects of pathogens, resistant transgenic plants of yellow passionfruit (*P. edulis* f. *flavicarpa*) have been generated. For instance, transgenic plants have become resistant to the virus passionfruit woodiness virus (PWV), introducing the gene codifying for the virus coat protein (CP) (Trevisan et al. 2007).

In another study, an antiapoptotic gene (*p35*) from a baculovirus, which is supposed to lead resistance to environmental stress and to a broad spectrum of diseases, was introduced to the genome of *P. edulis* f. *flavicarpa* by biobalistics (Freitas et al. 2007). Although it failed to confer resistance against the cowpea aphid-borne mosaic virus (CABMV) to transgenic passionfruit plants, it was able to increase their resistance against the bacterium *Xanthomonas axonopodis* pv. *passiflorae*, and their tolerance against the herbicide, glufosinate (Syngenta) (Freitas et al. 2007).

7.7 Genomics Resources Developed

7.7.1 Gene Sequences

In the NCBI genebank database (NCBI 2009), there are 1,648 *Passiflora* entries corresponding to 1,196 nucleotide sequences and 452 translated protein sequences. Approximately 95% of these entries were produced in phylogenetic studies and correspond to the sequences of plastid fragments, such as *rcb*L, the spacer *trn*H–*psb*A, the spacer *trn*L-F, and nuclear sequences, such as Internal Transcribed Spacer (ITS) and glutamine synthetase (GS2) (Muschner et al. 2003; Yockteng and Nadot 2004a, b; Krosnick and Freudenstein 2005; Hansen et al. 2006).

Population genetic studies have also generated molecular sequences. In a phylogeographical study, internal transcribed spacers (ITS) sequences of 32 individuals of *P. actinia* and 20 individuals of *P. elegans* from 41 different localities were obtained (Lorenz-Lemke et al. 2005). Besides, the study of genetic variation of populations of *P. alata* has provided new nuclear and chloroplast sequences (Koehler-Santos et al. 2006a). ITS of a fragment of glyceraldehyde 3-phosphate dehydrogenase (G3pdh) gene, the chloroplast intergenic spacers *trn*L–*trn*F and *psb*A–*trnL* and the intron *trn*L were sequenced to study 88 individuals of *P. alata* from 52 different localities of Brazil. For the first time, the second intron of gene *LEAFY*, which regulates the establishment of the floral meristem and flowering time in *Arabidopsis,* was sequenced in a *Passiflora* species. One novelty of this study was also the use of the first set of 12 microsatellite markers in a *Passiflora* species (Padua et al. 2005; Koehler-Santos et al. 2006a). A set of ten microsatellites specific *to P. edulis* f. *flavicarpa*, the yellow passionfruit, is also available (Oliveira et al. 2005) and was already used to construct a genetic linkage map (Oliveira et al. 2008) (see details in Sect. 7.5). Twenty-eight unpublished microsatellites specific to *P. alata* are also accessible in the NCBI database.

Several chloroplast and mitochondrial sequences of hybrids, product of interspecific crosses of *Passiflora* species, have been deposited in the database and used to determine the process of organellar inheritance in *Passiflora* (Muschner et al. 2006).

Besides, eight sequences of homolog isoforms of the allergenic compound of the pollen of Betulaceae (Bet v1) from *Passiflora morifolia* Mast., *Passiflora* sp., *P. suberosa*, *P. misera* and *P. organensis* Gardner are accessible in NCBI (Finkler et al. 2005). The aim of the study of Finkler and co-workers was to make a first characterization of Bet v1 homologs in *Passiflora* species and to identify the utility of this gene for future evolutionary and applied researches.

7.7.2 Expressed Sequence Tags

A few expressed sequence tag (EST) sequences of *Passiflora* are available in the NCBI database and all are obtained from the cultivated species *P. edulis*. Mita et al. (1998) were first to deposit five cDNA sequences of molecules playing an important role in the ethylene pathway. Their study generated two cDNA sequences of the ethylene receptor of *P. edulis*, called *PE*-ERS1 and *PE*-ETR1, which have a length of 2,297 bp with an open reading frame (ORF) encoding 637 residues of amino acids and 2,715 bp with an ORF encoding 738 amino acids. They also obtained cDNA sequences from two copies of the ACC synthase (*PE*-ACS1 and *PE*-ACS2) and one copy of the ACC oxidase (*PE*-ACO1), each having a sequence length of 1,054 bp with an ORF encoding for 351 amino acids, 1,063 bp with an ORF encoding for 354 amino acids, and 785 bp with an ORF of 261 amino acids. A subsequent study of ethylene pathway identified a third cDNA sequence of an independent copy of an ethylene receptor (*PE*-ERS2) measuring 2,357 bp that include an ORF codifying for 634 amino acids (Mita et al. 2002). The cDNA sequence of an antifungal protein (*PE*-AFP1) with a length of 25 amino acids residues was purified from seeds of *P. edulis* and constitutes the first defense peptide with antifungal properties identified in *Passiflora* (Pelegrini et al. 2006). Besides, the Myo-inositol-1L-phosphate synthase (MIPS) cDNA, component of Inositol phosphate biosynthesis pathway, as well as two cDNAs of translation elongation factor 1a-1 of *P. edulis* was sequenced (Abreu and Aragao 2007). MIPS cDNA isolated from developing passionfruit seeds measures 1,951 bp in length contains one open reading frame of 1,533 bp encoding 510 amino acids. Recently, the cDNA of the chloroplast-targeted allene oxide synthase from *P. edulis* (Pf-AOS), which is one of the components of the jasmonate pathway, was isolated. The coding region of Pf-AOS contains 1,512 nucleotides and encodes a protein of 504 amino-acids flanked by a 50-UTR of 155 nucleotides and a 30-UTR of 116 nucleotides (Siqueira et al. 2008). This study indicates that PfAOS may play an important role in systemic wound response against chewing insect attack (Siqueira et al. 2008).

7.7.3 Complete Genome Sequences

So far, no genome sequence is available from any *Passiflora* species. Instead, the genome of three pathogenic virus affecting *Passiflora* species has been totally sequenced (Iwai et al. 2006b; Song et al. 2006; Spiegel et al. 2007). The full maracuja mosaic tobamovirus (MarMV) genome consists of 6,794 nucleotides and contains four open reading frames (ORFs) coding for proteins (Song et al. 2006). The genome of carlavirus *Passiflora* latent virus (PLV) isolated from *P. edulis* plants measures 8,386 nucleotides and contains six ORFs (Spiegel et al. 2007). The genomic RNA of East Asian *Passiflora* virus (EAPV) isolated from *P. edulis* is composed of 10,046 nucleotides and contains a single ORF encoding a polyprotein of 3,220 amino acids (Iwai et al. 2006a). The availability of complete genome sequences of *Passiflora* virus will permit to understand better the mechanisms used by the pathogen to generate the disease in the host-plant. The continuous development of the molecular techniques will permit to obtain hopefully the first complete genome sequence of a *Passiflora* species.

7.8 Scope for Domestication and Commercialization

7.8.1 Wild Species as Resistant Rootstock

In passionflower, grafting appears to be a good and more immediate solution than breeding to reduce the heavy impact of soil pathogens. In French Guiana, Delanoë (1992) tested nine species as potential rootstocks for *P. edulis* f. *flavicarpa*. None showed incompatibility, but grafting success varied from 40 to 60% for *P. coccinea*, *P. glandulosa* and *P. garckei*, to 60–80% for *P. nitida*, *P. laurifolia* and *P. cirrhiflora*, or more than 80% for *P. serrato-digitata*, *P. fuchsiiflora* and *P. candida*. However, the plants grafted on *P. fuchsiiflora* and *P. candida* died after 17 months. In terms of quality and development of the rootstock and the scion, the best results were obtained with *P. coccinea*, *P. glandulosa* and *P. laurifolia*, followed by *P. nitida* and *P. garckei*. Junqueira et al. (2005) reported similar grafting experiments using *P. nitida*

or a *P. edulis* f. *flavicarpa* × *P. setacea* hybrid as rootstock. The grafted materials yielded as much as seedlings of yellow maracuja, but only half of its production in cuttings. Grafted plants were effectively protected from soil pathogen attacks. However, the economic application of grafting is an issue because of the low availability of wild species germplasm, their erratic seed germination, and the elevated maintenance cost of a crop with short cycle (less than 3 years) (Meletti et al. 2005). In Colombia, Campos (1992) successfully used *P. manicata* as a rootstock for adapting *P. tripartita* var. *mollissima* to lower altitudes and dry conditions. However, the impact on economic parameters has not yet been tested.

7.8.2 New Commercial Fruit Crops

Among the very high diversity of *Passiflora* species producing an edible fruit, there are many potential candidates for developing new fruit crops. However, a more realistic approach would be giving priority to those species that have been partially domesticated, and thus rescuing their cultivated germplasm. These crops include *P. incarnata*, for temperate climates, species of series *Laurifoliae* such as *P. maliformis* and *P. nitida*, species of series *Tiliifoliae* such as *P. platyloba* and species of supersection *Tacsonia* such as *P. cumbalensis*, *P. pinnatistipula* and *P. antioquiensis* for tropical highlands. The gene pools for these species have been briefly presented in Sect. 7.4.

McGuire (1999) proposed to develop the maypop, *P. incarnata* into a fruit crop. This fruit was "abundant in Indian gardens" since its archeological remains are common in the southeast of the United States. However, the level of domestication reached in that time is uncertain (Gremillion 1989). The fruits of *P. incarnata* are good sources of vitamin A and niacin (Martin and Nakasone 1970).

The reputation of *Passiflora maliformis* suffers from its vernacular name of "stone granadilla." However, the epicarp solidity is present only in part of its germplasm and the fruits are sold well on regional markets in Colombia. Some germplasm of this species shares interesting traits with the purple form of *P. edulis*. Indeed, the resemblance is such that they have received the same vernacular names of *gulupa* or *chulupa*. They share a yellow and highly aromatic pulp, similar fruit size and a deep red shell. But *P. maliformis* is superior to *P. edulis* in two aspects: the fruit shell does not wrinkle at maturity keeping its smooth appearance, and it is considered generally resistant or tolerant to most passionfruit pathogens. These interesting traits, found also in the closely related *P. serrulata* and *P. multiformis*, could make *P. maliformis* a strong competitor in the passionfruit market. Lowland species of series *Tiliifoliae* are already cultivated on a very small scale in forest regions of western Colombia. Their fruits could benefit from their resemblance with those of *P. ligularis*, which are appreciated in the national market. But their potential is wider since their fruits could be produced in many other rain forests. As for the highland sweet granadilla, their smooth and brittle epicarp makes them both attractive and easy to consume. The cultivated populations could currently provide an excellent basis for the commercial development of these species.

Species of the series *Laurifoliae* offer similar or even better perspectives. The genetic improvement should aim at equilibrating their resource allocation between generative and vegetative parts in order to reduce the overall development and promote a longer harvesting season. Breeding should easily maintain the general quality of the sweet and aromatic pulp, aril size, and fruit coloration, while the reduction of the fruit mesocarp would enhance pulp proportion.

The rosy passionfruit, *P. cumbalensis*, is cultivated only around Bogota, and this crop will certainly get lost in very near future if no particular attention is paid to it; this species is simply being forgotten. Indeed, it is very similar to its cousin *P. tripartita* var. *mollissima*, with which it shares the large and abundant orange arils of excellent flavor and aroma. The fruit is generally bright red, although some types give yellow fruits, and the plant seems less affected by anthracnose than *P. tripartita* var. *mollissima*. An effort should be done to promote the two fruits on the same market. The botanical varieties of supersection *Tacsonia* found from Colombia to Peru constitute a wide gene pool for the sustainable future of this potential crop (Holm-Nielsen et al. 1988). A similar case of a species that is becoming rare in home gardens is *P. antioquiensis*. Its germplasm is in high danger of extinction while it is still present in the wild. In cultivated materials, the flavor of the cream orange fruit pulp is a special and highly aromatic

blend, matching the best fruits of supersections *Passiflora* and *Tacsonia*. But its fruiting may not be abundant enough for commercial production. The species should be restored not only as fruit but also as garden ornamental because of the exceptional beauty of its carmine and fusiform flowers hanging from 30 to 60 cm peduncles. *Passiflora pinnatistipula* is a very particular species of supersection *Tacsonia* because of its round fruits and its flowers, which have a relatively short hypanthium and a filamentose corona. The whitish pulp is extremely sweet; however, the seeds are relatively large, round and hard, so breeding could help to solve this problem. Another potential problem is that the species hybridizes very easily with *P. tripartita* var. *mollissima*, giving sterile hybrids (*P.* × *rosea*) that are difficult to distinguish from *P. pinnatistipula* until they flower. Therefore, these two species cannot be closely cultivated. *Passiflora pinnatistipula* may be grown at higher latitudes (up to 4,000 m), allowing fruit production in cold mountain climates.

7.8.3 Source of Therapeutic Compounds

Since prehistoric periods, *Passiflora* species have been used for therapeutic purposes (Dhawan et al. 2004). Although one species, *P. incarnata*, is the mostly recognized in pharmaceutics, many species have been used in traditional folk medicine in several countries (Table 7.3). After an exhaustive review, Dhawan et al. (2004) concluded: "This genus is a boon and blessing and a panacea for the ailing masses". The medicinal properties of passionflowers are consequence of their diversity in phyto-constituents among which are flavonoids, glycosides, alkaloids and phenol compounds.

Several experimental studies performed in mice or rats give evidence of the anxyolitic, sedative, antioxidant and anti-inflammatory properties of *Passiflora* extracts such as *P. incarnata, P. edulis, P. alata, P. quadrangularis* extracts (Wolfman et al. 1993, 1994; Soulimani et al. 1997; Zanoli et al. 2000; Petry et al. 2001; Dhawan et al. 2003; Frode et al. 2004, 2005; Rudnicki et al. 2004; Santos et al. 2005; Bezerra et al. 2006; Garros et al. 2006; Gomes et al. 2006; Oliveira et al. 2006; Reginatto et al. 2006; Silva et al. 2006; Beninca et al. 2007; Castro et al. 2007; Montanher et al. 2007; Rudnicki et al. 2007; Vargas et al. 2007; Barbosa et al. 2008; Lorencini et al. 2008). Most of the studies have also shown an absence of secondary adverse effects of *Passiflora* extracts. No adverse effect of *P. incarnata* doses was found in female pregnant mice or their litter during gestational, fetal and post-natal periods (Mello et al. 2007). One experimental research in humans compared the effect of an oral dose of 500 mg of *P. incarnata* (Passipy™ IranDarouk) in a group of 30 patients with pre-operatory anxiety versus the effect of an oral dose of placebo in other 30 patients (Movafegh et al. 2008). The results show that anxiety was significantly lower in patients that took the *Passiflora* dose and the recovery of psychomotor function was comparable in both the groups. This study would support the efficacy of *P. incarnata* extracts as a remedy against anxiety. Other *Passiflora* medicinal uses are as antidote to counteract the venom of the scorpion *Heterometrus laoticus* (Uawonggul et al. 2006), and antimicrobial activity (Bendini et al. 2006).

The principal bioactive compounds described in this genus are the *C*-glycosil flavonoids (vitexin, isovitexin, orientin, isoorientin and apigenin) and β-carbolinic alkaloids (harman, harmin, harmalin, harmol and harmalol). The high concentration of isovitexin in *P. actinia* extracts would produce sedative and anxyolitic effects in animals (Santos et al. 2006). In addition, vitexin from various *Passiflora* species has an antithyroid effect in mice (Dhawan et al. 2004). Another active compound, the monoflavonoid chrysin purified from *P. caerulea* and *P. incarnata,* is possibly responsible for the anxyolitic and anticonvulsive effects on mice (Dhawan et al. 2004; Brown et al. 2007). This flavonoid is a ligand of benzodiazepine receptors and GABA receptors that mediates biochemical processes in the body. *Passiflora incarnata's* chrysin has also been reported to attenuate the suppression of activity of natural killer (NK) cells after a surgery (Beaumont et al. 2008). The NK cells are lymphocytes responsible for killing virus-infected cells and tumor cells during metastasis. Surgical procedures, anesthetics and pre- and post-surgery stress would affect the activity of NK cells. Therefore, *Passiflora*'s chrysin could have an indirect effect against cancer metastasis. Other anticancer activity was reported in extracts from *P. edulis* f. *flavicarpa* that influence the death of transformed foci, potential tumor cells (Rowe et al. 2004). In addition, extracts from fruit of *P. edulis* and *P. foetida*

L. var. *albiflora* inhibit the activity of two enzymes, the gelatinase MMP-2 and MMP-9, two metallo-proteases involved in the tumor invasion, metastasis and angiogenesis (Puricelli et al. 2003). Chrysin has also reported to have some negative effects such as hyperalgesia, which is increased sensitivity to pain stimulation (Zhai et al. 2008). Another compound isolated from *P. edulis* is a defense peptide (*Pe*-AFP1) that inhibits the development of filamentous fungi *Trichoderma harzianum*, *Fusarium oxysporum* and *Aspergillus fungigatus*. The discovery of *Pe*-AFP1 could contribute, in a near future, to the development of biotechnological products as antifungal drugs against pathogenic fungi (Pelegrini et al. 2006). Besides, a hemolysin, probably a saponin, was isolated from the leaves of *P. quadrangularis*. Hemolysins are transmembrane receptors and are potential bactericidal and anticancer drugs (Yuldasheva et al. 2005).

The concentration of phytoconstituents in plants apparently depends on their mineral nutrition. The absence of minerals such as potassium, phosphor and nitrogen, in the nutrition of *Passiflora* plants increments their concentration in vitexin (Freitas et al. 2008). Populations of the same species located in different geographical areas would have different profiles in their phytoconstituents. For instance, the concentration of vitexin in *P. foetida* varies among samples collected in different geographical regions (Pongpan et al. 2007).

Despite all the beneficial pharmaceutical effects of passionflowers, precaution is needed about the presence of toxic compounds such as cyanogenic constituents. The plants of *Passiflora* produce secondary metabolites, especially cyanogenic glycosides as protection against herbivores. The unripe fruits of *P. adenopoda* DC. have already caused poisoning due to the presence of HCN (Dhawan et al. 2004). *Passiflora manicata* is called *diablito* (little devil) in Ecuador, for similar reasons.

Although a few studies are based on non-cultivated species, wild *Passiflora* species have been reported to be diverse in their phytoconstituents (Abourashed et al. 2002, 2003). Wild species also produced flavonoids and alkaloids. Sometimes, the concentration of these phytoconstituents is larger in wild species than in pharmaceutical ones. For example, the concentration of vitexin is seven times higher in the wild species *P. holosericea* L. than in *P. incarnata* (Abourashed et al. 2002). Wild species constitute, therefore, an important source of potential therapeutic compounds. Hence, it is necessary to conduct studies in order to detect their pharmaceutical properties and their active compounds.

7.8.4 Psychoactive Drugs

In certain tropical regions of South America, the dried leaves of *P. edulis* f. *flavicarpa* are sometimes smoked in the same way as those of *Cannabis sativa* L. Specialized websites indicate similar uses for *P. incarnata*, although the effect is said to be weaker than that of marijuana. Tea made with dried flowers also has a slight psychotropic effect. Indeed, *P. incarnata* may contain small amounts of harmala alkaloids, which are used in the preparation of a highly psychotropic drink, the ayahuasca (Recreational Drug Information Website 1999). Besides, harmala alkaloids reduce the breakdown of the psychoactive drug DMT (*N*, *N*-dimethyltryptamine) in the digestive system. In traditional medicine, *P. incarnata* has been used for drug deaddiction of morphine, cannabinoids, nicotine and alcohol, which was successfully tested on mice (Dhawan et al. 2004).

7.8.5 Dietary Supplements

Once again, dietary supplements are mainly reported in cultivated species. However, wild passionflowers constitute a latent source of dietary supplements waiting to be explored. The yellow passionfruit rinds, usually discarded after juice production, have been reported to be a source of variable dietary supplements. Firstly, their cell wall material rich in non-starchy polysaccharides constitute a possible source of dietary fiber, which could protect against cardiovascular diseases, diabetes, obesity, colon cancer and diverticular diseases (Yapo and Koffi 2008). Furthermore, yellow passionfruit rinds are also a potential source of pectin, a compound widely used in many foodstuffs as gelling agents (Yapo and Koffi 2006). The pectin isolated from *P. edulis* f. *flavicarpa* is comparable to the commercial citrus low-methoxyl pectin. Finally, the yellow passionfruit rinds have been also reported as a rich source of lycopene that is an

antioxidant carotenoid which probably reduces risk of chronic diseases (Mourvaki et al. 2005). Few sources of lycopene are available in diet such as tomatoes.

7.8.6 Other Uses

Passiflora species could be a source of ecofriendly materials. For instance, a new polysaccharide, xiloglucan biopolymer, is extracted from peels of *P. ligularis*. This biopolymer could be used to produce a biodegradable film using the discards of passionfruit industry (Tommonaro et al. 2007). Seeds of *P. edulis* are also a source of vegetable oils useful to produce ecofriendly polymeric material (Lopes et al. 2008). Moreover, *P. incarnata* extracts have been described as useful to develop organic sunscreens with a protective defense against UV radiation. The use of plant compounds would reduce the concentration of chemical UV filters in sunscreens (Velasco et al. 2008). In addition, the enzyme system of *P. edulis* fruit barks has permitted the biotransformation of carbonyl compounds such as ketones and aldehydes (Machado et al. 2008). These compounds used in perfumery and in paints do not degrade quickly over time, hence the utility of passionfruit preparations to reduce compounds produced in the industry. In addition, peels of *P. edulis* have also permitted the reduction of synthetic dyes such as methylene-blue (Pavan et al. 2008).

Among the numerous alkaloids, phenolics, flavonoids and volatiles substances found in passionflowers, kavain, yagonin, dihydromethysticin and coumarin have properties that completely suppress the barnyardgrass growth. Hence, the use of the allelopathic plant *P. edulis* as a natural herbicide would reduce the dependency on synthetic herbicides and other agrochemicals (Khanh et al. 2006, 2008).

7.9 Invasive Species and Weeds

In a general sense, a weed is a native or exotic plant that grows and reproduces aggressively causing nearly always economic, environmental or ecological loss (Randall 2002). Instead, invasive species is an exotic species (e.g., plant or animal) that adversely affects economically, environmentally or ecologically the habitats that it invades. Human activities provoke the transportation of species beyond their native range. The introduced species are sometimes able to establish and spread in a foreign habitat. Among *Passiflora* species, 19 species have been reported as invasive in 37 countries (Table 7.5).

Islands, which usually present a great number of endemic species, are particularly susceptible to invasions because the introduced species lack natural competitors and predators that control populations in their native ecosystems. The probability of successful invasions is also increased in islands because of the availability of unfilled ecological niches. For instance, the entire island chain of Hawaii has been devastated by foreign insects, plants and animals among which 12 *Passiflora* species with two cause a documented impact. The introduced Andean species *P. tarminiana* (banana poka in Polynesian local language; often confused with *P. tripartita* var. *mollissima*) invaded at least 50,000 ha of upper-elevation rainforests of Hawaii (La Rosa 1992; Trujillo et al. 2001; Trujillo 2005), threatening the survival of the native Hawaiian koa (*Acacia koa* A. Gray) and other 20 native endangered Hawaiian plant species (Trujillo et al. 2001). To control this weed, the US government spent $90,000 per year for uprooting plants and $500,000 to find adequate biological control agent against it (Waage et al. 1981; Causton et al. 2000; Causton and Ranget 2002). A fungal pathogen *Septoria passiflorae* (Coelomycetes: Sphaeropsidales), noxious against *P. tarminiana,* was found in the plant native range (Trujillo et al. 1994). Inoculations of this biological agent permitted to eliminate 95% of the biomass of banana poka in 4 years and allowed the revitalization of the high-elevation forests of Kauai, Maui, and Hawaii (Trujillo et al. 2001; Trujillo 2005).

A native species can become a weed by the effects of natural events or human modifications to the habitat. For example, the Brazilian species, *P. alata* also known as maracuja doce, is colonizing unoccupied regions of southern Brazil (Koehler-Santos et al. 2006a, b). The colonization process of *P. alata* may have been facilitated by the presence of dispersion and pollination agents of other *Passiflora* species already present in the colonized area. In addition, high genetic diversity among and within the populations of *P. alata* probably promotes the success of the colonization process. Therefore, *P. alata* can, in these circumstances, be considered a native weed. In addition to

Table 7.5 *Passiflora* invasive species and native weed[a]

Species	Country	Weed type	Character
Invasive			
P. alata	La Réunion (France)		
P. biflora	United States	EW	N
P. caerulea	Australia (continental), Hawaiian Islands, New Zealand, South Africa	NW	C, T, N
P. coccinea	United States		C, T, N
P. edulis	Australia (continental and Pacific offshore islands), Cook Islands, Ecuador (Galápagos Islands), Federated States of Micronesia, Fiji Islands, Hawaiian Islands, La Réunion (France), New Caledonia, New Zealand (Continental and offshore islands), Niue, South Africa, Tonga, United States	EW	C, T, N
P. filamentosa Cav.	Australia (continental)		N
P. foetida	American Samoa, Australia (continental and Indian Ocean offshore islands), Cambodia, Chile, China, Commonwealth of the Northern Mariana Islands (US), Federated States of Micronesia, Fiji Islands, French Polynesia, Guam, Hawaiian Islands, Indonesia, Japan, Kiribati, La Réunion (France), Malaysia, Naurui, Negara Brunei Darussalam, New Caledonia, New Guinea, Niue, Palau, Palau (main island group), Philippines, Seychelles Islands, Singapore, South Africa, Thailand, Tonga, United States, Vietnam, Wallis and Futuna (Horne) Islands, western Samoa Islands	EW	C, T, N
P. incarnata	Fiji Islands, French Polynesia, Hawaiian Islands, New Caledonia, Niue, Tonga, United States, western Samoa Islands		C, T
P. ligularis	Ecuador (Galápagos Islands), Hawaiian Islands, Indonesia, United States, western Samoa Islands	EW	C
P. maliformis	American Samoa, Cook Islands, Fiji Islands, French Polynesia, Hawaiian Islands, New Caledonia, Niue, Pitcairn Islands, Tonga		
P. mixta	New Zealand	EW, QW, NW	C, N
P. morifolia	Australia, United States		C, N
P. pulchella Kunth (*P. bicornis*)	Hawaiian Islands, United States	QW, NW	C, N
P. quadrangularis	Australia, Ecuador (Galápagos Islands), Federated States of Micronesia, Guyana, Hawaiian Islands, United States, western Samoa Islands		C, T, N
P. rubra	Cook Islands	EW	C, T, N
P. suberosa	Australia (continental), Commonwealth of the Northern Mariana Islands (US), Cuba, Fiji Islands, French Polynesia, Guam, Hawaiian Islands, Japan, La Réunion (France), New Caledonia, Palau (main island group), Seychelles Islands, South Africa, Tonga, United States, western Samoa Islands	EW, QW, NW	C, T, N
P. subpeltata	Australia (continental), Hawaiian Islands, Tonga	EW, QW, NW	C, T, N
P. tarminiana (*P. mollissima*)	Australia (continental), Australia (Indian Ocean offshore islands), Hawaiian Islands, New Zealand, New Zealand (offshore islands), South Africa, United States	EW, QW, NW	C, N

(*continued*)

Table 7.5 (continued)

Species	Country	Weed type	Character
Native Weed			
P. alata	Brazil		C, T
P. cincinnata	Brazil		
P. cinnabarina Lindl.	Australia (continental and Indian Ocean offshore islands)		T, N
P. lutea	United States		C

[a]Randall (2002), Koehler-Santos et al. (2006a), US Forest Service (2007). The species accounted as weed and introduced are considered here as invasive species. The type of weed are also signaled (*EW* Environmental Weed, *NW* Noxious Weed, *QW* Quarantine Weed). Few characteristics of each species are also listed, when information is available (*C* Cultivated, *N* Naturalized, *T* Toxic)

P. alata, two other passionflowers are reported to be native weed, *P. cinnabarina* Lindl. in Australia and *P. cincinnata* also in Brazil (Randall 2002).

Weed and invasive species can be a threat for human health if the species are toxic. For instance, the weed *P. alata* induces occupational respiratory allergic disease (Giavina-Bianchi et al. 1997), and should be considered a risk because it could induce allergic problems in new colonized areas. Ten weedy *Passiflora* are also accounted as toxic (Table 7.5). Programs for the identification of *Passiflora* weeds and prevention of dispersal of these plants are essential to maintain the equilibrium of ecosystems.

7.10 Conclusion

Wild *Passiflora* species constitute a large source of genetic resource for the improvement of cultivated species and the introduction of new fruit crops and tropical garden ornamentals. Moreover, new pharmaceutical, organic and ecofriendly products can be developed from these wild passionflowers. Although the majority of studies have been conducted on cultivated species, in particular in *P. edulis*, they constitute a useful reference to conduct similar studies in wild species

The fragmentation and loss of habitat caused by human activities endanger many tropical plants, such as wild *Passiflora* species. Many passionflowers are endemic and certainly threatened; thus it is indispensable to ensure their conservation. The determination of their geographical distribution is required to further develop in situ conservation programs. In addition, ex situ *Passiflora* germplasm collections should be increased in number of species and individuals to ensure the conservation of a great range of the genetic variability of wild species and its economic valorization.

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Chapter 8
Persea

Vanessa E.T.M. Ashworth, Haofeng Chen, and Michael T. Clegg

8.1 Introduction

Avocado (*Persea americana* Mill.) is an economically valuable tree crop that originated in the New World but is today grown in many parts of the world. Global production has seen a constant rise for many years due to the popularity of avocado as a nutritious fruit rich in beneficial oils and vitamins. What distinguishes avocado from its wild relatives is primarily a far larger fruit that consists of a greater proportion of edible flesh in relation to the size of the seed and a thinner exocarp. Many attributes of avocado are subject to considerable variability, including fruit size, shape and composition, tree shape and size, vegetative features, and ecological and edaphic tolerances, reflecting its origin in multiple domestication centers, and a breeding system that favors outcrossing. Cultivars have been developed for use as scions or rootstocks or occasionally both. The interest in wild germplasm lies in its rich repository of characteristics that could be tapped for avocado improvement and for the study of the avocado genome. Graft incompatibility and sterility barriers within the genus *Persea* have so far tempered exploration in search of wild germplasm, but as micropropagation methods improve this hurdle will eventually be mitigated. With this in mind, efforts are underway to capture the genetic diversity remaining in the native range of avocado before commercial concerns replace the rich germplasm resources of traditional home gardens with cultivar monocultures.

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8.2 Taxonomy, Distribution, and Domestication

8.2.1 *Taxonomy of Genus* Persea

Avocado belongs to the order Laurales in the magnoliids, an assemblage of angiosperms with "primitive" features (Cronquist 1988; Takhtajan 1997). The ancestral placement of Lauraceae is underscored by the fact that Magnoliidae are sister to (predate) the separation of eudicots and monocots (Jansen et al. 2007; Moore et al. 2007). Lauraceae are a large pantropical family of some 50 genera (up to 3,000 species), almost all of which are trees. A fairly high base chromosome number ($x = 12$) and the presence of duplicated gene sets – ca. 25–29% of loci are reported to be duplicated (Soltis and Soltis 1990; Cui et al. 2006) – hint at ancient polyploidy in the family. Most members of Lauraceae, including avocado, have $2n = 24$ chromosomes (Garcia 1975). The genome size of avocado is 907 Mbp (Arumuganathan and Earle 1991).

The genus *Persea* has seen many taxonomic realignments, and the generic circumscription followed herein is that of *Persea sensu stricto*, in which species native to Asia (ca. 80) are considered part of other genera (mainly *Machilus* spp.). This makes genus *Persea* an exclusively New World taxon of some 70 species, although possibly not a monophyletic one (Campos-Rojas et al. 2007; Rohwer et al. 2007). *Persea indica,* although often included in subgenus *Persea*, is endemic to the Macaronesian Islands of the west coast of Africa (including the Canary Islands). It is now seen as a relictual species of the ancestrally Gondwanan genus *Persea*, surviving in its Macaronesian refugium after the climate in Africa became too dry; it likely merits placement in its own subgenus.

