Mohammad Miransari *Editor*

Use of Microbes for the Alleviation of Soil Stresses, Volume 1

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Preface

The issue of "stress" is important influencing plant growth and crop production in different parts of the world. Stress is a situation with unfavorable conditions for plant growth. There have been different methods of alleviating stress such as use of plants, which are naturally tolerant or plants which have been tolerant using the related stress genes. However, the use of microbes including arbuscular mycorrhizal (AM) fungi and plant growth promoting rhizobacteria (PGPR) has also been proved to be effective under stress. Such microbes can symbiotically or non-symbiotically enhance plant growth under different conditions including stress. Hence, different microbial species and strains are being tested, produced, and used as microbial inoculums in different parts of the globe. The great contribution of soil microbes to the growth of plant and production of crop plants can be of significance environmentally and economically. It is, hence, recommendable to persuade the farmers use microbial inoculums (biological fertilization) for plant growth and crop production. New research work contributes to the utilization of newer and more efficient microbial strains and species indicating the importance of literature, which has to be updated on a regular basis.

Mohammad Miransari

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Chapter 1
Plant-Growth-Promoting Rhizobacteria: Potential Candidates for Gibberellins Production and Crop Growth Promotion

Sang-Mo Kang, Muhammad Waqas, Abdul Latif Khan and In-Jung Lee

Introduction

Rhizosphere, the layer of soil influenced by plant root (Saharan and Nehra 2011; Antoun and Prévost 2005), is known to play pivotal role in plant growth and development (Hrynkiewicz and Baum 2012). Highest proportion of microbial groups such as bacteria, fungi, nematodes, protozoa, and microarthropods inhabit rhizosphere (Lynch and Whipps 1990; Raaijmakers 2001; Morgan et al. 2005). Members of these microbial groups have beneficial, neutral, or harmful effects on the plant growth (Nihorimbere et al. 2011; Bais et al. 2006). The rhizosphere is diversely populated by bacteria known as rhizobacteria. Rhizospheric bacteria feed on the available soil nutrients and root exudates of plants (Bais et al. 2006; Rovira 1991; Dodd et al. 2010). Currently, the term plant-growth-promoting bacteria (PGPB) is used to encompass all those bacteria that enhance plant growth (Tarkka et al. 2008; Brencic and Winans 2005). However, among PGPB, plant-growth-promoting rhizobacteria (PGPR) are studied more because of their ability to colonize the plant roots (Kamilova et al. 2005; Sturz and Nowak 2000). Due to potential application of the beneficial effects of PGPR, scientists from multiple discipline have been involved to elucidate the underlying mechanisms of plant growth. PGPR influence the plant growth through direct or indirect mechanisms. In direct mechanism, PGPR facilitate the growth promotion by nutrient acquisition and alter the physiological signaling by synthesizing bioactive constituents (Welbaum 2004; Brimecombe et al. 2007), while in indirect mechanism, PGPR enhance plant growth via a set of biocontrol mechanisms. Some PGPR decrease or combat the adverse effects of pathogenic microorganisms, by colonizing plants in

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high population during pathogen attack (Nihorimbere et al. 2011). These PGPR are capable of producing antagonistic metabolites such as antibiotics (Compant et al. 2005; Haas and Défago 2005), siderophores (RodrÍgueza and Fragaa 1999), HCN (Ahmad et al. 2008), phenazines (Pierson and Pierson 2010), pyoluteorin (Nowak-Thompson et al. 1999), pyrrolnitrin (Hwang et al. 2002). Furthermore, the PGPR must be able to deliver the chemical constituents in right amount, time, and place to effectively combat the adverse effects of pathogenic attack (Lugtenberg and Kamilova 2009).

In case of direct mechanism, PGPR can stimulate plant growth in the absence of pathogenic attack by secreting plant growth substances. Nitrogen-fixing bacteria such as *Bradyrhizobium* and *Rhizobium* fixes atmospheric N_2 by reducing it into ammonia that can be used by legume plants as a nitrogen source (Franche 2009). Some PGPR help in plant growth by their enhanced potential to solubilize soil phosphate (Bertrand et al. 2000). PGPR have also recently known to produce phytohormones such as auxin, cytokinin, and gibberellins which are synthesized through plant-secreted precursors (Baca and Elmerich 2003). These bacteria-derived phytohormones subsequently facilitate plant growth by promoting cell division under varying environmental conditions. In abiotic stresses, like salinity, drought, and heavy metal, the ethylene production is stimulated in plants, which subsequently inhibits plant growth. Some PGPRs have shown the ability to stimulate the activity of enzymes called 1-aminocyclopropane-1-carboxylate deaminase (ACC) that can hydrolyze ACC into 2-oxobutanoate and ammonia via modulation of plant hormonal level (Glick 2005; Mayak et al. 2004). Glick et al. (1998) previously reported that the continuous exudation of ACC from plant roots under abiotic stress is converted by PGPRs containing ACC deaminase and might be used for their own growth (Siddikee et al. 2010; Nadeem et al. 2010).

Looking at the great potential of PGPR, in this chapter, we focused on gibberellins producing PGPR and its role in abiotic stress particularly drought and salinity stress. Gibberellins (GAs) are ubiquitous plant hormones that elicit various metabolic function required during plant growth like seed germination, stem elongation, sex expression, flowering, formation of fruits, and senescence (Hedden 1997; Hedden and Kamiya 1997). Exogenous applications of GAs (GA_3 and GA_4) have been reported to improve plant growth and biomass while counteracting abiotic stresses in plants (Hedden and Kamiya 1997). The production of such plant growth regulators like auxin, cytokinin, and gibberellins by PGPR can give an additional support to the growth of host plants (Joo et al. 2004, 2005, 2009; Kang et al. 2009, 2010). There are few previous studies (Table 1.1) which elucidated the GA production by PGPR (Joo et al. 2004, 2005, 2009; Kang et al. 2009, 2010; Atzhorn et al. 1988; Bastian et al. 1998; Bottini et al. 1989; Gutierrez-Manero et al. 2001; Janzen et al. 1992; Mansour et al. 1994); here, we further elaborated the role of PGPR in plant growth regulation during abiotic stress.

Table 1.1 PGPR species reported for producing gibberellins

PGPR species	GAs potential	References
Acetobacter diazotropicus	GA_1, GA_3	Bastian et al. (1998)
Azospirillum lipoferum	GA_1, GA_3	Bottini et al. (1989)
Azospirillum brasilense	GA_1, GA_3	Janzen et al. (1992)
Bacillus licheniformis	GA_1, GA_3, GA_4, GA_{20}	Gutierrez-Manero et al. (2001)
Herbaspirillum seropedicae	GA_3	Bastian et al. (1998)
Rhizobium phaseoli	GA_1, GA_4	Atzhorn et al. (1988)
Bacillus pumilus	GA_1, GA_3, GA_4, GA_{20}	Gutierrez-Manero et al. (2001)
Bacillus pumilus CJ-69	GA_1, GA_3, GA_4, GA_5, GA_7, GA_8, GA_9, GA_{12}, GA_{19}, GA_{20}, GA_{24}, GA_{44}	Joo et al. (2004)
Bacillus cereus MJ-1	GA_1, GA_3, GA_4, GA_7, GA_9, GA_{12}, GA_{19}, GA_{20}, GA_{24}, GA_{34}, GA_{36}, GA_{44}, GA_{53}	Joo et al. (2004)
Bacillus macroides CJ-29	GA_1, GA_3, GA_4, GA_7, GA_9, GA_{12}, GA_{19}, GA_{20}, GA_{24}, GA_{34}, GA_{36}, GA_{44}, GA_{53}	Joo et al. (2004)
Acinetobacter calcoaceticus	GA_1, GA_3, GA_4, GA_9, GA_{12}, GA_{15}, GA_{19}, GA_{20}, GA_{24}, GA_{53}	Kang et al. (2009)
Burkholderia cepacia	GA_1, GA_3, GA_4, GA_9, GA_{12}, GA_{15}, GA_{20}, GA_{24}	Joo et al. (2009)
Promicromonospora sp.	GA_1, GA_4, GA_9, GA_{12}, GA_{19}, GA_{20}, GA_{24}, GA_{34}, GA_{53}	Kang et al. (2012)

Gibberellin Biosynthesis in PGPR

Phytohormones are organic in nature and effective in very low amount. They are usually synthesized in tissues of plants and are transported to their specific site of action. Upon transport to the targeted tissues, the hormone causes physiological changes in plants such as fruit ripening, lateral root formation, flowering, and bud initiation. Each response is often the result of antagonistic or synergistic action of two or more hormones. Plant physiologists had categorized the hormones into five major groups: auxins, gibberellins, ethylene, cytokinins, and abscisic acid. Recently, two new hormones have also been recognized and known as brassinosteroids and strigolactones. Gibberellin is responsible for active role in seed germination, seedling emergence, stem and leaf growth, floral induction, and flower and fruit growth. Similarly, gibberellin production by PGPR promotes the growth and yield of many crop plants. A small number of PGPR have been identified to produce gibberellins (GA). These PGPR regulate the plant hormone level in three ways either by direct synthesis of GA itself, de-conjugation of glucosyl gibberellins, and change of inactive status of gibberellins into active GA (Lucangeli and Bottini 1997; Piccoli et al. 1997, 1999; Cassán 2001).
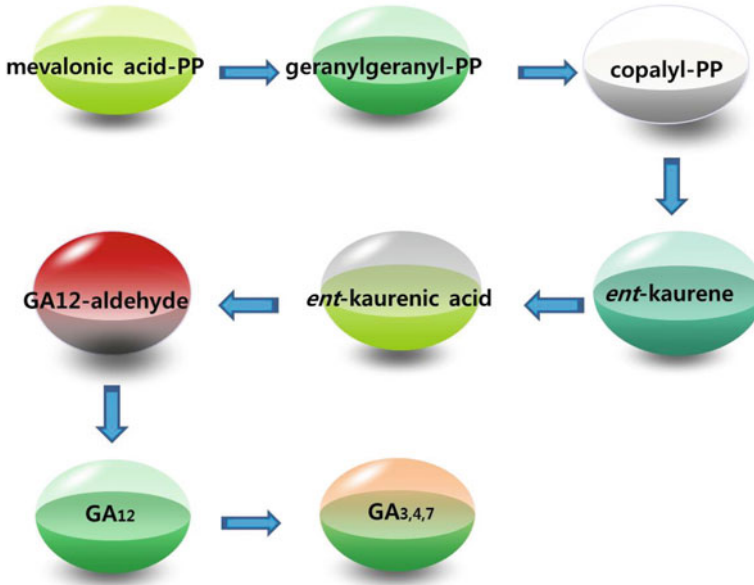
Fig. 1.1 Proposed and comparative GA biosynthesis pathway in bacteria based on the current knowledge from plant and fungi

In bacteria, the elucidation of GA biosynthesis pathway is based upon the knowledge from plants and fungi. Usually, GAs are biosynthesized from geranylgeranyl-PP (Fig. 1.1), which is converted into ent-kaurene via ent-copalyl diphosphate, and ent-kaurene is converted into GA_{12}-aldehyde via ent-kaurene oxidase and ent-kaurenoic acid oxidase. GA_{12}-aldehyde is then oxidized into GA_{12} and metabolized into other GA (Fig. 1.1; Baca and Elmerich 2003; Bomke and Tudzynski 2009). Morrone et al. (2009) have also reported the involvement of *operan* whose enzymatic composition indicates that gibberellin biosynthesis operate a third independent assembled pathway relative to plants and fungi. The reported pathway has superficial similarity to plants instead of fungi. GAs have been identified and isolated from higher plants, fungi, and bacteria. Up until now, 136 GAs from higher plants (128 species), 28 GA from fungi (7 species), and only 4 GA (GA_1, GA_3, GA_4, and GA_{20}) from bacteria (7 species) have been identified (Table 1.1; Hedden and Thomas 2012).

Gibberellin Quantification and Analysis in Microbial Culture

Until now, universal methods to quantify and analyze the gibberellins from microbes does not exist. However, modern analytical techniques such as GC-MS and LC-MS have enabled the plant physiologist to analyze and quantify the minute

quantities of GA in any culture sample. These advance equipments are sufficiently sensitive and selective to measure any phytohormones including GA at low concentrations. For gibberellin quantification and analysis, the microbes are grown initially in specific cultural broth. After a period of time (one week or ten days), the pure cultural filtrate (CF) is separated from growing cells by centrifugation or filtration. Onward several tedious steps are involved to remove interfering substances and bring it to a stage to be analyzed for the presence of GA. The concentrations of GA are very low (ng ml^{-1}) in the cultural broth of bacteria and require very sensitive methods for their detection. The analytical procedure must be able to identify the GA from other components of secondary metabolites. Furthermore, the choice of extraction and purification depends on analyte, type of analysis to be performed, and the equipments available. For GA characterization, extensive purification and standardization with pure substances are needed. The steps followed for GA analysis must eliminate potential impurities from analyte.

Extraction and Purification of Microbial Cultural Filtrate for Gibberellins

For extraction and purification of microbial cultural filtrate, the required strains are grown in nutrient broth (100 ml) for 7 days at 30 °C (shaking incubator at 200 rpm) (Kang et al. 2009, 2010; Lee et al. 1998). The culture and bacterial biomass are separated by centrifugation (2,500× g at 4 °C for 15 min). The culture medium (50 ml) is used to extract and purify GA as described by Kang et al. (2009). GAs have functional groups, highly oxidized and may be relatively labile to extreme pH in aqueous solutions. In alkaline conditions, epimerization is another reason due to which the extraction and purification procedures should be performed within certain range of pH like 2.5–8.5 (Urbanova et al. 2011). All the process of purification and especially the aqueous solution containing GA must be handled at temperature below 40 °C. Therefore, the pH of CF is adjusted to 2.5 using 6N HCl and partitioned with ethyl acetate (EtOAc) to obtain the extract. Before partitioning, deuterated stable GA internal standards (20 ng; [17, 17-^{2}H$_2$] GA$_1$, GA$_3$, GA$_4$, GA$_7$, GA$_{12}$, GA$_{19}$, GA$_{24}$, GA$_{34}$, and GA$_{53}$) are added in the CF. Tritiated GA, i.e., [1, 2-^{3}H$_2$] GA$_9$ and [1, 2-^{3}H$_2$] GA$_{20}$ are also added (can be obtained from Prof. Lewis N. Mander, Australian National University, Canberra, Australia). The organic layer is vacuum dried and added with 60 % methanol (MeOH), while the pH is adjusted to 8.0 ± 0.3 using 2N NH$_4$OH. The bacterial cultures are subjected to chromatographic and mass spectroscopy techniques for identification and quantification of GA (Table 1.2).

Table 1.2 GC-MS analysis of HPLC fractions from ethyl acetate fractions of bacterial culture

Fraction no.	GA	KRI[a]		m/z (%, relative intensity of base peak)[b]				
6–8	GA_8	2,818	Sample	594(100)	448(25)	379(20)	375(15)	238(28)
		2,818	standard	596(100)	450(24)	381(21)	375(11)	240(26)
12–14	GA_1	2,674	Sample	506(100)	448(20)	313(17)	491(13)	377(12)
		2,674	standard	508(100)	450(19)	315(14)	493(11)	379(13)
24,25	GA_{20}	2,485	Sample	418(100)	375(45)	403(14)	359(12)	301(13)
		2,485	standard	420(100)	377(45)	405(13)	361(10)	303(11)
26–28	GA_{44}	2,789	Sample	432(63)	238(41)	417(12)	373(17)	207(100)
		2,789	standard	434(62)	240(39)	419(10)	375(16)	209(100)
29–31	GA_{19}	2,600	Sample	434(100)	374(59)	402(41)	462(10)	375(57)
		2,600	standard	436(100)	376(57)	404(40)	464(9)	377(55)
37,38	GA_{53}	2,450	Sample	448(47)	251(30)	235(30)	389(25)	241(18)
		2,450	standard	450(47)	253(28)	237(28)	391(25)	243(19)
42–44	GA_{12}	2,335	Sample	300(100)	240(31)	328(31)	360(2)	285(19)
		2,335	standard	302(100)	242(32)	330(29)	362(2)	287(20)

[a] *KRI* Kovats retention index
[b] Identified as methyl ester trimethylsilyl ether derivatives by comparison with reference spectra and KRI data (Gaskin and MacMillan 1991)

Chromatography for Purification

The extracts are passed through a Davisil C18 column (90–130 μm; Alltech, Deerfield, IL, USA). The eluent is reduced to near dryness at 40 °C in vacuum. The samples are then dried onto celite and then loaded onto SiO_2 partitioning column (deactivated with 20 % water) to separate the GA as a group from more polar impurities. GAs are eluted with 80 ml of 95:5 (v / v) EtOAc: hexane saturated with formic acid. This solution is dried at 40 °C in vacuum, redissolved in 4 ml of EtOAc, and partitioned three times against 4 ml of 0.1 M phosphate buffer (pH 8.0). Dropwise addition of 2N NaOH is required during the first partitioning to neutralize residual formic acid. One gram of polyvinylpolypyrrolidone (PVPP) is added to the combined aqueous phases, and this mixture is slurried for 1 h. The pH is reduced to 2.5 with 6N HCl. The extract is partitioned three times against equal volumes of EtOAc. The combined EtOAc fraction is dried in vacuum, and the residues are dissolved in 3 ml of 100 % MeOH. This solution is dried in a Savant or a steam of nitrogen. The dried samples are subjected to preparative high-performance liquid chromatography (HPLC) for fractionations. To improve the purification efficiency, a 3.9 × 300 m Bondapak C18 column (Waters Corp., Milford, MA, USA) is used and eluted at 1.0 ml/min with the following gradient: 0–5 min, isocratic 28 % MeOH in 1 % aqueous acetic acid; 5–35 min, linear gradient from 28 % to 86 % MeOH; 35–36 min, 86–100 % MeOH; and 36–40 min, isocratic 100 % MeOH. Forty-eight fractions of 1.0 ml are collected.

GC/MS: SIM for Hormonal Analysis

Qualitative and quantitative analysis is very important for the GA produced by bacterial strains. GA identification requires physicochemical detectors having the ability to distinguish structurally unique compounds from each other. Only nuclear magnetic resonance (NMR) and mass spectrometry (MS) are commonly used techniques to fulfill this condition. MS is more useful than NMR as it is very sensitive to analyse the extremely low concentration of GA. However, NMR is useful for identification of unidentified GA and completes structure elucidation of known GAs. Liquid chromatography has also been remained a choice of qualitative analysis of derivatized GA. Moreover, the lack of efficiency to selectively detect (UV or fluorescence) the carboxylic acid derivatization has limited its use (Urbanova et al. 2011; Crozier and Durley 1983; Reeve and Crozier 1978; Heftmann et al. 1978; Morris 1978). Another great achievement of MS in terms of tandem instruments has improved the identification of GA and made easy the qualitative analysis (Urbanova et al. 2011). Here, we will focus on the qualitative analysis of GA through MS in combination with gas chromatography, and the scheme of whole process is described in Fig. 1.2. In GC-MS, the samples are injected and converted into gas form and then introduced into mass spectrometer ion source serving as a highly versatile GC detector (Urbanova et al. 2011; Hedden 1986).

The fractions are then prepared for gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring (SIM) system (6890N Network GC System, and 5973 Network Mass Selective Detector; Agilent Technologies, Palo Alto, CA, USA). Inside GC, derivatization of GA is important for enhancing their volatility to reproduce good peaks. Before analysis with GC-MS, the ethereal diazomethane and BSTFA (N,O-bistrimethyl silyltrifluoroacetamide) or MSTFA (N-methyl-N-trimethyl silyltrifluoroacetamide) are added to GAs to decrease the polarity of the emergent molecule and, more importantly, improve its mass spectral characteristics (step 1, Fig. 1.2). For each GA, 1 µl of sample is injected in GC/MS (step 2, Fig. 1.2); inside GC column, GA are separated (step 3, Fig. 1.2) and introduced
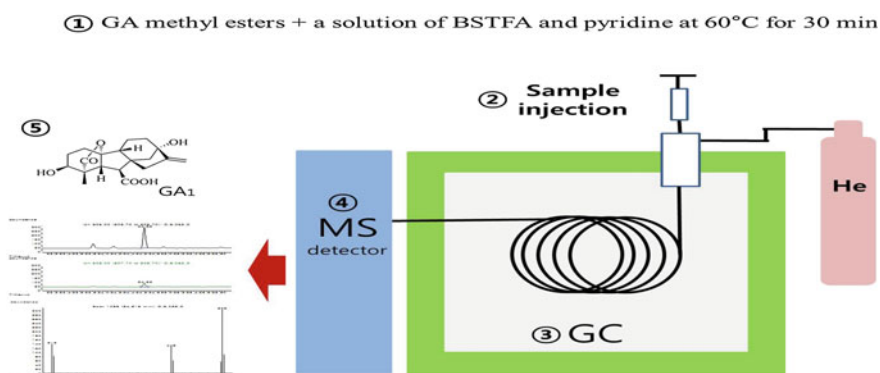

Fig. 1.2 Schematic process of GA identification through GC/MS SIM analysis

into the mass spectrometer (step 4, Fig. 1.2), where they undergo extensive fragmentation (Table 1.2).

The bacterial CF containing GA are calculated from the peak area ratios of sample GA to corresponding internal standards (step 5, Fig. 1.2). The retention time/identity of GA is determined using hydrocarbon standards to calculate the Kovats retention index (KRI) value. The KRI confirms the identity of GA. The GA quantification is based on the peak area ratios of non-deuterated (extracted) GA to deuterated GA (Kovats 1958).

Crop Growth and Abiotic Stress

Crop growth is the accumulative irreversible increase in crop plants. Abiotic and biotic stresses, mostly due to anthropogenic activities, cause losses to the crop yield. This is impossible until we understand inside the plant knowledge that how it interacts with outside environment including beneficial microbes (Mittler 2006) in abiotic stress. In abiotic stresses drought, salinity, and extreme temperature are most common all over the world (Khan et al. 2011). The interaction in such harsh conditions is very complex and may vary from crop to crop and growth stages. The impact is also highly variable on plant growth and biomass production (Tuteja 2007). Drought stress reduces the plant cell water potential and turgor pressure followed by increase in solute concentrations in the cytosol. In response to drought, increase in ABA, compatible osmolytes, and overproduction of reactive oxygen species occur. Overall, the important process for growth and development like acquisition of mineral and cellular metabolism are arrested (Khan et al. 2011; Lisar et al. 2012; Christensen et al. 2007; Munns and Tester 2008).

Salinity has devastated the crop production on more than 45 million hectares of irrigated land around the globe (Munns and Tester 2008; Carrillo et al. 2011). Salinity stress creates osmotic stress, ion toxicity, nutritional disorders, oxidative stress, change in metabolic functions, membrane disintegration, genotoxicity, and negatively influences cell division and expansion (Mittler 2006; Carrillo et al. 2011; Zhu 2007; Hossain et al. 2007, 2008; Türkan and Demiral 2009). The fluctuation in climatic conditions due to global warming has tremendously changed the general pattern of crop plant growth (Mahajan and Tuteja 2006; KohIba 2002; Shah et al. 2011). A high temperature exposure can injure the plant cell and cause cell death in a minute (Schöffl et al. 1999; Wahid et al. 2007). Overall, combination of such stresses cause starvation, growth retardation, abridged ion flux, and production of toxic compounds and reactive oxygen species (Wahid et al. 2007; Howarth 2005; Smertenko et al. 1997; Heidarvand and Amiri 2010; Wang et al. 2003), hence reducing the crop yield.

Different crop plants have devised various strategies to cope abiotic stresses and possess a cascade of signals ranging from primary to secondary responses. In primary response, plant maintains cell ionic and osmotic balance, which is followed by secondary response of activation of hormone, and secondary metabolites
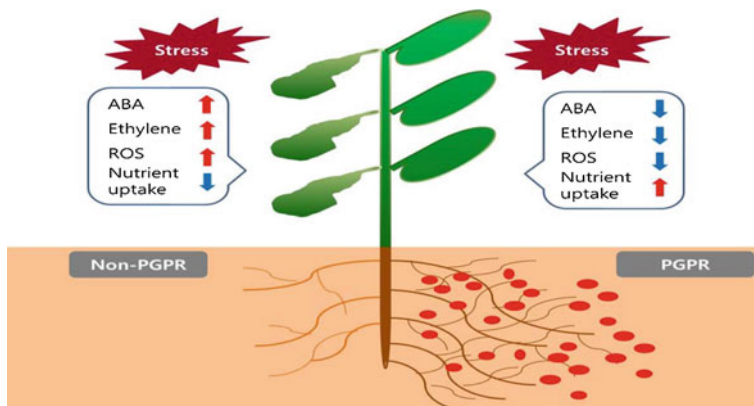
Fig. 1.3 Mechanism involved in PGPR role in crop tolerance against abiotic stress. *The upward arrow* indicates activation of effects, while *the downward* shows reduction

formation occurs. As we know, different abiotic stresses share some common symptoms and mitigation strategies (Hossain et al. 2007; Mahajan and Tuteja 2006; Wang et al. 2003). For example, drought and salinity cause ionic and osmotic stress, and in both cases, plant activates genes related to stress resistance and brings ionic and osmotic homeostasis through salt overly sensitive genes pathway or other related pathways. Drought and low temperature cause the same damage (disintegration of membrane, dehydration, and solute leakage). In perception of both stresses, crop plants either turn on detoxification signaling or activate stress genes which control damage and repair of cell membrane (Fig. 1.3; Lisar et al. 2012; Carrillo et al. 2011; Mahajan and Tuteja 2006; Wahid et al. 2007).

Sustainability of agricultural production is very important to fulfill the growing demands of food for human population. However, there is a need to minimize such abiotic stress in an eco-friendly way (Wang et al. 2003). Use of PGPR as a biocontrol and a biofertiliser seems an ideal strategy to mitigate various extreme environmental conditions like salinity, drought, and temperature stress (Fig. 1.3).

GA-Producing PGPRs and Crop Growth Amelioration

The ability of PGPR to produce phytohormones is one of the most important mechanisms by which many rhizobacteria promote plant growth (Spaepen et al. 2007; Martínez-Viveros et al. 2010). Several fungal and bacterial species are reported for phytohormone production (Tsavkelova et al. 2006). The phytohormone producing ability is widely distributed among bacteria associated with soil and plants. Research on PGPR has established that it can stimulate plant growth through the production of auxins, gibberellins, and cytokinins (Spaepen et al. 2008; Bottini et al. 2004; Timmusk et al. 1999), or by regulating the high levels of endogenous ethylene in the plant (Table 1.3; Glick et al. 1998).

Table 1.3 Reported PGPR species and their role in plant growth and development

PGPR species	Target plants	Observed effects	Reference
Pseudomonas fluorescens	Bean	Higher lignin content	Anderson and Guerra (1985)
Serratia plymuthica	Cucumber	Against disease	Benhamou et al. (2000)
Pseudomonas aeruginosa	Bean	Increased activity of phenlyalanine ammonia lyase	De Meyer et al. (1999)
Pseudomonas corrugata	Cucumber	Induced peroxidase (PO) activity	Chen et al. (2000)
Azospirillum brasilense *Azospirillum lipoferum*	Maize and rice	Gibberellin production	Cassán et al. (2001)
Bacillus subtilis	*Arabidopsis*	Elevated levels of L-malic acid	Thimmaraju et al. (2008)
Bacillus cereus	Tomato	Induced systemic resistance	Bernardo de et al. (2006)
Variovorax paradoxus	Indian mustard	Cadmium tolerant	Belimov et al. (2005)
Acinetobacter calcoaceticus	Cucumber, Chinese cabbage, crown daisy	Gibberellin production—Phosphate solubilization	Kang et al. (2009)
Rhizobium	Rice	Produced auxin (IAA) and gibberellins	Yanni et al. (2001)
Bacillus amyloliquefaciens	Tomato	Nutrient (nitrogen and phosphorus) uptake	Adesemoye et al. (2009)
Azotobacter	Wheat	Antifungal activity produced IAA	Zarrin and Sharon (2010)
Brevibacterium iodinum *Bacillus licheniformis* *Zhihengliuela alba*	Pepper	ACC deaminase producing	Siddikee et al. (2010)
Stenotrophomonas maltophilia	*Arabidopsis*	Production of siderophores and chitinases	Domenech et al. (2007)
Pseudomonas monteilii *Cronobacter dublinensis* *Bacillus* spp.	Sweet basil	Nutrient uptake, antagonist	Rakshapal et al. (2013)
Azospirillum lipoferum	Maize	Accumulation of free amino acids, soluble sugars, proline, and soluble protein contents	Qudsia et al. (2013)

Table 1.3 (continued)

PGPR species	Target plants	Observed effects	Reference
Azospirillum sp., *Pseudomonas* sp.	Canola	Antioxidant enzymes and Microelements	Noorieh et al. (2013)
Azospirillum brasilense *Gluconacetobacter diazotrophicus* *Herbaspirillum seropedicae* *Burkholderia ambifaria*	Tomato	Fixing atmospheric nitrogen, protecting the host plant from pathogens	Anna et al. (2013)
Bacillus pumilus *Micrococcus* spp. *Mesorhizobium* sp.	Brassicaceae	Effective metal immobilizing	Wafae et al. (2013)
Pseudomonas aeruginosa	Chickpea	Uptake of nitrogen and phosphorus (P) Production of phytohormone (IAA)	Jay et al. (2013)

Gibberellin-producing PGPR are very little known for their plant growth promotion. In PGPR, the phytohormones are secondary products and are suggested for beneficial effects in plant growth. Several types of PGPR have been identified for their potential to produce gibberellins. These are isolated from rhizosphere and preliminarily selected for plant growth promotion. Plant growth promotion by PGPR species that produce GAs has been previously reported (Bastian et al. 1998; Gutierrez-Manero et al. 2001; Atzhorn et al. 1988). In cultures of wild-type and mutant strains of *Rhizobium phaseoli*, Atzhorn et al. (1988) found GA_1 and GA_4 along with smaller quantity of GA_9- and GA_{20}-like compounds. In another experiment, Bastian et al. (1998) detected phytohormones indole-3-acetic acid and gibberellins GA_1 and GA_3 from chemically defined cultures of *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae*. Both bacteria are associated with Gramineae species in endophytic mode of life and were found to promote plant growth and yield (Table 1.1).

Gutierrez-Manero et al. (2001) isolated the plant-growth-promoting rhizobacteria (PGPR), *Bacillus pumilus* and *Bacillus licheniformis*, from the rhizosphere of alder (*Alnus glutinosa* [L.]). Full-scan gas chromatography–mass spectrometry analyses on extracts of these media showed the presence of GA_1, GA_3, GA_4, and GA_{20} in addition to the isomers 3-epi-GA_1 and iso-GA_3. Bioassay data showed that all the three strains have a strong growth-promoting activity in alder seedlings.

Joo et al. (2004) isolated *Bacillus cereus*, *B. macroides*, and *B. pumilus* and found the production of GA_5, GA_8, GA_{34}, GA_{44}, and GA_{53} for the first time by bacteria. The newly identified PGPR were also evaluated for growth promotion in red pepper which showed that they not only enhanced different plant growth parameters but also increased endogenous gibberellin level (Joo et al. 2004, 2005).

PGPB are also investigated in vegetables. In one experiment, Kang et al. (2012) investigated the symbiotic effect of gibberellin and organic acids producing PGPR (*Acinetobacter calcoaceticus*) on cucumber plant growth. In symbiotic association, the PGPR has significantly ameliorated cucumber plants to higher growth. The PGPR application had higher shoot length, plant biomass, and chlorophyll contents as compared to distilled water and nutrient broth-treated control plants. The bacterial culture-treated plants have also increased the amino acid and crude protein contents as compared to control plants. The improved effects were also observed by the regulation of stress-related abscisic acid which was significantly lower in PGPR-inoculated plants as compared to controls. Contrarily, the endogenous GA quantity was up-regulated, indicating the activation of GA biosynthesis pathway by which it increased the shoot lengths of cucumber plant.

Similar studies were also investigated in tomato plants. *Promicromonospora* sp. SE188 was producing gibberellins and had higher phosphate solubilisation potential. Its inoculation to the tomato plants resulted in higher plant biomass and shoot length as compared to distilled water-treated control plants. The presence of *Promicromonospora* sp. SE188 significantly up-regulated the non-C-13 hydroxylation GA biosynthesis pathway ($GA_{12} \rightarrow GA_{24} \rightarrow GA_9 \rightarrow GA_4 \rightarrow GA_{34}$) in tomato plants as compared to the control plants. Endogenous abscisic acid was significantly down-regulated in the presence of *Promicromonospora* sp. SE188. Contrarily, endogenous salicylic acid was significantly higher in the tomato plant after *Promicromonospora* sp. inoculation as compared to the control.

Karako and Aksoz (2006) isolated the potent *Pseudomonas* sp. from soil of olive waste. The *Pseudomonas* sp. was capable of producing gibberellins. However, no investigation was reported on plant growth promotion. Furthermore, on optimization of nutrient broth, the *Pseudomonas* sp. yielded the highest level of gibberellic acid (285.06 mg/l) upon incubation at 30 °C for 72 h at pH 7 using rotary shaker under dark conditions.

The role of ecological significance must be considered when using PGPR. Barea et al. (1976) isolated fifty phosphate-dissolving bacteria from rhizospheres of various crop plants. Assessing their potential to secrete gibberellins, IAA, and cytokinins, only 29 rhizobacterial strains were active to produce gibberellins.

Another study showed that mutualistic symbiosis of maize and *Pseudomonas* fluorescent enhanced the drought stress tolerance of the host (Ansary et al. 2012). Results showed that drought stress triggered a change in plant phytohormonal balance, including an increase in leaf proline and abscisic acid content, and a decline in auxin, gibberellin, and cytokinin synthesis. In comparison with control, plants inoculated with *P. fluorescens* showed highest level of proline, abscisic acid, auxin, gibberellin, and cytokinin in the leaves. This study indicates that application of PGPR can enhance phytohormone content of maize under water-deficit stress conditions. In addition to maize, *Pseudomonas* strains associated with rapeseed exhibited higher growth and more oil yield in drought stress (Arvin et al. 2012). Results showed that drought stress reduced yield up to 152.5 %, oil content, and yield components. It was also concluded that inoculation treatment had better effects than either no inoculation (control) or co-inoculation.

From the semi-arid ecosystem of south-east Spain, Kohler et al. (2008) isolated PGPR along with arbuscular mycorrhizal fungi and rhizobium bacteria. The symbiotic association was evaluated alone or in combination with each other using *Anthyllis cytisoides* L., a test plant. The parameters evaluated were biomass accumulation and allocation, N and P uptake, N_2-fixation (15N), and specific root length. Many microbial combinations were effective in improving plant development, nutrient uptake, N_2-fixation, or root system quality. It was also concluded that beneficial microbes native to the environment are more effective than the exotic species and instead of selecting a multifunctional microbial inoculum. Appropriate microbial combinations can be recommended for a given biotechnological input related to improvement of plant performance.