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The taxonomic complexity of *Persea* and other lauraceous genera means that wild relatives of avocado potentially may be found in genera other than *Persea*. Members of *Beilschmiedia* have sometimes been included in *Persea*, and in a recent phylogenetic analysis based on morphological characters (Campos-Rojas et al. 2007) *Persea* emerged as paraphyletic in relation to *Nectandra* and *Ocotea*. Some of these genera are sympatric with *Persea* (Table 8.1) and often resemble the wild avocado relatives. *Beilschmiedia anay* (Blake) Kosterm. produces edible fruit of similar taste and consistency to avocado (Borys et al. 1993). Morphological resemblance to avocado has even been noted for certain *Endiandra* species of the northeastern Australian rainforest (Schroeder 1995).

Species of *Persea sensu stricto* are assigned to the subgen. *Persea* (Mesoamerican) and *Eriodaphne* (primarily South American), with *P. americana* (cultivated avocado), *P. parvifolia,* and *P. schiedeana* residing in subgen. *Persea* (Table 8.2). This infrageneric taxonomy of *Persea* has been questioned, most recently in light of a morphology-based phylogenetic study suggesting subgen. *Persea* to be more closely related to *Nectandra* and *Ocotea* than to subgen. *Eriodaphne* (Campos-Rojas et al. 2007). In a preliminary molecular study based on the nuclear ribosomal internal transcribed spacer region (Rohwer et al. 2007), only genus *Machilus* and *Persea* subgen. *Eriodaphne* were monophyletic. Significantly, species in subgen. *Persea* are interfertile, but crosses between the two subgenera are incompatible. As yet, no large-scale molecular phylogenetic studies exist for the genus as a whole, and its relationship with other lauraceous genera is also unclear. It is advisable, therefore, to target a wide taxonomic spectrum when prospecting for wild avocado relatives.

Table 8.1 Species of *Persea* in subgenus *Eriodaphne* and related genera mentioned in the text. Geographic distributions are taken from Tropicos (2010)

Taxon	Geographic distribution
P. alpigena Spreng.	Jamaica
P. borbonia (L.) Spreng.	USA: Alabama, Arkansas, Delaware, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Texas, Virginia
P. caerulea (Ruiz & Pav.) Mez	Honduras; El Salvador; Nicaragua; Costa Rica; Panama; Colombia; Venezuela; Ecuador; Peru; Bolivia
P. cinerascens Blake	Mexico: Veracruz
P. donnell-smithii Mez	Mexico: Chiapas; Guatemala; Honduras; Nicaragua; Costa Rica
P. indica (L.) Spreng.	Macaronesian Islands (including Canary Islands)
P. lingue (Ruiz & Pav.) Nees	Peru; Chile
P. longipes (Schltdl.) Meisn.	Mexico
P. pachypoda Nees	Mexico
P. palustris (Raf.) Sarg.	USA: Alabama, Delaware, Florida, Georgia, Louisiana, Maryland, Mississippi, North Carolina, South Carolina, Texas, Virginia; Bahamas
P. skutchii C.K.Allen	675–900 m; Costa Rica; Panama
Beilschmiedia anay (S. F.Blake) Kosterm.	Mexico: Puebla, Veracruz; Guatemala; Honduras; Costa Rica; Panama; Colombia
Beilschmiedia mexicana (Mez) Kosterm.	Mexico: Chiapas, Guerrero, Hidalgo, Puebla, Querétaro, San Luis Potosí, Veracruz; Belize; El Salvador
Machilus Rumph. ex Nees	Asia
Mutisiopersea Kostermans	Central America
Nectandra Rol. ex Rottb.	Mexico: Jalisco, Oaxaca, Veracruz; Central America; Caribbean; South America
Ocotea Aubl.	Mexico: Chiapas, Nayarit, Oaxaca, Veracruz; Central America; Belize to South America; Africa and Madagascar

Countries are arranged from North to South

8.2.2 Taxonomy and Distribution of P. americana

P. americana, the species comprising cultivated avocado, is exceedingly variable and is split up into about nine botanical varieties (Table 8.2), three of which together comprise today's avocado (Fig. 8.1). This arrangement creates the unusual situation where some of the wild relatives of avocado are actually part of the same species (Fig. 8.1). A suite of morphological and ecological characteristics distinguishes each of the three botanical varieties (also known as ecological races) making up avocado, var. *americana* (lowland "West Indian" avocado), var. *drymifolia* (Mexican avocado), and var. *guatemalensis* (Guatemalan avocado). These varieties are thought to have undergone domestication in different parts of Mesoamerica, the

Table 8.2 Summary of the main botanical characteristics of selected *Persea* Mill. taxa in subgenus *Persea*

Taxon	Characteristics	Notes	Geographic distribution
P. americana var. *americana* Mill.	Fruit 10–25 cm long; skin thin and leathery, color green through purple; flesh whitish	*Cultivated: West Indian race* Two distinct subtypes in Mesoamerica and South America (Rhodes et al. 1971; Ben-Ya'acov et al. 1992)	<1,200 m, MX: Chiapas, Michoacán, Nayarit, Nuevo León, Oaxaca, Quintana Róo, San Luis Potosí, Sonora, Tabasco, Tamaulipas, Veracruz, Yucatán; Belize; Guatemala; Honduras; El Salvador; Nicaragua; Costa Rica; Panama; Ecuador; Peru; Bolivia
P. americana var. *costaricensis*[a]	Fruit round, sometimes pyriform, ca. 4 cm in diameter; skin pale green, peelable and thin; pyriform fruit edible (Ben-Ya'acov et al. 2003a)	Combines West Indian and Guatemalan traits; possibly ancestor of West Indian race (Ben-Ya'acov et al. 2003a)	1,200–2,000 m, Costa Rica
P. americana var. *drymifolia* (Schltdl. & Cham.) Blake	Fruit 4–12 cm long; skin very thin, dark green, purple or black; anise-scented flesh and leaves (usually); (see Storey et al. 1986)	*Cultivated: Mexican race* Higher- and lower-elevation ecotypes (Chen et al. 2009)	>1,000 m, MX: Chiapas, Guerrero, Jalisco, México, Michoacán, Nuevo León, Oaxaca, Puebla, San Luis Potosí, Sinaloa, Tamaulipas, Veracruz; Belize; Guatemala; Honduras; El Salvador; Costa Rica; Panama
P. americana var. *floccosa* (Mez.) Scora[a]	Fruit 4–7 cm long; skin >4 mm thick, dark green	Rare; related to vars. *drymifolia* and *nubigena*	2,000–2,800 m; MX: Chiapas, Puebla, Veracruz; Honduras
P. americana var. *guatemalensis* (Williams) Scora[a]	Fruit 10–18 cm long; skin (rind) >4 mm thick, green or black; small seed tight in cavity	*Cultivated: Guatemalan race* Originated from var. *nubigena* (Williams 1976), var. *steyermarkii* (Schieber & Zentmyer 1978), or related to vars. *steyermarkii*, *nubigena*, *tolimanensis* and *zentmyeri*; only in cultivation or as an escape	100–2,300 m, MX: Chiapas, Guerrero, Hidalgo, Jalisco, México, Michoacán, Nuevo León, Veracruz; Guatemala; Honduras; El Salvador; Nicaragua; Costa Rica; Panama; Venezuela
P. americana var. *nubigena* (Williams) Kopp	Fruit 2.5–5 cm long; skin (shell) >4 mm thick, green	Edible but not cultivated; related to var. *guatemalensis* together with *P. steyermarkii* (also Kopp 1966; Furnier et al. 1990; Scora and Bergh 1990)	1,500–3,000 m, Guatemalan and Central American mountains, MX: Chiapas, Oaxaca; Guatemala; Honduras; El Salvador; Nicaragua; Costa Rica
P. americana var. *steyermarkii* (C.K.Allen) Scora[a]	Fruit 2.5–4 cm long; skin (rind) >4 mm thick, dark green	Rare montane taxon; related to var. *nubigena*	500–1,000 m (MX), 1,300–4,000 m (Guatemala), 2,200 m (El Salvador); MX: Chiapas, Oaxaca; Guatemala; El Salvador; Venezuela
P. americana var. *tolimanensis* (Zentmyer & Schieb.) Scora[a]	Fruit 6–8 cm in diameter; skin 4–5 mm thick, persistent at maturity, dark green; flesh up to 2 cm thick; taste often bitter (in Nicaragua some edible forms)	Link between vars. *nubigena*, *steyermarkii* and *guatemalensis*	Distribution similar to var. *nubigena*: MX: Chiapas to Costa Rica
P. americana var. *zentmyeri* (Schieb. & Bergh) Scora[a]	Fruit 3.5–5 cm long; skin ca. 5 mm thick	Related to vars. *guatemalensis* and *steyermarkii*	1,200 m, single collection in northern Guatemala
P. parvifolia L.O. Williams	Smallest-leaved taxon in subgenus; fruit ca. 3.5 × 2.5 cm; flesh 4 mm thick; skin green	Related to var. *drymifolia* based on fruit characters	2,300–2,600 m; MX: Veracruz
P. schiedeana Nees	Fruit variable, 5–10 cm long; skin <3 mm thick; variable fruit color	In warm and humid locations, similar to West Indian race	250–2,500 m; eastern and southern MX and Central America to Colombia

Information and putative relationships adapted from Scora et al. (2002) and/or other sources as indicated. Countries are arranged from North to South
[a]Name not validly published (USDA–GRIN 2010)
MX Mexico

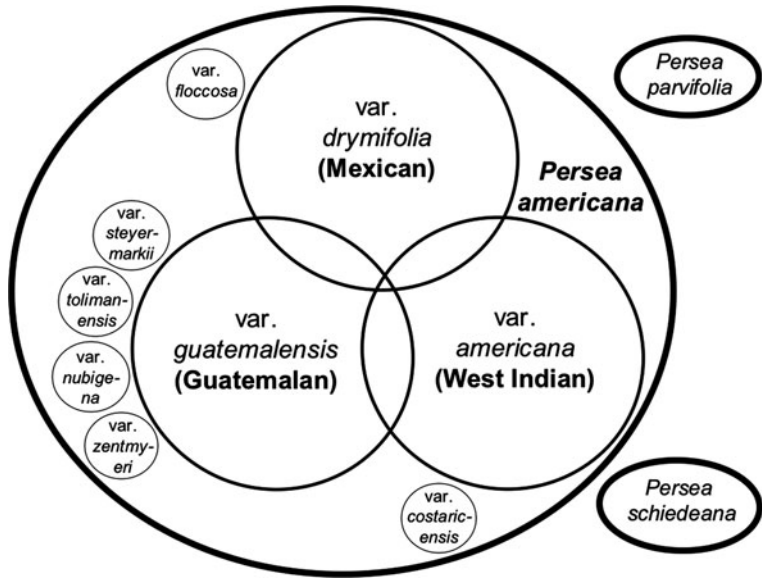

Fig. 8.1 Diagrammatic representation of *Persea* subgenus *Persea*. *Ovals* represent species and *circles* denote varieties of *P. americana*. *Persea americana* varieties *americana*, *drymifolia,* and *guatemalensis* collectively contribute to cultivated avocado. All other varieties of *P. americana* occur exclusively in the wild. *Persea parvifolia* and *P. schiedeana* are separate species in subgenus *Persea*

Mexican race in highland Mexico, the Guatemalan race in upland Guatemala, and the West Indian race in lowland coastal Guatemala. Although originating in lowland Guatemala, var. *americana* is widely known as the West Indian avocado, and this designation will be used herein.

The relationships between the six wild *P. americana* varieties *costaricensis*, *floccosa, nubigena*, *steyermarkii*, *tolimanensis,* and *zentmyeri* and their cultivated counterparts – vars. *americana*, *drymifolia,* and *guatemalensis* – are speculative. In particular, it is unclear whether the wild varieties represent separate derivatives of the same ancestral wild taxon that gave rise to avocado, or whether they experienced some early selection before escaping back into the wild. Some of these varieties are confined to very few or a single collection locality (Scora et al. 2002; Ben-Ya'acov and Barrientos-Priego 2003).

The two other taxa at the rank of species in subgen. *Persea* are *P. parvifolia* and *P. schiedeana*, the latter also a morphologically very variable species with an expansive New World distribution (Table 8.2). *Persea schiedeana* is of interest to avocado breeding owing to its edible fruits and tolerance of fungal pathogens.

Germplasm relevant to avocado breeding and genomics therefore includes, in order of most to least distant relationship (1) species of *Persea* in subgen. *Eriodaphne* and *Persea* (and perhaps of other genera), (2) wild botanical varieties of *P. americana*, (3) semi-domesticated forms representing botanical varieties *americana*, *drymifolia,* and *guatemalensis*, especially those from regions of avocado domestication (cultigens), and (4) cultivars. Cultivars themselves, as representatives of three distinct botanical races and retaining abundant phenotypic variability, are also an important resource for breeders and molecular geneticists.

8.2.3 Domestication

The domestication history of avocado has been pieced together from many lines of evidence, including archeology, linguistics, and historical records. Collectively, these sources firmly place the center of domestication in pre-Columbian Mesoamerica. The onset of avocado consumption has been dated back as far as 7000–8000 BC (Smith 1966) from archeological findings in the Puebla region of Mexico, and separate evidence exists for the use of avocado by the Mesoamerican Mokayas, predecessors of the Mayas and Olmecs (Galindo-Tovar et al. 2007). Archeological, iconographic, and linguistic research (Smith 1966, 1969; Gama-Campillo and Gomez-Pompa 1992) suggest human selection and utilization from about 4000 BC in Mesoamerica. The three ecological races arose at the hands of a succession of different cultures across

Mesoamerica, presumably by selective retention of desirable trees and removal of undesirable ones in forest gardens or home gardens. Ample evidence suggests that the three races were firmly established before European contact, as noted by numerous chroniclers in the fifteenth to seventeenth centuries (Anonymous 1997; Popenoe and Zentmyer 1997), and their distinctness was acknowledged by horticulturalists and botanists, including Popenoe (1963) and Kopp (1966). Occasional admixture between them undoubtedly occurred prior to the twentieth century, but to this day little evidence of intercrossing is observed in the centers of origin (Ben-Ya'acov and Barrientos-Priego 2003). Only much later, in the early twentieth century, did deliberate hybridization between the three races occur in earnest. The salient characteristics that distinguish the three ecological races comprising avocado are well documented elsewhere, but a summary is provided here for convenience (Table 8.3).

Table 8.3 Main features differentiating the three cultivated races of *Persea americana*

Characteristics	Mexican race (var. *drymifolia)*	Guatemalan race (var. *guatemalensis)*	West Indian race (var. *americana)*
Center of domestication	Highland Mexico	Upland Guatemala	Lowland Guatemala
Climatic adaptation	Subtropical	Subtropical	Tropical
Cold tolerance	High	Moderate	Low
Salinity tolerance	Low	Moderate	High
Shoots: flush color	Reddish	Green	Yellow–green
Leaves: anise scent	Present	Absent	Absent
Time from flowering to fruit maturity	5–7 months	10–18 months	6–8 months
Fruit: skin color	Purple–black	Green or black	Pale green, maroon
Fruit: skin thickness	Thin	Thick	Moderately thin
Seed : fruit ratio	High	Mostly low	High
Seed tightness in cavity	Often loose	Tight	Often loose
Flesh: flavor	Rich, slight anise scent	Often rich	Sweetish, mild
Flesh: oil content	High	Moderately high	Low

The transformation of the wild avocado ancestor into three semi-domesticated forms apparently occurred without an appreciable loss of genetic diversity (Chen et al. 2008). Whether by chance or due to differential selection priorities pursued by the peoples of Mesoamerica, the problem of a genetic bottleneck was avoided. Many of today's avocado cultivars harbor levels of genetic diversity that in other crops would need to be recruited from the wild gene pool.

The Guatemalan and Mexican avocados did not experience much human dispersal from their respective centers of domestication until after European contact, presumably due to their origins in dissected and inaccessible mountainous regions far away from the coastal trade routes. By contrast, avocados of the West Indian race were already spread far beyond their center of origin in pre-Columbian times (Popenoe and Zentmyer 1997; Knight 2002), probably because of their coastal presence that encouraged distribution by raft or boat. West Indian avocados were the first to reach Peru, and several distinct forms of this race have been reported from Ecuador (Scora et al. 2002; Ben-Ya'acov and Barrientos-Priego 2003). The misnomer "West Indian" avocado may arise from this earlier spread, the West Indian archipelago presumably having served as a destination on pre-Columbian trade routes, and avocado trees had already been planted on these islands by the time of European contact. Since then, spurred by the era of world exploration, avocados have reached destinations far beyond the New World (Knight 2002; Scora et al. 2002).

8.3 Breeding

Open-pollination and hybridization, followed by selection of promising material among the progeny, have been the only methods available to avocado breeders. Compared with the carefully orchestrated genetic composition of many major crops, where inbred and isogenic lines and other breeding tools are available, the makeup of contemporary avocado cultivars is often a stochastic mixture of two or occasionally all three botanical races. Indeed, it is customary to infer racial composition of a hybrid cultivar retrospectively by comparing its phenotypic traits against those of racially uniform cultivars, when parentage is unknown. Wild germplasm and cultigens, though not

actively used in breeding programs at the moment, are being preserved for the future as repositories of valuable traits.

8.3.1 History

Prior to the twentieth century, selection of avocado is thought to have occurred in a seedling orchard environment by growing seeds from superior trees and selectively retaining superior progeny, principles used to this day in home gardens and other non-commercial situations. The techniques of clonal propagation and grafting onto a rootstock were not developed until later (Ruehle 1963). Breeders in the twentieth century sought to combine desirable attributes of the three ecological races of *P. americana* and focused on open-pollination and hybridization among them. This was unavoidable due to the impracticability of performing controlled pollination, a consequence of the great abundance of tiny flowers on any given tree and low seed set that makes floral bagging futile (Davenport 1986). Desirable progeny from these crosses were either maintained by clonal propagation, or their seeds were included in future rounds of selection. Despite occasional reports to the contrary (the Rodiles Orchard of Atlixco, Mexico: Scora et al. 2002), there is ample evidence that the segregating progeny from a cross between superior genotypes will result in many undesirable forms (e.g., Lahav and Lavi 2002) and that none of the traits studied to date is controlled by a single gene (e.g., Lavi et al. 1993a: fruit skin color, flowering group and anise scent do not show Mendelian segregation). In the absence of alternatives, however, open-pollination and interracial hybridization have been the methods by which new avocado varieties have been developed. In both California and Florida, the main growing regions within the US, introduction of cultivars from (primarily) Mexico and Cuba, respectively, resulted in interracial hybrid cultivars: Mexican × Guatemalan forms in California and West Indian × Guatemalan forms in Florida. Early twentieth century avocado improvement in California was characterized by the import of avocado seeds and budwood from mostly Mexican sources, usually by private individuals, but records of where the material came from have often been lost. Many of today's cultivars arose as chance seedlings in backyards. An account of attempts to trace the possible origin of cultivar Duke (Zentmyer et al. 1963) is a case in point, invoking germplasm imported from different parts of Mexico or possibly as far as Chile. The origin of cultivar Hass is also vague, having been discovered in the backyard of Rudolf Hass in Southern California where it was apparently destined for use as a rootstock. It arose as a chance seedling from a cross between unidentified Mexican and Guatemalan material. Molecular genetic data are starting to shed light on the origins of some of these cultivars.

Hybridization among the members of subgen. *Persea* is successful, although attempts generally meet with limited success due to the recovery of inferior attributes in the progeny. Rootstock improvement initially attempted hybridization between *P. americana* and wild germplasm from subgen. *Eriodaphne*, members of which have strong disease resistance, but failed due to sterility barriers between the two subgenera. This property has been used as an argument in favor of assigning species of subgen. *Eriodaphne* to another genus (Kostermans 1993: *Mutisiopersea*). Graft incompatibility between cultivated avocado and wild-collected rootstock material has been an informal criterion for assigning new accessions to subgen. *Eriodaphne*.

Discouraged by sterility barriers and graft incompatibility between avocado and wild relatives, breeders until recently restricted their efforts to hybridization among mainstream cultivars. As molecular techniques become more powerful, wild relatives and cultigens are receiving renewed interest as sources of important and novel traits.

8.3.2 Valuable Phenotypic Traits

Many traits are of interest to avocado breeders, but comprehensive studies that sample from across subgen. *Persea* and more distant germplasm are scarce. Instead, comparative data are often gathered from cultivars representing the three botanical races or their hybrids. Such studies are instructive in that they narrow down which race should be the focus of selection efforts for a particular trait and which ecological habitat or domestication center to target in search of wild germplasm.

8.3.2.1 Tree Habit

The shape and size of a tree is relevant to the quest for reduced tree spacing and simplified canopy management. Mexican race cultivars are generally smaller than cultivars from the other two races, but tree size plays a subordinate role to canopy shape in determining suitability for narrow spacing. Branching patterns are key to canopy shape, and the tendency for a given cultivar to form a more columnar or more spreading canopy is largely a function of the prevalence of functionally indeterminate versus functionally determinate flowering shoots (Thorp and Sedgley 1993). Canopy shape is generally cultivar-specific, with "Fuerte," for example, being more spreading and "Bacon" more columnar. Spreading forms that branch readily are less suited to high-density planting than columnar forms. A study by Soto et al. (2007) revealed a higher proportion of verticillate (whorled) than irregular branching in West Indian cultivars and their hybrids compared with Guatemalan cultivars.

Dwarfing phenotypes are desirable especially for use as rootstocks to curb the growth of the scion cultivar and facilitate orchard management. Cultivars with a dwarfing habit include Colin V-33, Jalna, Mt 4, and Wurtz Nowels and has also been reported in vars. *floccosa* and *nubigena* and in *P. schiedeana* (Bergh 1975), although many factors modulate the dwarfing habit (Ben-Ya'acov and Michelson 1995). Dwarfing is claimed to have a genetic component in *P. schiedeana* (Litz et al. 2007b), and wild relatives are of incipient interest in the search for dwarfing rootstocks (Thorp and Hallett 1999). Inspired by findings from apple dwarfing rootstocks, efforts are concentrated on testing wood-anatomical markers (vessel density and the ratio of bark transectional area to stem or root transectional area; Meza-Castillo et al. 2007) in the segregating progeny of "Colin V-33." Such an approach enables rapid screening of material. Another feature that facilitates propagation from cuttings is the natural tendency to produce adventitious roots. This tendency is known from cultivated avocado and *P. americana* var. *steyermarkii* (Borys 1991; Barrientos-Priego et al. 1995).

8.3.2.2 Leaves

A comparative study of leaf oils in *P. americana* and a range of *Persea* species was conducted by Scora and Bergh (1992). The chemical composition of the leaves of the Mexican botanical race is distinctive by its anise scent that is absent from the other two races. The monoterpene responsible is estragole that attains 60% of the total essential oils in leaves of Mexican race cultivars. A related compound, anethole, was encountered in a wild relative of the Mexican botanical race (aguacate de anis). The leaf oil profiles of West Indian and Guatemalan races were comparable, consisting of monoterpenes, sesquiterpenes – especially caryophyllene – and esters. Unlike the Guatemalan profile, leaves of the West Indian race virtually lacked the sesquiterpene esters but exhibited small amounts of *cis*-ocimene. The wild relatives *P. americana* vars. *floccosa, tolimanensis,* and *steyermarkii* had leaf oil profiles that resembled those of the Guatemalan race. In subgen. *Eriodaphne, P. borbonia* leaves were enriched in *cis*-ocimene, while *P. caerulea* contained primarily caryophyllenes. Camphor was detected in *P. pachypoda*. No anise scent is present in *P. schiedeana* (Bost 2009). In a study of the segregating progeny of 14 cultivars, Lavi et al. (1993a) concluded that presence of anise in the leaves is controlled by multiple genes.

8.3.2.3 Fruits

Avocado has been termed an "overbuilt, extravagant fruit" in the context of seed dispersal (Barlow 2000), reflecting its large size and nutritious oily content, probably a coevolutionary holdover from adaptation to a now extinct neotropical megafauna. Its composition is indeed of commercial interest in that it includes an array of antioxidants (e.g., Kim et al. 1998, 2000; Ding et al. 2007), carotenoids including lutein (Lassen et al. 1944; Slater et al. 1975; Heinonen et al. 1989; Lu et al. 2005), D-mannoheptulose (Shaw et al. 1980), persenones (Kim et al. 2000), phenolics (Vinson et al. 2001), β-sitosterol (Duester 2001), terpenoids (Moreno et al. 2003), and vitamins B, C and E (Lassen et al. 1944), with manifold beneficial properties, including cholesterol reduction (Lopez-Ledesma et al. 1996) and anticarcinogenicity (d'Ambrosio 2007; Ding et al. 2007). By and large, studies of phytochemistry have focused exclusively on cultivated avocado or, rarely, on readily available (often ornamental) species of *Persea*, such as *P. indica* and *P. borbonia*. The exception is *P. schiedeana* that

bears fruit with a fairly high fatty acid content (up to 36%) and good fatty acid composition (Martinez et al. 2007). A growing interest in the composition of the avocado fruit has paved its entry into the field of nutrigenomics. Baseline studies are starting to accumulate information on the composition of phytonutrients in different parts of the avocado fruit (skin and various flesh layers; Ashton et al. 2006) with a view to the production of high-quality cooking oil. Lower-grade avocado oil is used in lotions, creams and hair products.

The color of avocado skin is important to breeders since black avocados such as "Hass" fetch a higher price in many parts of the world. Black skin is often characteristic of Mexican race avocados, but is less diagnostic than some of the other distinguishing traits (Table 8.3). In a study of three traits in the progeny (selfed and outcrossed) of 14 cultivars, Lavi et al. (1993a) noted that green skin color predominated among both selfed and outcrossed progeny, ruling out control by a single gene. *P. schiedeana* exhibits considerable variation with regard to skin color (Martinez et al. 2007). Skin thickness and consistency are additional features important to breeders. Both attributes vary among the three races of cultivated avocado (Table 8.3) and especially among the wild relatives in subgen. *Persea*, ranging from paper-thin to rind- or shell-like (Table 8.2). Peelable skin (as in the Mexican cultivar Fuerte) or a firm skin permitting the flesh to be scooped out (a trait reflecting Guatemalan race ancestry) are both desirable properties for cultivated forms.

Fruit set in cultivated avocado is extremely low in comparison to the profusion of flowers produced on a tree. A far higher fruit set was noted in *P. floccosa* (Lahav and Lavi 2002), but progeny from a cross between a commercial cultivar and *P. floccosa* failed to combine characteristics of the two parents in a desirable way.

8.3.2.4 Pests

Among the pests that adversely affect avocado production are persea mite (*Oligonychus perseae* Turtle, Baker and Abatiello), avocado thrips (*Scirtothrips perseae* Nakahara), red-banded whitefly (*Tetraleurodes perseae* Nakahara), and avocado lace bug (*Pseudacysta perseae* Heidemann) (Hoddle 2004). So far, wild *Persea* relatives have not been examined for their abilities to withstand or deter pests. *Persea borbonia* and certain Lauraceae (*Cinnamomum camphora*) serve as alternate hosts to avocado lace bug (Hoddle et al. 2005), and persea mite is known to attack *C. camphora* (Hoddle 2004). Efforts so far have concentrated on comparing insect feeding preferences for different cultivars, studying their biology, and searching for natural enemies in the native range (Hoddle et al. 2002).

8.3.2.5 Diseases

Probably the most serious fungal disease that causes root dieback in avocado is *Phytophthora cinnamomi,* the causal agent of root rot, with *Phytophthora citricola* (collar rot) and avocado sunblotch viroid constituting additional problems. Since 2002, avocado in the southwestern USA has been threatened additionally by laurel wilt (*Raffaelea lauricola*). Its inexorable spread by the beetle *Xyleborus glabratus*, coupled with a broad host range (infection of *P. borbonia, P. palustris,* and several other Lauraceae), are factors predicted to impact avocado far beyond the southwestern states (Fraedrich et al. 2008). Other pathogens are specific to certain regions only, such as *Rosellinia necatrix* in southern Spain.

Breeders are continually searching for resistance sources by screening progeny of promising genotypes, but progress is slow as the inheritance of resistance appears to be polygenic. Horticulturalists in the nineteenth and first half of the twentieth centuries traveled to Central America and Mexico in search of suitable rootstock germplasm with good disease resistance, tolerance of poor soils, and capable of conferring other beneficial attributes on the scion (tree size, dwarfing, fruit yield). Their selections were either imported back to the US as graftable budwood or as seeds. Later imports of germplasm were almost exclusively motivated by the search for root rot resistance.

Unfortunately, *Persea* relatives with the highest root rot resistance are almost always from subgen. *Eriodaphne* and hence graft incompatible, although some strains of the graft-compatible *P. schiedeana* carry moderate levels of resistance (Coffey et al. 1988). Even compatible breeding material rarely gives rise to suitable progeny, which often exhibit instead susceptibility to other diseases, poor tolerance of soil stresses, or an adverse impact on scion yield in the absence of disease pressure (Coffey et al. 1988;

Newett et al. 2002). Contemporary selection strategies (Douhan 2008) concentrate on exploring diseased or otherwise heavily stressed orchards for so-called escape trees – individuals that appear to be surviving under intense disease pressure and other forms of adversity. The pedigree of escape trees is generally unknown, but the trees are most likely extant – or perhaps outdated – cultivars and rootstocks or their seedlings. Use of escape trees circumvents the problem of graft incompatibility with taxonomically distant germplasm, but selection progress also tends to be slow. Rootstock breeding is poised to embrace tissue culture and micropropagation methodology when it becomes available in order to take advantage of disease resistance in subgen. *Eriodaphne*.

8.3.2.6 Salinity and Adverse Edaphic Conditions

West Indian cultivars and rootstocks show greater tolerance of saline or calcareous soils than their Guatemalan and Mexican counterparts (Lahav and Lavi 2002; Newett et al. 2002). However, this tolerance does not extend to waterlogging, a condition under which Mexican selections perform better. Moreover, rootstocks tolerant of edaphic stresses often do not combine this property with disease resistance, nor may they impart enhanced scion productivity, and even productivity can vary widely in different locations. Consequently, rootstock breeding must simultaneously address a multitude of factors, and to date no single rootstock selection excels in all respects.

In California and regions subject to cold soil temperatures, rootstocks are typically drawn from Mexican stock, whereas in tropical regions West Indian rootstocks and their hybrids are the norm. Guatemalan race material rarely plays a part in rootstock trials, although moderately successful hybrid Guatemalan rootstocks have been developed in the past ("Edranol" and "Velvick"). Until recently, the only wild germplasm tested for tolerance of soil stresses was the Mexican × *P. schiedeana* hybrid G755 (= "Martin Grande") that shows salinity tolerance (Newett et al. 2002). Among Mexican rootstocks, "Borchard" shows good tolerance of salinity and alkali soils, combined with resistance to *P. citricola* but high susceptibility to *P. cinnamomi*. The *P. cinnamomi*-resistant Mexican cultivar Thomas (Chen et al. 2009), by contrast, is highly sensitive to salinity and susceptible to *P. citricola* and *Dothiorella gregaria* canker (Rose 2003). "Zutano," though traditionally a scion cultivar and a Mexican × Guatemalan hybrid (Chen et al. 2009), is used as a salinity-tolerant rootstock in Australia and New Zealand (Newett et al. 2002).

Rootstocks suitable for Israel growing conditions were developed during a major breeding program involving West Indian material. The program sought to combine soil tolerances with scion productivity. Attention is now focused on exploring the center of diversity of the West Indian race for new germplasm. Ben-Ya'acov and Barrientos-Priego (2003) reported that trees thought to be West Indian × Guatemalan race hybrids from along the Pacific coastal belt of Costa Rica exhibited drought, salt, and flooding resistance. They also noted that West Indian germplasm from the Cienega Grande in Colombia was growing in saline soils and that Mexican race material collected in Mexico and Ecuador was tolerant of high soil lime contents. The assignment methodology developed by Chen et al. (2009) to assess racial composition and provenance of avocado selections may guide breeders in the choice of breeding material from both cultivated and wild sources.