To assess the effects and intensity of abiotic stress tolerance of GA-producing PGPR, Kang et al. (2012) applied novel strains, viz., *Promicromonospora* sp. SE188, *Burkholderia cepacia* SE4, and *A. calcoaceticus* SE370 to cucumber plants. The experimental design comprised of eight sets of cucumber (*Cucumis sativus* L) plants with (1) PGPR interactions; (2) non-PGPR interactions; (3) PGPR interactions salt; (4) non-PGPR interactions salt; (5) PGPR interactions drought; and (6) non-PGPR interactions drought. *B. cepacia* SE4, *Promicromonospora* sp. SE188, and *A.calcoaceticus* SE370 were assessed for their potential to resist high salinity (120 mM) and drought (15 % PEG) stress continuously for 7 days. Parameters like plant growth parameters, relative water content, electrolytic leakage, antioxidant activities, and endogenous hormonal regulation were studied. Other functional biochemicals like crude protein contents, amino acids, and nitrogen content were also evaluated. Overall, the effect was very satisfactory, and the application significantly enhanced the growth parameters of the plants. However, *B. cepacia* SE4 was more prominent to extend the abiotic stress tolerance in cucumber plants. Such kind of studies should be extended to other important agronomic crops to save the agriculture loss during harsh climatic conditions.

Future Perspectives

Our current knowledge about PGPR is still very limited, and to understand it better, we have to explore, isolate, and screen the PGPR wealth available with different agricultural crops. More investigations are needed to analyze and assess the role of active PGPR in crop growth under various abiotic environmental circumstances like salinity and drought. Furthermore, the mechanism needs to be explored in phytohormonal regulation (abscisic acid, salicylic acid, jasmonic acid, and gibberellins) during the PGPR interaction with crop host plants under abiotic stress, to further improve strategies for sustainable crop production.

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Chapter 2
Mycorrhizal Fungi to Alleviate Drought Stress on Plant Growth

Francesca Rapparini and Josep Peñuelas

Introduction

Plants are frequently subjected to different abiotic environmental stresses that determine geographic distribution and adversely affect growth, development, and agronomic yield. Drought is one of the major constraints on plant productivity worldwide and is expected to increase with climatic changes (IPCC 2007 and EEA 2011). The symbiotic relationship between arbuscular mycorrhizal (AM) fungi and the roots of higher plants is widespread in nature, and several ecophysiological studies have demonstrated that AM symbiosis is a key component in helping plants to cope with water stress and in increasing drought resistance, as demonstrated in a number of host plant and fungal species (Augé 2001; Ruiz-Lozano 2003; Smith and Read 2008; Ruiz-Lozano and Aroca 2010).

The alleviating effect of AM symbiosis in response to drought generally relies on the positive effects of AM fungi on the uptake and transport of water and on an improved uptake of nutrients, especially of available soil phosphorus (P) and other immobile mineral nutrients, resulting in the hydration of plant tissues, a sustainable physiology and a clear promotion of growth (Fig. 2.1; Augé 2001). AM

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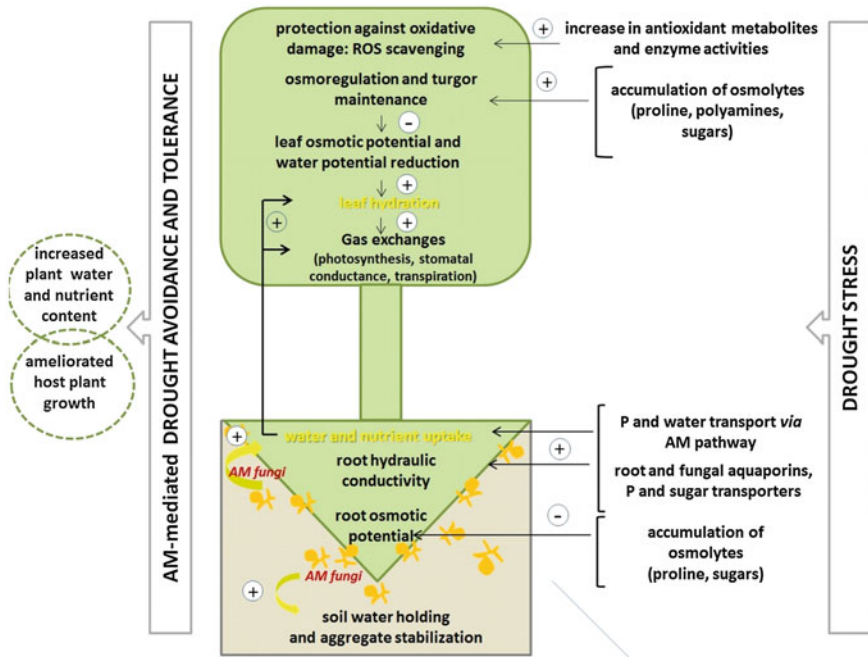
Fig. 2.1 Arbuscular mycorrhizal (*AM*) symbiosis can help plants to cope with the detrimental effects of soil water deficit acting, directly or indirectly, on plant functionality both above- and belowground. At the levels of both leaves and roots, the osmotic stress usually caused by drought is counteracted by mycorrhizal plants through biochemical changes that mostly include increased biosynthesis of metabolites (mainly proline and sugars) that act as osmolytes. These compounds contribute to the lowering of the osmotic potential, and in turn, of the leaf water potential. These lower potentials allow the plants to maintain high organ hydration and turgor that sustain overall cell physiological activity, mainly related to the photosynthetic machinery. AM plants withstand drought-induced oxidative stress by the increased production of antioxidant compounds that scavenge ROS and enhance the activities of antioxidant enzymes. AM root colonization can enhance root growth, architecture, and hydraulic properties and can thus induce the formation of a highly functional root system for nutrient/water uptake. At the same time, AM fungal hyphae in the soil provide an efficient pathway for nutrient/water uptake and transport, allowing a more efficient exploitation of the water and nutrient reservoirs in the soil where only fungal hyphae can grow, thereby bypassing the zones of water and nutrient depletion around the roots. Molecular mechanisms activated by AM symbiosis to counteract drought include gene activation of functional proteins, such as the membrane transporter aquaporins and, potentially, ion and sugar transporters, in both roots and fungi. Improved nutrient/water uptake and transport in roots translates into enhanced hydration of the aboveground organs that in turn affects physiological and biochemical processes. In addition, AM symbiosis can increase the resistance of plants to drought through secondary actions such as the improvement of soil structural stability that in turn increases the retention of soil water

symbiosis has a variety of effects on the defensive responses of host plants, depending on the species of host plant and the AM fungus involved (Bezemer and van Dam 2005).

In addition, the numerous confounding influences and system feedbacks inherent to the nature of AM symbiosis must be differentiated when describing the effects of AM on water balance. The AM-mediated response of many physiological and biochemical traits to changes in water availability may be confounded by concurrent changes in plant growth and nutrient availability (Smith and Read 2008) and can cause some drawbacks that limit our ability to clearly understand how AM fungi enable drought resistance in plants.

The comparison of plants of similar size and nutritional status is thus recognized as a fair requirement when evaluating the function of AM fungi during drought stress. Extensive study has demonstrated AM-mediated plant resistance to drought conditions, but the underlying mechanisms have not yet been clearly elucidated. Our incomplete understanding of how AM symbiosis affects the ability of plants to withstand conditions of limited water represents an important challenge to meeting the goal of improved plant productivity under the projected critical global scenarios.

AM-Mediated Plant Strategies to Cope with Drought: Avoidance Versus Tolerance

Despite the large variability in the effects of water stress on plants, they are able to respond to drought through two major strategies: avoidance of water stress and drought tolerance (Bray 1997). According to accepted terminology (Levitt 1980; Ludlow 1989; Turner 1997), plants can be classified as drought avoiders or as drought tolerant based on the absolute value of leaf water potential: drought avoidance allows the plant to withstand water-limiting conditions by maintaining a higher water status, mainly through enhanced water uptake and/or minimized water loss; tolerance to dehydration is associated with survival and sustained physiological activity when the leaf water potential is low, resulting in the ability of leaves to endure dehydration.

AM symbiosis protects host plants against the detrimental effects of drought stress through mechanisms of drought avoidance (Augé 2001; Ruiz-Sanchez et al. 2010). Strategies of drought avoidance in mycorrhizal plants rely on the ability to maintain an adequate hydration status on the level of whole plants as characterized by relative water content, although a thorough review of the literature indicates that leaf water potential was not measured in some experiments (Augé 2001; Augé and Moore 2005).

The improved capability of drought avoidance mediated by AM colonization has often been associated with the AM promotion of plant growth through enhanced nutrition. However, when not considering shoot size and nutritional effects, the influence of AM symbiosis on leaf hydration, mainly via the increased water uptake characteristic of mycorrhizal plants, may be the basis for their improved drought resistance. On top of being drought avoiders, mycorrhizal plants

have also been characterized as drought tolerant, mainly because of more improved osmotic adjustment, which allows the hydration and turgor of leaves to be sustained when leaf water potentials are low.

This distinction between drought strategies related to the AM-mediated responses of plants is fundamental for a better comprehension of the ecological and agricultural consequences for a plant species, because the AM-mediated response to drought is a complex process involving numerous metabolites and metabolic pathways. Studies to date investigating the role of AM symbiosis in ameliorating plant responses to drought stress have suggested the up-regulation and down-regulation of several physiological and biochemical processes. (1) The direct uptake and transfer of water and nutrients by AM fungi, (2) increased osmotic adjustment, (3) enhanced gas exchange and water use efficiency, and (4) better protection against oxidative damage when water is limiting may ameliorate, mitigate, and compensate the negative impacts of water stress in mycorrhizal plants.

Augé (2001) compiled a comprehensive review of the literature covering subjects such as plant strategies for controlling water status under drought and the metabolic processes underlying responses of mycorrhizal plants to oxidative stress. Ruiz-Lozano (2003) reviewed several aspects in need of investigation at the molecular level for understanding the different mechanisms by which AM symbiosis protects host plants from the detrimental effects of water deficit in terms of osmotic stress. These authors have provided new perspectives for molecular studies that could contribute to a global understanding of the different mechanisms by which AM symbiosis protects host plants against water deficit. Progress has also been made on the interpretation of the relationships between the different pathways regulated by the host plant or by the AM symbiotic relationship (Smith et al. 2010).

The aim of the present review is to outline the recent advances in the study of drought resistance by AM symbiosis with a particular focus on nutrient and water uptake/transport and on the lesser-known protective metabolites.

Biochemical–Metabolomic Responses of AM Plants to Drought

Role of Metabolic Changes in Osmoregulation

When water is limiting, decreased stomatal conductance and increased diffusive resistance to CO_2 could lead to increased plant water potential. To maintain water uptake from the soil, though, the water potential must be reduced. To achieve such an effect, plants can rely on mechanisms of 'osmotic adjustment' or 'osmoregulation' that decrease the osmotic potential resulting from the accumulation of compatible solutes or osmolytes (Munns 1988; Serraj and Sinclair 2002).

Osmolytic accumulation in plant cells can act as a mechanism of osmotic adjustment for decreasing the cellular osmotic potential and thus for maintaining water absorption and turgor. Osmolytic accumulation can also protect cellular components, such as cell membranes and proteins, and sustain the physiological activity of plants (Serraj and Sinclair 2002).

The accumulation of metabolites alone, however, may not always be sufficient to account for their effect on osmotic adjustment under drought stress. An alternative role for osmolytes as scavengers of reactive oxygen species (ROS) has been suggested (Hoekstra et al. 2001). Typical metabolites that can prevent the negative effects of drought include amino acids such as proline, other nitrogenous compounds such as polyamines, and a wide range of sugars and alcohol sugars. AM-mediated biochemical changes under conditions of drought stress principally involve the accumulation of protective metabolites such as osmolytes.

The colonization of roots by AM fungi in various plant species induces proline accumulation when water is limiting (Ruiz-Lozano et al. 1995; Azcón et al. 1996; Goiochea et al. 1998; Yooyongwech et al. 2013). The enhanced accumulation of proline in these studies was linked to AM-induced drought resistance with proline acting as osmoprotectant. Conversely, in several studies, while proline content increased in response to water deficit, a lower accumulation of proline has been observed in mycorrhizal plants relative to nonmycorrhizal counterparts (Ruiz-Lozano and Azcón 1997; Wu and Xia 2006; Aroca et al. 2008; Ruiz-Sánchez et al. 2010; Abbaspour et al. 2012; Fan and Liu 2011; Asrar et al. 2012; Doubková et al. 2013), suggesting that AM symbiosis enhanced host plant resistance to drought.

In fact, proline could also be considered as a marker of the potential injury caused by water deficit, indicating that mycorrhizal plants, characterized by lower proline accumulation, were less stressed than the nonmycorrhizal plants. Furthermore, proline can act as an effective scavenger of ROS in the protection against denaturation and in the stabilization of membranes and subcellular structures (Kishor et al. 2005). The levels of free polyamines, other soluble nitrogenous compounds, increased in the leaves of drought-stressed mycorrhizal plants, and this increase was interpreted as indicating that free polyamines could serve as osmoprotectants under drought conditions, conferring drought resistance to mycorrhizal plants (Goicoechea et al. 1998).

AM symbiosis can increase the drought tolerance of plants if the commonly observed higher rates of photosynthesis lead to an increased accumulation of nonstructural carbohydrates that, acting as osmoprotectants, can lower the osmotic potential (Augé 2001; Porcel and Ruiz-Lozano 2004; Khalvati et al. 2005). Several studies have reported the accumulation of carbohydrates when plants are subjected to water stress in both woody species such as *Citrus* (Wu and Xia 2006) and *Macadamia* cultivars (Yooyongwech et al. 2013) and in herbaceous species such as lettuce cultivars (Baslam and Goicoechea 2012) and pistachio (Abbaspour et al. 2012). Carbohydrate accumulation in these studies was correlated with improved plant performance under drought stress, but the leaf osmotic potential was not evaluated; so correlating changes in carbohydrate levels with differential capacities of osmoregulation in mycorrhizal plants was thus not possible.

In addition, other reports have observed an AM-mediated decrease in soluble sugars in *Erythrina variegata* (Monoharan et al. 2010) and *Casuarina equisetifolia* (Zhang et al. 2010) exposed to drought stress, and this pattern was correlated with lower amounts of drought injury in the host plant. Furthermore, considering the positive mycorrhizal effect on plant growth, transport to sink organs and higher turnover rates of carbohydrates are likely to occur and suggest that the increased net assimilation rates do not necessarily indicate the accumulation of carbohydrates. In addition to the dynamic balance between the demands of growth and osmotic adjustment, another significantly relevant and competitive sink for carbohydrates is represented by the AM fungi themselves, which commonly drain photosynthate from the plants.

New insights are emerging into the regulation of uptake, exchange, and competition for carbohydrates by membrane transporters at the cellular plant–fungus (symbiotic) interface (see review by Doidy et al. 2012). Genes encoding transport proteins specific to the uptake of sucrose and hexoses have been identified by transcriptomic and genomic analyses at the AM symbiotic interface, in both plants and fungi (Doidy et al. 2012). The future application of combined physiological and molecular genetic approaches will open promising perspectives for a better understanding of the regulatory role of sugar transporters in the partitioning and allocation of carbohydrates between plants and AM fungi and hence of the effects of AM symbiosis in response to environmental limiting conditions, such as drought.

The above findings support the assessment that the direct measurement of physiological parameters such as soil and/or leaf water potential and turgor are fundamental for clearly interpreting the significance of AM-induced biochemical changes and for unambiguously interpreting the data (Augé 2001). Changes in metabolite levels could then be more confidently attributed to the strategies of drought tolerance or avoidance. Decreases in osmolytes have previously been interpreted as a mechanism of drought avoidance, while accumulation of osmoprotectants has been associated with drought tolerance (Augè and Moore 2005, 2010; Ruiz-Sánchez et al. 2010). Metabolic profiling of plants exposed to stress conditions is an important tool for studying stress-induced changes in metabolites, including osmolytes, but we lack knowledge of the regulation of the genes encoding enzymes of the osmolytic biosynthetic pathway.

Protection Against Oxidative Stress: Antioxidant Metabolites

Protection against oxidative damage by various antioxidants is another fundamental mechanism that can enhance drought resistance in mycorrhizal plants (Ruiz-Lozano 2003). Drought involves the production of excess ROS, such as singlet oxygen, superoxides, hydrogen peroxide, and hydroxyl radicals, leading to cell damage or death (Smirnoff 1993). Plants are characterized by a complex response network of antioxidant compounds and enzymes that defend plant cells

against excess ROS. Direct reactions can quench ROS activity, as can indirect responses such as hormone-mediated signaling to up-regulate primary and activate secondary defense genes (see review Apel and Hirt 2004; Kwak et al. 2006).

Oxidative stress occurs when the antioxidant defense system is overloaded and is unable to maintain an adequate cellular redox balance. The antioxidant system includes both enzymatic (e.g., superoxide dismutases, ascorbate peroxidases, and catalases) and nonenzymatic molecules (e.g., ascorbate, glutathione, flavonoids, carotenoids, and tocopherols; Mittler 2002). Antioxidants act not only as direct ROS scavengers but also as key sensors of the cellular redox status, so they trigger a number of signaling events for tightly controlling cellular ROS levels.

The amelioration of stress resistance by AM symbiosis is often related to the enhancement of antioxidant levels or activities in plants (Wu et al. 2006a, b; Wu and Zou 2009; Ruiz-Sánchez et al. 2010; Baslam and Goicoechea 2012). Ruiz-Sánchez et al. (2010) found that AM symbiosis ameliorated the response of plants to drought by improving photosynthetic performance but mainly through the accumulation of the antioxidant compound glutathione, which was concomitant with a reduction in oxidative damage to membrane lipids and to low cellular levels of hydrogen peroxide. In the same study, while glutathione levels increased, ascorbate levels decreased in mycorrhizal plants compared to nonmycorrhizal counterparts. This comprehensive study further supports the premise that mycorrhizal protection against drought-induced oxidative stress may be a crucial mechanism by which AM symbiosis increases the resistance of host plants to drought (Ruiz-Lozano 2003). In addition, it suggests differential up-regulation of the various antioxidant systems, with preferential activation of the systems that are more effective in protecting plants against drought. As already discussed in the case of changes in proline levels in response to drought, these antioxidant compounds can also be viewed as markers of drought stress: low accumulations of both glutathione and ascorbate in mycorrhizal plants of lavender under drought conditions were correlated with a high level of resistance to plant drought (Marulanda et al. 2007).

Among other potential ROS scavengers, flavonoids might also play a role in protecting mycorrhizal plants against oxidative damage: AM-mediated increases in the amounts of these compounds were sometimes found when plants were exposed to drought conditions (Abbaspour et al. 2012). Several studies suggest that AM symbiosis helps plants to alleviate drought stress by enhancing the activities of antioxidant enzymes, such as superoxide dismutase, guaiacol peroxidase, peroxidase, and catalase (Ruiz-Lozano et al. 1996; Wu and Zou 2009). Increased activity of superoxide dismutase was confirmed by transcriptomic analysis of the genes encoding this enzyme (Ruiz-Lozano et al. 2001). Protection against drought stress in soybean plants may originate from an enhanced activity of glutathione reductase concomitant with lower glutathione levels and decreased oxidative damage to biomolecules (Porcel et al. 2003; Ruiz-Lozano et al. 2001). Increases in the activities of several antioxidant enzymes induced by mycorrhizae have been associated with an AM-mediated enhancement in photosynthetic activity, plant biomass, and nutrient status (Alguacil et al. 2003; Roldán et al.

2008). The response of the specific antioxidant compound or enzyme, however, may depend on the host plant and the fungal species.

Even though most research on plant antioxidants has focused on nonvolatile compounds, volatile organic compounds emitted by leaves may contribute to an additional protective system against abiotic stresses (Kesselmeier and Staudt 1999; Peñuelas and Munné-Bosch 2005). Accumulating evidence supports the hypothesized role of volatile isoprenoids, in particular isoprene, in the protection against oxidative stress by mediating the oxidative status of plants through direct ROS scavenging, indirect alteration of ROS signaling, and/or membrane stabilization during abiotic stress, including drought (Peñuelas and Munné-Bosch 2005; Vickers et al. 2009).

Many plants that form AM mycorrhizae emit isoprenoids involved in the protection against several stresses, but the contribution of the mycorrhizae to the production of isoprenoids by plants has been scarcely investigated, especially under drought stress (Rapparini et al. 2008; Asensio et al. 2012). Moreover, the roots of mycorrhizal plants produce high amounts of specific isoprenoid-derived apocarotenoids (Walter and Strack 2011) and strigolactones (Lopez-Ráez et al. 2008).

We recently tested whether AM symbiosis affected the allocation of carbon resources to different classes of isoprenoids such as the volatile nonessential isoprenoids (monoterpenes and sesquiterpenes) and the nonvolatile essential isoprenoids (abscisic acid (ABA), chlorophylls, and carotenoids; Asensio et al. 2012). By subjecting tomato plants to stressors such as drought and to an exogenous application of jasmonic acid, we examined the AM symbiotic interaction in conditions where isoprenoids usually play a role in resistance to stress and in plant defense. Our results suggested that mycorrhizal plants use complex feedback responses associated with the activation of different pathways of isoprenoid production. Root colonization favored the production of essential rather than nonessential isoprenoids, especially under conditions of drought stress or after the application of jasmonic acid. In an overall view of the mycorrhizal plant system, carotenoids are both a sink of the universal precursors of isoprenoids and a concomitant source of important growth regulators such as apocarotenoids, ABA, and strigolactones, which are specifically produced when plant roots are colonized by AM fungi (Bouwmeester et al. 2007; Cazzonelli and Pogson 2010). We, accordingly, proposed that a more important demand of carotenoid-derived compounds and pigments is expected to increase in AM plants, especially under stress conditions where these isoprenoid compounds might play a role in plant protection and defense. The accumulation of carotenoids has also been found in lettuce plants (Baslam and Goichoechea 2012). Increased production of strigolactones in host plants during nutrient deficiency and salt stress (Yoneyama et al. 2007; Lopez-Ráez et al. 2008; Aroca et al. 2013) promoted AM fungal development and symbiotic establishment, suggesting a potential function of these compounds in enabling plants to overcome these abiotic constraints.

Physiological Responses of AM Plants to Drought

Aboveground Processes Affecting Plant–Water Relations

The physiological effects of AM symbiosis include aboveground modifications of water relations and physiological status in terms of leaf water potential, relative water content, stomatal conductance, CO_2 assimilation, and efficiency of photosystem II as compared to nonmycorrhizal plants (Augé 2001; Barzana et al. 2012). Many studies have shown an enhancement of the rates of gas exchange (stomatal conductance, transpiration, and photosynthetic rates) in mycorrhizal plants over nonmycorrhizal counterparts under water-limited conditions, independently of growth- or nutrition-mediated effects (see review Augé 2001; Ruiz-Lozano 2003; Sanchez-Blanco et al. 2004; Khalvati et al. 2005; Lee et al. 2012).

The mechanism by which AM symbiosis affects these physiological parameters is still unclear. The role played by ABA has been suggested as one of the nonnutritional mediated mechanisms by which AM symbiosis influence stomatal conductance and other physiological traits when plants are drought stressed (Ludwig-Müller 2010). In support of this hypothesis, recent studies have shown that ABA levels increased in response to water deficit and increased more in nonmycorrhizal plants than in mycorrhizal plants, suggesting that AM plants experience less intense drought stress (Doubková et al. 2013). Furthermore, these physiological processes may vary depending on host plant and especially on fungal species. Both stomatal conductance and photosynthesis varied widely during drought depending on the AM fungal species, even when comparing plants of similar size.

Several studies have reported that gas exchange in host plants is often related to the effect of AM symbiosis on the hydration of leaves (Augé 2001). Despite the numerous findings showing the positive effects of AM symbiosis on foliar gas exchange, the influence of these processes on leaf water potential in mycorrhizal plants subjected to drought is still unclear. In several studies, leaf water potential did not differ between mycorrhizal and nonmycorrhizal plants under drought stress (Augé 2001). Nevertheless, recent studies have demonstrated a higher (less negative) leaf water potential in mycorrhizal plants in water-limited conditions, which was interpreted as an AM-mediated mechanism of avoidance to mitigate the negative impact of drought on plant growth (Porcel and Ruiz-Lozano 2004; Asrar et al. 2012).

Leaf water potential is recognized as an index of the water status of an entire plant and hence represents a fundamental trait revealing a potentially improved resistance of plants to drought through better hydration. Hence, measurements of water use efficiency (WUE) provide an integrated measure of plant water use and thus allow a further dissection of the plant–water relations of mycorrhizal plants when water is limiting.

The extensive survey of the literature by Augé (2001) covered repeated attempts to examine the impacts of AM symbiosis on WUE. At the time these studies were conducted, however, the response in WUE was highly variable under

water stress: increases or decreases in WUE with AM symbiosis were observed. A sampling of the recent literature confirmed this variable response, showing an increase in WUE in *Antirrhinum majus* L. (Asrar et al. 2012) and the lack of a positive AM effect on this trait in *Knautia arvensis* during drought (Doubkóva et al. 2012).

A large part of plant resistance to drought is the ability to manage excess radiation resulting from limitation of photosynthesis by drought (for a review see Chaves et al. 2003) and reduced CO_2 availability leading to an inefficient use of incident light and to an increased susceptibility to photodamage (Powles 1984). Photoprotective mechanisms regulate the excitation energy that reaches the reaction centers of the photosystem by the dissipation of thermal energy (Demmig-Adams and Demmig 2006); the mechanisms also scavenge oxidative molecules and repair oxidative damage (Fernandez-Marın et al. 2009).

Recent reports have indicated that AM symbiosis under drought conditions enhances the photochemical efficiency of photosystem II, given by Fv/Fm, assessed by chlorophyll fluorescence in rice plants (Ruiz-Sánchez et al. 2011) and in woody tree nut species (Yooyongwech et al. 2013). Such results indicate the improved performance of the photosynthetic machinery and the absence of photoinhibition when mycorrhizal plants were exposed to water deficit. These findings are consistent with those of another recent study investigating the effect of root inoculation of different tree species with a combination of both AM and ectomycorrhizal fungi (Fini et al. 2011). The dynamics of photosystemic function and the potential forms of thermal dissipation, including those regulated by xanthophylls, however, have not yet been studied in detail.

Belowground Role of Root Systems and AM Fungi

Drought resistance in plants is strongly affected by their nutritional status. Soil–water deficit is tightly linked to low nutrient availability and to poor soil structure, so various hypotheses have been formulated to explain the underlying plant nutrition mechanisms involved in AM-induced resistance to drought. Improved nutrient uptake by AM fungi is a fundamental mechanism that can alleviate the adverse effects of water stress on plant growth.

One of the most common explanations for the improved nutrient status in mycorrhizal plants is the enhanced absorbing surface provided by the hyphae in the soil together with the ability of fungi to take up water from soil with low water potential (Augé 2001; Ruiz-Lozano 2003). The diameter size of hyphae (2–5 μm) is one or two times smaller than the diameter size of roots (10–20 μm), a trait conferring the ability to access very small soil pores that retain water and nutrients as soil dries. This allows to bypass the zones of water and nutrient depletion around the roots and, thus, a more extensive exploration of the soil (Miransari et al. 2007; Smith et al. 2010, 2011) that in turn may induce dense growth of roots (Miransari et al. 2007; Subramanian et al. 2006).

AM symbiosis is considered the most common strategy for enhancing P availability in the soil or P uptake capacity (Smith et al. 2011). Recent findings have provided new evidence for the contribution of the two well-recognized pathways (roots and fungal hyphae) by which P can be absorbed in mycorrhizal plants. These results suggest a pivotal role of a 'hidden P uptake' into plants via the AM fungal pathway (AM fungal hyphae; Smith et al. 2011), including when mycorrhizal plants experience conditions of drought stress (Smith et al. 2010). The authors suggested that the AM pathway may be active in P uptake even in plants that do not grow during drought conditions.

The relative contribution of the AM pathway to P uptake by plants and hence the contribution of direct uptake by roots under water stress has not yet been estimated. New molecular genetic studies investigating the expression of genes encoding high-affinity P transporters in the root cells of mycorrhizal plants will provide further information on the functional relevance of the direct pathway in P uptake and on the interplay of these two pathways of P uptake in AM plants when exposed to environmental conditions of stress (Smith et al. 2009; Smith and Smith 2011).

The fundamental contribution of P nutrition in the promotion of plant growth by AM symbiosis is well documented, but little information is available on the role of nitrogen (N) nutrition in the AM-mediated responses of plants to environmental limiting conditions, including drought. Even though few studies have investigated N uptake, an increased uptake of ammonium by fungal hyphae and the significant transfer of N from the fungus to the roots have been demonstrated (He et al. 2003), especially under drought conditions (Subramanian and Charest 1999). This increase was concomitant with increased activities of the main N-assimilating enzymes (Ruiz-Lozano and Azcón 1996).

Improved N uptake and assimilation have been associated with enhanced P nutritional status or is independent of P nutrition (Ruiz-Lozano and Azcón 1996). A recent review (Smith and Smith 2011) suggested that mycorrhizal plants could benefit from N uptake and transfer to the roots via the AM fungal pathway when exposed to water-limited conditions. Lee et al. (2012) recently investigated the role of N uptake and assimilation in the promotion of AM-mediated growth of perennial ryegrass using an N-labeled tracing technique. They found that AM symbiosis improved plant fitness under drought mainly by improving the plant water status and N uptake that, together with an enhancement of the activities of N-assimilating enzymes, resulted in increased amounts of proteins and amino acids.

The role of AM fungal hyphae in water uptake when water is limiting, as with P uptake, is still a matter of debate (Augé 2001; Smith et al. 2010). Difficulties in clearly interpreting the physiological and biochemical outcomes of AM symbiosis under drought conditions are due to the nature of AM symbiosis, because differentiating the effects of roots alone or of AM fungi alone from their combined effects is difficult (Ruth et al. 2011). This distinction becomes crucial when investigating the plant–fungus water relations where isolating the direct effect of AM symbiosis and understanding the real contribution of the AM fungi to the water balance of entire plants are also difficult. Specialized compartmented pot

systems have been designed for separating whole plants, including the root system, from the hyphal structure, but only a few attempts have been made to estimate the relative contribution of AM fungi to the total water uptake of the plant and the bulk flow velocity within the hyphae (Faber et al. 1991; Ruiz-Lozano and Azcón 1995).

In a recent study on barley plants inoculated with *Glomus intraradices*, Ruth et al. (2011) used a compartmented 'split plant-hyphal' chamber together with a specifically adapted online system for monitoring the soil water content to provide an accurate estimate of the water content of the two compartments and thus to derive the hyphal water flow. They monitored the presence of the water flow in the fungal hyphae and estimated the hyphal water flow at approximately 20 % of the total water uptake of the plant. These findings are consistent with earlier results that suggested a direct uptake and transfer to the host plants via the AM hyphae (Ruiz-Lozano and Azcón 1995; Marulanda et al. 2003; Khalvati et al. 2005), confirm previous estimates of the hyphal water flow (Faber et al. 1991; Cui and Nobel 1992) and support the premise of a significant contribution of fungal hyphae to plant water uptake (Allen 1982; Ruiz-Lozano and Azcón 1995). Discrepancies with other studies that found a low (Khalvati et al. 2005) or negligible contribution of the hyphae to the water balance of the plant (Cooper and Tinker 1981; Fitter 1985; George et al. 1992; Koide 1993) may be due to functional differences in the experimental designs of the compartmented systems.

In light of the enhanced water uptake by AM symbiosis during drought from improved P nutrition or growth, both of these mechanisms may also affect root hydraulic conductivity (Koide 1993). The hydration of leaves is indeed caused by the balance between the transpiration stream and water uptake by roots. AM symbiosis improves the plant water content by regulating the properties of plant hydraulics, including root hydraulic conductivity, although some authors have reported an enhanced (Sanchez-Blanco et al. 2004; Aroca et al. 2008) or reduced (Aroca et al. 2007; Ruiz-Lozano et al. 2009) effect of AM fungi on this trait. The role of the membrane transporter aquaporins in root hydraulic conductivity at the cellular level and their contributions to the transpiration stream have been investigated (Conner et al. 2013; Maurel et al. 2008) and will be discussed in the next section.

In addition to the effects of AM symbiosis on plant–water relations where AM fungi act independently and directly on nutrient and water uptake, AM symbiosis could increase drought resistance in plants through secondary actions such as the improvement of soil structural stability that in turn increases the retention of soil water (Augé 2001; Ruiz-Lozano 2003). AM fungal hyphae can enhance soil structure through the entanglement of soil particles to form aggregates and through the production of the glycoprotein glomalin (Rillig and Mummey 2006; Singh et al. 2011). AM fungi, in part due to their filamentous structure, also influence the development of soil structure both in the rhizosphere and in bulk soil (Miransari et al. 2007).

Augé et al. (2001) reported that the soil in which mycorrhizal plants were grown was characterized by more water-stable aggregates and substantially higher extraradical hyphal densities than the soils of nonmycorrhizal plants, and this

pattern correlated well with the improved retention of moisture of the mycorrhizal soil. By binding roots to the soil, fungal hyphae may even maintain liquid continuity and limit the loss of hydraulic conductivity caused by air gaps (Augé 2001; Augé et al. 2001).

New Insights into the Molecular Genetic Basis of Water Relations in AM Symbiosis Under Drought: Membrane-Protein Water Transporters

The physiological responses of mycorrhizal plants to drought stress can be regulated by the expression of drought-related plant genes, e.g., those involved in signaling and regulatory pathways or those encoding enzymes that synthesize functional or structural metabolites. Emerging insights are provided by studies on the regulation of important genes that encode significant components of the cellular water transport system, such as the aquaporins. These components are membrane proteins that channel water, uncharged molecules, across cell membranes in both roots and leaves (Conner et al. 2013; Maurel et al. 2008). These proteins may even increase root hydraulic conductivity and leaf water potential and decrease the transpiration rate in the leaves of mycorrhizal plants (Ruiz-Lozano et al. 2006, 2009; Aroca et al. 2008).

Both regulation and activity of aquaporin genes are modulated by conditions of water stress and thus have a potential role in the symbiotic exchange of water and nutrients between AM partners. Aquaporins are generally considered to be involved in the processes of symbiotic exchange at the plant–fungus interface, suggesting a fine regulation of water relations and the determination of the transport properties of the two partners (Maurel and Plassard 2011).

AM regulation of plant aquaporin genes under drought stress generally improves plant water status and drought tolerance (Aroca et al. 2007; Aroca and Ruiz-Lozano 2009; Li et al. 2012). In particular, the expression of genes encoding aquaporins has been demonstrated (Uehlein et al. 2007), and an aquaporin has been identified in AM fungal structures, both in the periarbuscular membrane and the extraradical mycelia (Aroca et al. 2009; Li et al. 2012). Both plant and fungal aquaporins are affected by stresses, including drought (Uehlein et al. 2007; Aroca et al. 2009; Li et al. 2012).