8.4 Molecular Genetics

8.4.1 Genetic Markers

Genetic markers are valuable tools for inferring taxonomic relationships, assessing and verifying parentage, and for marker-assisted selection. Furnier et al. (1990) and Davis et al. (1998) employed restriction fragment length polymorphism (RFLP) markers to identify avocado cultivars and close relatives. Their findings supported morphological studies (Kopp 1966; Scora and Bergh 1990) recommending realignment of *P. floccosa, P. nubigena,* and *P. steyermarkii* as varieties of *P. americana*. They also suggested that the Guatemalan race originated from hybridization between vars. *steyermarkii* and *nubigena*. Results by Davis et al. (1998) lent further support to earlier work that Guatemalan and West Indian races are more closely related to one another than to the Mexican race, although this was not evident in a large germplasm survey by Schnell et al. (2003) where all three races were equally distinct.

Considerable molecular heterozygosity has been revealed using a range of markers, including RFLPs (42%; Ashworth and Clegg 2003), microsatellites (64%, Ashworth and Clegg 2003; 83%, Schnell et al. 2003; 38–70%, Lavi et al. 1994), and single nucleotide polymorphisms (SNPs) (40–70%; Chen et al. 2008). The microsatellite study by Ashworth and Clegg (2003) included *P. americana* var. *steyermarkii*, *P. schiedeana,* and several wild rootstock selections from Guatemala and Costa Rica of uncertain racial origin. Amplification failure, presumably due to mutation at one of the primer annealing sites, was noted for the wild material at numerous microsatellite loci, most notably so for the rootstock G875, suggesting its distant relationship to today's cultivars (Ashworth and Clegg 2003). *P. americana* var. *steyermarkii* grouped with West Indian cultivars, contrary to its postulated status as progenitor to the Guatemalan ecotype (Furnier et al. 1990). Rootstock selection G810 also associated with West Indian cultivars, suggesting that it may have been obtained from coastal Guatemala, the center of diversity of the West Indian race. Rootstock selection G6, however, clustered with Mexican race cultivars. Interestingly, microsatellite profiles for G755A, a rootstock selection presumed to be a seedling from a cross between a Guatemalan type and *P. schiedeana* (Ellstrand et al. 1986), exhibited frequent amplification failure or multiple bands, forcing its exclusion from some analyses (Ashworth et al. 2004; V. Ashworth personal observation).

As data from molecular techniques accumulated, conclusions about relationships have sometimes changed or been tempered as a result of statistical and analytical concerns. For example, the sensitivity of many phylogenetic and distance-based analytical methods to sample composition and the unpredictable effect of hybrids in a dataset were voiced in Ashworth and Clegg (2003). While genetic markers continue to be useful tools, methods employing DNA sequence data to characterize relationships are providing new perspectives on avocado cultivars and their wild relatives (Chen et al. 2009).

8.4.2 DNA Sequencing

In a recent resequencing study (Chen et al. 2009), SNP data derived from almost 6,000 bp of DNA sequence at four nuclear loci were used to study population structure in 21 wild-collected avocado cultigens. Genetic differentiation in racially uniform groups of wild cultigens was analyzed to delimit racial boundaries de novo and then used to examine racial composition of 33 cultivars. The genetic clustering and assignment programs Structure (Pritchard et al. 2000) and Structurama (Huelsenbeck and Andolfatto 2007) assigned popular cultivars to one or more wild germplasm-inferred clusters and estimated admixture where cultivars were of hybrid origin. For the first time, an analysis was able to quantify the relative contribution of each of the three races to the genome of cultivars, sometimes contradicting prevailing racial assignments. Thus, wild germplasm and cultigens are being used to calibrate the contribution of the three botanical races to the genomes of existing cultivars.

In addition to corroborating the distinctness of the three ecological races, the approach also provided new insights into the composition of each race. In particular, it discerned population substructure in the accessions from central Mexico (Mexican race) based on elevation and latitude and revised our perception of the putative racial composition of certain cultivars. For example, although "Duke 7" and "Duke 6" are known as Mexican race rootstock selections, their nucleotide composition reflected an origin from contrasting elevations, "Duke 7" from the higher elevation stock (M^h) and "Duke 6" from the lower elevation stock (M^l). Similarly, in the two Guatemalan × Mexican cultivars Hass (42% Mexican) and Zutano (43% Mexican), the Mexican component was from higher elevation and lower elevation germplasm, respectively. Overall, the higher elevation Mexican race avocados have left a greater genetic footprint in hybrid cultivars than their lower elevation counterparts.

In general, this study showed that cultivars maintained 80–90% of the nucleotide diversity present in the cultigens, reflecting both the effect of multiple domestications and the role of subsequent hybridization. The assignment methodology applied by Chen et al. (2009) will be a useful tool for genetic resource managers as they seek to collect genotypes that maximize the amount of genetic diversity. Percentage racial composition of hybrid cultivars and rootstocks is indicated below, where available, based on Chen et al. (2009).

8.4.3 Quantitative Genetics

To date, quantitative genetic studies have been confined to progeny of mainstream cultivars. Chen et al. (2007) measured growth- and yield-related traits in ca. 200 open-pollinated progeny from cultivar "Gwen" (99% Guatemalan) that had been genotyped using microsatellite markers to determine the pollen parent. The study revealed broad-sense heritability values to be low, ranging from 23% to 35.5% for growth traits, flowering profusion, and fruit set, probably due to uncontrolled environmental error associated with initial planting date. Moderate positive correlations were detected between growth rates and fruit set. The influence of the pollen parent was evident in that height growth was significantly slower and canopy diameter significantly larger in progeny sired by "Fuerte" (99% M^h), consistent with the somewhat spreading growth habit of this cultivar. Progeny sired by "Bacon" (94% Guatemalan) had significantly greater flower abundance than those of "Fuerte," "Zutano" (43% M^l), or a mixed category composed of miscellaneous known – or unidentified – pollen sources. The appreciable number of unidentified pollen donors, comprising about 25% of the total number of progeny, is attributed to the location of the maternal "Gwen" tree in an experimental field station where wild and semi-cultivated germplasm and many different cultivars coexist in relatively close proximity and participate in open-pollination. This study, and earlier ones by Lavi et al. (1991, 1993b), demonstrate the high level of heterozygosity and non-additive genetic variance present in avocado that encumbers breeding advance by the traditional open-pollination process.

8.4.4 Linkage

In avocado, contrary to many other crops, wild germplasm is not essential to boost polymorphism in crosses destined for the construction of genetic linkage maps. The polymorphism present in contemporary cultivars is sufficient. A preliminary genetic linkage map in avocado (Sharon et al. 1997) was based on 91 markers (microsatellites, RAPDs [random amplified polymorphic DNA], and DNA fingerprint fragments) distributed across the 12 linkage groups at a density of 2–5 markers per group. The map was developed from the progeny of a cross between cultivars "Pinkerton" (98% Guatemalan) and "Ettinger" ("Fuerte" progeny). Another linkage map was constructed from a reciprocal cross between "Simmonds" and "Tonnage" (West Indian and West Indian × Guatemalan cultivars) using microsatellite markers (Borrone et al. 2009). Genetic linkage was reported for DNA fingerprint fragment P8 and a locus regulating fruit skin color (Sharon et al. 1998). The same authors also found microsatellite marker AVAO4 to be linked with a locus controlling fibers in fruit flesh on linkage group 3. Researchers in Spain recently used microsatellites and amplified fragment length polymorphism (AFLP) markers to develop a linkage map from the half-sib progeny of a Spanish genotype that transmits tolerance of *R. necatrix* (Viruel et al. 2007).

Association mapping has received much attention in human genetics and is a promising approach for long-lived tree crops as well. The idea is to identify linkage disequilibria (LD) between markers and phenotypic traits of interest. High LD may be treated as a surrogate for close linkage, so the map position of markers exhibiting high LD with valuable traits is used to infer the map location of genes determining the trait. Selection for a trait can then be applied indirectly by selecting on markers showing high LD with the trait. This approach allows the investigator to exploit population data, thus avoiding the time and expense associated with the construction of genetic crosses. Association approaches appear to be especially promising for crops such as avocado where costs associated with creating experimental populations are high and where generation times are long. Preliminary experiments with *Arabidopsis* have successfully applied association approaches to identify known genes underlying traits for disease resistance and flowering time (Aranzana et al. 2005), so the method has some level of validation.

It is important to note that high LD is not a perfect predictor of close linkage between a trait and a marker. One source of error arises when subpopulations are pooled and LD is calculated from the pooled data. This error arises because LD is induced independent of linkage, as a consequence of pooling frequencies across genetically differentiated subpopulations. Given the high levels of genetic heterogeneity across geographic subpopulations of avocado (Chen et al. 2009), it will be important to characterize

subpopulation structure before attempting association mapping in avocado. A second source of error arises from the process of recombination. Recombination is composed of two processes: cross-over events that have a frequency proportional to map distance, and gene conversion events that recombine markers that reside within a few hundred base pairs, while leaving more distantly linked markers unaffected. Recent estimates of the frequency of gene conversion reveal that these events occur at least as frequently as cross-overs, thereby causing nearby markers to appear more weakly linked than is actually the case (Morrell et al. 2006). Finally, recent estimates of LD within genes in avocado (Chen et al. 2008) reveal that LD decays to half of its initial value within about 1,000 bp, suggesting little residual LD in wild accessions of *P. americana*.

8.4.5 Expressed Sequence Tags

Almost all *Persea* gene sequences deposited in the public databases are derived from cultivated avocado. The Floral Genome Project (Chanderbali et al. 2008) has led to the development of some 10,000 expressed sequence tags (ESTs), including 6,183 unigenes. An extension of this research is currently in progress as part of the US NSF-funded Ancestral Angiosperm Genome Project that will boost the number of ESTs by a further 85,000 (Chanderbali et al. 2008). These ESTs have been generated from flowers of cultivated avocado.

A UC-funded project at UC Irvine (M. Clegg et al. in progress) is generating ESTs from ripening fruit tissue of a "Gwen" progeny genotype. This work is devoted to the discovery of candidate genes involved in fruit biochemical composition. A proprietary EST library based on fruit of "Hass" was developed by HortResearch in New Zealand and includes close to 7,000 sequences averaging a length of 588 bp (G. S. Ross, HortResearch USA, Davis, CA, personal communication).

Researchers from LANGEBIO – the Mexican National Laboratory of Genomics for Biodiversity of the Center for Research and Advanced Studies (CINVESTAV; López-Gómez et al. 2007) – are generating ESTs from a cDNA library of the fruit mesocarp of a Mexican race avocado. At the time of publication, they had generated 4,612 sequences, with an average length of 722 bp. Of the sequenced genes, 42% were related to metabolism, 20% to unknown function, 14% to fruit ripening, 8% to lipid synthesis, 6% to pathogen response, and 4% were involved in senescence. Interestingly, 6% of the genes showed no similarity to any sequence reported in the databases, perhaps reflecting the genetic distance of avocado to other taxa in the public databases.

8.4.6 Gene Expression

Avocado relatives have been employed in the study of contrasting gene expression levels. Chanderbali et al. (2006) compared the expression of floral genes between *P. americana* and the wild relative, *P. borbonia*, that differ respectively by having an undifferentiated perianth (tepals) and differentiated perianth (sepals and petals), respectively. Two genes emerged as responsible for differential perianth development, *AG* and *SEP3*. Their expression levels were initially at comparable levels in both species, but subsequent interruption of expression in the outer tepals of *P. borbonia* resulted in sepaloid morphology and a dimorphic perianth, whereas expression remained constant in *P. americana* (Chanderbali et al. 2006).

8.4.7 Tissue Culture and Transgenic Plants

The promise of somatic hybridization, tissue culture, and genetic transformation to overcome the problems of infertility and graft incompatibility between avocado and subgen. *Eriodaphne* is clearly appealing but has proved challenging. Preliminary success with somatic hybridization between embryogenic avocado protoplasts and mesophyll protoplasts of *P. borbonia, P. cinerascens, P. pachypoda*, and *Nectandra*, all of which share high *P. cinnamomi* resistance, yielded successful hybrids with *P. pachypoda* and *Nectandra* (Witjaksono 1997). Although somatic embryogenesis is readily achieved (Witjaksono and Litz 1999a, b), genetic transformation suffers from very reluctant shoot and root development in the somatic embryos, requiring elaborate plant recovery methods (Litz et al.

2007a; Raharjo et al. 2008). The rate of genetic transformation itself has also been of variable success. Raharjo et al. (2008) successfully incorporated the defensin gene *pdf1.2* via *Agrobacterium tumefaciens* but concluded that all transgenic lines had arisen from a single transformation event. Similarly, Palomo-Ríos et al. (2007) incorporated the gene *neomycin phosphotransferase* II into embryogenic lines of cultivar Duke 7 at a transformation efficiency of 0.8–3.3% with the ultimate goal of generating avocados with improved resistance to *R. necatrix*. One of the transformants is being further transformed with the gene encoding the NPR-1 protein of *Arabidopsis thaliana*. The vast pool of genetic variation present in wild germplasm will be harnessed as soon as micropropagation and genetic transformation protocols have become routine.

8.5 Conservation Initiatives

Many ex situ repositories of wild and cultivated avocado accessions exist, with major collections in North America located in California and Florida, USA, and in several states in Mexico. The Avocado Germplasm Collection of the University of California at Irvine was begun in 1958. An active germplasm collection is also housed at UC Riverside. These collections contain wild relatives cultigens, heirloom and reference cultivars, and rootstocks. Primarily West Indian race avocados and West Indian × Guatemalan types suited to Florida conditions are maintained at the USDA–ARS National Germplasm Repository at Miami, Florida. Backup collections of the Florida avocado germplasm are being established elsewhere in the wake of the laurel wilt outbreak in the southwestern USA (Kendra et al. 2009).

In Mexico, the Universidad Autonoma de Chapingo maintains an ex situ collection of some 180 accessions of *Persea* (mainly *P. americana*, plus other taxa from both subgenera of *Persea*). It is established at two locations of contrasting elevation in the state of México ("El Potrero," Coatepec Harinas, 2,240 m and "El Salitre," Ixtapan de la Sal, 1,920 m). Diversity is being assessed using geographic information systems (GIS) in conjunction with herbarium inventories (Aguilar-Gallegos et al. 2007). Another collection is the National Institute for Forestry, Agriculture and Livestock Research Germplasm Collection in the state of Guanajuato. Ex situ germplasm collections that include *Persea* accessions are also present in Mesoamerican countries (Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica and Panama) that, together with Mexico, have formed the Mesoamerican Network of Plant Genetic Resources (REMERFI 2009).

Many other countries have germplasm collections of various sizes, mostly of cultivars but also some with interesting historical collections. The Spanish germplasm collection "Estación Experimental La Mayora" in Málaga, for example, retains some very old trees grown from seed brought back by Spanish explorers from the New World (Alcaraz and Hormaza 2008).

Conservation initiatives focus especially on wild or semi-domesticated taxa of subgen. *Persea* and on disease-resistant germplasm suitable for use in rootstock development from subgen. *Eriodaphne*. Emphasis is placed also on acquiring germplasm with novel features of horticultural interest and tolerance of salinity and other soil conditions. Almost 200 accessions are also maintained ex situ at the Volcani Center in Bet Dagan, Israel (Ben-Ya'acov et al. 2003b). The importance of ex situ collections is growing as the natural vegetation in the centers of domestication has been disappearing rapidly over the past 20–30 years. Reliable funding sources are necessary to prevent abandonment of existing collections. International collaboration promises to safeguard coordinated collecting efforts and to maintain duplicate material at different locations to avoid losses of valuable germplasm due to pest and disease outbreaks, natural disasters, or political instability.

8.6 Conclusions

Although contemporary avocado cultivars and rootstocks still harbor considerable genetic diversity, concerns are growing about the loss of wild germplasm and cultigens from the Mesoamerican centers of diversity. Forest clearances due to population pressure and economic priorities that favor cultivar monocultures have been eroding the rich variation that used to characterize seedling orchards and home gardens. Mindful of the characteristics of avocado in need of improvement, such as resistance to pathogens and tolerance of adverse soil conditions, the scientific community and

horticulturalists are realizing that germplasm is the key to facing current and future challenges. Given the taxonomic uncertainly of *Persea*, exploration should extend to germplasm from related genera, in the expectation that improvements in the field of micropropagation and genetic transformation will overcome incompatibility problems associated with wide crosses and grafting.

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Chapter 9
Poncirus

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9.1 Basic Botany of the Genus

Poncirus trifoliata (trifoliate orange or Japanese bitter orange) belongs to the subtribe Citrinae, tribe Citreaea, subfamily Aurantioideae of the family Rutaceae and is a close relative of citrus. It is thought to be a native of China and Korea. It was brought to North America as an ornamental plant. It is widely found in all citrus-growing regions of the world, as it is commonly used as a rootstock for citrus and it improves frost hardiness and fruit quality. It is usually propagated from seeds. It is a small, fast-growing, deciduous, perennial tree, which grows upto a height of 3–10 ft (2–3 m) and has a green bark with brown streaks. The twigs are covered with sharp spines. It is a very frost hardy tree and can withstand temperatures upto −5°F (−21°C) and lower. The leaves are trifoliate rarely having four or five leaflets, alternate with leaflets being glabrous, elliptic, oblong to obovate, sessile, and 2–6 cm long. The rachis is broadly winged. Flowers are simple, fragrant, sessile, white, borne on axillary inflorescence on previous year's growth, and have five sepals and petals, eight to ten stamens, which are entirely free, and a six-celled pubescent ovary. Though the flower buds are formed in early summer, they pass the winter in a dormant condition and are protected by bud scale. Fruits are small, pubescent, round to pear-shaped, yellow when ripe, and sour to bitter in taste with numerous seeds. Unlike citrus, the fruits of *Poncirus* are inedible in USA. However, in China, the dried mature fruits are used medicinally. The peel is candied and is used as a spice while the juice is used as flavoring syrup. It is used as a source of pectin and grown as an ornamental in the European countries. Due to its spines, it is also grown as a hedge plant in Brazil.

Poncirus species are mostly diploid with $2x = 2n = 18$ chromosomes. Chromomycin A 3 (CMA) and 4,6-diamidino-2-phenylindole (DAPI) have been used for chromosome banding for characterization of chromosomes of *P. trifoliata* (Moraes et al. 2008). This has allowed the separation of the nine chromosome pairs into three groups (4B + 8D + 6F) of which only one F chromosome pair could be distinguished (Befu et al. 2000; Brasileiro-Vidal et al. 2007). In situ hybridization demonstrated that two B chromosome pairs were different from those previously found in citrus as they displayed a 45S rDNA (ribosomal DNA) site colocalized with a CMA + proximal band and a 5S rDNA site adjacent to this band while only one pair of the four D chromosome pairs showed adjacent 5S and 45S rDNA sites, with a 45S rDNA site colocalized with a CMA + band (Brasileiro-Vidal et al. 2007). Bacterial artificial chromosome (BAC) in situ hybridization has also been attempted in *P. trifoliata* and allowed the identification of seven chromosome pairs while the other two were recognized by the presence of 45S rDNA associated with the CMA + band in the first one, and lack of any single copy signal and the presence of a terminal CMA + band in the second one (Moraes et al. 2008). All chromosome pairs were homomorphic, indicating a high level of chromosomal homozygosity. Thus, a combination of chromosome morphology, fluorochrome banding, and fluorescent in situ hybridization (FISH) with rDNA probes distinguished the *Poncirus* chromosomes successfully (Roose et al. 1998).

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9.2 Conservation Initiatives

Though *Poncirus* is widely used as a rootstock for citrus, very little information is available on the extent and distribution of its genetic diversity. It has traditionally been conserved in very small scale in clonal orchards belonging to botanical gardens or scientific institutions, where citrus is primarily conserved. It has been conserved along with citrus in large ex situ collections in Argentina, Australia, Brazil, France, Morocco, New Zealand, South Africa, Spain, Turkey, and USA (Krueger and Navarro 2007). No information is available on in situ conservation of *Poncirus*. Shoot tips from juvenile plants of *P. trifoliata* have been cryopreserved using encapsulation–dehydration method (Gonzalez-Arnao et al. 1988). Attempts have been made to cryopreserve seeds and embryonic axes of *P. trifoliata* in vitro. The seeds were found to be sensitive to desiccation, whereas the excised embryonic axes could be easily desiccated and successfully preserved in liquid nitrogen (Radhamani and Chandel 1992). Shoot tips excised from axillary buds of Troyer citrange (*P. trifoliata* × *C. sinensis*) have been cryopreserved using encapsulation–dehydration and encapsulation–vitrification methods (Wang et al. 2000).

9.3 Molecular Mapping in *Poncirus*

Poncirus has been extensively used as a parent in intergeneric crosses to facilitate optimum polymorphism to construct a number of molecular genetic linkage maps (Cai et al. 1994; Gmitter et al. 1996; Tozlu et al. 1999a, b; Ling et al. 2000); however, the hybrids obtained have inedible fruits making these mapping populations of limited use for fruit traits (Gmitter et al. 2007). A population obtained from an intergeneric backcross of *C. grandis* cv. "Thong Dee" and *P. trifoliata* cv. "Pomeroy," using the former as the recurrent (female) parent, has been used for performing genetic linkage analysis using restriction fragment length polymorphism (RFLP) and isozyme (Durham et al. 1992) markers. Another study used isozymes and RFLP for the construction of a genetic map based on the segregation of 8 isozyme, 1 protein, and 37 RFLP loci in 60 progeny of a cross of two intergeneric hybrids, "Sacaton" citrumelo (*C. paradisi* × *P. trifoliata*) and "Troyer" citrange (*C. sinensis* × *P. trifoliata*), often used as rootstocks (Jarrell et al. 1992). Linkage maps have also been constructed using various molecular markers from intergeneric crosses such as *C. grandis* × (*C. grandis* × *P. trifoliata*), *C. sunki* × *P. trifoliata* cv. Rubidoux, *C. grandis* cv. "Thong Dee," and *P. trifoliata* cv. "Pomeroy" (Cai et al. 1994; Cristofani et al. 1999; Sankar and Moore 2001; Table 9.1). Five genetic linkage maps have been constructed for the parents of progenies of *C. aurantium* × *P. trifoliata* var. Flying Dragon, *C. volkameriana* × *P. trifoliata* var. *Rubidoux* and a self-pollination of *P. trifoliata* var. Flying Dragon using simple sequence repeats (SSRs) for genome comparison (Ruiz and Asins 2003). Recently, an F_1 intergeneric population of *C. sinensis* × *P. trifoliata* was used to construct genetic maps in which 11 linkage groups with 113 markers in *C. sinensis*, nine with 45 markers in *P. trifoliata*, and 13 with 123 markers in the cross-pollinator consensus of both, were constructed (Chen et al. 2008).

Resistance to citrus tristeza virus (CTV) was found to be dominant by performing enzyme-linked immunosorbent assay (ELISA) on several *Poncirus*-derived populations. Using bulked segregant analysis (BSA) approach (Michelmore et al. 1991) and RAPD markers, a map was developed and the *Ctv* gene was identified from *Poncirus* (Gmitter et al. 1996; Fang et al. 1998). Two BAC contigs with integrated fine maps were constructed that resulted in the full-length sequencing of the locus spanning several hundreds of kilobases and identification of the candidate genes (Deng et al. 1997, 2001; Yang et al. 2001, 2003). Prolonged CTV challenge led to the suggestion that more than one gene may be involved in CTV resistance (Mestre et al. 1997). One CTV-resistant gene was later mapped in a different location within linkage group 4 of *Poncirus* from a population of citradias (derived from the cross between sour orange and *Poncirus*) suggesting a deviation from the single gene hypothesis, which could be quantitative trait loci (QTLs) (Bernet and Asins 2003; Asins et al. 2004). It was found that a major QTL, designated *Tyr1*, controls resistance to citrus nematode (Ling et al. 2000) and was adjacent to the *Ctv* region (Ling et al. 1999). In the *C. volkameriana* and *P. trifoliata* progeny, 11 putative QTLs have been detected in *P. trifoliata* that control the number of fruits per tree (Garcia et al. 2000). A *C. grandis* × *P. trifoliata* F_1 pseudo-testcross population was used to map QTLs associated with

Table 9.1 *Poncirus* linkage maps

Mapped parent	Type of cross	Population size	Markers used	Total markers	Map length (cM)	Linkage groups	Reference
C. grandis cv. Chandler × *P. trifoliata* cv. Webber-Fawcett	Intergeneric F_1	35	Isozymes	3	–	2	Torres et al. (1985)
C. grandis cv. Thong Dee × USDA 17–40 (*C. grandis* cv. Thong Dee × *P. trifoliata* cv. Pomeroy)	Intergeneric BC_1	65	Isozymes, RFLP	52	533	11	Durham et al. (1992)
Sacaton (*C. paradisi* × *P. trifoliata*) × Troyer (*C. sinensis* × *P. trifoliata*)	Intergeneric F_1	60	Isozymes, RFLP	38	351	10	Jarrell et al.(1992)
C. grandis cv. Thong Dee × USDA 17–40 (*C. grandis* cv. Thong Dee × *P. trifoliata* cv. Pomeroy)	Intergeneric BC_1	60	Isozymes, RAPD, RFLP	189	1,192	9	Cai et al. (1994)
C. reshni × *P. trifoliata*	Intergeneric F_1	52	RAPD	97	1,503	12	Luro et al. (1996)
Sacaton (*C. paradisi* × *P. trifoliata*) × Troyer (*C. sinensis* × *P. trifoliata*)	Intergeneric F_1	57	ISSR, RFLP	48	410	12	Kijas et al. (1997)
P. trifoliata	F_1	80	RAPD	62	866	8	Cristofani et al. (1999)
C. volkameriana × *P. trifoliata* cv. Rubidoux	Intergeneric F_1	80	CAPS, Isozymes, RAPD, RFLP, SSRs	38	–	3	Garcia et al. (1999)
C. grandis cv. Thong Dee × USDA 17–40 (*C. grandis* cv. Thong Dee × *P. trifoliata* cv. Pomeroy)	Intergeneric BC_1	65	AFLP, RAPD	337	1,031	14	Ling et al. (1999)
Sacaton (*C. paradisi* × *P. trifoliata*) × Troyer (*C. sinensis* × *P. trifoliata*)	Intergeneric F_2	57	ISSR, RAPD, RFLP	153	701	16	Roose et al. (2000)
C. grandis cv. Thong Dee × USDA 17–40 (*C. grandis* cv. Thong Dee × *P. trifoliata* cv. Pomeroy)	Intergeneric BC_1	60	Isozymes, ISSR, RAPD, RFLP	310	874	9	Sankar and Moore (2001)
P. trifoliata	Self F_1	80	IRAP, RAPD, SSR	48	10	342	Ruiz and Asins (2003)
P. trifoliata	F_1	97	EST-SSRs	45	426	8	Chen et al. (2008)

AFLP amplified fragment length polymorphism, *CAPS* cleaved amplified polymorphic sequence, *EST-SSR* expressed sequence tag-simple sequence repeat, *IRAP* interretrotransposon amplified polymorphism, *ISSR* intersimple sequence repeat, *RAPD* randomly amplified polymorphic DNA, *RFLP* restriction fragment length polymorphism, *SSR* simple sequence repeat

freezing tolerance (Weber et al. 2003). The dominant trifoliate leaf character of *Poncirus* has also proven to be advantageous in developing mapping populations, as it allows the direct identification of zygotic hybrids from true nucellar seedlings. F_1 progeny of *C. sunki* × *P. trifoliata* were evaluated for the detection of QTLs linked to citrus *Phythophthora* gummosis resistance. Two QTLs linked to gummosis resistance were detected in linkage groups 1 and 5 of the *P. trifoliata* map and one in linkage group 2 of the *C. sunki* map (Siviero et al. 2006). QTL analysis of morphological traits in an intergeneric BC_1 progeny of *C. grandis* × *P. trifoliata* under saline and non-saline environments has also been attempted (Tozlu et al. 1999b).

9.4 Role in Crop Improvement Through Traditional and Advanced Tools

9.4.1 Traditional Breeding Efforts

Poncirus is commonly used as a rootstock for most citrus species and is also the most valuable genetic resource for the genetic improvement of citrus (Gmitter et al. 2007). It produces fertile hybrids with citrus and is an important source of useful genes for citrus rootstocks (Roose et al. 1998). It is resistant or tolerant to CTV, Phytophthora root rot, citrus nematode, cold accumulation, and other environmental stresses and has been explored for use in citrus scion and rootstock genetic improvement programs via conventional breeding and molecular approaches (Cai et al. 1994; Gmitter et al. 1996; Tozlu et al. 1999a, b; Ling et al. 2000). Sexual hybridization using *P. trifoliata* as one of the parents has been used to produce genetically improved combinations of rootstocks for use in citrus propagation. Carrizo and Troyer citranges (*C. sinensis* × *P. trifoliata*) and Swingle citrumelo (*C. paradisi* × *P. trifoliata*) rootstocks were selected from intergeneric hybrid progeny and were found to have *Phythophthora*, virus, and nematode tolerance inherited from *P. trifoliata*. "US-852," a hybrid obtained from sexual hybridization of *C. reticulata* × *P. trifoliata*, exhibited outstanding effects on sweet orange fruit yield, producing fruit with high soluble solids on medium size trees (Bowman et al. 1999). Four new rootstocks, two (Forner Alcaide 5 and Forner Alcaide 13) obtained by sexual hybridization between Cleopatra mandarin × *P. trifoliata*, one (Forner Alcaide 418) of Troyer citrange × *P. trifoliata*, and one (Forner Alcaide 517) of King mandarin × *P. trifoliata*, resistant or tolerant to CTV and salinity have been released (Nicotra 2001; Forner et al. 2003). "X639", a hybrid between "Cleopatra" mandarin × *P. trifoliata*, though susceptible to nematodes and root pathogens, proved to be an excellent rootstock for lemons and mandarins (Miller et al. 2003).

9.4.2 Ploidy Manipulation

Anther culture has been used to recover haploid plantlets from *P. trifoliata* (Hidaka et al. 1979). Deng et al. (1992a) were able to obtain only heterozygous plantlets from anther culture of *P. trifoliata*. In *P. trifoliata,* pollen culture has also been attempted; however, plantlets were not obtained (Germana et al. 1996). The pollen developmental stages, genotype used as well as the tissue culture parameters affect the success of anther culture. The effect of different developmental stages of *P. trifoliata* pollen grains on the formation of embryoids, pseudobulbils, and calli has been studied (Hidaka et al. 1979). For embryoid production, the early uninucleate stage was the most suitable while anthers at other developmental stages from pollen mother cell to bicellular stage produced only calli (Germana 2007). Hidaka (1984) studied the effects of sucrose concentration (1, 3, 5, 7, and 9%) on embryoid and callus formation and found that 3% sucrose was ideal for embryoid formation in *P. trifoliata*. Medium supplemented with 0.2 mg/l of both indole-3-acetic acid (IAA) and kinetin (Kn) was found to be efficient for embryoid formation while the addition of 2,4-dicholorphenoxy acetic acid (2,4-D) induced callus formation in *P. trifoliata*. Deng et al. (1992b) found that the addition of α-naphthaleneacetic acid (NAA) and activated charcoal in the medium induced embryoid formation in *P. trifoliata*.

Hybrid embryo rescue has also been exploited for the genetic improvement of *Poncirus*. *P. trifoliata* is cold hardy and resistant to root-rot, CTV, and citrus-browning nematode. However, it is susceptible to citrus exocortis viriod (CVC). Controlled crosses, followed by triploid hybrid embryo rescue, were carried out between Red tangerine (which is resistant to

CVC) × *P. trifoliata* as well as Satsuma mandarin (which is citrus canker tolerant) × *P. trifoliata* to introduce these new characters into *P. trifoliata* (Tan et al. 2007). To produce triploid intergeneric hybrids, gametosomatic fusion between *P. trifoliata* tetrads and somatic protoplasts of *C. sinensis* has been reported (Deng et al. 1992b).