A relevant decrease in the expression of aquaporin genes in mycorrhizal plants compared to nonmycorrhizal plants has been observed under conditions of drought stress (Porcel et al. 2006 and Aroca et al. 2007), but other properties of these membrane proteins may also play a relevant role in the overall water relations of AM plants when water is limiting. An earlier study in *Phaseolus vulgaris* inoculated with *G. intraradices* found the commonly observed positive AM-mediated effect on plant water content but also found different effects of AM plant responses to drought on the regulation of aquaporins (Aroca et al. 2007). The authors

observed a lower expression of aquaporin genes in roots of mycorrhizal plants compared to nonmycorrhizal plants under drought conditions, suggesting that a mechanism of water conservation was employed by the AM plants. In the same experiment, AM symbiosis did not affect the phosphorylation state and amount of aquaporins and in particular the abundance of those proteins more functionally active in water transport, and this pattern was associated with a concomitant decrease in root hydraulic conductivity and foliar transpiration rates. The regulation of root hydraulic properties by AM symbiosis was strongly correlated with the regulation of aquaporin levels and phosphorylation state, and the authors suggested that down-regulating the activity of these proteins might provide a better explanation for these changes during water deficit. The drought-induced decrease in the transpiration stream observed in *Phaseolus* mycorrhizal plants, however, was concomitant with an increased free exuded sap flow, suggesting a higher water uptake from the soil in mycorrhizal plants compared to nonmycorrhizal plants under water-limited conditions and explaining the overall AM-improved water status (Aroca et al. 2007).

Li et al. (2012), however, recently reported an enhanced expression of two functional genes encoding aquaporins in both the roots of maize plants and in AM fungi when plants were subjected to drought stress. Since this pattern was concomitant with protein accumulation and a significant increase in root water content, the authors suggested that AM fungi improved plant water status by regulating the expression and activity of aquaporins in both plants and fungi. These studies provide molecular support for potential water transport via AM fungi to the host plant, suggesting that the simultaneous regulation of both expression and activity of aquaporins in host plants and fungi might represent a mechanism for enhancing plant tolerance to drought.

Another recent study used an appropriate inhibitor of aquaporin activity and an apoplastic tracer dye to separately measure the flow of water through the apoplastic pathway and via the root aquaporins ('cell-to-cell' pathway; Bárzana et al. 2012). The authors found an enhanced apoplastic water flow in the mycorrhizal roots that was competitive to the 'cell-to-cell' pathway during drought stress. The ability of AM plants to switch between the two transport pathways has thus been hypothesized as a mechanism that confers a higher flexibility in drought responses compared to nonmycorrhizal plants.

The mechanisms of nutrient exchange between the symbionts are not well defined, so the study of these membrane proteins should also provide a better understanding of the preferred mechanism of nutrient exchange in this symbiotic association. Recent findings suggest a potential involvement of the aquaporins themselves. Uehelin et al. (2007) identified various transmembrane aquaporins in the periarbuscular membranes of *Medicago truncatula* and found an AM-induced expression of specific aquaporin genes. They also suggested that aquaporins could act as low-affinity transporters of ammonia and/or ammonium. Further research is evidently necessary to fully understand the contribution of aquaporin genes to the enhanced drought resistance of AM plants.

Ecological Effects of AM Symbiosis: Ecosystem Services

Plants in ecosystems perform a series of functions (defined as 'ecosystem services') that are beneficial to the well-being of humans, providing multiple resources and processes (Daily 1997). Trade-offs and links between plants and soil microbial communities can act as drivers of a wide range of processes in ecosystems (Lavorel 2013 and Grigulis et al. 2013). Given the beneficial functions of AM fungi on plant fitness, resilience against environmental stresses, nutrient cycling, and soil quality, AM symbiosis is now recognized to play a fundamental role as a provider of ecosystem services.

Various ecosystem services delivered by AM have been identified: biofertilization from the AM promotion of plant growth, which in turn reduces fertilizer requirements, stabilization of soil structure, and bioregulation consequent to the plant metabolic modifications by AM fungi (Gianinazzi et al. 2010). Linking functional traits of plants and soil microbes, such as AM fungi, with their delivery of multiple ecosystem services is currently considered a rational mean for assessing the functioning of a given ecosystem (De Bello et al. 2010).

Less attention, however, has been given to beneficial soil organisms in general and AM in particular and their influence on the processes of ecosystems that contribute to the ecosystem services in agroecology. In this context, Gianinazzi et al. (2010) recently examined several aspects of plant–AM combinations that should be investigated further for appropriately managing the contribution of mycorrhizal fungi to ecosystem services and thus for optimizing the impact of these beneficial organisms while guaranteeing plant productivity and quality in agrosystems. The positive effect of AM on the ability of plants to counteract the conditions of drought confers to AM a pivotal role as a valuable technology not only for the sustainability of agricultural systems, but also for the restoration of degraded natural arid and semi-arid areas, where multiple environmental stresses, including drought, occur (Gianinazzi et al. 2010; Barea et al. 2011).

In light of the assessment of the multiple ecosystem services provided by AM, critical advances are required for elucidating the functional importance and value of plant and mycorrhizal diversity that are necessary for the functioning of ecosystems. These are also required for clarify the links among plant traits and their associated AM fungal characteristics to quantify the contribution of plant–AM fungi associations to ecosystem services under various environmental constraints (Barea et al. 2011; Grigulis et al. 2013; Lavorel 2013).

The role of AM symbiosis in the functional traits of both plants and microbes that could characterize above- and belowground ecosystem services has not yet been explored. Despite the recent advance in knowledge on mycorrhizal functioning, further research is necessary to better understand the significance and value of AM symbiosis in delivering ecosystem services in both agrosystems and natural environments.

An appropriate assessment of plant–AM feedbacks is therefore essential for predicting the effects of environmental constraints such as drought on ecosystem processes and, thus, for the provision of ecosystem services. Various advanced approaches can provide new insight to this field. The application of a trait-based approach to both plant and AM fungal communities represents a promising opportunity to understand how functional AM feedbacks between plant and AM fungi translate into interactions between ecosystem services (Lavorel 2013).

The new field of system biology that investigates plants at an ecological level, including all relationships and networks of plant communities, benefits from the different 'omic' technologies, from transcriptomics to proteomics, functional genomics, and metabolomics. New advances are represented by the emerging 'ecometabolomic' approach that aims to dissect the global metabolomic response of an organism to environmental changes (Sardans et al. 2011; Peñuelas et al. 2013). In particular, this new 'omic' system will allow the detection of the main metabolic pathways responsible for organismic responses and could provide improved knowledge of plant and mycorrhizal genes and their regulatory networks involved in the responses.

These integrated studies should provide the possibility of extrapolating plant responses from individual components to the level of ecosystems and of taking a step forward in our knowledge of the mechanisms and processes underlying the changes in resource use under future global change (Peñuelas et al. 2013). These pioneering approaches provide interesting perspectives and a very valuable framework for further studies focusing on integrated analyses of the effects of AM symbiosis under abiotic constraints for better quantifying the ecosystem services delivered by symbiosis, which has important implications for ecosystems in water-limited environments under future climatic changes.

Concluding Remarks

To summarize, mycorrhizal plants employ various protective mechanisms to counteract drought stress. Considerable progress has been made in understanding the role of AM symbiosis in conferring drought resistance to plants, but different aspects still require attention for unraveling novel metabolites and hidden metabolic pathways. The accumulated physiological, biochemical, and molecular data based on classical approaches will benefit from the various 'omic' techniques and their combinations. An in-depth investigation using the advanced methodologies could help to elucidate the mechanisms of drought avoidance and/or tolerance induced by AM symbiosis and to discriminate the drought-induced processes of the protective mechanisms regulated by AM symbiosis.

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Chapter 3
Role of Arbuscular Mycorrhizal Fungi in Alleviation of Acidity Stress on Plant Growth

Thangavelu Muthukumar, Perumalsamy Priyadharsini, Eswaranpillai Uma, Sarah Jaison and Radha Raman Pandey

Introduction

A large number of abiotic and biotic factors influence the establishment, health, and productivity of plants in both natural and agroecosystems. Among these, soil factors influence various plant processes to a greater extent since soil is the natural substrate for plants to anchor and take up nutrients and water. Around 30–40 % of the 1.44 billion ha arable land worldwide has suboptimal conditions for crop growth and thus has an adverse influence on agriculture (FAO 1992). Soil fertility is one of the major determinants for plant growth in both natural and agricultural ecosystems.

The adverse effects of soil fertility on plant growth and yield are mainly due to the deficiency of one or more essential nutrients necessary for plant growth. Factors such as acidity, alkalinity, salinity, erosion, and farming practices are the main causes for the decline in the availability of nutrients in the soil. Among the various factors that influence soil fertility, soil acidity is an important factor affecting plant growth worldwide (Iqbal 2012).

Soil pH is a highly sensitive factor, as it determines plant's survival, distribution, and its interactions with microorganisms, which are rather vital for the availability of essential nutrients and soil fertility (Marschner 1995). An increase in the H^+ ion concentration in the soil solution results in a decrease in soil pH, and soils with a pH < 5.5 or lower are categorized as acid soils. These soils occupy around 30 % (4 billion ha) of the world's total land area and 50 % of the world's cultivable lands (Von Uexküll and Mutert 1995; Baligar et al. 2001). Further, more than half of the world's acid soils (60 %) occur throughout the tropics and

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subtropics (Baligar and Fageria 1997; Fischer 1998). Therefore, acid soils affect crop yields in many 'hunger hot spots' of the world.

In natural ecosystems, soil acidity determines the availability of mineral nutrients such as phosphorus (P) and also determines the level and severity of phytotoxic elements such as aluminum (Al), manganese (Mn), and iron (Fe) (Kochian et al. 2004). When Al concentration increases in the soil solution in response to a reduction in pH, induction of reactive oxygen species and lipid peroxidation damage of root plasma membrane occur reducing root growth and plant's response to stress conditions (Yamamoto et al. 2001, 2002). Though Al ions present in acidic soils prevent the intrinsic toxicity of H^+, it can concurrently cause an extrinsic toxicity through calcium (Ca) and magnesium (Mg) deficiency (Kinraide et al. 2005).

Causes for Soil Acidity

Natural causes for acid soils include high rainfall, resulting in leaching of basic cations, acidic parental material, and decomposition of organic matter. Biological processes such as root and microbial respiration and uptake of cations such as ammonium (NH_4^+) also influence soil pH. Cultivation of legumes acidifies soils more as they take up more cations than anions compared to non-legumes. In addition to these above-mentioned natural causes, human activities, such as the extensive use of NH_4^+ fertilizers for crop production, industrial emission of nitrogen oxides and sulfur di-oxide resulting in acid rain and mining activities, all contribute to the acidification of soils.

Acid rain, an environmental hazard, is one of the primary reasons for soil acidification. Acid rain results in the leaching out of basic cations, reduces evaporation, releases bound Al into the soil solution, and increases the oxidative biological activities (Carver and Ownby 1995). During precipitation, water percolates through the soil particles washing away the basic cations from the soil, which are replaced by acidic cations such as Al^{3+}, Mn^{2+}, and H^+ ions (Sumner et al. 1991). However, the CO_2 containing water molecules entering the soil profile replaces the free salts quite rapidly in contrast to basic cations, which are replaced rather more slowly. This results in acidic soils under high rainfall regions (Brady 1990). Increased presence of SO_4^{2-} ions in rain water leads to the considerable eradication of H^+ and other cations from the soil profiles (Overrein et al. 1980). Biological oxidation of carbon (C), nitrogen (N), and sulfur (S) in the way of burning fossil fuels also results in acid rain.

Modern agriculture mainly focuses on higher yields with large inputs of synthetic fertilizers. However, the chemicals present in these fertilizers react with the soil mineral nutrients, resulting in changes in the soil pH. This indirectly affects plant growth and health. It has also been shown that the forms of N present or applied could influence soil pH (Marschner 1995). A significant correlation between soil pH and Al^{3+} was reported by Rout et al. (2001) in acidic soils in response to trim down the basic ions.

Results of Soil Acidity

Soil acidification leads to changes in the soil environment as well as in plant growth and metabolism, which can be summarized as follows:

1. Increase in the availability of Al, Mn, and H^+ ions in the soil solution (Kochian et al. 2004, 2005).
2. Reduction in the availability of essential nutrients such as P, N, Mg, Ca, molybdenum (Mo), and zinc (Zn) (Kochian et al. 2004).
3. Negative effects of Al and other ions on plant growth especially the root system resulting in reduced nutrient and water uptake (Barcelo and Poschenrieder 2002).
4. Defects in shoot growth and appearance of necrotic spots due to Mn toxicity (Schier and McQuattie 2000).
5. Changes in plant physiology, metabolic, and biochemical activities leading to mortality (Heijne et al. 1996; Kochian et al. 2005).
6. Accumulation of organic acids in the roots (Adams et al. 1999; Kinraide et al. 2005).
7. Changes in microbial populations and their activities, which are known to affect plant growth (Miller and Kissel 2010; Kaps and Kering 2011; Chen et al. 2012).

Influence of Soil Acidity on Al Availability and Toxicity

The third most ubiquitous element Al is a light metal comprising of 7 % of the earth's crust and usually represented in the form of oxides and aluminosilicates (Ma et al. 2001). In the soil solution, Al is present as $Al(OH)^{2+}$ and $Al(OH)_2^+$ at pH 4–5, Al^{3+} at pH 5.5–7, and $Al(OH)_4^-$ at pH 7–8 (Drabek et al. 2003). Nevertheless, soils differ in their potential to sustain it (Scancar and Milacic 2006). Forms of Al such as $AlSO_4^+$ and $Al(SO_4)_2^-$ or Al–F lack rhizotoxicity.

According to Kochian (1995), toxicity has been convincingly demonstrated only for Al_{13} and Al^{3+}. Consequently, when the soil pH drops to below 5.5, Al containing compounds tend to dissociate, resulting in the abundance of aluminum-hydroxy cations and Al^{3+} in soil solutions. In soils, the soluble forms of Al are present in two forms: monomeric in the form of Al^{3+}, Al–OH, Al–F, and Al–SO_4 (highly toxic) and acid-soluble Al in the form of polymer state (less toxic) (Xu and Ji 1998).

The Al^{3+} also forms mononuclear species that are more toxic in nature (Kochian 1995; Panda and Matsumoto 2007). Even at micromolar level, Al^{3+} ions can modify the morphology and physiology of plant roots as well as alter the activities of certain enzymes (Simon et al. 1994; Alvarez et al. 2012). Under acidic conditions, the complex forms of Al dissociate, resulting in the release of toxic form of Al as shown below:

$$Al\ (OH)_3 + 3H^+ \leftrightarrow Al^{3+} + 3H_2O$$
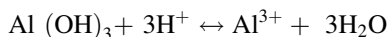
Aluminum toxicity is one of the key factors that are harmful for plant growth in acidic soils. Acid soils generally have high amounts of the mineral oxides, which readily inactivate or fix P by precipitation or forming complexes of Al and Mn oxide radicals, thus making it unavailable. The symptoms of Al toxicity in plants include inhibition of root growth, decline in the uptake of water and other essential nutrients (N, P, and Ca), and overall stunting of plant growth (Matsumoto 2000; Purcell et al. 2002; Fukrei et al. 2011). Formation of both primary and lateral roots is affected by high concentrations of Al in the soil solution, and even when the roots are formed, they are devoid of root hairs, thickened, brittle, and brown in color (Wang et al. 2006; Claudio et al. 2008; Gazey and Davies 2009; Bhalerao and Prabhu 2013). Aluminum is strongly adsorbed onto the plant root surface either by the exchange process or by formation of complexes.

Influence of Soil Acidity on Mn Availability and Toxicity

Manganese is an essential micronutrient that plays a vital role in plant metabolism but toxic when present in excess. Manganese aids in the synthesis of chlorophyll and assimilation of nitrate and activates enzymes involved in the fat biosynthesis. Functional role of Mn involves the formation of riboflavin, ascorbic acid and leaf carotene. Normal or adequate level of Mn in plants is 30–500 mg/kg dry mass (Clarkson 1988), and deficiency occurs when the levels drop below 10–20 mg/kg dry mass (Marschner 1995). Manganese toxicity is an important factor limiting plant growth in acidic soils and especially in poorly drained soils (Horst 1988a, b; Delhaize et al. 2004). Manganese toxicity is possibly the second most important metal toxicity limiting crop production in acid soils next to Al (Foy et al. 1973; Sumner et al. 1991).

Manganese availability in the soil solutions is strongly dependent on soil pH. The availability of Mn increases in the soil as pH decreases. Soils tend to become deficient in Mn at pH 6.5 and toxic when the pH drops below 5.5 (Hue et al. 2001; Kochian et al. 2004; Ducic and Polle 2005). The Mn toxicity symptoms in plants include stunted growth and necrotic spots on shoots (Alam et al. 2000), but the physiological mechanisms for these symptoms are still elusive. Greenhouse experiments carried out to determine the adequate and toxic levels of Mn in five different crop species [rice (*Oryza sativa*), common bean (*Phaseolus vulgaris*), maize (*Zea mays*), soybean (*Glycine max*), and wheat (*Triticum aestivum*)] in an Oxisol indicated that 60–520 mg/kg of Mn was adequate for plant growth and 720–4,560 mg/kg of Mn was toxic to plant species (Fageria 2001).

Plants Tolerant to Acid Soils

Intense research has been carried out over the past two decades to identify, characterize, and understand the mechanisms adopted by plants to survive and thrive in acid soils. The results of these investigations reveal that three possible group of mechanisms appear to operate in plants to tolerate acidic conditions. These include the following: (1) exclusion of toxic ions such as Al and Mn from the root apex, (2) tolerance to toxic levels of Al and Mn through detoxification in the plant symplasm, and (3) enhanced efficiency in the uptake of limiting nutrients from acid soils (Kochian et al. 2004; Bhalerao and Prabhu 2013) (Fig. 3.1).

In the exclusion of toxic ion mechanism, roots tend to release organic acids such as maltate, citrate, and oxalate in response to the presence of metal ions in the soil solution (Hue et al. 1986; Adams et al. 1999; Kinraide et al. 2005; Iqbal 2012). These organic acids complex with the toxic ions in the rhizosphere and prevent their entry into roots. Therefore, tolerant crop genotypes such as wheat, maize, and sorghum (Sorghum bicolor) accumulate toxic ions several folds less in their tissues than the sensitive genotypes. Plants with internal detoxification mechanism complex toxic metal ions with organic acids (e.g., Al-oxalate) and store them in the vacuoles. Thus, plant like buck wheat can accumulate Al as high as 15,000 ppm in their leaves when grown on acid soils (Ma et al. 2001).

Phosphorus availability is one of the major constrains for plant growth in most of the tropical soils. Generally, the low availability of P in the soils is due to its low mobility, fixation into organic forms, and high adsorption to soil particles (Marschner 1995). In acid soils, P availability is limited due to its fixation with Al and Fe oxides on the clay particles (Kochian et al. 2004). Therefore, P is one of the major limiting factors for plant growth in acid soils. Nevertheless, plants have developed several morphological, physiological, and biochemical adaptations to acquire P from such acid soils. These include mechanisms for increased P mobilization and uptake, changes in root structure, and association with arbuscular mycorrhizal (AM) fungi.

Plant Mechanisms for P Mobilization in Acid-Stressed Soils

Exudation of organic acids is one of the most common mechanism plants adopt to mobilize P in acid soils. Phosphorus deficiency triggers the exudation of organic acids such as malate and citrate from the roots which dissociate bound P from mineral surfaces, solubilizes it from Al, Fe and Ca oxides and hydroxides through metal complexation. Such type of organic acid exudation also occurs in cluster roots, which are produced in response to P stress (Wasaki et al. 2003). There is also enhanced mobilization of sparingly available P through proton secretion (Yan et al. 2002; Gao et al. 2010; Yang et al. 2013). In addition, many plants exude phosphatases and RNAases under P stress. Plant phosphatase activity is not
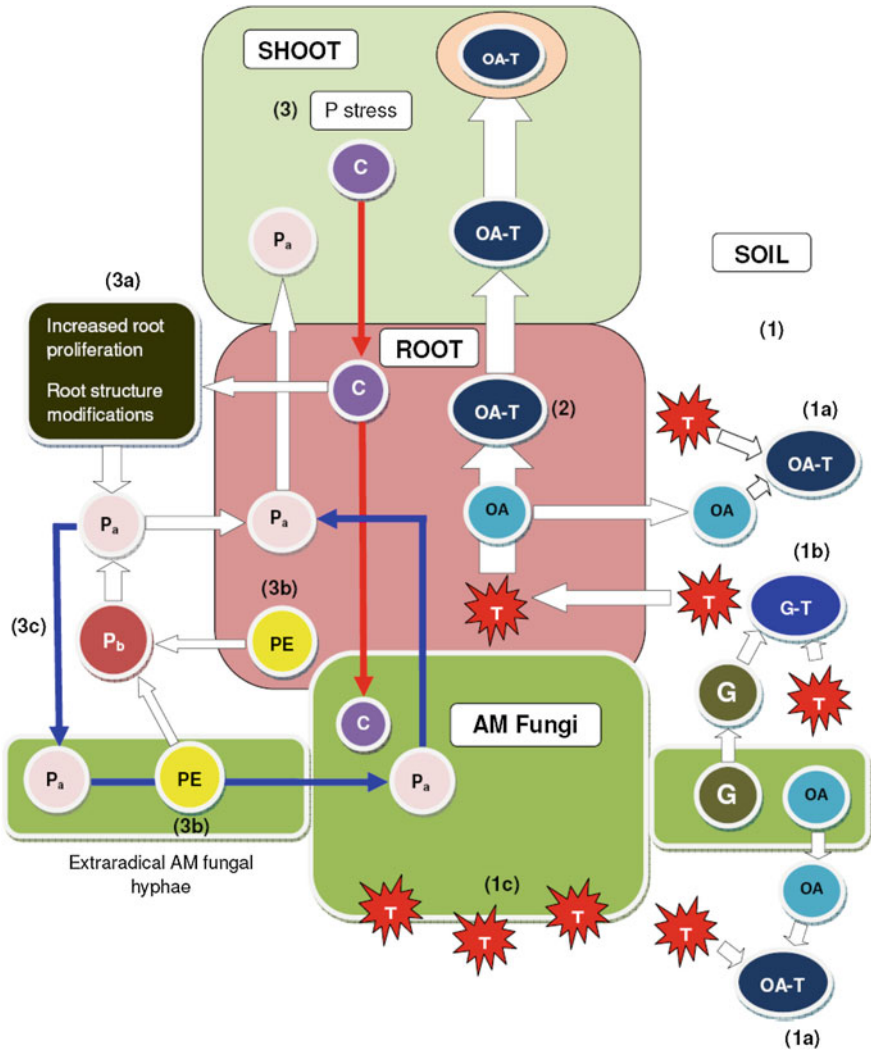
Fig. 3.1 Schematic presentation of the different mechanisms involved in plants acid tolerance. *1*. Exclusion of toxic ions, *1a*. organic acid (*OA*) complexation with toxic ions (*T*) to form *OA-T* complex, *1b*. binding of toxic ions with glomalin (*G*) of arbuscular mycorrhizal (*AM*) fungus forming a complex (*G-T*), and *1c*. binding of toxic ions to AM fungal structures; *2*. internal detoxification of toxic ions. Toxic ion complexation with organic acids (*OA-T*) and their storage in vacuoles; *3*. tolerance to phosphorus (*P*) stress: increased translocation of carbon (*C*) to roots, *3a*. changes in root structure and function, *3b*. phosphatases (*PE*) produced by roots and hyphae of AM fungi dissociates the bound P(P_b), and *3c*. uptake of available P(P_a) by AM fungal hyphae and their transfer to plant roots

constant, but may vary greatly across plant species and environmental conditions (Venterink 2011). These enzymes catalyze the hydrolysis of organic P, thereby enabling its uptake by roots. Furthermore, overexpression of transcription factor genes such as *OsPTF1*, *AtPHR1*, and *OsPHR2* enhances P uptake and accumulation under P-limiting conditions (Nilsson et al. 2007; Zhou et al. 2008). Likewise, plants with overexpression of the regulatory element miR399 tend to accumulate more P in plant parts under P-limiting conditions (Chiou et al. 2006; Lin et al. 2008; Gao et al. 2010).

One of the efficient strategies plants adopt to improve P uptake in the soils low in available P is to modify the architecture and morphology of their roots, thereby increasing the surface area of roots that are in contact with the soil. These could be achieved in several ways: (1) increasing the root:shoot ratio through modified allocation of carbon to the root system, (2) increased branching and production of thinner roots, (3) production of more profuse and long root hairs, and (4) formation of special type of roots such as cluster or proteoid roots (Niu et al. 2013).

Arbuscular Mycorrhizal Fungi

Another most common strategy plants adopt to uptake P from acid soils is to associate with the most common and widespread AM fungi. Belonging to Glomeromycota, AM fungi associate with roots of over 80 % of the wild and cultivated plant species (Selosse and Rousset 2011). Plants depend on AM fungi for the uptake of nutrients, especially P from nutrient-stressed soils, and the fungi in turn depend on plants for carbon (Smith and Read 2008).

Arbuscular mycorrhizal fungi contain two distinct phases: one within the roots (intraradical) that enables the transfer of nutrients taken up from the soil in exchange for carbon and another in the soil that is involved in nutrient exploration and reproduction. The extraradical fungal hyphae can be further distinguished into runner and absorptive hyphae. The runner hyphae grow externally to the root system and run between the root segments of single or multiple hosts. The main function of the runner hyphae is to initiate new colonization points (appressoria) on the root epidermis. The absorptive hyphae arising from the runner hyphae extend beyond the nutrient depletion zone and take up the inorganic minerals, especially P from the soil and translocate it to the host (Marschner 1991). Phosphorus is not easily accessible to plants in acidic soil due to its sparingly or insoluble nature. However, under such P-deficient conditions, mycorrhizal roots or AM fungal hyphae secrete phosphatase or phytase enzyme to solubilize insoluble P (Khalil et al. 1994; Tawaraya et al. 2006).

The AM fungi shield the root system of the host plant from the toxicity of Al and other ions under acidic conditions (Marschner 1995). It is well known that the effectiveness of AM fungal species in supporting P transfer to the host plant differs in response with the extent of colonization (Abbott and Robson 1981; Kittiworawat et al. 2010). Likewise, plant genotypes also exhibit variation in

tolerance to acidic conditions similar to AM fungal isolates (Sieverding 1991; Clark et al. 1999a, b). The AM fungal species tends to differ in their response to varying soil pH (Sano et al. 2002). Nevertheless, most investigations on the influence of soil acidity on AM fungi have focused on selecting suitable AM fungal species for growing plants in acidic soils (Cavallazzi et al. 2007).

Distribution of AM Fungal Spores and Phylotypes in Acid Soils

Though fewer or no spores have been found in acid soils with pH less than 5.5 (Wang et al. 1993), AM fungal spores have been found in acidic soils with pH as low as 2 (Cano et al. 2009). Distribution of certain AM fungal species appears to be strongly influenced by soil acidity. For example, spores of *Funneliformis mosseae* do not occur in soils with pH < 5.5 (Sieverding 1991). Although taxa of acidic soils mostly comprise species belonging to *Acaulospora* in soils with pH < 3.6–6.2 (Morton 1956; Oehl et al. 2006), spores of other taxa such as *Glomus* (Cano et al. 2009) and *Scutellospora* (Walker et al. 1998) have also been reported in low-pH soils with pH < 3.0. Further, spores of particular taxa could also occur in a wide range of acidic pH levels. For example, spores of *Glomus corymbiforme* have been reported to occur in acid soils, with pH ranging from 3.8 to 6.7 (Blaszkowski 1995).

A study on the distribution and abundance of AM fungi in Western Australian soils indicated that *Acaulospora* was the predominant fungus in low-pH soils or was the only species to be present in soils with pH < 5.0 (Nicolson and Schenck 1979). The influence of soil acidity on the restricted distribution and diversity of AM fungi do not always hold true. An assessment of AM fungal spore populations associated with sugar maple (*Acer saccharum*) in Eastern Canada showed that AM fungal species richness (number of spore morphotypes) and abundance were maximum in high acidic soils (pH 4.3) compared to moderate (pH 5.6–5.7) and low acidic soils (pH 6.0–6.3) (Moutoglis and Widden 1996).

An assessment of the community structure of AM fungi associated with *Miscanthus sinensis* in acid sulfate soils (pH 2.7–5.4) by An et al. (2008) suggested that soil pH could be the driving force for shaping up the community structure. In a later study, Higo et al. (2011) found that seven operational taxonomic units of AM fungal both from roots of *Wedelia* and from spores belonging to *Acaulospora*, *Glomus*, *Paraglomus*, and *Entrophospora* were reported in acid sulfate soils with a pH of 3.24 from Thailand.

Siqueira et al. (1990) showed that AM fungal spore production and species compositions were highly affected by changes in soil pH. As liming of acid soils favored the presence of *Claroideoglomus etunicatum*, *Rhizophagus diaphanus* and *Paraglomus occultum* originating from non-acidic soils predominated unlimed soils. Further, sporulation of *Gigaspora margarita* was abundant in unlimed soils,

but was rare in limed soils. A similar observation was noted by Coughlan et al. (2000) while examining the pH-induced changes in the diversity and sporulation of AM fungi associated with healthy and declining maple forests. While species such as *Rhizophagus clarus* and *Acaulospora* spp. sporulated in a wide range of soil pH from 4 to 7, certain species such as *Scutellospora calospora* sporulated only in soils with pH 5 or above.

Influence of Soil Acidity on AM Colonization

Many plants thrive at soil pH < 4 (Falkengren-Grerup 1994), and roots of these plants either lack or are minimally colonized by AM fungi (Higo et al. 2011). Arbuscular mycorrhizal fungal colonization has been observed in plants growing in soils with pH as low as 2.7 (Daft et al. 1975) and 3.5–3.9 (An et al. 2008). Studies by Clark et al. (1999a, b) have shown that root colonization of switchgrass (*Panicum virgatum*) by species of *Acaulospora*, *Claroideoglomus*, *Gigaspora*, *Glomus*, and *Rhizophagus* tended to decline with increasing soil acidity (Fig. 3.2). In contrast, root colonization in *M. sinensis* was maximum when raised on high acidic sulfate soils (up to 63 %, pH 3.5–3.9) compared to those raised under less acidic conditions (1.9 to 15.6 %, pH 5.4–6.1). However, the reduction in AM fungal colonization of root with increasing soil acidity has been reported in a number of species such as *Leucaena leucocephala* (Habte et al. 2011), *A. saccharum* (St Clair and Lynch 2005), barley (*Hordeum vulgare*) (Borie and Rubio 1999), *Clusia multiflora* (Cuenca et al. 2001), apple (*Malus prunifolia*)
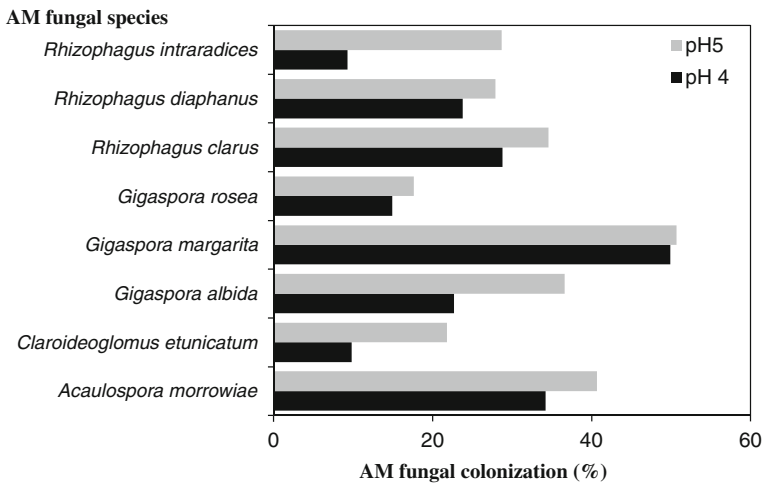

Fig. 3.2 Influence of soil acidity on the extent of root colonization by different arbuscular mycorrhizal (AM) fungi in switchgrass (data from Clark et al. 1999a)

(Cavallazzi et al. 2007), *Maianthemum bifolium, Glium odoratum, Mericurialis perennis, Stellaria memorum* (Postma et al. 2007), *Bupleurum falcatum, Cinidum officinale, Gentiana lutea* (Ueda et al. 1992), mung bean (*Phaseolus radiata*), and crotalaria (*Crotalaria mucronata*) (Lin et al. 2001).

Studies on the influence of soil acidity on root colonization by AM fungi also indicate the levels of total colonization, and root length with different AM fungal structures could vary with both the host and fungal genotypes. Root colonization of barley by *C. etunicatum* was found to be higher for the cultivar that was tolerant to Al (37.4 %) than for the cultivar that was sensitive to Al (26.9 %) raised on acidic soils (pH 5.15–5.70). Similarly, Habte et al. (2011) also showed that colonization of *L. leucocephala* roots by *G. aggregatum* varied with cultivars raised on acidic soils (pH 4.5).

Like the host genotypes, AM fungi also vary in their response in colonizing host roots under acidic conditions. For example, root colonization of switchgrass by different *Gigaspora* species (*G. albida, G. rosea,* and *G. margarita*) in acidic soils indicates differences in the extent of colonization (Clark et al. 1999a, b) (Fig. 3.2). Production of intraradical hyphae by *Rhizophagus* species and arbuscule production by *Gigaspora* species in switchgrass was found to be higher at low pH (Clark et al. 1999a) (Fig 3.3).

The influence of soil acidity on root colonization by AM fungi could be due to its effect on spore germination (Lambais and Cardoso 1989) and/or hyphal regrowth from mycorrhizal roots (Abbott and Robson 1985). However, the tendency of root colonization to increase with pH can be either due to the increase in the number of taxa involved in colonization or due to an enhanced ability of the associated taxa to colonize host roots (Yoshimura et al. 2013). The first possibility is supported by the observations of An et al. (2008) where the number of AM phylotypes detected in roots of *M. sinensis* increased with increasing soil pH. The second possibility is supported by a study by Clark (2002) who showed that five of the eight AM fungal species showed higher colonization levels in switchgrass with increasing soil pH.