Somatic hybridization allows the production of somatic hybrids that incorporate genomes of the two parents without recombination, thus avoiding the problem of the high heterozygosity (Navarro et al. 2004). Production of tetraploid somatic hybrids that combine complementary diploid rootstock germplasm via protoplast fusion has become a practical strategy with the overall objective of packaging necessary disease and pest resistance into horticulturally desirable, widely adapted rootstock such as *Poncirus*. In *Poncirus*, the first somatic hybrid was obtained between *C. sinensis* and *P. trifoliata* (Ohgawara et al. 1985). These results allowed the establishment of rootstock breeding programs in several countries. A number of somatic hybrids with *Poncirus* as one of the parents have been generated (Table 9.2) and are at different stages of field trial (Grosser et al. 2000). Somatic hybrid rootstocks are showing good potential to reduce tree size, as needed, for more efficient high-density plantings with good yields of high quality (Grosser 2003). Seed trees of most of these somatic hybrid rootstocks are also producing adequate nucellar seeds for standard propagation (Grosser et al. 2000).

9.4.3 Genetic Engineering

Genetic transformation of *Poncirus* and its hybrids has been achieved (Table 9.3). The first efficient protocol for transformation of seedling explants of *P. trifoliata* was established by Kaneyoshi et al. (1994), which was subsequently used by many groups (Kobayashi et al. 1996; Kaneyoshi and Kobayashi 1999; Wong et al. 2001; Iwanami et al. 2004; Endo et al. 2005). A similar protocol was used to transform Carrizo citrange (*C. sinensis* × *P. trifoliata*) that did not respond as well as *P. trifoliata* (Pena et al. 1995a). Hence, several factors affecting transformation and regeneration were critically studied (Cervera et al. 1998a). Cocultivation of epicotyl or internodal stem segments with *Agrobacterium tumefaciens* has been the most commonly used systems to efficiently produce transgenic plants of *P. trifoliata* (Kaneyoshi et al. 1994) and citrange (*C. sinensis* × *P. trifoliata*, Pena et al. 1995b; Gutierrez et al. 1997; Cervera et al. 1998b). To enhance both regeneration and transformation frequency, Yu et al. (2002) proposed cutting longitudinally the epicotyl segments of Carrizo citrange in two halves. Thin layers of about 1–2 mm cut transversally from etiolated epicotyls were found to be highly organogenic in *P. trifoliata,* Swingle citrumelo and Carrizo citrange transformation (Le et al. 1999; Molinari et al. 2004a, b).

Several authors have proposed the use of *rol* genes from the Ri plasmid as transgenes to produce dwarf *P. trifoliata* and citrange rootstocks (Gentile et al. 1998; Kaneyoshi and Kobayashi 1999). *rolC* gene from *A. rhizogenes* has been successfully incorporated into *P. trifoliata* (Kaneyoshi and Kobayashi 1999). Human epidermal growth factor (hEGF) has also been incorporated into *P. trifoliata* (Kobayashi et al. 1996). A citrus gibberellin (GA) 20-oxidase cDNA (*CcGA20ox1*) gene that controls the plant architecture and a halotolerance gene *HAL2* have been introduced into Carrizo citrange (Cervera et al. 2000a; Fagoaga et al. 2007). *Arabidopsis* genes such as *LEAFY* or *APETALA1* that alter the growth habit, reduce juvenility, and regulate vegetative and other behavior have been introduced into juvenile Carrizo citrange seedling explants (Pena et al. 2001). Regenerants of Carrizo citrange obtained under selective conditions after *Agrobacterium*-mediated transformation have been used for the characterization of these regenerants into silenced and/or chimeric plants (Dominguez et al. 2004). Gene constructs have been created for various types of CTV-derived genes and have been introduced into Carrizo citrange in efforts to induce resistance to the CTV virus (Gutierrez et al. 1997). A citrus blight-associated gene has also been introduced into Carrizo citrange (Kayim et al. 2004). Coat protein gene from citrus mosaic virus (CiMV) has been incorporated into *P. trifoliata* (Iwanami et al. 2004). Another gene *FLOWERING LOCUS T* that reduces the time of flowering has been incorporated into *P. trifoliata* and the transformed regenerants flowered in less than 8 months with four out of six transgenic lines developed normal fruits with intact seeds (Endo et al. 2005). In Spain, 16 transgenic plants of Carrizo citrange, with two plants each from eight independent transgenic lines, have been released under an experimentally controlled field for further evaluation (Pena et al. 2008).

Table 9.2 Somatic hybrids regenerated using *Poncirus* as one of the parents

Embryogenic parent	Leaf parent	Somatic hybrids regenerated	Reference
Trovita sweet orange	*Poncirus trifoliata*	–	Ohgawara et al. (1985)
Trovita sweet orange	Troyer citrange	–	Kobayashi and Ohgawara (1988)
Trovita sweet orange	*P. trifoliata*	–	Kobayashi and Ohgawara (1988)
Navel orange	Troyer citrange	>5	Ohgawara et al. (1991)
Valencia sweet orange	Carrizo citrange	7	Louzada et al. (1992)
Milam lemon	*C. depressa* × *P. trifoliata*	23	Tusa et al. (1992)
Cleopatra mandarin	Argentine trifoliate orange	6	Grosser et al. (1994)
Succari sweet orange	Argentine trifoliate orange	>200	Grosser et al. (1994)
Sour orange	Flying Dragon trifoliate orange	210	Grosser et al. (1994)
Shirayanagi navel orange	*P. trifoliata*	12	Kaneko et al. (1995)
Red Marsh grapefruit	Argentine trifoliate orange	33	Grosser et al. (1996)
Red Marsh grapefruit	Flying Dragon trifoliate orange	46	Grosser et al. (1996)
Willow leaf mandarin	Pomeroy trifoliate orange	24	Ollitrault et al. (1996)
P. trifoliata	*Fortunella hindsii*	17	Miranda et al. (1997)
Milam lemon hybrid	Swingle citrumelo	>100	Grosser et al. (1998)
Milam lemon hybrid	Carrizo citrange	15	Grosser et al. (1998)
Cleopatra mandarin	Carrizo citrange	>100	Grosser et al. (1998)
Sour orange	Carrizo citrange	>100	Grosser et al. (1998)
Page tangelo	*P. trifoliata*	>150	Deng et al. (2000)
Red tangerine	*P. trifoliata*	>30	Deng et al. (2000)
Red tangerine	*Citrange*	10	Deng et al. (2000)
Hamlin sweet orange	Flying Dragon trifoliate orange	>800	Grosser et al. (2000)
Valencia sweet orange	Carrizo citrange	>75	Grosser et al. (2000)
Cleopatra mandarin	Flying Dragon trifoliate orange	>300	Grosser et al. (2000)
Cleopatra mandarin	Swingle citrumelo	>300	Grosser et al. (2000)
Murcott tangor	Cohen citrange (pentaploid)	3	Grosser et al. (2000)
Nova tangelo	Cohen citrange (pentaploid)	>30	Grosser et al. (2000)
Cleopatra mandarin	Cohen citrange (pentaploid)	>30	Grosser et al. (2000)
Sour orange	*P. trifoliata* 50-7	>100	Grosser et al. (2000)
Washington Navel sweet orange	*P. trifoliata* 50-7	>100	Grosser et al. (2000)
Changsa mandarin	*P. trifoliata* 50-7	>100	Grosser et al. (2000)
Duncan grapefruit	*P. trifoliata* 50-7	>50	Grosser et al. (2000)
Sour orange	Benton citrange	>50	Grosser et al. (2000)
C. sunki	Carrizo citrange	–	Ollitrault et al. (2000)
C. aurantifolia	Carrizo citrange	–	Ollitrault et al. (2000)
Amblycarpa mandarin	Benton citrange	<50	Medina-Urrutia et al. (2004)
Amblycarpa mandarin	Carrizo citrange	<50	Medina-Urrutia et al. (2004)
Amblycarpa mandarin	C-35 citrange	<50	Medina-Urrutia et al. (2004)
Amblycarpa mandarin	Flying Dragon trifoliate orange	<50	Medina-Urrutia et al. (2004)
Amblycarpa mandarin	Rubidoux trifoliate orange	<50	Medina-Urrutia et al. (2004)
Amblycarpa mandarin	Somatic hybrid of sour orange + Flying Dragon trifoliate orange	<50	Medina-Urrutia et al. (2004)

Table 9.3 Summary of *Agrobacterium*-mediated transformation of *Poncirus trifoliata* and its hybrids

Genotype used	Explant used	Gene(s) introduced	Reference
Carrizo citrange	In vitro internodal stem segments	*nptII*, *uidA*	Moore et al. (1992)
P. trifoliata	In vitro epicotyl segments	*nptII*, *uidA*	Kaneyoshi et al. (1994)
Carrizo citrange	In vitro epicotyl segments	*nptII*, *uidA*	Pena et al. (1995b)
P. trifoliata	In vitro epicotyl segments	*hEGF*	Kobayashi et al. (1996)
Carrizo citrange	In vitro internodal stem segments	CTV-*CP*	Gutierrez et al. (1997)
Carrizo citrange	In vitro epicotyl segments	*nptII*, *uidA*	Cervera et al. (1998c)
Troyer citrange	In vitro epicotyl segments	*nptII*, *rolA, rolB, rolC*	Gentile et al. (1998)
Carrizo citrange	In vitro epicotyl segments or greenhouse internodal stem segments	*nptII*, *gfp*	Ghorbel et al. (1999)
P. trifoliata	In vitro epicotyl segments	*rolC*	Kaneyoshi and Kobayashi (1999)
Carrizo citrange	In vitro epicotyl segments	*nptII*, *uidA*	Cervera et al. (2000b)
Carrizo citrange	In vitro epicotyl segments	*HAL2*	Cervera et al. (2000a)
Troyer citrange	In vitro epicotyl segments	*nptII*, *gfp*	LaMalfa et al. (2000)
Carrizo citrange	In vitro epicotyl segments	CS-*ACS1*	Wong et al. (2001)
P. trifoliata	In vitro epicotyl segments	CS-*ACS1*	Wong et al. (2001)
Carrizo citrange	In vitro epicotyl segments	*AP1, LFY*	Pena et al. (2001)
Carrizo citrange	In vitro internodal stem segments	*nptII*, *uidA*	Yu et al. (2002)
Swingle citrumelo	In vitro epicotyl thin sections	*nptII*, *uidA*	Molinari et al. (2004a)
P. trifoliata	In vitro epicotyl thin sections	*nptII*, *uidA*	Molinari et al. (2004a)
Carrizo citrange	In vitro epicotyl segments	*p5cs*	Molinari et al. (2004b)
Carrizo citrange	In vitro epicotyl segments	*p12*	Kayim et al. (2004)
P. trifoliata	In vitro epicotyl segments	CiMV-*CP*	Iwanami et al. (2004)
P. trifoliata	In vitro epicotyl segments	Ci-*FT*	Endo et al. (2005)
P. trifoliata	Greenhouse internodal stem segments	CTV-*p23*	Fagoaga et al. (2005)
Carrizo citrange	In vitro epicotyl segments	*hpt, bar, gfp*	Cervera et al. (2006)
Carrizo citrange	In vitro epicotyl segments or greenhouse internodal stem segments	*ipt, R/RS* recombinase system	Ballester et al. (2007)

9.5 Genomics Resources Developed

Poncirus and its hybrids have been extensively used in EST-sequencing efforts (Talon and Gmitter Jr 2008). They have also been used to generate ESTs from several libraries under biotic (*Xylella fastidiosa*, CTV, citrus leprosis virus, *Phythophthora*, mite) and abiotic (drought) stresses, and during fruit development (Machado et al. 2007). Around 62,344 ESTs have been generated from *P. trifoliata*, and 9,791 from its hybrids with citrus (http://www.int-citrusgenome.org/usa/) using various tissues as seed, leaf, bark, greenhouse and field-grown plants, etc. An Affymetrix citrus GeneChip has been developed, which contains probe sets for detection of several pathogens and commonly used transgenes, and a representation of the region of the *P. trifoliata* genome containing *Ctv*, the CTV resistance allele (Close et al. 2006; Talon and Gmitter Jr 2008).

9.6 Conclusion

Poncirus is found in all citrus-growing regions of the world. It is known to have originated in China, where it is also used for its medicinal properties. In certain parts of Europe, it is grown as an ornamental plant. It is propagated by seeds and is a useful rootstock for citrus. Its genetic signatures of resistance to major diseases (such as CTV, nematode, etc.) and environmental stresses (such as salinity, temperature, etc.)

have been exploited by citrus breeders in their various rootstock improvement programs. It is also highly amenable to plant tissue culture techniques. Haploid plants have been produced via anther culture, hybrid embryo rescue has been utilized to produce triploids, and somatic hybridization has given rise to tetraploid plants in *Poncirus*. These haploids, triploids, and tetraploids are highly beneficial for citrus scion and rootstock breeding programs. It has also been genetically transformed to establish and standardize the protocol for use in citrus improvement.

Poncirus has been extensively used as a parent in intergeneric crosses with citrus. This has given rise to optimum polymorphism, which has facilitated the construction of a number of molecular genetic linkage maps. The identification, tagging, and cloning of the economically important genes will provide new information and gene targets for genetic manipulation, and hence will be of great use in citrus genetic improvement. The mapping populations of the intergeneric crosses between citrus and *Poncirus* have also been used to construct BAC libraries, develop ESTs, used in microarrays, and to develop sequence-based maps. Their gene sequence divergence, synteny, orientation, and possible probable functions have been annotated and compared. These genomic resources will have a great impact on the whole-genome sequencing of citrus. As the whole citrus genome is sequenced, subsequent exploration, comparison, and utilization of that data would be beneficial for the genetic improvement of *Poncirus*.

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Chapter 10
Spondias

Allison Miller

10.1 Introduction

The independent origins of agriculture in at least seven regions of the world ("centers of domestication") have produced a great variety of crops, many of which have become global staples that are cultivated well beyond their original distributions (e.g., banana, cassava, corn, mango, rice, wheat) (Vavilov 1992). In addition, in each of the centers of agricultural origins exists a plethora of cultivated plants that have not become global staples. These "companion" crop species play a crucial role in their native areas, contributing to both regional food security and economic stability (Tuxill 1999).

One of the centers of domestication is Mesoamerica (Mexico and Central America). The diverse flora of this region has contributed several globally important species to agriculture, including agaves (*Agave* spp.), avocados (*Persea americana* Mill), corn (*Zea mays* ssp. *mays*), cotton (*Gossypium hirsutum*), beans (*Phaseolus* spp.), and squashes (*Cucurbita* spp.). Significant components of the companion crop species of Mesoamerica are the cultivated fruit trees. In Mexico and Central America, several native fruit trees have been taken into cultivation including anona (*Anona cherimola*), avocado (*Persea americana*), cas (*Psidium friedrichsthalianum*), jocote (*Spondias purpurea*), nance (*Byrsonima crassifolia*), sapodilla (*Manilkara achras*), sapote (*Pouteria sapota*), and possibly guava (*Psidium guavaja*) and papaya (*Carica papaya*) (Rehm and Espig 1991). Today these fruits are sold in local markets and are consumed widely in the region, although the bulk of cultivation comes from backyard gardens and small multicrop farms. Large-scale production from trees planted in orchards, historically rare, is becoming more common.

The gene pool of a crop species comprises the cultivated populations (landraces, varieties, cultivars), as well as the wild (uncultivated) populations and close relatives. It has been estimated that in recent times (the last 100 years), the diversity within crop species and their wild relatives has declined by as much as 80% (Nabhan 1992; Tanksley and McCouch 1997). Native forests harboring the ancestors of cultivated plants have been lost, and more formal, centralized agriculture has promoted the adoption of a few high-yielding uniform cultivars over broad areas resulting in the abandonment of genetically variable indigenous varieties by subsistence farmers (Altieri and Merrick 1988).

Companion crops that are grown in their native ranges are occasionally cultivated in fields and orchards but are more commonly found in informal agricultural habitats, such as small multicrop farms, backyard gardens, and living fences. In addition, the wild relatives of the crops (individuals that are not cultivated and have not been domesticated) are generally found in these areas as well. Classical studies have demonstrated that traditional (informal) forms of agriculture nurture a diversity of cultivated varieties in a small area (e.g., Anderson 1952). Considering that the informally cultivated populations may be functioning as reservoirs of genetic diversity for these locally important species, and that extant native populations occur in the area, understanding the geographic distribution of genetic diversity both in wild populations and in informal agricultural habitats is vital to the sustainability of local agriculture and the conservation of crop genetic resources.

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10.2 Basic Botany of the Genus

S. purpurea (Anacardiaceae) is a small fruit tree (2–10 m in height) native to Mexico and Central America. In this region, both native populations as well as domesticated populations are found. *S. purpurea* trees produce plum-like fruits, some of which are eaten fresh, sold in local markets, and made into jams and beverages throughout the Neotropics (Mandujano et al. 1994; Avitia-García 1997; Baraona-Cockrell 2000; Mitchell 2000; Pimienta-Barrios and Ramirez-Hernandez 2003). Like plums, *S. purpurea* fruits have a single stone surrounded by a fleshy layer (mesocarp) and a thin skin, which varies widely in color.

10.2.1 Taxonomic and Morphological Framework

Spondias L. was described as a monotypic genus by Linnaeus (1753). Subsequent revisions delimited ~17 species (Table 10.1) including seven taxa in the Neotropics (Mexico–Brazil) and ~10 species in the Asian tropics (Linnaeus 1762; Airy Shaw and Forman 1967; Ding Hou 1978; Kostermans 1991; D Daly and JD Mitchell personal communication). *Spondias* is one of the basal-most genera in the Anacardiaceae, although neither the relationship of *Spondias* to closely allied genera, nor the relationships among the species of *Spondias* are well understood (Pell 2004). At least six *Spondias* species have been taken into cultivation: three Asian species (*S. borbonica, S. cytherea*, and *S. pinnata*) and three American species (*S. mombin, S. purpurea,* and *S. tuberosa*) (Table 10.1). This chapter focuses on the wild populations of the cultivated species *S. purpurea* and its sympatric congeners.

Spondias species are trees (rarely vines) ranging from 2 to 35 m in height. Their floral structure is characterized by having stamens that are twice the number of the petals. The carpels are united and range in number from 1 to 12 (usually 4–5), with terminal styles. The ovules are pendulous from an apical funicle. The primary synapomorphies for *Spondias* are an endocarp that is completely surrounded by a capsule of strong, intertwined longitudinal fibers, and leaflets with a marginal vein (Kostermans 1991). Variable breeding systems are common in the Anacardiaceae. In *Spondias*, several species are known to exhibit unisexual flowers (e.g., *S. purpurea*), bisexual flowers (e.g., *S. mombin*), or both (e.g., *S. bipinnata*, *S. mombin*, *S. pinnata*, and *S. purpurea*) (Kostermans 1991).

Table 10.1 *Spondias* species and their geographic distributions (Williams 1930; Airy Shaw and Forman 1967; Cavalcante 1976; Popenoe 1979; Brücher 1989; Kostermans 1991; D Daly, personal communication)

Species	Native range
S. acida Bl.	Malay Penninsula
S. acuminata Roxb.	India, Burma, Thailand
S. bipinnata Airy Shaw & Forman	Thailand (limestone hills)
S. bivenomarginalis K.M. Feng & P.Y. Mao & P. Me.	Yunnan, China
S. dulcis Parkinson	Asian; Cult. In New World, Brazil, Caribbean
S. macrocarpa Engl.	Eastern Brazil
S. malayana Kosterm.	Malesia, Philippines
S. mombin L. var. *mombin*	Mexico to Bolivia and eastern Brazil, Central America, Brazil
S. mombin L. var. *globosa*	Colombia, Venezuela, Ecuador, Peru, Bolivia
S. novoguineensis Kosterm.	New Guinea, Solomon Islands, etc.
S. pinnata (Koenig ex Linn. F.) Kurz	India, Himalayas, Burma, Sri Lanka
S. purpurea L.	Mexico – Central America, Ecuador
S. radlkoferi Donn. Sm.	Mexico, Central America, NW Venezuela, W. Ecuador
S. testudinis J.D. Mitch & Daly	SW Amazonia
S. tuberosa Arruda	NE Brazil
S. venulosa Mart. ex Engl.	E. Brazil
S. tonkinensis Kosterm.	Tonkin, Langson Province
S. xerophila Kosterm.	Sri Lanka

In the Neotropics, six *Spondias* taxa are recognized: *S. macrocarpa* Engl., *S. mombin* L. var. *mombin*, *S. mombin* L. var. *globosa*, *S. purpurea* L., *S. radlkoferi* Donn. Smith, *S. testudinis* J.D. Mitch. & Daly, and *S. tuberosa* Arruda (D Daly, personal communication). Of these, three occur naturally in Mexico and Central America: *S. mombin* var. *mombin*, *S. purpurea*, and *S. radlkoferi*. *S. mombin* var. *mombin* and *S. radlkoferi* share many similarities; both are large trees (≤35 m) that are found primarily in tropical wet forests. Inflorescences of large hanging panicles carry numerous small, cream-colored, white, yellow, or greenish, primarily hermaphroditic, outcrossing flowers (Stacy et al. 1996). *S. mombin* is distinguished from *S. radlkoferi* by its fruits. The fruits of *S. mombin* are yellow–orange spheres, while those of *S. radlkoferi* are more oblong in shape, and are green at maturity. In addition, the leaves of *S. radlkoferi* are pubescent with stellate hairs. Croat (1974) observed that the species flower at different times.

Although all three Mesomerican *Spondias* species are cultivated to some degree, *S. purpurea* is the most widespread and economically important. In most cases, *S. purpurea* trees are easily distinguished from *S. mombin* var. *mombin* and *S. radlkoferi*. Unlike its sympatric congeners, *S. purpurea* is a small tree (~2–10 m) native to the dry forests (Rzedowski 1978). *S. purpurea* flowers are cauliflorous; the inconspicuous unisexual or hermaphroditic flowers occur in fasicles or small, appressed panicles on the stem, and develop before the leaves appear. Unisexual flowers appear nearly perfect (Avitia-García 1997) or with reduced vestiges of the non-functional sex. The petals are cream to yellow or more commonly reddish-purple in color. The cauliflorous fruits are cylindrical to oblong drupes (2–6 cm in length) that range in color (red, orange, yellow, green, purple), taste (sweet, acidic), and size (3.0–5.5 cm). The leaflets of *S. purpurea* are smaller and more numerous than *S. mombin* var. *mombin* and *S. radlkoferi*.

Native *S. purpurea* populations are dioecious or polygamodioecious, a condition that has been shown to be relatively common in tree species in the dry forests of tropical regions (Bawa 1974; Bawa et al. 1985; Bawa and Opler 1975). Most likely, *S. purpurea* pollination is entomophilous, although the pollination mechanisms in native populations have not been studied. The fruits are usually bright red or yellow, and are smaller and more acidic than the fruits of cultivated *S. purpurea*. The fruits of native *S. purpurea* trees are an important water and food resource for forest animals (Mandujano et al. 1994). In Jalisco, Mexico, seeds of native *S. purpurea* trees are dispersed by coyotes (*Canis latrans*), coati (*Nasua narica*), gray fox (*Urocyon cinereoargenteus*), iguana (*Ctenosaura pectinata*), collared peccary (*Pecari tajacu*), Collie's squirrel (*Sciurus colliaei*), chachalaca (*Ortalis poliocephala*), and white-tailed deer (*Odocoileus virginianus*) (Mandujano et al. 1994). The endocarp must be kept wet or germination is unlikely. Mandujano et al. (1994) observed that only seeds covered with litter germinated, and that endocarps dispersed by iguanas were less likely to produce germinating seedlings than those dispersed by white-tailed deer (35% to 72%, respectively).

10.2.2 Distribution and Geographical Locations of Genetic Diversity

Native populations of *S. purpurea* are found in the dry forests along the Pacific side of Mexico and Central America below 1,300 m (Rzedowski 1978; Mandujano et al. 1994; Avitia-García 1997). Cultivated *S. purpurea* trees were grown widely from Mexico to the northern region of South America when the Europeans arrived in Mesoamerica, as recorded by the early chroniclers (Oviedo, Sahagún) (Estrada Lugo 1989; Cuevas 1994). The ancient Council Book of the Quiché Mayans, the *Popul Vuh*, lists *S. purpurea* (common name in Quiché: Q'inom) together with other native fruit trees valued and consumed by the Maya: anonas or cherimoya (*Annona* spp.), cacao (*Theobroma cacao*), matasanos (*Casimiroa edulis*), nance (*Byrsonima crassifolia*), and zapotes (*Manilkara zapota*) (Tedlock 1996). Ethnobotanical studies have shown that *S. purpurea* fruits are known and consumed by most indigenous groups in Mexico and Central America [e.g., the Huichol people of Jalisco and Nayarit, Mexico (Bauml 1994), the Mixteca people of Guerrero (Casas et al. 1994), the Huastec Maya of Veracruz and San Luis Potosi (Alcorn 1984), the Zinacantec Maya of Chiapas (Breedlove and Laughlin 2000)]. There are over 180 common names in more than 20 languages for *S. purpurea* including abal, ciruela mexicana, hog plum, jocote, and purple mombin (Miller 2004). Today, *S. purpurea* trees are grown throughout the Neotropics.

Analyses of cultivated and native populations based on Geographic Information Systems (GIS) data have revealed significant differences in the environmental attributes that characterize the geographic distributions of native *S. purpurea* populations relative to cultivated *S. purpurea* populations in Mesoamerica (Miller and Knouft 2006). Specifically, the areas inhabited by wild and cultivated *S. purpurea* populations differ significantly in mean diurnal temperature range, annual temperature range, precipitation seasonality, annual precipitation, and precipitation in the driest quarter. Areas where wild *S. purpurea* populations are found are characterized by significantly higher mean diurnal temperature range, higher annual temperature range, higher precipitation seasonality, lower annual precipitation, and lower precipitation in the driest quarter relative to areas in which cultivated *S. purpurea* populations reside. In addition, the variances of these five environmental variables were significantly less for native populations compared to cultivated populations (Miller and Knouft 2006). These data suggest that during the course of domestication, wild *S. purpurea* populations native to the dry forests were preferentially cultivated in regions that were less seasonal, cooler, and wetter relative to the dry forests. Further, these data demonstrate that the geographic distribution of native *S. purpurea* populations is remarkably narrow relative to the geographic range of their cultivated descendants (Miller and Knouft 2006).

10.2.3 Reproductive Biology

Native *S. purpurea* populations are dioecious (Mandujano et al. 1994; Avitia-García 1997; AJ Miller, personal observation), although there are some reports of wild populations with hermaphroditic flowers on female trees (Raymundo Ramirez, personal communication). Reproductive structures of the opposite sex are present in unisexual flowers, but are highly reduced and non-functional. In February 2005, the author visited four wild *S. purpurea* populations near Guadalajara, Jalisco (Mexico), and observed that wild populations include mostly male trees. To confirm these observations, the author collected and dissected five flowers per tree for nine wild *S. purpurea* trees. Eleven percent of wild trees surveyed were females (1 of 9 trees). Low numbers of female trees in wild populations may represent a common phenomenon in dioecious species; natural dioecious plant populations generally have male-biased populations (Lloyd and Webb 1977; Delph 1999). A second explanation is that decreased numbers of females in the wild populations may be the result of tree harvesting by humans; *S. purpurea* is readily propagated from large cuttings, and perhaps female trees were preferentially removed from the forest for cultivation.

In striking contrast to male-biased native *S. purpurea* populations, apparently all cultivated *S. purpurea* individuals in this dioecious species produce fruit, indicating a preponderance of flowers with female components in cultivated populations. As part of the study described in the previous section, the author visited five cultivated *S. purpurea* populations near Guadalajara, Jalisco (Mexico), and observed that cultivated populations appeared to be entirely female. To confirm these observations, the author collected and dissected five flowers per tree for ten cultivated *S. purpurea* trees. As expected, 100% of cultivated trees surveyed were females (A Miller, unpublished data). These observations underscore the importance of crop wild relatives as a source of sexual diversity. Many dioecious species have been domesticated for their fruits (Zohary and Spiegel-Roy 1975), and in these species, males are found only in native populations.

Native *S. purpurea* populations reproduce sexually and consist of seedling, sapling, and mature trees. This is not the case for cultivated *S. purpurea* populations, which are propagated exclusively vegetatively. Indeed, field observations and interviews with farmers from seven Mesoamerican countries failed to uncover a single case of *S. purpurea* propagation from seed (Miller 2004). Empirical studies have shown that seeds of cultivated *S. purpurea* trees do not form viable embryos (Juliano 1932; Avitia-García 1997). It appears that native *S. purpurea* populations may be the only remaining populations of *S. purpurea* which are capable of sexual reproduction; it is not clear if cross-pollination between cultivated and native *S. purpurea* results in viable offspring.

Fruits of native *S. purpurea* trees vary geographically. For example, native populations in central western Mexico produce small, bright red fruits before or just as the leaves are emerging in late spring. Native populations in Central America (e.g., Costa Rica, El Salvador) have yellow fruits. Artificial selection

focused primarily on fruit production, which led to clear differences in the fruits of cultivated and wild *S. purpurea* trees; cultivated *S. purpurea* fruits are larger than wild fruits, and have a thicker, juicier mesocarp, exhibit a greater range of colors (mature cultivated fruits can be red, orange, yellow, green, or purple; wild fruits are either red or yellow), and are much sweeter than the acidic wild fruits.

10.2.4 Agricultural Status

S. purpurea trees are cultivated for their fruits, which are eaten fresh, stewed, and made into jams and beverages (Baraona-Cockrell 2000; Pimienta-Barrios and Ramirez-Hernandez 2003). *S. purpurea* trees are cultivated throughout Mexico, Central America, the Caribbean, portions of South America, and the Philippines (Juliano 1932; Kostermans 1991; Macía and Barfod 2000). Fruits of *S. purpurea* are an excellent source of vitamin C (Koziol and Macía 1998). They are eaten fresh, candied, and made into jelly, wine, and vinegar. In Mexico, green fruits are made into a tart sauce. The fresh shoots are eaten raw or cooked as a vegetable (Kostermans 1991). The bulk of propagation occurs in traditional agricultural habitats (e.g., fruits are harvested from wild trees or from trees grown in backyard gardens and living fences). Commercial plantings are relatively uncommon, but have been developed in Mexico, Guatemala, Honduras, Costa Rica, and El Salvador (Kostermans 1991; Cuevas 1994, AJ Miller, personal observation). *S. purpurea* trees are cultivated exclusively vegetatively. Large branch-sized cuttings are used to propagate new trees, which usually set fruit within 2–3 years.

10.3 Conservation Initiatives

It has been estimated that less than 2% of the native habitat of *S. purpurea*, the Mesoamerican dry forests, remains (Janzen 1988). Little research has been conducted on specific native taxa in these threatened forests (Sánchez-Azofeifa et al. 2005). Unfortunately, there are no known in situ or ex situ conservation efforts explicitly designed for native *S. purpurea* populations or other *Spondias* species. The conservation of crop genetic resources must include both the wild relatives of the cultivated species (*Spondias* spp.) and the native populations from which the cultivated populations are derived (native, uncultivated *S. purpurea* populations). In addition, although many crop populations are found in modern agricultural environments (e.g., orchards in the case of trees), conservation initiatives must take the cultivated populations found in more traditional agricultural habitats into account (e.g., home gardens, living fences). Because traditional agricultural habitats can include domesticated plants as well as propagules derived directly from wild individuals, it has been suggested that these populations may represent one portion of a continuum of genetic differentiation ranging from wild to domesticated variants (Harris 1989; Tanksley and McCouch 1997; González-Soberanis and Casas 2004; Miller and Schaal 2006).

10.3.1 Evaluation of Genetic Erosion

Genetic variation in native *S. purpurea* populations has been quantified using amplified fragment length polymorphism (AFLP) data, chloroplast sequence data, and nuclear sequence data (Miller and Schaal 2005, 2006; Miller 2008). Analyses of chloroplast sequence data based on the spacer region *trnS–trnG* revealed that 71% of total allelic diversity was recovered in native populations, whereas only 53% of the total allelic diversity was recovered in cultivated populations (some alleles were found only in native populations, whereas other alleles were carried exclusively by cultivated individuals) (Miller and Schaal 2005). The extent of genetic erosion in *S. purpurea* was addressed directly in a study that compared native *S. purpurea* populations to cultivated populations collected from three different types of agricultural habitats: orchards, home gardens, and living fences (Miller and Schaal 2006). Thirty-four populations were sampled including 15 native populations, ten backyard populations, five living fence populations, and six orchard populations a total of 216 individuals. DNA was extracted and molecular markers were generated using the dominant AFLP marker technique (Vos et al. 1995). Briefly, the AFLP technique fragments genomic DNA using restriction enzymes; then, two rounds of polymerase chain reaction (PCR) are

conducted with (1) pre-selective primers and (2) selective primers to amplify a subset of fragments. The selective primers are fluorescently labeled and resulting fragments are visualized using an automated sequencer. Fragments are sized (in base pairs) and fragments of the same size are scored as present or absent for each individual. In this study, two primer combinations resulted in 200 polymorphic fragments that ranged in size from 40 to 400 bp. Based on these data, population genetic diversity was estimated using standard parameters, percentage of polymorphic sites, Shannon's diversity index, Nei's (1973) gene diversity, and Bayesian methods (e.g., average panmictic heterozygosity). Wilcoxon two-group tests revealed that orchard populations harbored significantly less genetic variation than native populations; however, there were no significant differences between native populations and living fence populations, or between native populations and backyard populations. When native populations were compared to cultivated populations as a group (orchards + living fences + backyards), the cultivated populations were found to harbor significantly less genetic variation than the native populations. In addition to the levels of genetic diversity, the amount of population structuring varies in the different stages of domestication. Estimates of population structure (based on AMOVA and Bayesian estimators of F_{st}) reveal that in wild populations, a smaller proportion of genetic variance is attributed to differences among populations (30.19%, $\phi_{ST} = 0.302$) than in cultivated populations (39.76%, $\phi_{ST} = 0.398$). This likely reflects differences in reproduction in *S. purpurea* populations; wild populations reproduce exclusively from seed, while cultivated populations are propagated vegetatively. Populations of backyard trees exhibit only slightly more variation among populations (31.19%, $\phi_{ST} = 0.311$) than do the wild populations, whereas living fences and orchards harbor greater proportions of genetic variance among populations (48.31%, $\phi_{ST} = 0.483$; 44.82%, $\phi_{ST} = 0.448$, respectively) than do the wild and backyard populations. Elevated estimates of population structuring in living fences and orchards as compared with backyard trees and wild populations may reflect greater levels of vegetative propagation in living fences and orchards, human-dispersal of cuttings from backyard trees, or that some gene flow occurs between backyards, or backyards and wild populations, or both.

10.4 Role in Elucidation of Origin and Evolution of Allied Crop Species

Historically, circumscription of the *Spondias* lineage has proved difficult. In 1952, in his book *Plants, Man, and Life*, Edgar Anderson addressed the genus *Spondias* (mombins) in Mesoamerica:

> It is a little hard to talk about the mombins because they have so many different names, none of which are really widely used. They are popular fruits in various parts of the tropics, and are about the size of plums. In Latin America they are frequently called the "ciruela" that being the common Spanish name for the European plum. Botanically they go in the genus *Spondias*, and are not at all closely related to plums, though like that fruit they are more or less acid and have a large central stone. Some are red, some are yellow, some red with a flush of yellow. Most Europeans do not consider them much of a delicacy, but to the Indian populations they are one of the pleasures of life. One sees baskets and piles of them in every native market. Indians coming into town with bundles of produce on their heads munch them as they hurry along at a half trot, and when they are in season towns like Antigua have a superficial paving on mombin pits on top of the old cobblestones. How many species of mombin are there, and where do they come from? . . ."Who knows? There may be two kinds; there may be fifty. The Indians have been gathering them and spitting them out again for we don't know how long. They have been planting selected forms in their native gardens and these native gardens have reverted to woodland. How are we ever going to know where they all came from?" (Anderson 1952, pp 91–92).

Recent studies have focused on identifying the lineage to which cultivated *S. purpurea* populations belong (Miller 2008), and on identifying the geographic origins of cultivated *S. puprurea* populations in Mesoamerica (Miller and Schaal 2005, 2006). In addition to *S. purpurea* accessions, these studies included samples of other congeneric taxa including *S. mombin* var. *mombin*, *S. mombin* var. *globosa*, *S. radlkoferi*, and *S. testudinus*.

10.4.1 Relationships Between S. purpurea and Related Species

A thorough molecular phylogenetic analysis of *Spondias* is lacking. Evolutionary analyses based on molecular data to date have included only a subset of species in the genus (Miller and Schaal 2005; Miller 2008;

Santos and de Oliveira 2008), consequently providing limited insight into relationships between *S. purpurea* other *Spondias* species. Distance-based analyses of AFLP data revealed that *S. purpurea* is genetically more similar to *S. mombin* than to *S. tuberosa* or *S. cytherea* (Santos and de Oliveira 2008). Chloroplast haplotype data demonstrated that in some Central American populations, three *trnS–G* alleles are shared by both *S. purpurea* and *S. mombin* var. *mombin*, and one *trnS–G* allele is shared by both *S. purpurea* and *S. radlkoferi* (Miller and Schaal 2005). Haplotype sharing can indicate shared evolutionary history; alternatively, it can result from gene flow between two distinct lineages. In the chloroplast data set, the situation is further complicated by the mutational relationships among additional alleles carried by other *Spondias* species. For example, aside from the shared alleles described above, the *trnS–G* alleles carried by *S. purpurea* trees are most closely related to alleles recovered in *S. testudinus* and *S. mombin* var. *globosa* populations collected in Brazil. Network analyses of nuclear sequence data from the fourth intron of the gene encoding phosphoenolpyruvate carboxylase (*Pepc*) yielded no shared haplotypes between *S. purpurea* and *S. mombin* (Miller 2008).

10.4.2 Relationships Between Cultivated S. purpurea Populations and Their Wild (Native) Ancestors

Cultivated and native *S. purpurea* populations share several features that distinguish the species from others in the genus: flowers unisexual (occasionally hermaphroditic) cauliflorous, developing before the leaves appear, usually purple (but occasionally yellowish-white), fruits cylindrical to oblong drupes, fruit color variable, and leaflets small and numerous (see Sect. 2.1). On the basis of these morphological characters, as well as chloroplast and nuclear sequence data, cultivated and native *S. purpurea* populations form a monophyletic group (Miller and Schaal 2005; Miller 2008).

Native populations of *S. purpurea*, in conjunction with cultivated *S. purpurea* populations, have been the subjects of two distinct statistical analyses designed to investigate the geographic origins of cultivated *S. purpurea* populations. First, chloroplast sequence data were employed in a nested clade analysis (Miller and Schaal 2005). In this approach, mutational relationships among alleles were used to construct a haplotype network. Haplotypes within the network were grouped into nested clades (smaller clades are nested in larger clades); at each level of nesting, the null hypothesis was no association between geographical locations of individuals and the clades in which the alleles they carry are found (Templeton and Sing 1993; Templeton 1998). In the *Spondias* study, sequences of the chloroplast spacer *trnS–trnG* were used to construct a haplotype network and to identify clades. The null hypothesis of no association between geography and genealogical lineage was rejected for four clades; two clades consisted of alleles carried by individuals collected in southern Mexico and Central America (in the southern portion of the range of *S. purpurea*) and two clades included alleles carried by individuals in western central Mexico (the northern portion of the *S. purpurea* range). These results provide statistical support for two distinct groups of *S. purpurea* alleles, a southern group and a northern group. Because each group includes haplotypes recovered in both native populations and in cultivated populations, these results suggest that alleles recovered in cultivated populations in the southern portion of the range were derived native trees in the southern portion of the range, whereas alleles recovered in cultivated populations in the northern portion of the range were derived from native trees in the northern portion of the range. This phylogeographical analysis provides statistical support for two genetically and geographically distinct native *S. purpurea* gene pools, each of which gave rise to cultivated populations.

The second investigation of the origins of *S. purpurea* populations was based on AFLP data (200 fragments) generated for 13 native populations (85 individuals) and 21 cultivated populations (131 individuals) collected in Mexico and Central America (Miller and Schaal 2006; described in Sect. 3.1). Variation in the AFLP dataset was summarized using a principal components analysis. Analyses of the first three principal components indicated that trees from native populations cluster in two distinct groups (1) a southern group and (2) a northern group, and that trees from cultivated populations cluster geographically as well. Further analyses provided statistical support for the two groups of wild trees and the two groups of cultivated trees (Miller and Schaal 2006). The AFLP dataset corroborates the

results of the phylogeographic study based on chloroplast sequence data, providing further support for geographically and genetically distinct native *S. purpurea* populations from which cultivated *S. purpurea* populations were derived in at least two independent domestication events (Miller and Schaal 2005, 2006).

10.5 Role in Classical and Molecular Genetic Studies

Recently, molecular genetic techniques such as DNA sequence data and AFLPs have been applied to elucidate the evolutionary history of *S. purpurea* (Miller and Schaal 2005, 2006; Miller 2008). Although these studies have advanced the general understanding of domestication processes in trees, there have been no known studies applying classical genetic techniques in *Spondias*. Several aspects of the basic biology of trees complicate classical genetics initiatives (see Petit and Hampe 2006; Savolainen et al. 2007 for reviews). For example, trees are long-lived organisms that can take decades to reach maturity. Experimental crosses cannot be evaluated until offspring reach maturity, which can be several years at best. Trees are primarily outcrossers and while variants resulting from genetic exchange provide a wealth of options on which natural selection can act in wild populations, extensive variation impedes efforts to improve tree crops. Classical genetics is hindered further by self-incompatibility in many native tree populations. In the case of *S. purpurea*, although chloroplast and nuclear sequence data suggest that gene flow between *S. purpurea* and *S. mombin* is possible (Miller and Schaal 2005; Miller 2008), however, classical genetic approaches may be difficult due to low levels of viability observed in *S. purpurea* seeds (Juliano 1932), as well as the time to maturity.

10.6 Role in Crop Improvement Through Traditional and Advanced Tools

The success of traditional breeding efforts is evidenced by the abundance of fruit shapes, sizes, and colors in cultivated *S. purpurea* populations, and by the number of local names available for *Spondias*. Interviews with local residents and farmers during field studies in Mexico and including Central America uncovered about 100 unique names for native Mesoamerican *Spondias* species, 89 names for *S. purpurea*, and seven names for *S. mombin*. Data from field collections were combined with names obtained from literature sources to produce a comprehensive list of 247 common names for *Spondias* in Mesoamerica (Miller 2004). On the basis of this information, 183 names are used for *S. purpurea* and 64 common names have been applied to *S. mombin*.

Potential for crop improvement through modern breeding efforts with wild populations (native *S. purpurea* populations) or other closely related species (e.g., *S. mombin*) has not been thoroughly explored. The application of modern breeding techniques to *S. purpurea* may be met with limited success because (1) *S. purpurea* have relatively long juvenile phases (see discussion in Sect. 5); and (2) cultivated *S. purpurea* populations may have lost the ability to reproduce sexually (Sect. 2.3).

During the course of the domestication of many tree species, humans propagated desirable individuals through cloning (Zohary and Spiegel-Roy 1975; Ladizinsky 1998; Zohary 2004). In these species, the original cultivars were derived from their wild ancestors in two possible ways (1) cuttings of the wild trees were removed directly from native forests and placed in human-managed environments, or (2) an assortment of wild seeds were transferred from the forests to cultivated habitats. Once established in cultivated habitats, individuals were propagated vegetatively. New individuals were simply clonal reproductions of the most attractive individuals that were initially removed from the wild populations for cultivation (Zohary and Spiegel-Roy 1975; Zohary 2004). Additionally, it is likely that new genotypes were introduced periodically from local native populations.

Many of the world's oldest and most well-known fruit trees are propagated vegetatively, including almonds, olives, figs, breadfruit, and dates (Casas et al. 1999; Sonnante et al. 2002; Lumaret et al. 2004; Zerega et al. 2004; Bender and Whiley 2002; Morton 1987; Zohary and Spiegel-Roy 1975 respectively). The shift from sexually reproducing wild populations to vegetatively propagated cultivated populations has led to a suite of morphological changes that characterize the evolution of cultivated trees. These changes include, in self-incompatible

wild populations, a shift to self-compatible cultivated populations, and in dioecious wild progenitors, a shift to cultivated populations with hermaphroditic flowers (e.g., grapes), or with only female trees that produce fruit parthenocarpically (without fertilization) (e.g., figs) (Zohary and Spiegel-Roy 1975; Zohary and Hopf 2000; Zohary 2004). In the case of *S. purpurea*, cultivated trees are grown exclusively from cuttings, and seeds do not appear to form viable embryos (Juliano 1932; Avitia-García 1997; Miller 2004).

While traditional breeding alone may not be practical in *S. purpurea*, crop improvement may be a feasible option with the application of molecular genetic tools. To date, two studies have applied AFLP data to characterize diversity in *S. purpurea* and its relatives (Miller and Schaal 2006; Santos and de Oliveira 2008). AFLP markers can be used to generate molecular maps that, in concert with phenotypic data, can be used to identify markers associated with phenotypic traits of interest (e.g., Skøt et al. 2005). In the case of *S. purpurea*, the AFLP markers could be applied in marker-assisted selection programs designed to identify individuals with traits of agronomic importance.

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Chapter 11
Vasconcellea

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11.1 Basic Botany of *Vasconcellea*

11.1.1 Taxonomy

The Caricaceae is a small family of six genera and 35 species, all American, except for *Cylicomorpha*, a West African genus with two tree species. *Horovitzia* and *Jarilla* include herbaceous species with a limited distribution. The first is monotypic and endemic to the Mexican state of Oaxaca. The latter, with three species, is found in southern Mexico and Guatemala. *Jacaratia* has seven tree species that are widely spread under tropical climates in the Americas. *Vasconcellea* is the largest genus within the family, containing 21 species (Table 11.1), often called "highland papayas" or "mountain papayas" (National Research Council 1989) because of their resemblance with the common papaya and their typical, albeit not exclusive, ecological preferences for higher altitudes. Because of its outstanding economic importance, the unique species of *Carica,* the common papaya (*C. papaya* L.), has become pantropical (Badillo 1971, 1993, 2000, 2001).

Caricaceae have long been placed in the order Violales (Cronquist 1981). Recently, however, molecular data (APG 1998) and the presence of mustard-oil glucosides (Rodman et al. 1998) suggest that Caricaceae should be placed within the Brassicales.

Because of the strong similarity in their morphology and uses, common papaya and highland papayas have long been treated as close relatives and studied together. The taxonomic distinction between *Carica* and *Vasconcellea* has been essentially supported by the former presenting a one-celled ovary and the latter a five-celled one (Saint Hilaire, cited in de Mello and Spruce 1869). This distinction was questioned very early (see de Mello and Spruce 1869). As early as 1867, Bentham and Hooker concluded that the differences between *Carica* L. and *Vasconcellea* Saint Hilaire were so slight that they incorporated the latter into the former as a section. The number of sections was later increased to three (Solms-Laubach 1889; Harms 1925). In a preliminary presentation of his work, Badillo (1967) focused on the reduction of the genus *Carica* from 57 to 21 species and proposed to reduce infrageneric divisions to two, without much conviction on the number of ovary divisions. However, this trait was the first one used in his key and he formalized this division in 1971, re-establishing two sections, i.e., *Carica*, with one species, and *Vasconcellea*, with 21 species. In 1993, he suggested the possible rehabilitation of the latter as a distinct genus, as well as the re-evaluation of two complex species: *C. microcarpa* and *C. glandulosa*. His comments on macro- and micromorphological traits and their variation at both inter- and intraspecific levels called for caution in the application of identification keys. He also underlined that interspecific barriers were often more labile within section *Vasconcellea*, resulting in numerous observations of spontaneous hybridization. Finally, reacting to the results of the molecular phylogenetic study by Aradhya et al. (1999) and morphological differences, he fully restored *Vasconcellea* as a genus (Badillo 2000, 2001).

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Table 11.1 List of all described *Vasconcellea* species and the countries from where they have been collected (see also Fig. 11.2)

Species[a]	Country
V. candicans	Ecuador, Peru
V. cauliflora	Colombia, Costa Rica, Venezuela, Nicaragua, Panama, Guatemala Mexico, Honduras, El Salvador
V. chilensis	Chile
V. crassipetala	Colombia, Ecuador
V. cundinamarcensis[a]	Colombia, Ecuador, Venezuela, Peru, Bolivia, Panama, Chile, Costa Rica
V. glandulosa	Bolivia, Argentina, Peru, Brazil
V. goudotiana[a]	Colombia, Ecuador (recent introduction), Venezuela
V. horovitziana	Ecuador
V. longiflora	Ecuador, Colombia
V. microcarpa	Ecuador, Peru, Colombia, Venezuela, Brasil, Bolivia, Costa Rica, Panama
V. monoica	Ecuador, Bolivia, Peru, Colombia
V. omnilingua	Ecuador
V. palandensis	Ecuador
V. parviflora	Ecuador, Peru
V. pulchra	Ecuador, Colombia
V. quercifolia	Argentina, Bolivia, Brasil, Paraguay, Peru
V. sphaerocarpa	Colombia
V. sprucei	Ecuador
V. stipulata[a]	Ecuador
V. weberbaueri	Ecuador, Peru
V. × *heilbornii*[a]	Ecuador, Peru

[a]Cultivated at small commercial scale

11.1.2 Morphology and Floral Biology

Members of the Caricaceae family are sparsely branched and pachycaul trees, rarely herbs, with soft wood and a well-developed system of articulated laticifers. Leaves are alternate, often spirally arranged at branch tips. They are large, palmately veined, and lobed to palmate in shape. Stipules are absent or, when present, spine-like. Flowers can be found solitary or in cymes and are rarely bisexual. Species are mostly dioecious. The regular flower consists of five sepals and five petals with ten anthers in the smaller male flower and a superior ovary in the larger female flower. The ovary consists of five fused carpels with many anatropous ovules on parietal placentas. The style is short and crowned by five stigmas. Seeds show a gelatinous coat, the sarcotesta, a straight embryo, and an oily proteinaceous endosperm. The fruit is a berry. The stem of *Vasconcellea* and *Carica* species is very unusual in that there is little secondary xylem development. The wood is formed largely from the phloem, which gives the trunk its rigidity (Heywood 1985; Mabberley 1990).

In addition to the relative difference in ovary division, *Carica* is differentiated from *Vasconcellea* by a hollow stem, leaves with more than seven principal veins, and lamelliform protuberances on the seed sclerotesta. Unlike papaya, where different floral types, ranging from strictly pistillate to strictly staminate flowers with different intermediate forms, (Lassoudière 1969a) coexist, most *Vasconcellea* species are strictly dioecious and do not have bisexual flowers (Horovitz 1954). Only *Vasconcellea monoica* (Desf.) A.DC is, as its name indicates, monoecious. *V. cundinamarcensis* Badillo is polygamous, some plants showing both pistillate and staminate flowers (Badillo 1993). Polygamous plants of *V. parviflora* A.DC have been observed as well (X Scheldeman personal observation).

V. cundinamarcensis presents rare cases of andromonoecy, a trait shared only with *C. papaya*. Such plants are male, bearing numerous male flowers on long and highly ramified inflorescences, a varying proportion of these flowers, at the extremity of the ramifications, bearing ovules that are not completely sterile, so that they can develop into a few small fruits hanging far from the plant stem. Horovitz and Jiménez (1967) showed that this trait is under the double control of a Ypm chromosome and a *pm* cytoplasmic factor. Other species may bear the latter factor but lack the former.

Papaya pollination has not been clarified. Most authors (Badillo 1971; Ronse Decraene and Smets 1999) report insect pollination, although wind

pollination (PROSEA 1992) and self-pollination, in case of bisexual flowers (Morton 1987), have also been documented. Arguments in favor of insect pollination are the presence of potential attractants especially in male flowers. In *C. papaya*, these potential rewards consist of pollen, calcium oxalate crystals, and nectar (Ronse Decraene and Smets 1999).

All papaya species show some degree of parthenocarpy (Badillo 1993). This trait is particularly expressed in the babaco [*V.* × *heilbornii* (Badillo) Badillo], whose fruits are formed independently from pollination and/or seed formation (Horovitz and Jiménez 1967). Indeed, seeds are exceptional in this cultigen, which is propagated by cuttings.

11.1.3 Cytogenetics and Interspecific Hybridization

C. papaya and the limited number of *Vasconcellea* taxa investigated to date – (*V. cundinamarcensis, V. cauliflora* (Jacq.) A.DC, *V.* × *heilbornii*, *V. monoica*, *V. goudotiana* Tr. et Pl. and *V. quercifolia* Saint-Hil.) – present a somatic chromosome number of $2n = 18$ (Darlington and Wylie 1955; de Zerpa 1959; Magdalita et al. 1997). Preliminary studies with 4′-6-diamidino-2-phenylindole (DAPI) staining (T Kyndt unpublished data) indicate that *V. candicans* (Gray) A.DC, *V. stipulata* (Badillo) Badillo, and *V. weberbaueri* (Harms) Badillo share the same chromosome number. Arumuganathan and Earle (1991) report a small genome size for *Carica papaya* with a haploid DNA content of 372 Megabases (Mb). Similar or slightly larger genome sizes have been observed for some *Vasconcellea* species. *V. longiflora* (Badillo) Badillo seems to have a genome size about twice that of *C. papaya* (T Kyndt unpublished data).

A high degree of intergeneric incompatibility is ensured by post-zygotic barriers. This phenomenon is less common within *Vasconcellea*, where interspecific barriers are known to be labile, and cases of spontaneous hybridization have been reported (Badillo 1993). Meiosis is often regular in hybrids (de Zerpa 1959). *V.* × *heilbornii* and a hybrid between *V. monoica* and *V. cundinamarcensis* were explicitly described in detail by Horovitz and Jiménez (1967) and Badillo (1971). More hybrids may be expected in regions where species distributions overlap.

11.1.4 Agricultural Status and Current Uses

11.1.4.1 Caricaceae as Fruit Crops

Early in the sixteenth century, the Europeans spread the cultivation of the common papaya to all intertropical regions, where it has taken considerable importance. This fruit is now the fourth most important tropical fruit crop, covering 370,000 ha and reaching an annual world production in 2007 of almost 7 Mt (FAOSTAT 2008). Its social importance is even higher, as it is better adapted than other major fruits crops to small plots and it shows no marked seasonality in production, providing a regular source of food, vitamins, work, and income along the year.

Vasconcellea species are commercially and socially important only at a local scale; however, they can pretend to specific niches of the international market. In the Andes, *Vasconcellea* fruits are consumed fresh, roasted, processed in juices, marmalades, preserves, or dairy products, or else prepared in sauces, pie fillings, and pickles (National Research Council 1989; CAF 1992; Van den Eynden et al. 1999, 2003), mostly at household level. The two most important species are the babaco (*V.* × *heilbornii* "Babaco"), in Ecuador and southern Colombia, and the papayuela (*V. cundinamarcensis*) in all Andean countries, but more particularly in Colombia.

Only the cultigen with the largest mountain papaya fruits, i.e., babaco, has been the object of active global commercial development, albeit on a small scale, except in Ecuador, where it is permanently on the market. Endt (1981) describes the taste of babaco as a unique blend of strawberry, pineapple, and papaya, although, as Kempler and Kabaluk (1996) state, due to low soluble solids contents, sugar must be added to processed and even fresh fruits. It was introduced as a crop in New Zealand in 1973 (Endt 1981; Harman 1983) from where it spread during 1980s to Australia (Cossio 1988), Italy (Cossio and Bassi 1987; Ferrara et al. 1993), Spain (Merino Merino 1989), France (CTIFL 1992), South Africa (Wiid 1994), Switzerland (Evéquoz 1990, 1994), Canada (Kempler and Kabaluk 1996), and the Netherlands (Heij 1989) where greenhouse trials were undertaken. However, most of these introduction trials have failed due to commercialization problems (see Sect. 11.3.1). More recently,

higachos or baby babacos, fruits from other varieties of *V.* × *heilbornii*, have gained importance, thanks to their aroma and their smaller size, which have been shown to be better adapted to the urban market.

V. cundinamarcensis is mainly cultivated in the Colombian altiplano around Bogotá (departments of Boyacá and Cundinamarca), either in home gardens or in small plots intensively cultivated for commercial purposes. Although a minor fruit crop, its situation ensures a significant market as the population of this region is of the same order as that of all Ecuador. Taking into account the small garden production in Venezuela and Peru (National Research Council 1989), and the small but intensive production in Chile (probably 100 ha), the economic importance of *V. cundinamarcensis* might be compared to that of babaco in Ecuador. Other *Vasconcellea* species such as *V. candicans*, *V. crassipetala* (Badillo) Badillo, *V. goudotiana, V. microcarpa* (Jacq.) A.DC., V. *monoica*, *V. palandensis* (Badillo et al.) Badillo, *V. parviflora, V. quercifolia*, *V. sphaerocarpa* (García-Barr. et Hern.), and *V. stipulata* are also consumed locally (Badillo 1993; Van den Eynden et al. 1999; Scheldeman 2002). According to National Research Council (1989), *V. stipulata* fruits are the best highland papayas for jams and sauces.

11.1.4.2 Caricaceae as Sources of Papain

All members of family Caricaceae possess laticifers. When a plant is damaged, it releases latex that works as a defense mechanism to fend off predators (Dussourd and Eisner 1987; Konno et al. 2004) and helps in wound healing. This latex contains a diverse mix of proteins such as chitinases (Azarkan et al. 1997) and proteases.

Latex of different Caricaceae species shows different compositions of proteinases. Proteinases of the common papaya have been studied in detail, as this species is cultivated worldwide for this purpose. The most important ones are papain, chymopapain, caricain (formerly known as proteinase Ω), and glycyl endopeptidase (also known as papaya proteinase IV) (El Moussaoui et al. 2001). Although it is only one component, papain is often used as a general term to describe all proteinases extracted from papaya. Papaya latex enzyme research flourished during the last decade due to the application of better, often molecular, techniques that can study in detail the structure and function of each enzyme (reviewed in El Moussaoui et al. 2001). Papain, chymopapain, and caricain are broad-spectrum endopeptidases, whereas papaya proteinase IV is a highly specific proteinase acting on a peptide linkage with glycine (Buttle 1994).

Crude papain is the commercial name given to the dried latex, which consists of a mixture of several proteases (Madrigal et al. 1980). Purified papain is used in the textile industry to reduce shrinkage, in the beer industry for clarifying beer, in tanning for bating hides, and in the food industry mainly as a meat tenderizer but also for dough conditioning, cheese preparation, and protein enrichment of cereals (Becker 1958; Poulter and Caygill 1985). Papain has also a number of pharmaceutical applications. It is used as a digestive aid and for external treatment of skin diseases such as wart removal, scar treatment, and skin cleansing (Poulter and Caygill 1985). Starley et al. (1999) report the use of mashed papaya flesh as a cheap remedy to treat burns, while, chymopapain, another protease from *C. papaya*, has been shown to act therapeutically in patients suffering from spine disk disorders (Jaziri et al. 1994). Papain is used in preparation or manufacturing of adjuvants and reagents for antibiotics or vaccines.

Although latex is exudated by several parts of the plant including stems, branches, and petioles, the amount recoverable from such parts is much lower than that obtainable from immature fruits (Foyet 1972). Productivity and proteolytic activity of the latex is affected by variety and sex of the plants, fruit maturity, lancing operations, cultural practices, and other factors (Becker 1958). In practice, extraction is realized by making 2 mm deep incisions in the immature fruits (Skelton 1969). Upon harvest, liquid latex is sun-dried, oven-dried at temperatures of 50–55°C for 6 h (Ortiz et al. 1980), or preferably spray- or freeze-dried (Baines and Brocklehurst 1979; Baeza et al. 1990). According to Becker (1958), 1 kg fresh latex yields 200 g crude papain, while Krishnamurthy et al. (1960) report total solids' contents ranging from 11.1 to 54.9%. Liaquat and Mazumdar (1994) report an increase in both quantitative and qualitative latex yield by applying ethephon, indole-3-butyric acid (IBA), and gibberellic acid (GA_3). Lassoudière (1969b) reports yields of approximately 100 kg dried latex/ha per year.

In 1985, world market figures varied between 200 and 400 tons, including papain of all types and qualities, with the USA, Japan, UK, Belgium, and France as

major importers (Poulter and Caygill 1985). In the early 1990s, a decrease in production in the Democratic Republic of Congo due to political instability led to a shortage of papain on the world market. Several users (mainly brewers) who switched to cheaper and more readily available substitutes never went back to it when supplies picked up. In 2000, papain production was estimated to be around 800 tons, with prices averaging between US$10 and 20/kg, up to US$80 for high grade papain (Agribusiness Development Centre 2000).

11.1.4.3 Medicinal Uses

Fruits, seeds, latex, roots, and extracts of papaya have a very wide range of traditional and modern medical and dental uses, mostly against worms, dyspepsia, digestive disorders, bilious conditions, rheumatism, asthma attacks, syphilis, fevers, including malaria, skin defects, ulcers, and tumors, as a diuretic, as an anticonceptive, abortive, or emmenagog (Morton 1987; Hewitt et al. 2000; Sarma and Mahanta 2000; Dawson 2009). Most of these uses seem related to properties of papain and the alkaloid carpaine. Medical studies have documented their properties as anthelminthic (against both nematodes and cestodes, for human and livestock; Stepek et al. 2007); antiamoebic (To and Kyu 1934); enterobacteria antimicrobial (Osato et al. 1993); anticoagulant; blood pressure regulator; vasoconstrictor (Eno et al. 2000; Dawson 2009); and in wound healing (Anuar et al. 2008). Papaya may affect negatively female and male fertility, as well as fetal nidation and development, which have been validated in mice, rats, monkeys, and humans (Cherian 2000; Sarma and Mahanta 2000; Anuar et al. 2008; Lohiya et al. 2008). Antioxidant effects of extracts from papaya seeds have been used in the treatment of asthenoteratospermia (Piomboni et al. 2008). Fermented papaya preparations have shown therapeutical potential for the prevention of hypersensitive immunoresponse (Hiramoto et al. 2008).

Little is known about medicinal values of *Vasconcellea*, although FAO (1992) mentions use of fruit to treat arterial sclerosis and use of latex to cure skin mycosis and verruca plane. Medicinal uses clearly seem as interesting as those of *C. papaya*. Vernacular names of several species (*tapaculo*) refer to the antidiarrheal properties of their seeds. Recently, Mello et al. (2008) demonstrated the medical application of proteinases from *V. cundinamarcensis* in experimental rodent models in which they were shown to heal chemically induced gastric ulcers.

11.1.4.4 Wild and Feral Populations

Most papaya species still exist in the wild, whereas some have become naturalized out of their original range. Indeed, *V.* × *heilbornii* is the only cultigen in the family, as it is propagated artificially by vegetative methods.

Wild *C. papaya* still exists in Central America. Manshardt and Zee (1994) circumscribed its original distribution to eastern Mesoamerica, from the Yucatán peninsula to the Petén region of Guatemala. However, Coppens d'Eeckenbrugge et al. (2007) report similar wild plants in Costa Rica, on the Pacific Coast, as well as feral types that are intermediate between wild and cultivated materials on both coasts of the latter country. In addition, the species has shown a great potential to re-establish in the wild, and feral papaya plants are common in all the humid tropics, colonizing open areas, such as forest gaps and road edges. However, their populations tend to be sparse and the species can be considered neither a problematic weed nor an invasive species.

Concerning mountain papayas, only *V. cundinamarcensis* has been reportedly grown and consequently become naturalized in tropical highlands and cool subtropical areas of Sri Lanka, India, New Zealand, Puerto Rico (Morton 1987), and Zambia (Kew herbarium data).