Role of Soil pH on Extraradical Hyphae

The role of extraradical mycelium growing out from colonized roots in the symbiosis is well documented. In addition to initiating colonization of new roots, the extraradical mycelium acts as an extension of the root system in enhancing plants access to soil nutrients and water (Rohyadi 2008). Although the production of extraradical mycelium is an inherent characteristic of the fungi (Abbott and Robson 1985), it could be substantially influenced by soil conditions (Abbott and Robson 1981). In spite of their importance, studies on the effect of soil acidity on extraradical mycelium are limited. These limited studies suggest that the ability of AM fungi to form extraradical mycelium differs with substrate pH (Abbott and Robson 1985;
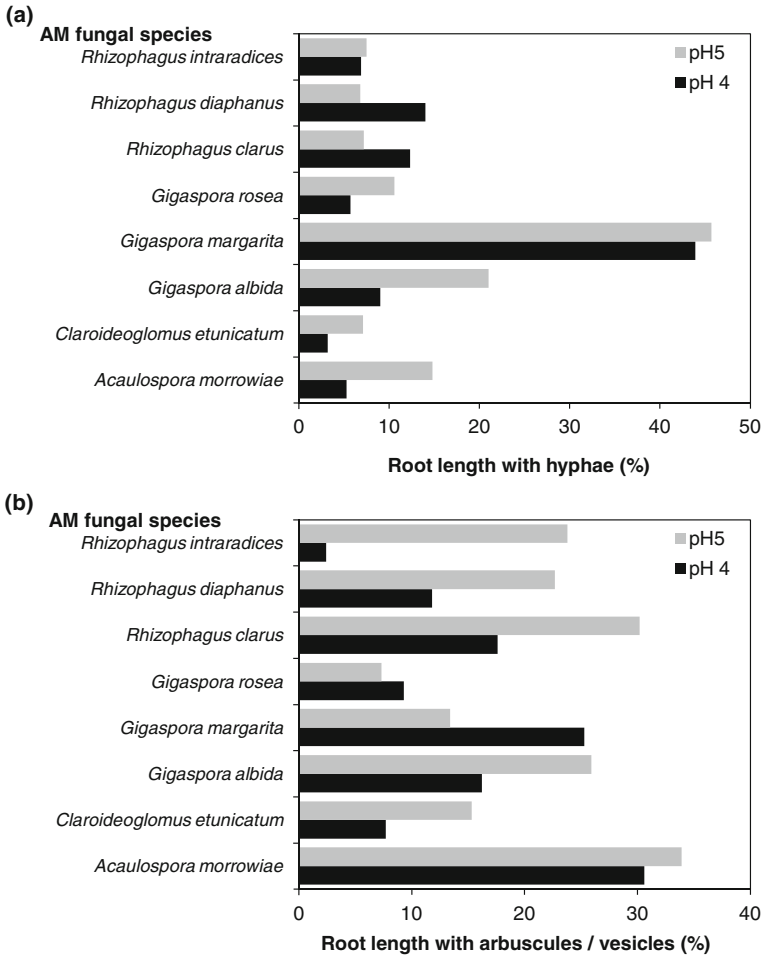
Fig. 3.3 Influence of soil acidity on root length containing hyphae **a** and arbuscules/vesicles **b** in switchgrass colonized by different arbuscular mycorrhizal (AM) fungi (data from Clark et al. 1999a)

Porter et al. 1987). van Aarle et al. (2002) tested the response of extraradical mycelia formation of two AM fungi, *S. calospora* and *Rhizophagus intraradices*, exposed to different acidic pH levels (4 and 5 or 6). The results of this study indicated that though both AM fungi were capable of forming extraradical mycelium at the higher pH, no detectable extraradical mycelium was detected for *R. intraradices* at lower pH.

Abbott and Robson (1985) showed that the spread of extraradical mycelium by a *Glomus* isolate was strongly inhibited at low soil pH, which was speculated to be caused by the aversion to the substrate (van Aarle et al. 2002). Similarly, extra-radical mycelia formation by *G. margarita* originating from an acid soil was found
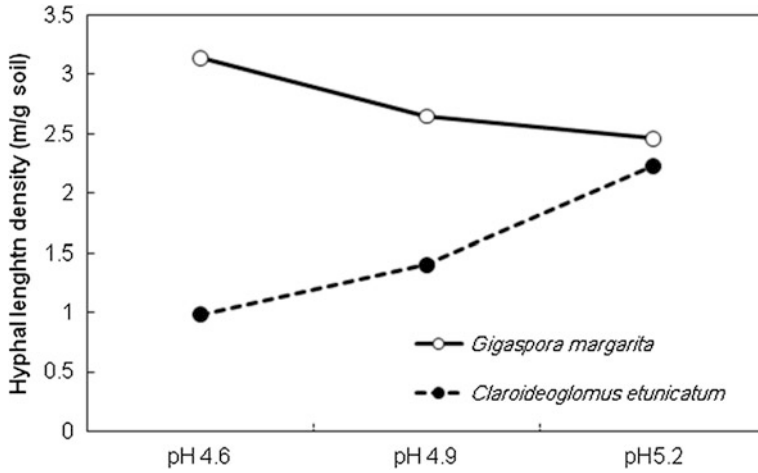
Fig. 3.4 Extraradical hyphal length density of *Gigaspora margarita* and *Claroideoglomus etunicatum* at different soil pH (data from Rohyadi 2008)

to be higher in low-pH conditions (4.6–5.6), whereas *C. etunicatum* also originating from an acid soil required a pH of 5.2 or higher for increased extraradical mycelia formation (Rohyadi 2008) (Fig. 3.4). These observations clearly suggest that the quantity of extraradical mycelium produced depends on specific pH ranges even for taxa originating from acid soils. In addition to these, host species could also influence the quantity of the extramatrical hyphae to certain extent as shown by Lin et al. (2001). Fungal species such as *Diversispora epigaea* and *Rhizophagus manihotis* produce more extraradical mycelium when associated with crotalaria than with mung bean (Fig. 3.5). The enzyme activities such as the alkaline
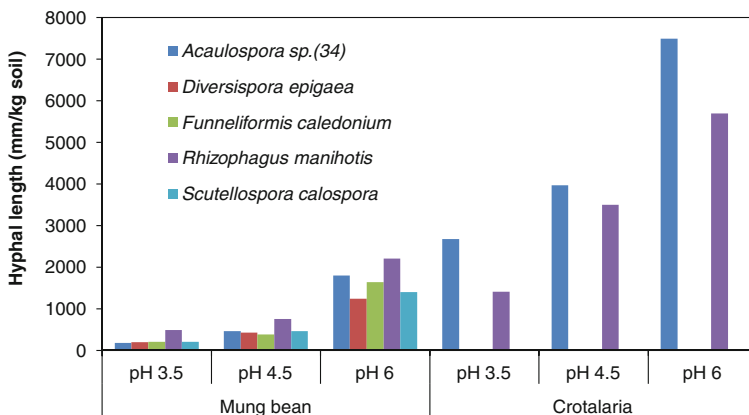
Fig. 3.5 Influence of soil acidity on extraradical hyphal length of different arbuscular mycorrhizal fungi in the rhizospheres of mung bean and crotalaria (data from Lin et al. 2001)

phosphatase and NADH-diaphorase activities in the external mycelium of AM fungi appear to be more sensitive to soil acidity (Vosatka et al. 1999; Malcova et al. 1999).

Effect of Soil Acidity on AM Spore Germination

Soil pH is one of the important soil factors that play a vital role in AM spore germination and presymbiotic hyphal growth. Most of the information on the influence of substrate acidity arises from the *in vitro* monoxenic cultures of AM fungi. A pH of 5.5 is usually maintained for standard monoxenic culture systems to maintain solubility and balance of the media components (Dalpé et al. 2005). However, the standard acidic pH maintained in monoxenic culture systems could affect the growth of certain AM fungal isolates. Maximum spore germination of *Acaulospora laevis* occurs between pH 4 and 5 and between pH 5 and 6 for *Racocetra coralloidea* and *Fuscutata heterogama* (Hepper 1984; Green et al. 1976).

The optimum pH for spore germination appears to be linked to the pH of the soil where the AM fungus originated. For example, the germination percentage of *A. laevis* spores originating from acidic soils tend to decline with increasing pH and the germination percentage drops to 10 % or less in neutral and alkaline soils (Hepper 1984).

Vosatka et al. (1999) tested the influence of simulated acid rain individually, or along with Al, amendment was on the germination of AM fungal spores belonging to *F. mosseae, Claroideoglomus claroideum,* and *Acaulospora tuberculata* associated with the rhizosphere of *Deschampsia flescuosa* seedlings (Vosatka et al. 1999). The results of this study suggested that *A. tuberculata* originating from high acidic soil exhibited greater tolerance to soil acidity than others.

Growth of AM Plants in Acid Soils

Compared with the amount of work done on the role of AM fungi on plant growth in non-acidic soils, less research has been done on acidic soils. The limited studies that have examined the role of AM fungi on plant growth in acidic soils have clearly revealed several benefits imparted by AM fungi on their associated host plants. In a greenhouse experiment, Heijne et al. (1996) determined the cause for the decline of two heathland herbs *Arnica montana* and *Hieracium pilosella* by growing them in the presence or absence of an AM fungus (*Rhizophagus fasciculatus*) on an extremely nutrient-poor sandy soil and irrigated with nutrient solutions with pH values ranging between 2.5 and 5.5. The results of the study showed that *A. montana* failed to survive and *H. pilosella* hardly grew in the absence of AM fungus, suggesting that AM symbiosis decreased the stress caused by soil acidity. Growth and mycorrhizal dependency of switchgrass varied with

AM fungal species and pH of the soil (Clark 2002) (Table 3.1). Shoot dry weights of switchgrass colonized by *G. margarita*, *G. albida*, and *C. etunicatum* were higher in low-pH soil than at a slightly higher-pH soil (Clark 2002) (Fig. 3.6).

Both shoot biomass and root biomass of *C. etunicatum*-colonized wheat plants were higher on an acid Andisol (pH 5.42) that was either unamended or amended with partly acidulated phosphate rock at the rate of 17, 43, or 86 kg/ha (Rubio et al. 2002). Nevertheless, *C. etunicatum* colonization was not effective in improving plant growth at any of these three levels when soluble P was added (Rubio et al. 2002). Grain and straw yield of wheat colonized by *R. intraradices* or two isolates of *F. mosseae* alone was higher in an acidic Alfisol (pH 5.2) soil treated with 50 and 75 % of recommended phosphorus pentoxide (P_2O_5) dose based on the targeted yield concept (Suri et al. 2011). Colonization of AM fungi along with increasing application rates of P_2O_5 resulted in consistent and significant improvements in straw and grain yields. All the three fungi along with 75 % P_2O_5 dose though produced acceptable yields; it was less than the yield at sole 100 % P_2O_5 dose (Suri et al. 2011).

Total biomass of broomsedge (*Andropogon virginatus*) colonized with isolates of *R. clarus*, *Acaulospora morrowiae*, and *R. heterogama* originating from acid or neutral soils was 2.3-, 2.0-, and 2.2-folds higher than the non-mycorrhizal plants when grown on sand culture and irrigated with nutrient solution at pH 4 (Kelly et al. 2005). The plant growth response was further amplified for *R. clarus* (12.89-folds) and *F. heterogama* (5.35-folds), but not for *A. morrowiae* when grown in sand culture containing 400 μm Al.

Shoot biomass of *L. leucocephala* cultivars (cv. K-8 and cv. K-636) colonized with *Glomus aggregatum* grown on Al-rich Oxisol and Mn-rich Vertisol acid soils increased with an increase in pH. Shoot biomass of mycorrhizal *L. leucocephala* cv. K-636 cultivar was higher than that of mycorrhizal cv. K-8 cultivar at pH 4.5 and 6.4, but was almost similar at the intermediate pH (Habte et al. 2011). Shoot and root dry mass of mung bean and crotalaria colonized by ten AM fungal species increased with increasing pH when grown on an acidic red soil. However, the growth response tends to vary with the AM fungi, host, as well as the growth period (Lin et al. 2001) (Fig. 3.7). Mycorrhizal dependency of both mung bean and crotalaria varied with soil acidity (Table 3.1). A reduction in shoot biomass was more prominent in crotalaria than for mung bean at pH 3.5. The increase in mycorrhizal dependency with increasing soil pH from 3.6 to 6.0 was more intense for crotalaria than mung bean (Table 3.1).

Plant dry weight of micropropagated apple rootstocks colonized by *C. etunicatum*, *S. pellucida*, *A. scrobiculata*, or *F. heterogama* was higher than non-mycorrhizal rootstocks when grown on acid soils with a pH of 4.0 or altered to pH 5.0 or 6.0 by adding $CaCO_3$ (Cavallazzi et al. 2007). However, root dry weights of apple rootstocks colonized by *F. heterogama* and *A. scrobiculata* were slightly less than the non-mycorrhizal rootstocks. The R/S ratios of mycorrhizal rootstocks were less than the non-mycorrhizal rootstocks. Mycorrhizal dependency of apple rootstocks colonized by *C. etunicatum* and *S. pellucida* was generally higher compared to those colonized by *A. scrobiculata* and *F. heterogama*

Table 3.1 Influence of soil acidity on mycorrhizal dependency[a] in different hosts

		pH 3.5	pH 4.5	pH 6.0
Mung bean (Lin et al. 2001)	*Acaulospora* sp. 34	−1.67	62.50	200.00
	Acaulospora sp. 53	1.67	−37.50	70.00
	Diversispora epigaea	0.00	−12.50	0.00
	Funneliformis caledonius	0.00	50.00	160.00
	Funneliformis mosseae	−3.33	75.00	90.00
	Fuscutata heterogama	−3.33	0.00	60.00
	Gigaspora sp.47	5.00	25.00	−10.00
	Rhizophagus manihotis 38	6.67	100.00	180.00
	Rhizophagus manihotis 49	0.00	25.00	30.00
	Scutellospora calospora	0.00	0.00	0.00
		pH 3.5	**pH 4.5**	**pH 6.0**
Crotalaria (Lin et al. 2001)	*Acaulospora* sp. 34	415.38	1946.67	1331.58
	Acaulospora sp. 53	23.08	53.33	178.95
	Diversispora epigaea	−15.38	33.33	252.63
	Funneliformis caledonius	−30.77	153.33	226.32
	Funneliformis mosseae	−15.38	120.00	421.05
	Fuscutata heterogama	69.23	106.67	52.63
	Gigaspora sp.47	−15.38	146.67	315.79
	Rhizophagus manihotis 38	69.23	346.67	794.74
	Rhizophagus manihotis 49	−30.77	40.00	236.84
	Scutellospora calospora	−38.46	33.33	78.95
		pH 4.6	**pH 4.9**	**pH 5.2**
Cowpea (Rohyadi 2008)	*Claroideoglomus etunicatum*	13	14	47
	Gigaspora margarita	81	65	53
		pH 4	**pH 5**	
Switchgrass (Clark 2002)	*Acaulospora morrowiae*	2075.00	2112.50	
	Claroideoglomus etunicatum	1743.75	3475.00	
	Gigaspora albida	518.75	1712.50	
	Gigaspora margarita	1856.25	3181.25	
	Gigaspora rosea	12.50	6.25	
	Rhizophagus clarus	4443.75	3018.75	
	Rhizophagus diaphanus	3731.25	3531.25	
	Rhizophagus intraradices	−25.00	1087.50	
		pH 5.15	**pH 5.7**	
Barley 'Carmen' (Borie and Rubio 1999)	*Claroideoglomus etunicatum*	719	98	
		pH 5.15	**pH 5.7**	
Barley 'teffi' (Borie and Rubio 1999)	*Claroideoglomus etunicatum*	6.67	−17.6	
		pH 4.48		
Chickpea (Alloush et al. 2000)	*Rhizophagus clarus*	18.39		
		pH 5.2		
Wheat (Suri et al. 2011)	*Funneliformis mosseae* (IARI)	15.39		
	Funneliformis mosseae (Local)	13.40		
	Rhizophagus intraradices (TERI)	14.02		

[a] Calculated from the cited studies according to Plenchette et al. (1983)
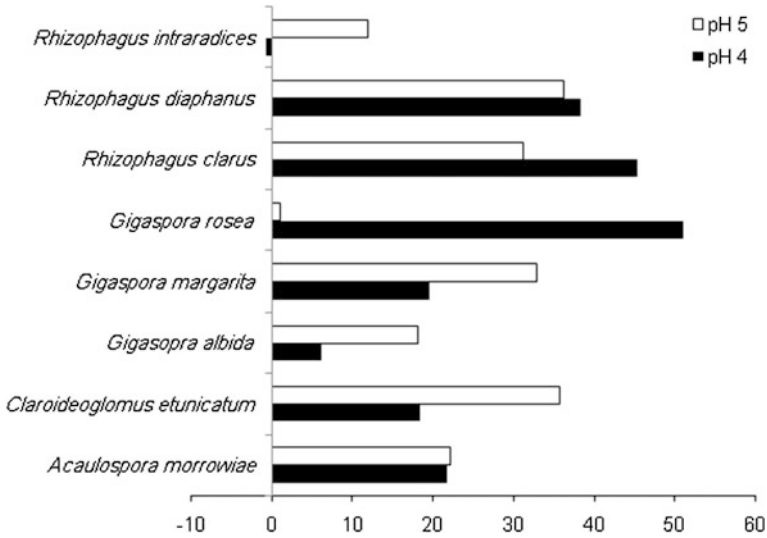
Fig. 3.6 Influence of soil acidity on shoot dry weight of switchgrass colonized by different arbuscular mycorrhizal fungi (calculated from Clark 2002)

(Cavallazzi et al. 2007). As the mycorrhizal dependency of apple rootstocks colonized by *C. etunicatum* and *S. pellucida* increased with an increase in soil pH from 4 to 6, a decline in mycorrhizal dependency was evident for rootstocks colonized by *A. scrobiculata* and *F. heterogama*.