11.1.5 *Geographic Distribution of* Vasconcellea *Diversity and Climatic Preferences*

Scheldeman et al. (2007) analyzed the distribution of *Vasconcellea* species and their climatic preferences. Observed and modeled distributions clearly confirm the association of the genus with the Andes and designate southern Ecuador as the center of its diversity. Indeed, the country holds 16 of the 21 species (Badillo 1993, 2000). Other countries with high observed species diversity are Colombia and Peru. Analysis of climate data on collection sites for each of the 21 *Vasconcellea* species (Fig. 11.1) shows that most

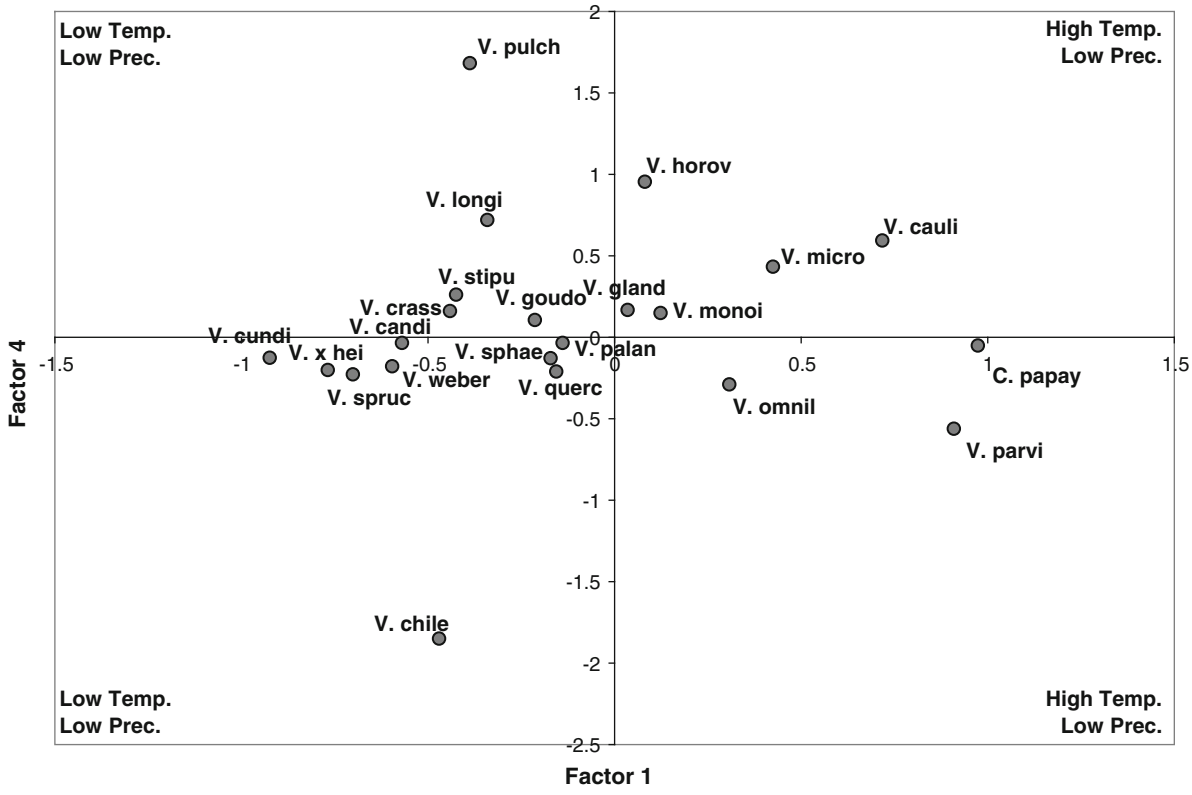


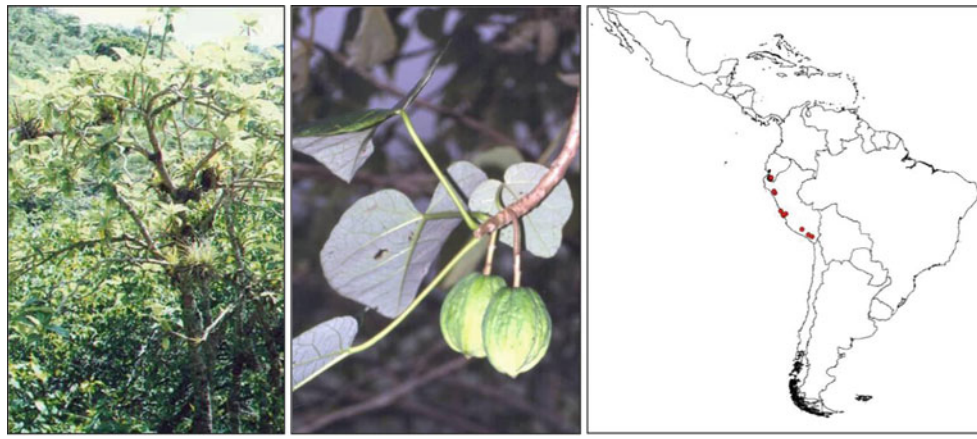
Fig. 11.1 Distribution of the species centroids for the first and fourth PCA axes (correlated to respectively annual precipitation and annual average temperature), for 1,702 *Vasconcellea* and *Carica* collection sites in Latin America based on 19 climatic parameters (see Scheldeman et al. 2007 for details on methodology)

species are adapted to zones with mild temperatures (hence their preference for higher altitudes) with average yearly precipitation figures ranging from 800 to 1,400 mm. Exceptions are *V. chilensis* (Planch. ex A.DC) A.DC, which is found in desert conditions, *V. pulchra* (Badillo) Badillo, which shows ecological preference for extremely wet conditions, and *V. parviflora*, which occurs on the Ecuadorian and Peruvian coasts in warm and relatively dry climates. Figure 11.2 shows the distribution of 17 *Vasconcellea* species.

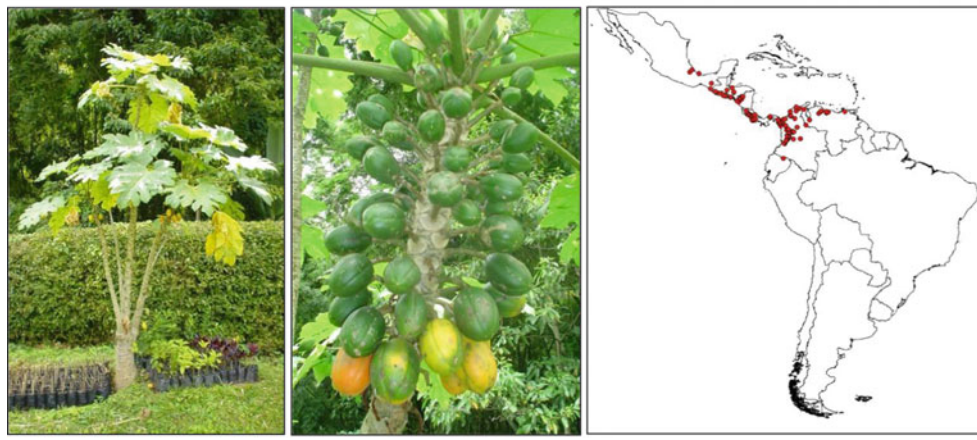
11.2 *Vasconcellea* Conservation and Genetic Erosion

The risk of genetic erosion in wild papaya species was long considered low because of their fast growth, their easy adaptation to disturbed habitats, numerous seeds, and their breeding system favoring outcrossing (IBPGR 1986). The study of the species distribution presented above imposes a revision of this statement. Indeed, besides differences in their ecoclimatic niches, the 21 described *Vasconcellea* species show significant differences in conservation status. Some species such as *V. cundinamarcensis* or *V. microcarpa* occur in regions representing wide distributions, while others are endemics, thus being more vulnerable to genetic erosion or even extinction.

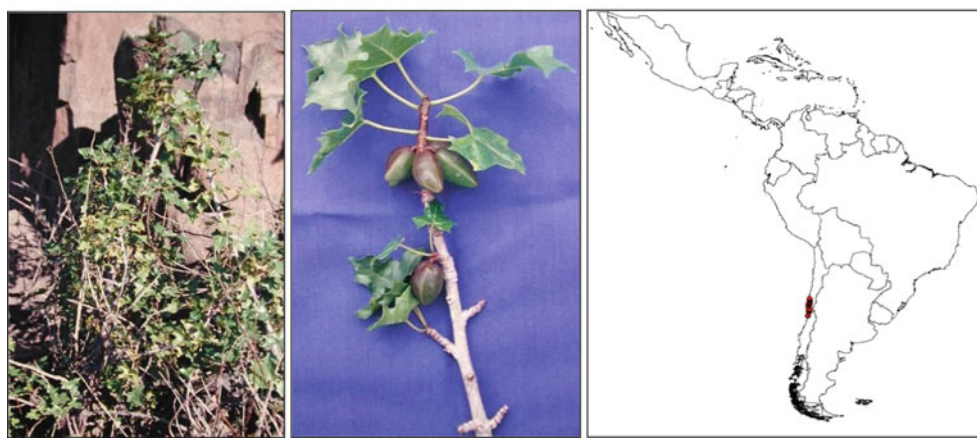
The International Union for Conservation of Nature (IUCN) is promoting the evaluation of the conservation status of species based on a standardized methodology (IUCN Standards and Petitions Working Group 2008). Species that are threatened (because of a limited distribution area or because of a sharp decline in documented populations) are assigned a threat category (Least Concern, Vulnerable, Near Threatened, Endangered, and Critically Endangered) and compiled into a Red List. Thus, these species are monitored more closely and given special attention in conservation activities. By 2008, five of the 21 *Vasconcellea* species had been included in the Red List (see



V. candicans (A. Gray) A. DC.



V. cauliflora (Jacq.) A. DC. (Photograph courtesy John Ocampo)



V. chilensis Planch. ex A. DC. (Photograph courtesy Tomas Fichet)

Fig. 11.2 (continued)

Table 11.2), with two species, *V. omnilingua* (Badillo) Badillo and *V. horovitziana* (Badillo) Badillo, listed as endangered.

The extent of occurrence (EOO) is an objectively verifiable and easily replicable parameter that quantifies the size of the distribution area of a species. It is

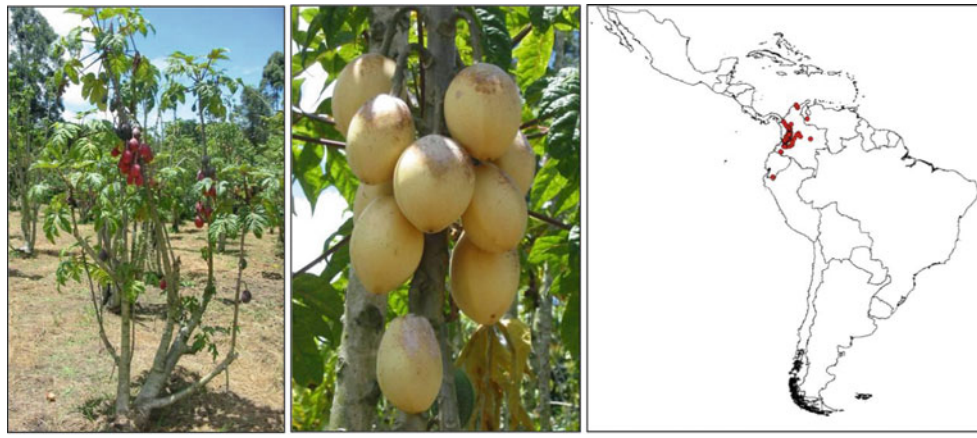
V. crassipetala (V.M. Badillo) V.M. Badillo

V. cundinamarcensis V.M. Badillo

V. glandulosa A. DC. (Photograph © VBRFMA-Bioversity, 2008-Moises Mendoza, MHNNK)

Fig. 11.2 (continued)

one of the parameters applied by IUCN to assess the conservation status of a plant species. Table 11.3 gives the EOO of each of the 21 *Vasconcellea* species based on the 1,553 *Vasconcellea* observations used in the first regional *Vasconcellea* study (Scheldeman et al. 2007). These values, and their accompanying conservation statuses, indicate that two additional *Vasconcellea* species (*V. chilensis* and *V. weberbaueri*) should be included in the Red List, resulting in one third of all known *Vasconcellea*



V. goudotiana Triana & Planch

V. longiflora (V.M. Badillo) V.M. Badillo (Photograph courtesy José Romero)

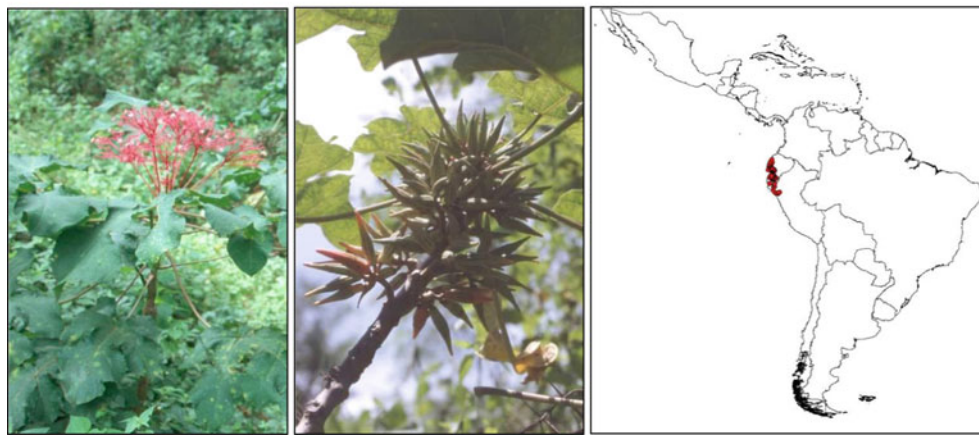
V. microcarpa (Jacq.) A. DC. (Photograph courtesy José Romero)

Fig. 11.2 (continued)

species facing some degree of risk. The recently described species *V. palandensis* should even be considered as Critically Endangered, a status that would correspond to current field observations, as no specimens were found in the area from which the species was first collected and described (Badillo

V. monoica (Desf.) A.DC.

V. palandensis (V.M. Badillo et al) V.M. Badillo (Photograph courtesy Veerle Van den Eynden)

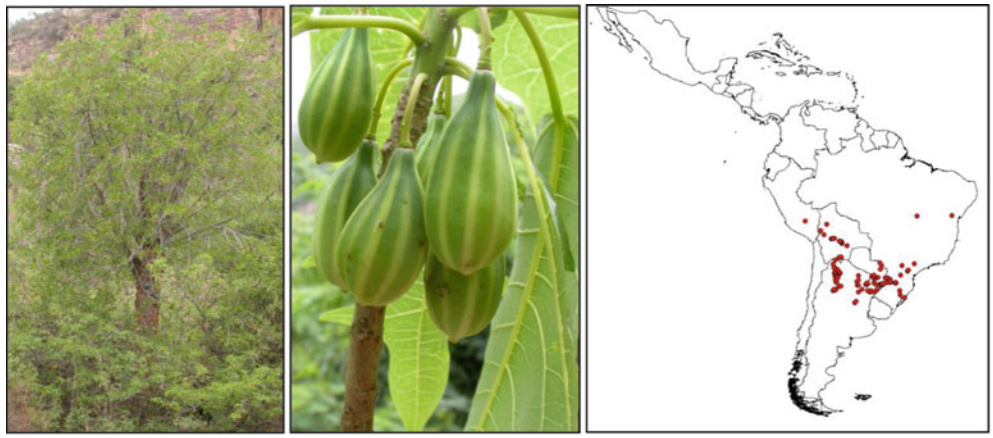


V. parviflora A. DC.

Fig. 11.2 (continued)

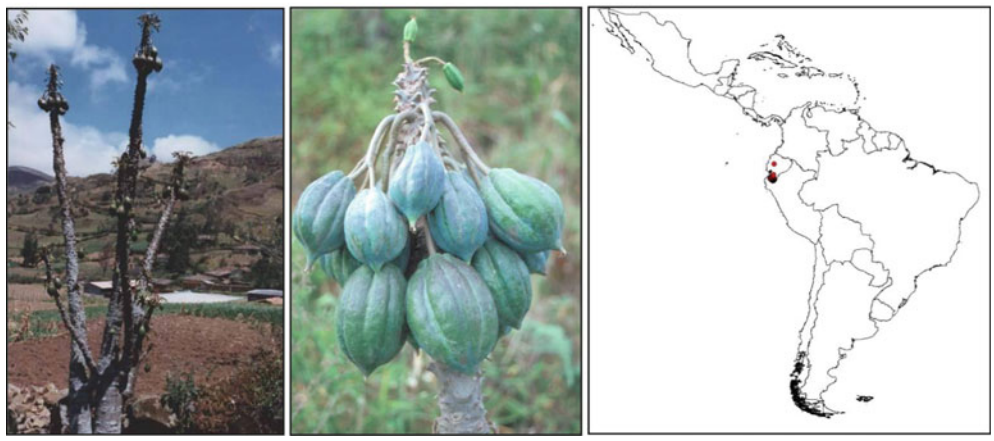
et al. 2000), and as most of the forest in which it occurred has been cut down (X Scheldeman and T Kyndt personal observation).

No formal studies on genetic erosion of *Vasconcellea* species have taken place to date. In southern Ecuador, farmers often eliminate local materials from



V. quercifolia A. St. Hill (Photograph © VBRFMA-Bioversity, 2008 – Nelly de la Barra, CBG)

V. sphaerocarpa (García-Barr. & Hern. Cam.) V.M. Badillo (Photograph courtesy John Ocampo)

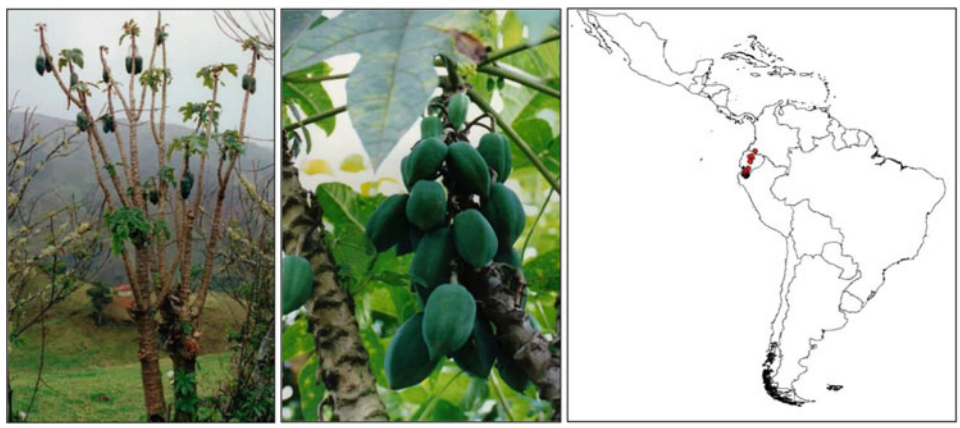


V. stipulata (V.M. Badillo) V.M. Badillo

Fig. 11.2 (continued)

home gardens and replace them by babaco. Increased pressure on forest, mainly driven by expansion of the areas dedicated to cattle grazing, is having a significant and negative effect on the presence of wild materials, which often prefer forest margins (Scheldeman et al. 2002). A Red Listing exercise from Bolivia also

V. weberbaueri (Harms.) V.M. Badillo



V. × *heilbornii* (V.M. Badillo) V.M. Badillo

V. × *heilbornii* (V.M. Badillo) V.M. Badillo, Babaco

Fig. 11.2 Plant and fruiting habit and distribution of 17 *Vasconcellea* species

reports that areas of natural occurrence of *Vasconcellea* species are subject to increased pressure from grazing, burning, and extension of the agricultural frontier, often leading to a decline in wild *Vasconcellea* populations (Nelly de la Barra personal communication).

Table 11.2 Conservation status of the 21 documented *Vasconcellea* species as published by IUCN completed with additional analysis of in situ conservation parameters based on the regional *Vasconcellea* database used by Scheldeman et al. (2007)

Species	IUCN status[a]	Extent of occurrence (EOO) (km^2)	IUCN Status according to EOO criterion[b]	No. of observations in protected areas	% of observations in protected areas
V. candicans		277,555		0	0
V. cauliflora		2,849,498		39	32
V. chilensis		16,565	VU	5	8
V. crassipetala		195,669		6	25
V. cundinamarcensis		4,323,047		21	8
V. glandulosa		2,410,183		4	9
V. goudotiana		715,484		5	4
V. horovitziana	Endangered (EN A4c; B1ab(iii))	55,561		1	13
V. longiflora		45,794		1	8
V. microcarpa		7,027,694		34	15
V. monoica		935,793		2	7
V. omnilingua	Endangered (EN A4c; B1ab(iii))	261	EN	0	0
V. palandensis	Vulnerable (VU D2)	6	CR	0	0
V. parviflora		110,199		6	7
V. pulchra	Near threatened (NT)	9,378	VU	0	0
V. quercifolia		4,697,606		2	2
V. sphaerocarpa		173,670		0	0
V. sprucei	Near threatened (NT)	10,894	VU	0	0
V. stipulata		22,176		0	0
V. weberbaueri		9,780	VU	0	0
V. × heilbornii		58,442		0	0

Extent of Occurrence (EOO) was calculated according to IUCN Standards and Petitions Working Group (2008) and data on protected areas based on UNEP-WCMC World Database on Protected Areas (United Nations Environment Programme 2008)
[a]IUCN (2010). IUCN Red List of Threatened Species. Version 2010.2. http://www.iucnredlist.org. Accessed 29 June 2010
[b]Critically Endangered (CR): EOO <100 km^2; Endangered (EN): EOO <5,000 km^2; Vulnerable (VU): EOO <20,000 km^2

11.2.1 In Situ Conservation

According to the Convention on Biological Diversity (United Nations Environment Programme 1992), in situ conservation is the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties. According to this definition, this conservation can consist of conservation of landraces or local varieties in farmers' fields (often also referred to as "on farm conservation") or of wild species, with protected areas offering the best options to do this (Heywood and Dulloo 2005).

Table 11.2 shows the number of species observed in protected areas for each of the 21 species. Nine of the 21 species have not been observed in protected areas so far. Of concern is that of these nine species, four are Red List species, species that should receive special attention to ensure their conservation.

11.2.2 Ex Situ Conservation

Table 11.3 provides an overview of ex situ conservation reported at the international level. It indicates adequate ex situ conservation for *V. cundinamarcensis* and to a lesser degree for *V. cauliflora*, *V. × heilbornii*, *V. goudotiana, V. monoica*, and *V. stipulata*. No collections of the threatened (red-listed) species, *V. horovitziana*, *V. omnilingua, V. palandensis, V. pulchra*, *and V. sprucei* (Badillo) Badillo, are found in genebanks (*V. palandensis* and *V. pulchra* are being conserved in botanical gardens), and this is a reason for concern and corrective measures. In total, 5 of the 21 *Vasconcellea* species are not reported to be conserved ex situ.

Table 11.3 Ex situ collections of *Vasconcellea* material based on published data and personal communications

Holding	*Vasconcellea* species	Source	Last update
Genebank			
USA: USDA (Hilo Hawaii)	Vcaul, Vcund, Vglan, Vgoud, Vmono, Vparv, Vquer, Vstip	Francis Zee (personal communication 2008)	2008
Peru: INIA (Cuzco, Arequipa)	Vcund	Llerme Rios Lobo (personal communication 2008)	2008
Ecuador: Universidad Técnica de Ambato, Ecuador	Vcund, Vheil, Vmono, Vstip	Jorge Vega (personal communication 2008)	2008
Ecuador: INIAP	Vcand, Vcaul, Vcund, Vgoud, Vheil, Vmicro, Vstip, Vwebe	Eddie Zambrano (personal communication 2008)	2008
Colombia: CORPOICA	Vcaul, Vcund, Vgoud, Vspha	Mario Lobo (personal communication 2008)	2008
Ecuador: UNL	Vcand, Vcund, Vgoud, Vheil, Vstip, Vwebe	Xavier Scheldeman (personal observation 1999)	1999
Peru: UNSAAC	Vcund	FAO-WIEWS (2010)[a]	1997
France: CIRAD-FLHOR	Vcund, Vcaul, Vgoud, Vstip	FAO-WIEWS (2010)[a]	1995
Peru: UNALM	Vcund, Vmon	FAO-WIEWS (2010)[a]	1984
India: IARI	Vcaul, Vcund, Vchil, Vheil, Vgoud, Vmicr, Vmono	FAO-WIEWS (2010)[a]	1984
Venezuela: CIBA UCV-FAGRO	Vgoud	FAO-WIEWS (2010)[a]	2008
Brasil: EMBRAPA	Vcaul, Vquer	FAO-WIEWS (2008)[b]	1999
India: IIHR	Vcund, Vcaul	FAO-WIEWS (2008)[b]	1991
Botanical garden			
Botanical gardens[a]	Vcund, Vheil, Vgoud, Vmicr, Vmono, Vparv, Vpulc, Vquer	Botanical Gardens Conservation International (2010)[c]	2010
National Botanical Garden of Belgium	Vcund, Vstip, Vwebe	National Botanic Garden of Belgium (2010) (LIVCOL)[d]	2010
Ecuador, Botanical Garden Loja	Vcand, Vcund, Vheil, Vpala, Vstip	José Romero (personal communication 2008)	2008
All	Vcand, Vcaul, Vchil, Vcund, Vglan, Vgoud, Vheil, Vmicro, Vmono, Vpala, Vparv, Vpulc, Vquer, Vspha, Vstip, Vwebe		

All published *Vasconcellea* collections have been contacted and previous data on them been updated in case of feedback received. In case of lack of feedback, sometimes old data were used

[a]http://apps3.fao.org/wiews/germplasm_query.htm. Accessed on 29 June 2010

[b]http://apps3.fao.org/wiews/germplasm_query.htm. Accessed on 10 Oct 2008

[c]http://www.bgci.org/plant_search.php/. Accessed on 29 June 2010

[d]http://www.br.fgov.be/RESEARCH/COLLECTIONS/LIVING/LIVCOL/index.html. Accessed 29 June 2010

There is limited information on the methods used for conservation. Most collections are maintained either as field or as seed collections. Only (part of) the United States Department of Agriculture (USDA) collection is conserved in vitro. Effective cryopreservation of *Vasconcellea* species have not been reported, although a vitrification-based shoot tip cryopreservation protocol was developed and successfully applied to *V. cundinamarcensis* (Ashmore et al. 2007).

As very little is known about the long-term conservation of *Vasconcellea* seeds, the question can be raised if some of the reported seed collections still contain viable seeds. A study on viability of *V. cundinamarcensis* seeds (Vanhove 2000) indicates that there was no significant loss of viability in seeds that had been conserved at 4°C for 2 years, indicating a likely orthodox seed behavior of this species. No information on conservation for longer periods could be found in the literature, however. Field collections often suffer from the lack of long-term funding, essential to ensure effective ex situ conservation. There have been several reports of loss of field collections, mainly in Ecuador, the country with the highest diversity.

Besides the typical germplasm collections in genebanks, some *Vasconcellea* material is also maintained in botanical gardens. These conservation activities

focus on conservation of the species giving less emphasis on intraspecific diversity.

Neither *Carica* nor *Vasconcellea* are included in Annex I of the International Treaty on Plant Genetic Resources for Food and Agriculture (Fowler et al. 2003). Although countries can decide to provide access according to the terms of the Treaty (through a Standard Material Transfer Agreement) to non-Annex I crops (SGRP 2007), in most cases access to conserved (wild or cultivated) material is taking place according to the provisions of the Convention of Biological Diversity (CBD), based on bilateral agreements between the competent organism in the country of origin of the material (often Ministry of Environment) and the requesting institute.

11.3 Scope for Domestication and Commercialization of *Vasconcellea* Species

Vasconcellea species show interesting potential and scope for domestication in different areas. First of all as a source of fruits with often appealing organoleptic properties (e.g., aroma, taste, and color), either based on existing species or varieties, or based on new varieties that make use of the different properties present in each of the different *Vasconcellea* species combined with their easy hybridization. Secondly, *Vasconcellea* species are also considered to be a potential source of papain, a proteolytic enzyme widely used in pharmaceutics and food industries, and other biologically active components of high pharmaceutical interest. The National Research Council (1989) states that the potential of highland papayas is currently only exploited at smallholder level in Andean countries and that even there, given adequate quality control, it might be possible to develop both fresh fruit export business and an Andean papain extraction industry.

11.3.1 *Use of* Vasconcellea *Species as Fruit Crop*

Most of the introduction trials of *Vasconcellea* species, i.e., babaco, outside of Ecuador have failed. This was always due to commercialization problems. In New Zealand, the main constraints were the fruit's novelty, consumer unfamiliarity with fruit utilization, and lack of promotion. The large fruit size made them too expensive for consumers wanting to try something new (Hewett 1993). In Italy, where in 1986 a massive plant sale promotion took place in an effort to launch its cultivation, fruit sale did not reach expected values due to quality issues (unfamiliar aroma and taste, elevated acidity, and low sugar content), oversized fruits, and a lack of promotion in the press and to consumers (Ferrara et al. 1993). Kempler and Kabaluk (1996) reported rapid overproduction and deteriorating fruit quality, partly due to open-air cultivation, as the main reason for commercialization failure in New Zealand and Italy. In Holland, where a greenhouse of 6,000 m^2 was planted with babaco, production was too high in comparison to demand, resulting in low prices and lack of profit (Stijger 2001). In Switzerland, production was not a problem, but elevated production costs, especially greenhouse heating, made babaco cultivation unprofitable so that it was cheaper to import fruits (Evéquoz 1994).

In spite of these failures there is still interest in babaco (Villarreal et al. 2003). As exotic fruits are increasingly popular, with kiwi and mango as well-known success stories, babaco still has the potential to develop into a niche crop with a solid market and fair producer prices. However, as Sabbe et al. (2007) were able to show, taste and aspect are important factors explaining acceptance of unknown tropical fruit species. In general, the latter are expected by western consumers to be healthier and tastier than most temperate zone species, thus offering ample scope for acceptance (Sabbe et al. 2008, 2009). Market development and quality control would be the most important pre-requisites to turn babaco into an economically rewarding crop (Kempler and Kabaluk 1996). Therefore, market chain analysis prior to babaco introduction and development seems to be a *conditio sine qua non* for developing this crop into a winner. The analysis should try and define weaknesses, but also strengths and opportunities, and threats that await product development, and should address such issues as strengthening all stakeholders involved in the production–commercialization chain.

Other *Vasconcellea* species have not yet received the same level of commercial attention as babaco. Pomological studies (Scheldeman et al. 2003) confirm that besides its attractive aroma, *V. stipulata* has the

highest levels of soluble solids of all consumed *Vasconcellea* species. Its smaller size makes it more attractive than the bigger and less aromatic babaco. In its reduced distribution zone in southern Ecuador, local consumers prefer this species over the more commercial babaco (Scheldeman 2002). *V. stipulata* should, therefore, be a priority species for further domestication work. A well-targeted promotion of *V. cundinamarcensis* and *V. goudotiana* could increase their market significantly, especially in Colombia, but in other parts of their traditional areas as well. The development of new products, better adapted to urban ways of life, such as preserves and health products, should also be envisaged.

Regarding breeding for fruit traits, natural hybridization of *V.* × *heilbornii* varieties with *V. stipulata* results in numerous intermediate forms with different flavors and varying amounts of seeds illustrating the ample use potential of this group (Horovitz and Jiménez 1967; National Research Council 1989). Indeed, diverse cultigens producing smaller fruits (higachos, now also called baby babacos) are becoming important on the urban markets of Ecuador, thanks to their smaller fruit, better adapted to current small family size, and their pleasant, strong aromas. The huge variability, especially in the species which are currently preferred by consumers, i.e., *V. stipulata* and *V.* × *heilbornii,* makes it relatively easy to select specific accessions. Hybridization may even enlarge this variability and make selection of highland papayas relatively easy. Currently, the lack of selection criteria is one of the main constraints in local breeding programs. A thorough consumer survey could help to establish these criteria. Furthermore, yield and pest and disease susceptibility of promising accessions should be evaluated under different environmental conditions.

Some of the non-commercial *Vasconcellea* species could also be used to improve babaco cultivation. In the case of Ecuador, where babaco (*V.* × *heilbornii* "Babaco") is still the only highland papaya that is cultivated at a significant commercial level, its production is seriously threatened, especially in greenhouses, by the root fungus *Fusarium oxysporum* and the nematode *Meloidogyne incognita*. As adequate chemical treatments are currently lacking (Ochoa et al. 2000), the use of resistant *Vasconcella* rootstocks in babaco cultivation could become another promising use of some highland papayas. The presumed progenitors of babaco, *V. cundinamarcensis* and *V. stipulata*, combine *F. oxysporum* resistance with high *M. incognita* susceptibility, which precludes their use as rootstock. On the other hand, *V. monoica* and especially *V. weberbaueri* show resistance against *F. oxysporum* and very low *M. incognita* susceptibility (Scheldeman et al. 2003). Evaluation of grafting techniques using different species combinations showed good compatibility with success rates ranging 50–100%, confirming earlier results obtained by Jiménez and Horovitz (1957), who tested successfully several combinations involving *Carica* and *Vasconcellea* species. The use of *V. cauliflora* rootstock allowed maintaining highland species under tropical lowland conditions.

Despite limited successes, attempts to establish successful commercial highland papaya cultivation should be pursued. Different species could provide a range of fruits in cooler parts of the developing world. With their extreme variability, they could become a successful backyard crop from Morocco to Papua New Guinea and develop from local trade over small- or large-scale domestic trade to export National Research Council (1989).

11.3.2 *Use of* Vasconcellea *Species as a Source of Papain*

Apart from the preliminary work done by Scheldeman et al. (2003), little research on commercial application of latex of *Vasconcellea* species has been realized. FAO (1992) mentions the use of fruit to treat arterial sclerosis and the use of latex of *V. cundinamarcensis* as a meat tenderizer, while the National Research Council (1989) speculates that *V. stipulata*, and other highland papayas, might be grown as a source of papain. Baeza et al. (1990) evaluated the latex of *V. cundinamarcensis* and found that the proteolytic activity of freeze-dried latex was between five- and eightfold higher than that of *C. papaya* latex. Spray-drying resulted in a 70% loss of activity, while oven-drying retained only 49% of proteolytic activity. Baeza et al. (1990) also reported a water content in fresh latex of 80% (weight percentage), which is 8% lower than that of papaya, and demonstrated that stem extracts showed even higher proteolytic activities. Horovitz and Jiménez (1967) advocated for the use of the whole plant and mentioned a particularly high proteolytic activity in a *V. stipulata* × *V. monoica* hybrid. Preliminary papain analysis of some Ecuadorian *Vasconcella* species revealed very high proteolytic activity, when compared to *C. papaya* (Table 11.4;

Table 11.4 Papain activity in some Ecuadorian *Vasconcellea* species according to Scheldeman et al. (2002)

Species	Papain activity (mU BAPNA/mg dried latex)
Carica papaya (reference)	10.4
V. × *heilbornii* "Babaco"	38.1
V. × *heilbornii* var. *chrysopetala*	127.6
V. stipulata	129.4
V. cundinamarcensis	57.0
V. monoica	55.1

Scheldeman et al. 2003), although caution is warranted as no results about exact enzymatic composition nor actual yields of most *Vasconcellea* latex are currently available. Dhuique Mayer et al. (2001) showed that proteolytic activity of babaco latex is equivalent to or slightly higher than that of papaya. Some accessions of *V.* × *heilbornii* and *V. stipulata* show particularly promising papain activity levels (Scheldeman et al. 2002; Kyndt et al. 2007).

More detailed studies on proteolytic enzymes in *V. cundinamarcensis* showed the presence of four proteinases, CC-I to CC-IV, whereby CC-I and CC-III correspond respectively to papain and chymopapain of *C. papaya* (Walraevens et al. 1993). Pereira et al. (2001) suggest the possibility that *V. cundinamarcensis* latex consists of six to seven cysteine enzymes, some of them with a transient existence. Teixeira et al. (2008) proved this by fractionating the latex of *V. cundinamarcensis* into 12 cysteine proteinase isoforms, whereby some of them displayed higher enzyme activities than the enzymes present in *C. papaya* latex. They separated them into two main groups of enzymes based on fractionation with carboxymethyl-Sephadex (CMS): CMS1 displayed the largest amidase activity, while the more acidic group (CMS2) displayed moderate to low activity. One of the members of the first group, a 23 kDa cysteine proteinase designated CMS1MS2, with about fourfold higher activity than papain, was purified for crystallization by Gomes et al. (2008). Several cDNA sequences coding for cysteine proteinases in *V.* × *heilbornii* and *V. stipulata* were identified using primers based on conserved sequences (Kyndt et al. 2007). Ion exchange chromatography and gel filtration procedures on latex of *V.* × *heilbornii* revealed five major protein fractions (VXH-I–VXH-V) showing very high amidase activities. Altogether, these studies confirm a higher degree of proteolytic activity in the latex of all *Vasconcellea* species investigated so far, in comparison to *C. papaya*. This is most probably due to higher protein contents and the presence of other, more active, cysteine proteinases in their latex.

The variable distribution of proteolytic activities in latex from different species reported in the literature must be regarded with caution, as it has been shown that amidase activity fluctuates during latex coagulation in *C. papaya* (Moutim et al. 1999). However, although the different studies are not consistent about the level of proteolytic activity due to varying experimental conditions, they confirm the potential of *Vasconcellea* for commercial proteinase production. On the other hand, the amount of latex that can be collected from the (generally smaller) fruits of the wild *Vasconcellea* plants is definitely lower than the current latex yield of the common papaya. Consequently, future *Vasconcellea* breeding for commercial latex production should focus on both latex activity and yield.

Recently, a first enzyme involved in sugar metabolism, i.e., an alpha-mannosidase, was characterized from the latex of *V.*× *heilbornii* (Blom et al. 2008), illustrating the complex mix of enzymes present in the latex of *Vasconcellea* species.

11.4 Role in Elucidation of Origin and Evolution of Cultivated Papayas

Several biochemical and molecular studies have not only supported an early evolutionary separation of genera *Carica* and *Vasconcellea*, but they have also shown that *Vasconcellea* and *Carica* are relatively distant genera in the family, as *Jacaratia* appears intermediate (Aradhya et al. 1999; Van Droogenbroeck et al. 2002; Kyndt et al. 2005c, 2006). The picture that has emerged in the last decade indicates the need to consider crop evolution in common papaya and cultivated highland papayas separately.

11.4.1 Evolution of the Common Papaya (*C. papaya*)

The long-lasting grouping of all papayas under the genus *Carica* influenced breeding strategies and the management of genetic resources in the common papaya significantly. Indeed, the high diversity of species of *Carica sensu* Bentham and Hooker in northern South America led several authors (e.g., Badillo 1971; Brücher 1989, cited in Smith et al. 1992) to consider this region as the center of diversity for papayas as a whole.

Wild papaya was long known as *C. peltata* Hooker et Arnott, a name that was downgraded to a synonym for *C. papaya* by Badillo (1967). Although its existence in Central America was recognized by other authors (Purseglove 1968; Storey 1976), it was often ignored (León 1987; Samson 1989) or overlooked and very poorly documented in the literature. The only brief descriptions of wild papayas in recent literature are that of Manshardt and Zee (1994) and Coppens d'Eeckenbrugge et al. (2007). Manshardt and Zee (1994) circumscribed their distribution to eastern Mesoamerica, from the Yucatan Peninsula to the Peten region of Guatemala. Coppens d'Eeckenbrugge et al. (2007) reported similar wild plants in Costa Rica, on the Pacific Coast, as well as feral types that are intermediate between wild and cultivated materials on both coasts of this country. Wild papaya diversity has only been studied with morphological and isozyme analyses of wild and feral papayas (Morshidi et al. 1995; Coppens d'Eeckenbrugge et al. 2007).

In fact, despite their incompatibility with the common papaya (see infra), highland papayas have received more attention as wild germplasm and potential sources of genetic resistance to diseases, in a series of hybridization experiments and studies of genetic diversity in the 1990s (Manshardt and Wenslaff 1989b; Chen et al. 1991; Chen and Chen 1992; Sharon et al. 1992; Magdalita et al. 1996; Jobin-Decor et al. 1997; Drew et al. 1998). Characteristically, the widest study of *C. papaya* genetic diversity (Kim et al. 2002) mentions its Central American origin and includes *Vasconcellea* accessions, but no wild material of the common papaya.

Despite the lack of knowledge on wild papayas, we can infer that the evolution of *C. papaya* as a crop plant involves a direct domestication process. Papaya was very common in all tropical America at the arrival of the first Europeans (Leal 2003). Glottochronological data indicate that papaya was an important crop in Mesoamerica more than 2,600 years ago (Brown 2010). Archeobotanical remains, dated 800–1200 BC (Pearsall 1992), give even more ancient dates for its presence in South America. But the best argument to consider that it is a very ancient crop plant is the species' ecology and its spectacular proteolytic properties. Indeed, as most Caricaceae, common papaya is a pioneer species, a very early colonizer of any open space in the forest. Early farmers clearing a land for cultivation could not ignore its presence. So we must envisage that papaya management by humans is at least as ancient as early lowland agriculture in Mesoamerica, i.e., somewhere around 10,000 years BP (Piperno 2006).

A comparison of fruits from wild *C. papaya* and unimproved Mesoamerican landraces indicates that selection under domestication increased fruit size, from a few tens of grams to several kilograms in some cultivars; widened the mesocarp, from a few millimeters to several centimeters; changed flesh color, from yellow to orange or red; improved taste by reducing latex content in fruit tissues; increased sugar content; and decreased papain content and activity, allowing direct consumption in fresh state. The domestication syndrome appears essentially limited to seed physiology. As for many pioneer trees, wild papaya seeds are small, remain dormant over extended periods of time, and show strong light requirement to promote germination. In contrast, cultivated papaya seeds are 33% larger and present higher germination rates, with no dormancy, no light requirement, and no sensitivity to fluctuating temperature (Paz and Vazquez-Yanes 1998). It has also been suggested that domestication affected the propagation system of the species. From the study of teratological flowers, Storey (1967, 1976) hypothesized progressive evolution of dioecy in the family, assuming an ancestral perfect flower, with the staminate flower evolving by elimination of the functional pistil and the pistillate flower deriving by a succession of anomalies. Consequently, the present-day sexual types were attributed to continuous artificial selection. But during the same scientific meeting, Horovitz and Jiménez (1967) proposed that dioecy is the primitive state, with sex determinism of the classical XX–XY type with a heterogametic male and lethal YY genome. The common hermaphrodite sexual type of *C. papaya* would have

been derived by a modified Yh chromosome conserving the lethal region of the Y chromosome. Recent molecular data favor the latter hypothesis (Ming et al. 2007a; Yu et al. 2008a).

Although not in the way proposed by Storey (1976), artificial selection seems to have favored hermaphroditism. Indeed, natural populations are uniformly dioecious (Manshardt and Zee 1994), whereas the hermaphrodite sexual type, favorable for fruit production, is common in many cultivars. According to Horovitz et al. (1953), the hermaphrodite type depends on humans, as it is eliminated in just a few generations in feral populations. This statement is contradicted by our observation of ancient feral populations in Cameroon forests, with plants still producing very small fruits on both female and hermaphrodite types. In any case, recent molecular data have seriously questioned the correlation between the appearance of the hermaphrodite flower and the domestication process, as Yu et al. (2008b) estimated that the Yh chromosome of this sexual type diverged from the male Y chromosome more than 73,000 years ago, well before the origin of agriculture.

11.4.2 Evolution of Main Cultivated Highland Papayas (*V. cundinamarcensis*, *V.* × *heilbornii*, and *V. goudotiana*)

11.4.2.1 *V.* × *heilbornii*

V. × *heilbornii* includes babaco and a number of diverse cultigens producing smaller fruits (higachos, now also called baby babacos). It is the only cultigen in the genus, as *V. cundinamarcensis* and *V. goudotiana* still exist in the wild. Propagation by stem cuttings appears easier in *V.* × *heilbornii* than in its putative parental species, *V. cundinamarcensis* and *V. stipulata*, with rooting rates of 50% versus 11 and 4%, respectively. In contrast, the hybrids present a low level of sexual fertility and germination rates below 5% (Jiménez et al. 1998). Thus, dependence on vegetative reproduction is clearly a key component of the domestication syndrome.

Outside of Ecuador, *V.* × *heilbornii* is mostly known from the large, five-ribbed babaco fruit, from which it took its first Latin epithet, as *Carica pentagona* Heilborn. It was long considered as a sterile crop plant, producing parthenocarpic fruits and reproducing vegetatively. Badillo (1967) described more diversity and studied experimental hybrids obtained by Horovitz in detail. By doing so, Badillo realized the contribution of *V. stipulata* and *V. cundinamarcensis* to this diversity [described by Badillo (1966)] and established a hybrid species: *C.* × *heilbornii*. This hybrid species, with several forms, included babaco as the *pentagona* notomorph, and *C. chrysopetala*, *C. fructifragans*, and Horovitz's artificial hybrid as three other notomorphs. The first three were later raised to botanical variety status in 1993 by Badillo, who then mentioned the existence of other, undescribed, variants of this hybrid, so recognizing a highly dynamic element in the evolution of the cultigen. This dynamic evolution also affects *V. stipulata* itself, as Badillo (1971) noticed morphological variation between its representatives from central and southern Ecuador that could be attributed to introgression of *V. cundinamarcensis* genes. Similarly, from their breeding experiments, Horovitz and Jiménez (1967) mention that pollination of var. *chrysopetala* by *V. cundinamarcensis* produces a progeny of female and andromonoecious forms (indicating the presence of cytoplasmic sexual factors of *V. cundinamarcensis*), while pollination by *V. stipulata* produces a dioecious progeny whose yellow flowers and spiny stipules are similar to those of *V. stipulata*. The authors underlined that fruit growth was independent of pollination in *V.* × *heilbornii* (which is not completely the case in other Caricaceae) and related this property to domestication, together with sexual sterility and vegetative propagation. Horovitz and Jiménez (1967) also suspected that other species than *V. stipulata* and *V. cundinamarcensis* might have contributed to the babaco genome (de Zerpa 1980). These views have been widely confirmed, and even enriched, by more recent morphological, biochemical, and molecular studies of other research groups.

Many studies have focused on elucidating the diversity and origin of *V.* × *heilbornii*. A first morphological study by Scheldeman (2002) included *V. cundinamarcensis* (13 accessions), *V. stipulata* (22 accessions), and *V.* × *heilbornii* (14 accessions of babaco, 30 accessions of var. *chrysopetala*, and 19 undetermined representatives), all from Loja (southern Ecuador). The analysis revealed an impressive

morphological variation, particularly in *V.* × *heilbornii*, blurring the distinction with *V. stipulata* and suggesting that sexuality was sporadically involved in its reproduction. Principal component analysis and cluster analysis differentiated *V. cundinamarcensis*, *V. stipulata*, and babaco. In the principal plane, *V.* × *heilbornii* var. *chrysopetala* formed a group between *V. stipulata* and the babacos, but closer to *V. stipulata*, with some overlap. The other specimens of *V.* × *heilbornii* formed a distinct group, between babacos and *V. cundinamarcensis*. Different amplified fragment length polymorphism (AFLP) and nuclear SSR marker studies on very similar sample sets from southern Ecuador confirmed a high genetic diversity in *V.* × *heilbornii*, with considerable AFLP variation even within babaco, and placed its accessions at an intermediate position between the presumed parental species, with one group of accessions closer to *V. stipulata*, and one group closer to *V. cundinamarcensis*, suggesting a bidirectional introgression with the putative parents of the hybrid species (Van Droogenbroeck et al. 2002; Kyndt et al. 2005a, 2006).

Another morphological study, by Restrepo et al. (2004a), included 14 accessions of babaco and 23 other representatives of *V.* × *heilbornii* (baby babacos, classified as var. *chrysopetala* and af. var. *chrysopetala*), mostly from Central Ecuador. Again, baby babacos appeared highly variable, surprisingly so for vegetatively propagated cultigens. Babaco plants clearly segregated into two groups, corresponding to at least two distinct clones, which definitely contradicts babaco classification as a cultivar (cultivars being defined on a morphological basis, independently from the genetic constitution and breeding system). Interestingly, a geographic structure appeared in the analysis. In agreement with the view of Badillo (1993), accessions of babaco and baby babacos from southern Ecuador appear closer to *V. stipulata*, the presumed parent that is endemic to this region, which suggests that introgression processes are still very active in the evolution of *V.* × *heilbornii*, despite the predominantly vegetative reproduction. This geographic structure was confirmed by an isozyme analysis of the highland papayas from the same Ecuadorian origin plus accessions from Colombia (Jiménez 2002). It was most spectacular for accessions of *V. cundinamarcensis*. On the Ecuadorian side of the principal plane, the babacos and *V. stipulata* formed two closely related uniform groups, the accessions of *V.* × *heilbornii* var. *chrysopetala* taking an intermediate position between these two groups and the one consisting of Ecuadorian accessions of *V. cundinamarcensis*. The most typical representatives of this variety were placed closer to *V. stipulata*, whereas those accessions with smaller stipules, greenish flowers, and more ramified inflorescences were placed at mid-distance from *V. cundinamarcensis*.

Chloroplast DNA studies have provided additional insights on the origin of *V.* × *heilbornii*. The first polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) study by Aradhya et al. (1999) revealed intraspecific diversity despite the small sample analyzed. In particular, the two accessions of babaco exhibited slightly different haplotypes, none of them corresponding to their putative parent species.

A wider sample including 18 *Vasconcellea* species, mostly collected from Loja province (southern Ecuador), was subjected to PCR-RFLP analysis for three organellar DNA regions: two chloroplastic and one mitochondrial (Van Droogenbroeck et al. 2004). These data were later compared with AFLP and morphological data (Kyndt et al. 2005a). Only 11 chlorotypes were observed for *Vasconcellea*, five of them being shared by several species. Only *V. microcarpa* showed intraspecific differentiation. Species with the same chlorotype also had the same mitotype (four different mitotypes among the sample set). The study results contrasted with those of Aradhya et al. (1999) in identifying two lineages in the Caricaceae, the first one including *Carica*, *Jacaratia*, and some *Vasconcellea* species, among them *V. stipulata* and *V.* × *heilbornii*, and the second one a number of other *Vasconcellea* species, among them *V. cundinamarcensis*. *V.* × *heilbornii* shared its haplotype with *V. weberbaueri*, while *V. stipulata* was placed at the base of the lineage, even further from the babaco than *C. papaya*. In a later study, the same research group observed the typical haplotype of *V. stipulata* in two *V.* × *heilbornii* genotypes (Kyndt et al. 2005a).

A third PCR-RFLP study of cpDNA (Restrepo et al. 2004b) involved 54 accessions from 12 *Vasconcellea* species, from Colombia and Ecuador (central and southern provinces). Ample intraspecific variation was observed, with, e.g., nine haplotypes for *V. cundinamarcensis* (13 accessions), seven for *V.* × *heilbornii* (13), and six for *V. stipulata* (8), only one haplotype being shared by two species

(*V.* × *heilbornii* var. *chrysopetala* and *V. stipulata*). As in the study of Aradhya et al. (1999), the different accessions of babaco show variation. Principal coordinate analysis (PCO) places *V. cundinamarcensis* in one large group, and *V.* × *heilbornii* and *V. stipulata* in the other, similar to Van Droogenbroeck et al. (2004). Again, *V. stipulata* is placed at the base of the second group, relatively distant from *V.* × *heilbornii*. However, two accessions of this species are grouped with *V.* × *heilbornii*. *V.* × *weberbaueri* is placed close to *V.* × *heilbornii*, at a short distance. The slight differentiation between *V.* × *heilbornii* and *V. weberbaueri* was later confirmed with cpSSR markers (Kyndt et al. 2006).

A phylogenetic study carried out by Kyndt et al. (2005c), involving a sample of each *Vasconcellea* species, confirmed the existence of two lineages for cpDNA in the genus. The smaller one consists of *V. parviflora*, *V. weberbaueri*, *V. stipulata*, and *V.* × *heilbornii*. The latter is closely associated with *V. stipulata* in the *psbA–trnH* tree, and with *V. weberbaueri* in the combined *matK/trnL–trnF* tree. In the same study, the nuclear internal transcribed spacer (ITS) phylogenetic tree showed a close association between the same four species, but did not further resolve their relationship. The incongruence between nuclear ITS and chloroplast *psbA–trnH* datasets underlined the frequency of reticulation events in the evolution of *Vasconcellea*, as expected from the relative frequency of interspecific hybridizations underlined by Badillo (1971, 1993), and provided further evidence of the hybrid or introgressed nature of different taxa, including *V.* × *heilbornii* varieties. Moreover, significant ITS sequence heterogeneity was found within the genome of the analyzed specimens of *V. stipulata*, *V. cundinamarcensis*, and *V.* × *heilbornii*, a very likely consequence of recent hybridizations.

This complicated situation led Van Droogenbroeck et al. (2006) to resume their AFLP/PCR-RFLP studies on a wider sample set, including 60 individuals from *V. stipulata*, *V. cundinamarcensis*, *V.* × *heilbornii*, *V. weberbaueri*, and *V. parviflora*, plus one hybrid between *V.* × *heilbornii* and *V. cundinamarcensis*, all from Ecuador. Special attention was given to detect species-specific fragments, except for *V.* × *heilbornii*. The AFLP dataset cluster analysis and PCO principal axis (Fig. 11.3) first distinguished *V. cundinamarcensis* and a wide group of *V. stipulata* and *V.* × *heilbornii*. A smaller group of the latter, including the artificial hybrid, took an intermediate position, closer to *V. cundinamarcensis*.

The three babaco accessions formed a small uniform cluster, placed close to the *V. stipulata*/*V.* × *heilbornii* group. The second PCO axis opposed *V. weberbaueri* to *V. cundinamarcensis*/*V.* × *heilbornii*/*V. stipulata*, while *V. parviflora* took an intermediate position. The combined analysis of chlorotypes/mitotypes and species-specific AFLP markers was particularly informative, so it is synthesized at Table 11.5. Most accessions of *V.* × *heilbornii*, including babacos, present the same chlorotype as *V. weberbaueri*, the others presenting the *V. stipulata* chlorotype. The latter group shows a tendency to bear a higher number of *V. stipulata*-specific AFLP markers. Babacos bear few of them, and in variable numbers. In all the *V.* × *heilbornii* groups, even those presenting a high number of *V. cundinamarcensis*-specific markers, some accessions present *V. weberbaueri*-specific markers, which strengthens the hypothesis of an ancient hybridization involving this species or some of its ancestors. This ancient role of *V. weberbaueri* appears quite opposite to that of *V. cundinamarcensis*. Indeed, the latter is still actively involved in the current evolution of *V.* × *heilbornii*, but it acts only as a pollen donor, as no *V.* × *heilbornii* accession bears its chlorotype or mitotype, and its contribution in terms of specific AFLP markers is limited, as surprisingly few accessions bear a significant number of them. The authors of the latter study suggest that there might be a mechanism leading to a disproportionate loss of *V. cundinamarcensis*-specific markers in the backcross progenies of *V.* × *heilbornii*.

Cytological work of De Zerpa (1980) already indicated the existence of such a mechanism in the backcross progenies of the artificial hybrids between *V. cundinamarcensis* and *V. stipulata*, the latter being the recurrent parent. Meiosis is strongly altered in the F_1 hybrid, with irregular pairing, indicating a significant lack of homology between the parental genomes. It is much more regular in the BC_1 generation, with a higher proportion of normal tetrads. In the BC_2 generation, phenotypically similar to *V. stipulata*, meiosis results in the formation of eight bivalents, out of a potential nine ($2n = 18$), and the final production of essentially normal tetrads and pollen grains. De Zerpa (1980) deduced that these eight bivalents result from complete homology, being constituted of

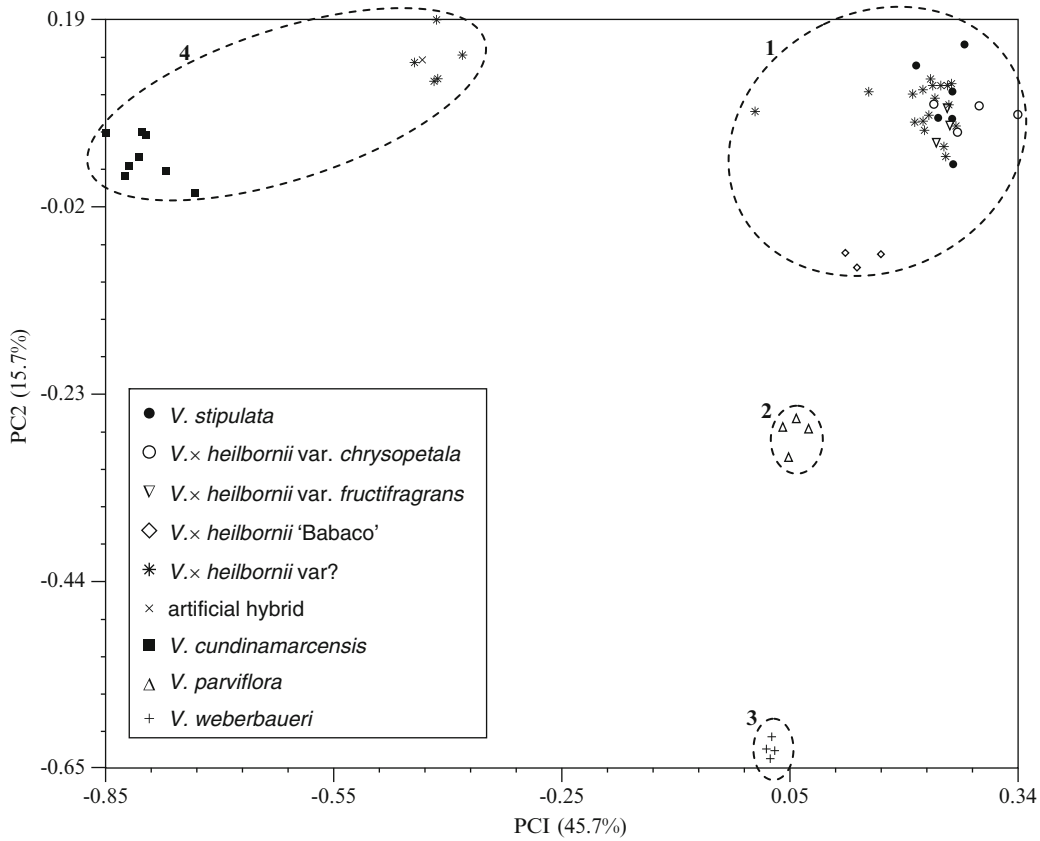


Fig. 11.3 Principal coordinate (PCO) plot showing the first and second principal coordinates estimated with 234 AFLP markers scored for 61 *Vasconcellea* individuals from southern Ecuador. Reproduced from Van Droogenbroeck et al. (2006)

Table 11.5 Summary of the results of the genetic analysis of *V.* × *heilbornii* and related species performed by Van Droogenbroeck et al. (2006)

Taxon (No of accessions)	cpDNA chlorotype	mtDNA mitotype	No of species-specific AFLP markers		
			V. stipulata	*V. cundinamarcensis*	*V. weberbaueri*
V. stipulata (4)	S	H	24	0	0
V. cundinamarcensis (8)	C	C	0	47	0
V. weberbaueri (5)	H	H	0	0	21
V. parviflora	P	P	0	0	0
V. × *heilbornii* (10)	S	H	19–24	0 (7cases), 1, 4, 47	0–2
V. × *heilbornii* (20)	H	H	10–21	0–1, 6 (1 case)	0–2
V. × *heilbornii* babaco (3)	H	H	8 or 12	0	2
V. × *heilbornii* (5)	H	H	10–21	46–47	0–1

The presence of species-specific organellar (chloroplast and mitochondrial) PCR-RFLP markers and nuclear AFLP markers is shown for several taxa

V. stipulata chromosomes, the ninth pair being the sex chromosome pair.

From this wealth of data, a general hypothesis can be proposed for the evolution of *V.* × *heilbornii*. Southern-central Ecuador is home to a small clade of *Vasconcellea*, originally constituted by three species endemic to southern Ecuador and northern Peru, i.e., *V. stipulata*, *V. weberbaueri*, and *V. parviflora*. The first two species spontaneously exchanged elements of their nuclear and organellar genomes, which seems to be a natural process between two sympatric and closely related species. These introgression events

occurred early enough to allow for some diversification of their chloroplast genome, as observed with PCR-RFLP (Restrepo et al. 2004b), the sequencing of *psbA–trnH* region (Kyndt et al. 2005c), and cpDNA SSRs (Kyndt et al. 2006). Then again, this differentiation may have pre-existed, as the same PCR-RFLP studies show such variation at the intraspecific level among different member of the genus. Then, representatives of *V. stipulata* have hybridized with *V. cundinamarcensis,* in a process under human influence. Indeed, in comparison with other possible hybrids of *V. stipulata*, hybrids with *V. cundinamarcensis* present four main advantages to be involved in *V.* × *heilbornii* evolution under domestication. The first one is that this species is common and adapted to a wide range of environments, so it was able to hybridize more frequently than many other *Vasconcellea* species. The second one is that the fruit of the hybrids is significantly larger and more regularly shaped than that of *V. stipulata*. The third one is that it is sterile, which considerably simplifies fruit processing and consumption. The fourth one is that it is parthenocarpic, which suppresses the need to maintain male plants in orchards. In fact, as a few seeds could still be produced through introgression with the two main parental species, sowing them would produce a mix of female and male plants, the former being subjected to drastic clonal selection, while the latter, being unproductive, would be rogued off, just as present farmers still do. Such processes of varietal creation through spontaneous sexual reproduction, followed by clonal selection, have also been documented for other vegetatively propagated crops, such as cassava (Elias et al. 2000, 2001). In the case of *V.* × *heilbornii*, a particular element is the combination of dioecy and parthenocarpy, resulting in male plant roguing. It explains why *V. cundinamarcensis* has only contributed to hybrids as a pollen donor. As babacos/higachos constituted the main crop, there were many more reasons for roguing *V. cundinamarcensis* plants from the orchard, tolerating it only in less intensively exploited areas, than to look for rare babaco seeds in the seedy fruits of *V. cundinamarcensis*. Roguing male hybrid plants had another consequence: it imposed a strong dominance of backcross over recombination between hybrids, as the male pool is essentially constituted by representatives of the parental species. Among them, *V. stipulata* was favored because of the backcross on this species is more than five times more fertile than the backcross on *V. cundinamarcensis* (Horovitz and Jiménez 1967). In the following backcross generations, a strong selection for genotypes presenting homologous pairing at meiosis further reinforced this trend. In this context, specific parts of the *V. cundinamarcensis* genome, lacking a homologous counterpart, were eliminated. Artificial selection also played a role in limiting the contribution of *V. cundinamarcensis* by discarding clones with too strong phenotypic resemblance with that parent, simply because they were too far from a babaco/higacho ideotype. Thus, only floral and vegetative traits of negligible importance to the farmer have been retained in the hybrids.

11.4.2.2 *V. cundinamarcensis*

V. cundinamarcensis, papayuelo (Colombia) chamburo, siglalón, or chihualcan (Ecuador), is widespread, with a highland distribution ranging from Panama to the Andes of Bolivia (Badillo 1993). As a crop, it is particularly developed in Colombia. The evolution of the papayuelo (the plant itself, papayuela designating the fruit) seems much simpler than that of the babacos. There is no apparent domestication syndrome. Cultivated types seem to result from selection for the fruit. This selection has been relatively intense, as can be appreciated from the morphological uniformity of the plants and fruits cultivated in Colombia. Wild populations of the species are not well documented, so original morphology is not well known. There is one wild or feral population in the mountain forest of the Cocora valley in the central coffee-growing zone of Colombia. Fruits are of irregular shape, but their size is similar to that of the cultivated papayuelo.

V. cundinamarcensis plants look very different in Ecuador, where the species is rarely cultivated. The typical dense pubescence of the species, which resulted in its synonym epithet of *pubescens*, gets much sparser and is even quite absent in some specimens collected in southern Ecuador. The fruits, which are green turning to yellow in Colombia and Venezuela, may exhibit very different colors, from pink to dark red, with a glossier appearance. They are smaller and their central constriction and ribs are more marked. These traits are the likely result of the introgression of *V. stipulata* genes in southern Ecuador populations of *V. cundinamarcensis*, with subsequent diffusion

northward, as was also observed via AFLP markers in materials from southern Ecuador (Van Droogenbroeck et al. 2004) and isozyme markers in materials from central and southern Ecuador (Jiménez 2002). Thus, the differentiation of Ecuadorian populations of *V. cundinamarcensis* would be the reciprocal phenomenon of its contribution to *V.* × *heilbornii*. The situation could be symmetric, *V. cundinamarcensis* exchanging genes with other *Vasconcellea* species in Colombia too. Indeed, the isozyme patterns of Colombian accessions of *V. cundinamarcensis* present similarities with those of other Colombian species, such as *V. goudotiana*, *V. sphaerocarpa*, and *V. crassipetala* (Jiménez 2002). Based on phylogenetic analysis of ITS sequences, *V. cundinamarcensis* is placed close to *V. sprucei* and *V. horovitziana*, while chloroplast sequences show a close relationship with *V. monoica* and *V. cauliflora* (Kyndt et al. 2005c). Hybrids with *V. monoica* have also been described (Badillo 1967) and were later confirmed on the basis of molecular data (Kyndt et al. 2005c, 2006). *V. cundinamarcensis* has been successfully used in interspecific crosses, in both directions, giving normal seeds, not only with *V. stipulata*, but also with *V. monoica*, *V. microcarpa*, and *V. horovitziana* (Horovitz and Jiménez 1967).

The future of the crop will depend on its adaptation to the needs of modern urban markets, particularly in Colombian highlands. Traditional home processing into candies and preserves is likely to decline, which leaves few possibilities to develop the crop. In contrast, Chile has developed a small canning industry, stimulating the consumption of the fruit, and even allowing some export.

11.4.2.3 *V. goudotiana*

V. goudotiana is \another species from Colombia, yielding midsize fruits, locally called papayuelas. The species has recently been introduced into Ecuador, where it has escaped from gardens (Romeijn-Peeters 2004). It is mostly cultivated in gardens, with only confidential commercialization in the modern sector. The fruit is more appealing than that of *V. cundinamarcensis*, as it is very regularly fusiform, slightly five-angled, smoother and glossier in appearance, and yellow, orange, or deep red at maturity. However, its pulp is considered less aromatic. *V. goudotiana* is cultivated in the highlands of Colombia, at the same altitudes as *V. cundinamarcensis*, although wild populations occur at lower elevations. In the Valle del Cauca department, they are found at altitudes of 1,000–1,500 m on the foothills of the Cordillera Central, down to 500 m on the Pacific side (Cordillera Oriental). Wild or feral plants and fruits appear quite similar to those under cultivation, indicating a simple and direct evolution through selection and, possibly, adaptation to higher altitudes.

V. sphaerocarpa appears morphologically and genetically very closely related to *V. goudotiana*, as indicated by PCR-RFLP studies of cp DNA (Aradhya et al. 1999; Restrepo et al. 2004b) and chloroplast and nuclear sequences (Kyndt et al. 2005c). The relationship with *V. palandensis* seems slightly more distant, according to the SSR study of Kyndt et al. (2006) and the cp DNA studies of Restrepo et al. (2004b) and Kyndt et al. (2005a).

According to Horovitz and Jiménez (1967), *V. goudotiana*, when used as a pollen donor in crosses with other *Vasconcellea* species, results in the production of seeds without endosperm. However, Torres and Ríos (1967) obtained viable seeds from a cross between *V. monoica* and *V. goudotiana*.

11.5 Role of *Vasconcellea* Species in Papaya Improvement Through Traditional and Advanced Tools

Highland papayas are fascinating raw materials from which new fruit crops can be created. Given their great variability and interfertility, they offer excellent opportunities for creating new taste combinations. Unfortunately, breeding programs have been limited in number, duration, and ambition, as is often the case for minor crops. The most advanced hybridization program was carried out in Venezuela by Horovitz and Jiménez (1967), who proposed to explore their potential for both fruit and papain production. Unfortunately, the latter program was discontinued after the retirement of Professor Horovitz (De Zerpa 1980). Smaller programs focused on babaco, with the aim of obtaining smaller and seedless fruits (Soria and Soria 1992). This situation contrasts with the much more important efforts to overcome barriers to hybridization at the intergeneric level to transfer tolerance/resistance traits from *Vasconcellea* to *Carica* (Manshardt and Wenslaff 1989a; Magdalita et al. 1997; Drew et al. 1998).

Indeed, interesting traits present in *Vasconcellea* germplasm, such as cold tolerance and disease resistance, could be introgressed into the papaya genome in order to extend the range of papaya cultivation and improve its yield. The pleasant fragrance of *V. stipulata* and babacos, the monoecious habit of *V. monoica*, the cold tolerance of *V. cundinamarcensis* and *V. stipulata*, and the ornamental qualities of pink-flowered *V. parviflora* are examples of traits of interest (Manshardt and Wenslaff 1989b). The wide application range of papain in beverage, food, and pharmaceutical industries makes the cysteine proteinase genes a worthwhile focus of study for future introgression. The latex of *V. cundinamarcensis* (Baeza et al. 1990; Jaziri et al. 1994; Walraevens et al. 1999; Pereira et al. 2001), *V.* × *heilbornii*, and *V. stipulata* (Scheldeman et al. 2002; Kyndt et al. 2007) contains a wide range of highly active cysteine proteinases with a proteolytic activity reported to be 1.25 to 17 times higher than that found in *C. papaya* and is a promising target for transfer into the papaya genome or for focused *Vasconcellea* breeding programs (for more details, see Sect. 11.3.2).

To date, the emphasis on intergeneric breeding programs between *Carica* and *Vasconcellea* has been disease resistance. The common papaya is particularly vulnerable to numerous viral and fungal diseases (Singh 1990; Ploetz et al. 1994), due to extensive monoculture, a narrow gene pool (Drew et al. 2005), and inbreeding among hermaphrodite varieties (Kim et al. 2002). Papaya ringspot virus type P (PRSV-P) is considered the worst pathogen of papaya, and all known varieties of papaya are susceptible (Manshardt and Drew 1998). Losses as high as 70% of expected yield have been reported in affected areas (Manshardt and Drew 1998). To withstand this threat, papaya cultivation has long been limited to the use of PRSV-tolerant lines: e.g., "Cariflora" in Florida (Conover et al. 1986), although the development of resistant papaya varieties is widely considered to be the best strategy for long-term control of PRSV-P (Gonsalves 1998). The susceptibility of papaya to this disease coupled with the difficulty of producing viable intergeneric crosses between *Carica* and *Vasconcellea* (of which some species exhibit resistance, see further) has led to the adoption of molecular biology tools as a means of improvement. The development of a papaya ringspot virus-resistant variety SunUp by genetic transformation (Fitch et al. 1992) was responsible for containing the spread of PRSV, which nearly destroyed Hawaiian papaya industry and negatively impacted papaya production worldwide (Gonsalves 1998). Nowadays, 80% of the Hawaiian papaya crop is transgenic (Stokstad 2008). Two Australian transgenic cultivars were also developed (Lines et al. 2002), but they were never cultivated commercially. SunUp, which was generated through transformation of Sunset that had undergone more than 25 generations of inbreeding, was the first commercial virus-resistant transgenic fruit tree to have its genome sequenced (Ming et al. 2008).

Although the transgenic approach has been very successful, it does not meet all the requirements of the different papaya-growing regions. To elicit transgenically induced silencing of viral genes, homology between the virus and the transgene must be high (>98%) (Gonsalves 1998). Since genetic divergence of the different PRSV-P strains correlates with their geographic distribution (Wang and Yeh 1997), the development of unique transgenes for different papaya-growing regions is required. In Australia, PRSV-P isolates have been shown to vary as much as 12% in the coat protein region (Bateson et al. 1994). Another problem facing transgenic papayas is that there is still considerable resistance to their acceptance for commercial production in most countries where papaya is grown and marketed.

Several *Vasconcellea* species, such as *V. cauliflora*, *V. cundinamarcensis*, *V. quercifolia*, and *V. stipulata*, exhibit complete resistance to PRSV-P and present a valuable source of resistance genes with potential for application in *C. papaya* (Magdalita et al. 1988; Manshardt and Wenslaff 1989b; Drew et al. 1998). Earlier studies of inheritance of an Australian strain of PRSV-P resistance in F_1 intergeneric hybrids of *C. papaya* and *V. cauliflora* suggested single gene regulation in this species (Magdalita et al. 1997). Also for *V. cundinamarcensis*, a single resistance gene (*prsv-1*) was identified (Dillon et al. 2005, 2006). Markers linked to these resistance genes are ideally suited for application in marker-assisted breeding programs because of their dominant inheritance, and because resistance to the Australian strain of PRSV-P imparted by *prsv-1* has been shown to be robust (Magdalita et al. 1997; Drew et al. 1998). Dillon et al. (2006) used bulked segregant analysis on the F_2 progeny from a *V. parviflora* (susceptible) × *V. cundinamarcensis* (resistant) interspecific cross (Dillon et al. 2005) to identify dominant

randomly amplified DNA fingerprint (RAF) markers linked to *prsv-1*. Sequence characterization of Opk4_1r, mapped adjacent to the *prsv-1* locus at 5.4 cM on chromosome 7 of *V. cundinamarcensis*, permitted its conversion into a codominant cleaved amplified polymorphic sequence (CAPS) marker (PsiIk4), diagnostic for the resistant genotype based on digestion with the restriction endonuclease *Psi*I. The codominant CAPS marker Psilk4 was shown to correctly identify resistant genotypes 99% of the time (Dillon et al. 2006). As a result, this marker is a potentially powerful tool for marker-assisted selection of homozygous resistant genotypes, which may be used in breeding programs to facilitate delivery of PRSV-P resistance from *V. cundinamarcensis* into *C. papaya*.

As discussed above, intergeneric hybridization of *C. papaya* and *Vasconcellea* species is an important mechanism for accessing wild germplasm, but the success of introducing alien variation into crop species from related species depends on the cytogenetic relations between the species. If there are no restrictions on chromosome pairing and recombination in species hybrids, a backcrossing program is generally very useful and straightforward to obtain the desired gene transfers. Unfortunately, incorporating desirable characters from the different genera of the Caricaceae family into the cultivated *C. papaya* by conventional breeding have met with little success.

In general, successful intergeneric hybridizations have been reported for *C. papaya* and at least six *Vasconcellea* species [*V. parviflora*] (Manshardt and Wenslaff 1989a; Drew et al. 1998), *V. cundinamarcensis* (Manshardt and Wenslaff 1989a; Drew et al. 1998), *V. quercifolia* (Manshardt and Wenslaff 1989a; Drew et al. 1998), *V. goudotiana* (Manshardt and Wenslaff 1989a, b), *V. stipulata* (Manshardt and Wenslaff 1989a), and *V. cauliflora* (Manshardt and Wenslaff 1989a; Magdalita et al. 1997; Drew et al. 1998). Importantly, the PRSV-P-resistant character was shown to be inherited by intergeneric progeny of three resistant species: *V. cauliflora* (Manshardt and Wenslaff 1987; Magdalita et al. 1997), *V. cundinamarcensis* (Manshardt and Wenslaff 1987; Drew et al. 1998), and *V. quercifolia* (Drew et al. 1998, 2006a). However, instabilities such as infertility, abortion of immature embryos, and poor hybrid vigor are observed to varying degrees in the progeny (Sawant 1958; Horovitz and Jiménez 1967; Manshardt and Wenslaff 1989a, b; Drew et al. 1998) and have impeded successful transfer of resistance genes until now.

These problems have been attributed to genetic incompatibility between the distantly related genomes of *Vasconcellea* and *Carica* (see Sect. 11.4 about evolution), whose species are described as sexually incompatible (Sawant 1958; Manshardt and Wenslaff 1989a, b; Magdalita et al. 1997; Drew et al. 1998). Manshardt and Wenslaff (1989a) observed that the major barriers to interspecific gene flow were post-zygotic and included ovule and embryo abortion, as well as lack of endosperm development. Magdalita et al. (1997) showed that genome incompatibility barriers prevented intergeneric hybridization, leading to abortion of hybrid seeds soon after their formation. Recently, 5% sucrose was shown to enhance pollen germination and tube growth in *C. papaya* × *V. cauliflora* crosses (Dinesh et al. 2007), but still a very low number of viable seeds were recovered (an average of 13.73 viable seeds per fruit in comparison with 300 in papaya). However, the major limitation of *C. papaya* × *V. cauliflora* hybridization is not production of plantlets but infertility of the resultant F_1 plants, which has halted further crossing or backcrossing.

These omnipotent genetic barriers need to be addressed before *Vasconcellea* spp. can be utilized in breeding with *C. papaya*. Although the technique of embryo rescue has been used to overcome some incompatibility barriers and to aid in the formation of some hybrid plants (Manshardt and Wenslaff 1989a; Chen et al. 1991; Chen and Chen 1992; Magdalita et al. 1996; Drew et al. 1998), most hybrid progeny still remains highly sterile or shows poor vigor in the field (Manshardt and Wenslaff 1989a, b; Drew et al. 1998). Recently, some fertility has been obtained between crosses of *C. papaya* and *V. quercifolia*, whereas backcross generations have been grown to maturity in the field in Philippines and Australia (Drew et al. 2006a, b). These results demonstrate that attempts to introgress PRSV-P resistance to *C. papaya* by wide hybridization are better directed toward crosses between *C. papaya* and *V. quercifolia* than with other *Vasconcellea* species. Heightened intergeneric compatibility between papaya and some *Vasconcellea* species, such as *V. parviflora* or *V. quercifolia* (Drew et al. 1998), may be exploited to bridge hybridization between papaya and other *Vasconcellea* spp., since many *Vasconcellea* species are intercompatible and spontaneously cross-pollinate to produce hybrids

with varying degrees of fertility (Jiménez and Horovitz 1957; Horovitz and Jiménez 1967; Mekako and Nakasone 1975; Badillo 1971).

Homozygous resistant hybrids of *V. cundinamarcensis* and *V. parviflora* may provide a possible bridge to mediate stable transfer of resistance genes from *V. cundinamarcensis* into *C. papaya* using the Psilk4 marker of Dillon et al. (2006) to assist the selection process at each cycle.

Although the number of chromosomes does not seem to play a role in their genomic incompatibility, a lack of homology between chromosomes of *Carica* and *Vasconcellea* probably results in the failure of adequate chromosome pairing, leading to chromosome or even whole-genome elimination in intergeneric hybrids (Manshardt 1992; Magdalita et al. 1997; Siar et al. 1998). Current advanced microscopical techniques such as fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH) will certainly be helpful in the further elucidation of the genomic constitution and chromosomal interaction of interspecific and intergeneric hybrids.

In the future, techniques of chromosome manipulation, involving the introduction of a chromosome of the donor species (e.g., *Vasconcellea*) into papaya, might be attempted. Transfer of complete *Vasconcellea* chromosomes into the papaya genome has not been attempted to date, but current advances in papaya in vitro culturing might open up possibilities for the future. Chromosome engineering is an established crop improvement tool and continues to make a significant impact in production agriculture (Gill and Friebe 1998). Backcrossing and selecting for the character being transferred in this situation result in the introduction of whole chromosomes as addition or substitution lines. In general, this can only be done in polyploid species because the genetic duplication inherent in the structure of such species allows the genotype to tolerate some loss and addition of chromosomal material.

In 1942, Hofmeyr and Van Elden described the generation of tetraploid papaya after colchicine treatment, whereas, more recently, haploid papaya plants were generated using anther culture (Litz and Conover 1978; Tsay and Su 1985; Rimberia et al. 2005). Rimberia et al. (2007) generated anther-derived triploid papayas with short stature and high yield of parthenocarpic fruits, showing high potential for breeding and commercial fruit production. However, the lack of seed production in triploids precludes conventional breeding and female dihaploid generation should be attempted in future experiments. Clarindo et al. (2008) recently reported the detection of aneuploid and polyploid plantlets in somatic embryogenesis cultures of papaya. In vitro regeneration of polyploid papaya was successful; however, aneuploid plantlets displayed a weak and inadequate development and could not be regenerated for multiplication (Clarindo et al. 2008).

11.6 Role in Classical and Molecular Genetic Studies

Primarily for economic reasons, genetic and genomic data were initially developed for *C. papaya*, while relatively few have been developed for *Vasconcellea* (Ming et al. 2005). Several genetic linkage maps have been constructed for *C. papaya*. Fundamental biological issues raised by the initial genomic research on *C. papaya* led to significant increases in developing genomic resources, ultimately resulting in whole-genome sequencing.

The first papaya genetic map, reported nearly 70 years ago, consisted of only three morphological markers: sex form, flower color, and stem color (Hofmeyr 1939). The second map developed was based on 62 random amplified polymorphic DNA (RAPD) markers and contained the sex determination locus *Sex1* on linkage group 1 (Sondur et al. 1996). Two flanking markers were mapped approximately 7 cM on each side of *Sex1*. Deputy et al. (2002) mapped RAPD markers tightly linked to *Sex1* and cloned three RAPD products to develop sequence-characterized amplified region (SCAR) markers that showed linkage within 0.3 cM of *Sex1*. The third map was constructed using 1,501 markers, including 1,498 (AFLP) markers, the papaya ringspot virus coat protein marker, morphological sex type, and fruit flesh color (Ma et al. 2004). These markers were mapped in 12 linkage groups and covered a total length of 3,294 cM, with an average distance of 2.2 cM between adjacent markers. Recently, a sequence-tagged high-density genetic map, consisting of the morphological marker for fruit flesh color, and 706 microsatellite markers, derived from bacterial artificial chromosome (BAC)-end sequences and whole-genome shotgun sequences, was constructed (Chen et al. 2007). The mapped microsatellite markers provided data for integration

of genetic and physical maps (Ming et al. 2008). A draft genome sequence of papaya has been produced and large quantities of sequence data from BAC ends and cDNA have been integrated into the genome (Lai et al. 2006; Ming et al. 2008) (for more details see further), from which Eustice et al. (2008) and Wang et al. (2008) deduced SSR markers for papaya. Although the cross-genus transferability of papaya SSR markers isolated by Ocampo et al. (2006) toward *Vasconcellea* spp. was found to be limited, the markers developed for *V.* × *heilbornii* (Kyndt et al. 2005b) were shown to be usable within genus *Vasconcellea* and in some cases even across the Caricaceae family (Kyndt et al. 2006). Their application is particularly promising for future *Vasconcellea* research as they save considerable costs and time compared with de novo development, thus allowing more efficient investigations in the future. For example, these SSR markers can be used in fine-mapping studies to locate genes of interest encoding important agricultural traits such as flower number, fruit size, fragrance, and pest resistance, which might be of importance for future *Vasconcellea* or *C. papaya* breeding. Analysis of the levels of polymorphism present between different parental mapping populations will further serve to elucidate the genetic variability between closely related varieties and provide an indication of genetic distances and heritance of varieties.

11.7 Genomic Resources Developed

11.7.1 Vasconcellea Genomic Resources

So far, sequence data generated from *Vasconcellea* spp. involve only small parts of the nuclear and/or chloroplast genome. Nucleotide sequences of the nuclear ITS of the ribosomal DNA were generated for 21 *Vasconcellea* spp. and some members of the related genera *Carica*, *Jarilla*, *Jacaratia*, and *Cylicomorpha* to study inter- and intrageneric phylogenetic relationships within Caricaceae (Olson 2002; Kyndt et al. 2005c). These studies also generated chloroplast sequence information from three fragments across the Caricaceae family: one coding sequence, *matK*, and two intergenic spacers: *trnL–trnF* and psb*A–trnH* (Kyndt et al. 2005c). The National Center for Biotechnology Information (NCBI) sequence database also contains (1) an expansin cDNA from *V. cundinamarcensis* (Gaete-Eastman et al. unpublished data); (2) two chloroplast fragments from *V. parviflora* containing part of the *matK* gene and part of the tRNA–Lys intron, *trnL–trnF*, tRNA–Leu, and tRNA–Phe; (3) cysteine proteinase sequences from *V.* × *heilbornii*, *V. stipulata* (Kyndt et al. 2007), and *V. cundinamarcensis* (Pereira et al. 2001; Junqueira et al. unpublished data); (4) nine microsatellite-containing sequences from *V.* × *heilbornii* (Kyndt et al. 2005b); and (5) the 5S ribosomal RNA gene from *V. quercifolia* (Singh and Yadav unpublished data). BAC libraries for two *Vasconcellea* species, *V. cundinamarcensis* and *V. monoica*, and for one *Jacaratia* species, *Jacaratia spinosa*, were constructed. Each of these three libraries was estimated to provide 10× genome equivalents with an average insert size of 100 kb (Q Yu, R Ming and P Moore unpublished data). Recent advances in DNA sequencing will make it possible to rapidly acquire more information about the *Vasconcellea* genome and transcriptome by sequencing expressed sequence tags (ESTs) and BAC libraries.

11.7.2 C. papaya *Genomic Resources*

11.7.2.1 BAC Cloning and Utilization

A BAC library was constructed from hermaphrodite plants of the transgenic papaya cultivar SunUp and consists of 39,168 clones with an average insert size of 132 kb (Ming et al. 2001). This library was estimated to provide 13.7× papaya genome equivalents, excluding the false-positive (empty clones) and chloroplast clones.

The entire set of 39,168 BAC clones fingerprinted to yield a total of 30,824 high-quality fingerprints, estimated as 11× genome equivalents, was used to construct a *C. papaya* physical map. After automated overlap evaluation and manual review, 26,466 papaya BAC clones were assigned to 963 contigs. A total of 4,358 singleton clones could not be assigned to the fingerprint contigs. The three largest contigs included over 200 BACs, whereas 204 contigs contained only two BACs. The remainder of the 756 contigs contained 3–199 BACs.

A high-density genetic map was recently reported (Chen et al. 2007) using SSR markers derived from BAC-end and whole-genome shotgun (WGS) sequences (Eustice et al. 2008; Wang et al. 2008). A total of 707 markers, including 706 SSR markers and one morphological marker, were mapped on nine major linkage groups corresponding to the nine chromosomes and to three minor linkage groups, which have subsequently been merged with the nine major groups based on fluorescent in situ hybridization (FISH; Wai, Q Yu, and Jiang unpublished data). A total of 1,259 overlapping oligonucleotide (overgo) probes, representing anchors to *Arabidopsis* and genetically mapped *Brassica* loci, have been incorporated into the current papaya physical map (Q Yu, A Paterson, P Moore, R Ming unpublished data). This map provides a framework for comparative structural and evolutionary genomic research in the order Brassicales.

11.7.2.2 Expressed Sequence Tag Resources

Five *C. papaya* flower cDNA libraries have been constructed: three from pre-meiosis (<4 mm) flower buds (male, hermaphrodite, and female) and two from mature flower buds (hermaphrodite and female). ESTs from these five libraries were sequenced from the 5′ end to produce 31,652 clean sequences with a minimum length of 200 nucleotides. The average read length of a clean sequence was 486 nucleotides with a minimum quality score of 20. Clusters containing only one sequence were grouped as singletons. The EST clusters were assembled into contigs (contiguous sequence) by multiple-sequence alignment that generates a consensus sequence for each of the clusters, with criteria of 95% identity over 30 nt overlap. A unigene set of 8,571 EST contigs and singletons was assembled. Blast analysis indicated that about 82% of the unigenes from these papaya libraries have homologous sequences in the protein database of *Arabidopsis* (Q Yu, P Moore, R Ming, unpubl data). In addition, a normalized and subtractive cDNA library was constructed using pooled RNA samples isolated from roots, leaves, seeds, calli, three sex types of flowers, and three ripening stages of fruit. Over 50,000 EST sequences were generated from this library, yielding an additional total of 16,432 unigenes for genome annotation (Ming et al. 2008).

11.7.2.3 Sex Chromosomes

A high-density linkage map of the papaya genome (Ma et al. 2004) was used to characterize the papaya sex determination locus. The sex determination locus was mapped to the middle of a large linkage group (LG1) having a large cluster of 225 sex cosegregating markers. This non-recombinant block accounted for 66% of the 342 markers on LG1 and 15% of all markers mapped on the genome. This map demonstrated severe suppression of recombination at or around the sex determination locus, indicating that this linkage group is the sex chromosome in papaya.

The sex determination locus was fine-mapped using 4,380 informative chromosomes (two each from 2,190 female and hermaphrodite plants of three F_2 and one F_3 populations) and six DNA markers. Despite the large populations screened, not a single recombination event was detected (Liu et al. 2004). Detailed sequence analysis of the male-specific region of the Y chromosome (MSY) BACs revealed much lower gene density and its perocentromeric location (Yu et al. 2007). A much higher rate (85.6%) of repetitive sequence was estimated using a papaya-specific repeat database (Ming et al. 2008). The physical map of the MSY now reached 8 Mb (Yu et al. 2008a), and the MSY is likely to have spread to both sides of the centromere with four Y-specific heterochromatic knob structures (Zhang et al. 2008).

Two slightly different Y chromosomes exist in papaya; one controlling males, designated as Y, and the other controlling hermaphrodites, designated as Y^h (Ming et al. 2007b). Direct comparison of homologous X- and Y^h-BAC sequences provided data to assess the antiquity of the sex chromosomes. Two pairs of X- and Y^h-BACs were sequenced and direct alignment of their sequences revealed three inversion events on the MSY and numerous indels and duplications (Yu et al. 2008a). Gene expression analyses indicated seven genes on the two X-BACs and four genes on the two Y-BACs. Comparison of the aligned sequences of the two X- and Y^h-BAC pairs showed 9.6–35.2% DNA sequence expansion on the MSY BACs. Gene expression analyses indicated seven genes on the two X-BACs and four genes on the two Y-BACs. The time of divergence between the four X–Y^h gene pairs was estimated to be between 0.5 and 2.2 million years ago (Mya), supporting the concept of recent origin of the sex chromosomes in papaya (Yu et al. 2008a).

A pair of dioecious X- and Y-specific BACs was sequenced and their sequences were compared to corresponding gynodioecious X- and Y^h-specific BACs (Yu et al. 2008b). DNA sequence expansion was also documented on the Y-BAC. Dioecious X and gynodioecious X^h-specific BACs were virtually identical sharing 99.97% sequence identity with only seven single nucleotide polymorphism and five single nucleotide indels. The Y- and Y^h-specific BACs shared high degree (98.6%) of DNA sequence identity, while the X- and Y-BACs shared about 84.4% sequence identity. Analysis of sequence divergence between three dioecious X and Y gene pairs resulted in the estimated ages of divergence from 0.6 to 2.5 million years, reinforcing the recent origin of the papaya sex chromosomes. The estimated age of divergence between Y and Y^h chromosomes was approximately 73,000 years, prior to the origin of agriculture about 1,000 years ago (Gupta 2004). The hermaphrodite Y^h chromosome is likely evolved from an ancestral Y chromosome naturally, and not resulted from human selection as once suggested.

11.7.2.4 Genome Sequencing

The genome of SunUp female was sequenced at 3× coverage using whole-genome shotgun approach with Sanger sequencers (Ming et al. 2008). It was assembled into contigs containing 278 Mb and scaffolds spanning 372 Mb including embedded gaps. The estimated residual heterozygosity of SunUp is 0.06%, confirming the highly inbred nature of this Solo variety. Of 16,362 unigenes derived from ESTs, 15,219 (92.5%) matched this assembly. Among 706 BAC-end and WGS sequence-derived SSR markers on the genetic map, 652 (92.4%) could be used to anchor 167 Mb of contigs or 235 Mb of scaffolds to papaya linkage groups in the current genetic map. Papaya has an AT-rich genome, with an overall G + C content of 35.3%, nearly identical to that of *Arabidopsis* at 35%. The papaya genome consists of about 52% repetitive sequences, mostly retrotransposons (40% of the genome). A total of 23,151 genes were predicted, less than any of the other sequenced five sequenced angiosperm genomes (*Arabidopsis*, rice, poplar, grape, and sorghum) (Wei and Wing 2008; Paterson et al. 2009). One possible explanation for the lower than expected number of papaya genes is that the papaya genome did not undergo the two rounds of recent whole-genome duplications observed in *Arabidopsis*. Another surprise is the extremely small number of disease-resistance genes of the nucleotide-binding site leucine-rich repeat (NBS-LRR) class. *Arabidopsis* has more than 200 NBS-LRR genes and rice more than 600. In contrast, there are only 55 NBS-LRR genes in papaya. This paucity of NBS-LRR genes might indicate that papaya has developed alternative strategies of host defense. It would be interesting to know how well the papaya situation exists among the *Vasconcellea* species.

11.8 Recommendations for Future Actions

There is little doubt that *Vasconcellea* species have a lot of untapped potential, both for direct use of its fruits and as a source of interesting genes in papaya breeding.

Most *Vasconcellea* species have not yet been studied in detail, e.g., one species was only described in the year 2000, despite the fact that there are concerns about the conservation of several species (see Tables 11.2 and 11.3). Therefore, enhancing the conservation of highland papayas should be a priority, especially in countries that hold a lot of diversity (i.e., Ecuador, Colombia, and Peru). Existing ex situ collections should aim at widening their coverage, focusing on adding the currently missing species (i.e., *V. crassipetala*, *V. horovitziana*, *V. longiflora*, *V. omnilingua, V. pulchra,* and *V. sprucei*), while in situ conservation strategies (both in wild as on farm) should include a focus on including *Vasconcellea* species in their management plans, especially for threatened species (*V. chilensis*, *V. horovitzia*, *V. omnilingua*, *V. palandensis*, *V. pulchra*, *V. sprucei*, and *V. weberbaueri*). Local public awareness campaigns, by local governments as well as NGOs, should inform the local population on the use of the *Vasconcellea* diversity and the need to conserve it.

Despite the failure to introduce babaco in the global (sub)tropical fruit market, the commercialization potential for *Vasconcellea* has not been fully exploited yet. Several of the constraints that babaco commercialization was facing (e.g., large size and limited aroma) can be addressed by tapping on the existing fruit diversity present in the center of origin.

Scheldeman et al. (2003) already highlighted the potential, especially that present in the aromatic *V. stipulata* and in the natural hybrids of the *V. stipulata*, *V. cundinamarcensis*, and *V.* × *heilbornii* complex found in southern Ecuador, stressing that this area still needs further research. National agricultural research centers and universities located in the center of origin should increase their efforts to carry out more studies on the diversity and the market potential of local materials, if possible with financial support of international donors. The commercial exploitation of *Vasconcellea* diversity is suffering not only from a lack of scientific studies on the diversity of fruit traits, but also from the often limited knowledge of the local population on its properties and uses. Increasing public awareness of cultural and gastronomical heritage will be needed to make enhanced commercialization a reality. The growing urban markets, where *Vasconcellea* has largely been lost, should especially be targeted. While in the first phase special attention should be given to study and selection of local materials, in the second phase breeding of *Vasconcellea* varieties should be envisaged, mainly focusing on aroma and fruit size, though other properties (e.g., orange color of *V. weberbauri* or the purple color of some *V. goudotiana* accessions) could be included as well. In the long term, analysis of *Vasconcellea* fruits for the presence of vitamins, minerals, and other valuable bioactive compounds (e.g., flavonoids) could enhance their status as "health food" and would promote their introduction on international markets.

The potential of *Vasconcellea* species as a source of papain is even less studied than their fruit potential. While recent studies on proteolytic enzymes activity have included material of *Vasconcellea* species, mainly *V. cundinamarcensis* and *V.* × *heilbornii*, most species have never been analyzed so far. Again local research institutes should start an extensive study on papain potential to confirm and eventually promote the use of *Vasconcellea* as a source of commercial papain. Some studies have already highlighted this potential, but so far no research on the economic aspect and financial viability of this potential has been carried out. Once the commercial viability of *Vasconcellea* cultivation as a source of primary material for the papain industry is confirmed, additional breeding targeting papain content and yield could be pursued.

Finally, the potential of *Vasconcellea* species in papaya breeding has to be further analyzed. Characters of interest include resistance or tolerance to a wide range of biotic and abiotic stresses (including cold and drought) as well as specific organoleptic traits. While this screening has already been initiated for some species, e.g., resistance to PRSV-P in *V. cauliflora*, the search for potentially interesting traits in other species still has to initiate.

Acknowledgments We dedicate this manuscript to the late Prof. Victor Manuel Badillo, 1920–2008, who was a pioneer in the taxonomy of Caricaceae.

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