Clusia multiflora seedlings inoculated with AM fungal inocula originating from acid or neutral soils accumulated more shoot and root masses and had increased root lengths than non-mycorrhizal seedlings grown on an acid humic Ultisol at pH 4.2 and irrigated with acidified water of pH 3, 4, and 5 (Cuenca et al. 2001). The shoot/root ratio of mycorrhizal seedlings was higher than that of non-mycorrhizal seedlings irrespective of pH levels and origin of AM inocula (Cuenca et al. 2001).

Sweet potato (*Ipomoea batatus*) plants colonized by *G. margarita* and raised on an acidic soil that was either unlimed (pH 4.2) or limed (pH 5.2) had significantly higher plant biomass than non-mycorrhizal plants at pH 4.2 and not at pH 5.2 (Yano and Takaki 2005). Shoot biomass of cowpea (*Vigna unguiculata*) colonized by *G. margarita* was higher than those colonized by *C. etunicatum* when grown on an acidic Podozole (pH 4.9) (Rohyadi 2008). As the AM fungal benefit on plant growth declined from 81 to 39 % with an increase in pH from 4.6 to 5.2 for plants colonized by *G. margarita*, it increased from 13 to 33 % for plants colonized by *C. etunicatum*. Such an inverse pattern was also evident for mycorrhizal dependency of cowpea plants colonized by *G. margarita* and *C. etunicatum* (Table 3.1).
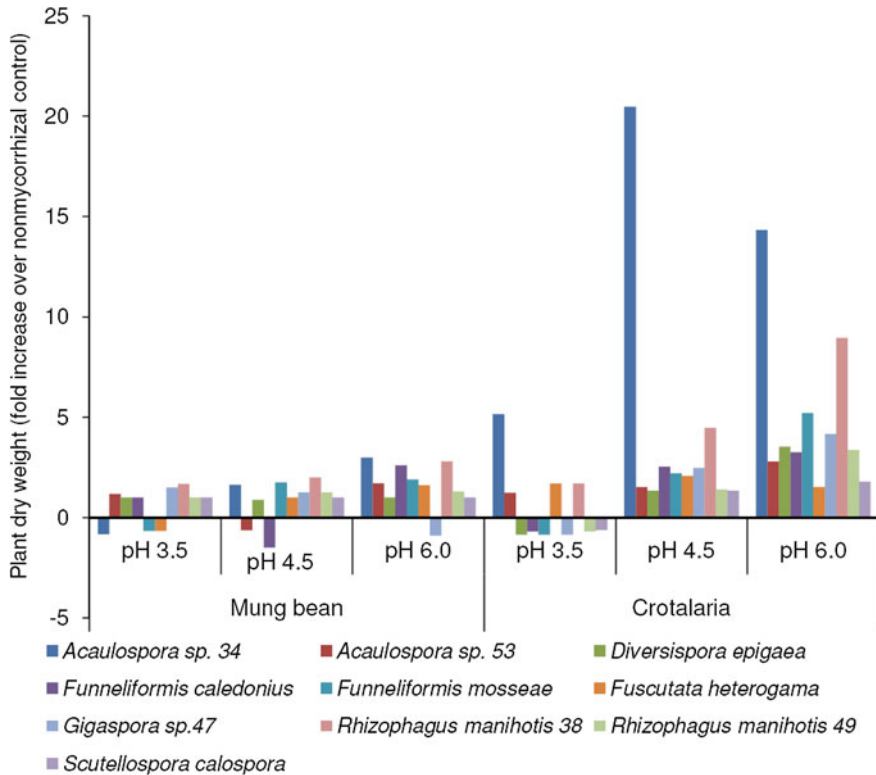
Fig. 3.7 Influence of soil acidity on growth of mung bean and crotalaria colonized by different arbuscular mycorrhizal fungi after 45 days of growth (calculated from Lin et al. 2001)

An investigation on the role of *C. etunicatum* on growth of barley cultivars that were either tolerant or sensitive to Al on an unlimed (pH 5.15) or limed (pH 5.70) Andisol indicated that the growth benefit of *C. etunicatum* association was more pronounced in Al-tolerant ('Carmen') than in Al-sensitive ('Steffi') barley cultivar (Borie and Rubio 1999).

Efficiency of AM Fungi in Ameliorating Al Toxicity

The AM fungal association can modify the interaction between plant and soil and also protect the host plant under stress environments such as heavy metals (Smith and Read 2008; Muthukumar and Bagyaraj 2010). The presence of high concentrations of Al^{3+} in the soil is deleterious to the survival and activity of the microorganisms (Rohyadi 2006). The uptake of Al by roots and its translocation within plants are greatly reduced by AM fungal association. The production of

exudates by the extraradical mycelium results in the chelation of heavy metals in the mycorrhizosphere (Tonin et al. 2001; Hall 2002).

Mycorrhizal tulip-poplar (*Liriodendron tulipifera*) seedlings had higher concentrations of P in their leaves and higher biomass in contrast with the non-mycorrhizal plants when raised on substrates amended with various concentrations of Al (Lux and Cumming 2001). Kelly et al. (2005) inoculated broomsedge with five isolates of three AM fungal species (*R. clarus*, *A. morrowiae*, and *F. heterogama*) in substrates amended with 400 μm Al. The results of this study indicated that *R. clarus* was more resistant to Al toxicity (22.4–92.7 %) and growth inhibition, followed by *F. heterogama* and *A. morrowiae* (Kelly et al. 2005). Rohyadi et al. (2004) also showed that plant growth especially the shoot and root dry weights of cowpea plants inoculated with *G. margarita* was higher compared to plants inoculated with *C. etunicatum* at different soil acidic levels (4.4, 4.9, and 5.2).

However, the resistivity for Al appears to be much higher for AM fungal isolates originating from Al-rich soils compared to those from non-contaminated soils. For instance, *C. multiflora* seedlings inoculated with natural inoculum of AM fungi originating from acidic as well as neutral soils and watered with acidic solution indicated that seedlings inoculated with AM fungal inoculum originating from acidic soil accumulated less Al and root growth was normal compared to seedlings inoculated with AM fungal inoculum from neutral soil (Cuenca et al. 2001). In general, the abundance of vesicles in the roots colonized by AM fungi originating from non-acidic soil indirectly indicates that the plants are under some sort of stress as vesicle production tends to peak under stress conditions (Cooke et al. 1993). Though several factors are shown to affect AM fungi under low pH, the crucial or dominant ones are still elusive.

Aluminum toxicity affects root architecture as mentioned earlier, which affects nutrient and water uptake (Foy et al. 1978). Compared to roots, the extraradical mycelium of AM fungi can spread beyond the nutrient depletion zone surrounding the root and take up low mobile nutrients like P from the soil and translocate it to the host (Smith et al. 2000). The extraradical hyphae of AM fungi can spread up to 10 cm from the root surface (Jakobsen et al. 1992), and the smaller diameter of the fungal hyphae than roots (Bolan 1991) increases the surface area for absorption by fourfold. This favors the efficient uptake of P and other soil nutrients by the mycorrhizal roots in nutrient-stressed soils.

Like plants, AM fungi also possess certain defense mechanisms to protect themselves against various stress conditions. There is enough evidence to believe that exudation of organic acids by AM fungal hyphae (Plassard and Fransson 2009) especially the citrate, malate, and acetate (Tawaraya et al. 2006; Toljander et al. 2007) could ameliorate the Al toxicity. Therefore, AM fungi-colonized roots are well protected from the deleterious effects of the metal toxicities (Clark and Zeto 1996; Maddox and Soileau 1991) through extensive hyphal network and root exudates.

In addition, root colonization by AM fungi could also influence the release of carbon by plant roots into the rhizosphere, increasing the availability of organic

acids and other substrates (Seguel et al. 2013). For example, Klugh and Cumming (2007) showed altered concentrations of organic acids in the root zones of AM tulip-poplar raised on sand culture and irrigated with a nutrient solution of pH 4.0. In addition, the organic acid production by AM plants was independent of the degree of colonization. A similar observation was also made in a later study by Klugh-Stewart and Cumming (2009) for AM broomsedge. The fungal hyphae bind the toxic metals like Al extracellularly to the cell walls or sequestrate intracellularly in vacuoles by phosphate granules (Toler et al. 2005; González-Guerrero et al. 2008; Zhang et al. 2009).

Certain studies indicate that AM fungi could also sequester Al in their vesicles and auxiliary cells (Yang and Goulart 1997; Cuenca et al. 2001). Investigations by Yano and Takaki (2005) and Cuenca et al. (2001) showed that sweet potato and *C. multiflora* could accumulate >200 % of the normal Al concentration in their roots without exhibiting any toxic symptoms when colonized by *G. margarita* and *Acaulospora* species, respectively. Likewise, a 51 % increase in tissue Al level was noted in the roots of tulip-poplar inoculated with *R. clarus* and *R. diaphanus*.

In addition, the production of glomalin, which is an abundant glycoprotein in the soil, is produced by the hyphal wall of AM fungi (Treseder and Turner 2007). The glomalin deposited in the soil when the hyphae senescence is reported to sequester toxic minerals considerably. Etcheverría (2009) showed that glomalin-related protein (GRSP) could bind around 4.2–7.5 % of Al in acidic soils of a temperate forest in southern Chile. The production of GRSP has been shown to be directly proportional to the adverse soil conditions, especially low pH (Vodnik et al. 2008; Cornejo et al. 2008). These mechanisms significantly reduce the deleterious effects of Al and improve the functionality of plants. Altogether, AM fungi play a vital role in ameliorating the effects of Al stress by various detoxifying mechanisms.

Efficiency of AM Fungi in Ameliorating Mn Toxicity

The concentration of Mn in shoots and roots of mycorrhizal plants is often lower than that in non-mycorrhizal plants (Kothari et al. 1991; Nogueira and Cardoso 2000; Nogueira et al. 2004). Similar concentrations of Mn have been reported in shoots (1.02 and 1.04 mg/g) and roots (0.38 and 0.33 mg/g) of non-mycorrhizal and mycorrhizal (*G. margarita*) sweet potato grown on an acid soil (pH 4.2) (Yano and Takaki 2005). Likewise, Mn toxicity was less severe in mycorrhizal plants than in non-mycorrhizal soybean plants in spite of similar concentrations of Mn in these plants (Bethlenfalvay and Franson 1989).

Habte et al. (2011) speculated that AM fungal colonization in *L. leucocephala* cultivars (cv. K-636 and cv. K-8) was low in Mn-rich acid Oxisol soil at pH 4.5 because of the high similarity in the reactivity of the host and the fungi to Mn toxicity. The tolerance of *L. leucocephala* seedlings to acid toxicity in Mn-rich Oxisol varied with the pretransplant mycorrhizal status of the seedlings. Tolerance level of *L. leucocephala* cv.K-636 that was less tolerant than cv. K-8 in Mn-rich

Oxisol improved when the seeds were mycorrhized prior to transplantation. Nogueira et al. (2002) also reported that soybean inoculated with *C. etunicatum* under different levels of Mn (0, 5, 10, 20, and 40 mg/kg) exhibited better growth and less Mn toxicity symptoms (callose deposition).

Earlier studies on the influence of AM in acid soils suggested that colonization by AM fungi generally enhanced the uptake of Mn^{2+} by host plants (Mederios et al. 1994; Clark and Zeto 1996; Clark et al. 1999b; Lux and Cumming 2001). Nogueira and Cardoso (2003) investigated the effectiveness of three AM fungi (*Glomus macrocarpum, C. etunicatum,* and *R. intraradices*) on soybean in two different soils (sandy and clay). The results of this study showed that soybean plants had lower Mn content and biomass in sandy soil compared to clayey soil. Nevertheless, plants inoculated with *C. etunicatum* and *R. intraradices*, and *G. macrocarpum* exhibited Mn toxicity symptoms and had reduced biomass in clayey soil indicating the soil-type influence on Mn toxicity.

Most of the studies on the role of AM fungi on plant nutrient uptake in acid soils indicate an enhanced Mn uptake by AM plants. Nevertheless, the influence of AM fungi on Mn uptake by plants in acid soils could be time dependent as shown by Nogueria et al. (2007) where soybean plants colonized by *C. etunicatum* or *G. macrocarpum* had higher concentrations of Mn during initial stages of growth and lower concentrations during later phase of plant growth. There are also studies indicating that AM fungi reduce the amount of Mn entering the roots by suppressing the activity of Mn oxidizing and reducing bacteria in the rhizosphere at pH 5.7 or higher (Bethlenfalvay and Franson 1989; Kothari et al. 1991; Nogueria et al. 2007).

AM Fungal Amelioration of Plant P Deficiency

In contrast to an increment in the concentrations of Al and Mn in the soil, there is a simultaneous decline in the availability of essential nutrients such as P, K, and Mo (Fageria et al. 1990). Depletion of these essential mineral nutrients inversely affects the plant growth, leading to reduction in crop production. As already mentioned, P fixation and availability depend mainly on soil pH (Hsu 1964). Minimal availability of P is one of the common and well-known limiting factors for plant growth and development in soils with a pH range of 2–4 (Bowden et al. 1980; Nian et al. 2003).

The worldwide distribution and causes for P-limiting soils have recently been discussed in detail by Lynch and Brown (2008). In acid soils, P exists in the form of insoluble mineral complexes such as Al–P and Fe–P and therefore is not available for uptake by plants (Sample et al. 1980). Complexolysis is a process in which the complexing agents such as the exudated organic acids liberate minerals from their complex insoluble forms through organic acidolysis, and complex and chelate formations (Courty et al. 2010). These processes are most suited for the solubilization of P adsorbed to Al or Fe oxyhydroxides.

The development of extensive hyphal network in the soil ameliorates the effects of extremely low pH through improved uptake of P. Smith et al. (2000) showed

that about 80 % of the total P acquired by AM *Medicago truncatula* were provided by the extraradical mycelium of the fungi associated with those plants. The functions of the fungal hyphae radiating out from the colonized roots are more important in acid soils as the development and proliferation of roots are severely affected in soils with low pH.

Rohyadi (2008) observed an increase in P uptake in maize colonized by *G. margarita* under acidic conditions and suggested that the enhanced P levels in AM maize tissues could be due to the greater exploration of soil by the AM fungal hyphae. This suggestion is supported by the observation where the P-uptake response of cowpea plants colonized with *G. margarita* was 104 and 46 % higher compared to plants colonized with *C. etunicatum* at pH 4.6 and 4.9. Similarly, the amount of P uptake per unit root length of *G. margarita-* and *C. etunicatum-* colonized cowpea plants were 75–144 % and 41–88 % higher compared to non-mycorrhizal plants.

Toro et al. (1998) stated that AM fungi not only had the access, but also could reach to the unexploited sources of P in deficient soils. The enhanced growth of maize colonized by *C. etunicatum, Glomus diaphanum,* and *R. intraradices* in spite of the low number of arbuscules in the roots in an acidic soil (pH 4.2–4.5) was attributed to the hyphal network in the soil reaching for the sparingly available P sources (Clark and Zeto 1996).

In a later study, Clark (2002) showed that the P inflow rates per unit root length of mycorrhizal switchgrass were around 18-fold higher compared to non-mycorrhizal plants when grown on soil with pH 4. However, the inflow rates declined to half when the plants were raised on a slightly higher pH of 5. The effectiveness of AM fungi on stress amelioration under acidic conditions could be attributed to the proliferation of external hyphae rather than colonization (Rohyadi 2008).

The AM fungi associated root system are highly efficient than non-mycorrhizal root systems as they could use various forms of phosphate such as inorganic and organic P sources (Tarafdar and Marschner 1994; Ravnskov et al. 1999), which are limited in acid soils. Colonization of switchgrass by four different AM fungal isolates (*R. intraradices* WV894, *R. clarus* WV751, *C. etunicatum* WV579A, and *Acaulospora mellea* BR152A) in five acid soils (Lily, Porters, Tatum, Rayne, and Pacolet) resulted in varied extractable plant P pools (Clark et al. 2005). These differences in P pools were attributed to the varied uptake of P by different AM plants, similar to the observations made by Graw (1979), Saif (1987) and da Silva et al. (1994).

Role of AM Fungi in the Uptake of Other Nutrients

Plants growing on acidic soils also have limited access to several essential mineral nutrients other than P such as Ca, Mg, potassium (K), copper (Cu), and zinc (Zn). Low levels of ions migrate to the exchange sites in the rhizospheric region under acidic conditions, rendering it less available for the plants (Sumner et al. 1991). These nutrient limitations are often compensated by extended extraradical hyphal

network of AM fungi. Enhanced acquisition of several mineral nutrients (including Zn and Cu) was reported in maize in response to colonization by *C. etunicatum, G. diaphanum,* and *R. intraradices* in acidic soils with a pH of 4.2–4.5 (Clark and Zeto 1996).

Alloush and Clark (2001) demonstrated a better uptake and translocation of Ca, Mg, and K by *R. clarus* in maize when grown on soils with a pH 4.7. A similar increase in K, Ca, and Mg uptake was also reported for maize plants in acidic soils (Liu et al., 2000). Siqueira et al. (1990) also reported higher concentrations of Ca in the tissues of *Brachiaria* grass (*Brachiaria decumbens*) colonized by AM fungal assemblage with taxa originating from different acidity compared with non-mycorrhizal plants when grown on soils with pH 4.5.

Certain studies in contrast to the above-mentioned observations have reported the lack of plant benefit from AM fungi in acid soils. Sweet potato plants colonized by *G. margarita* failed to improve the uptake of P, K, Ca, and Mg when grown on soils with pH ranging from 4.2 to 5.2 (Yano and Takaki 2005). A similar observation was made in wheat colonized by species of *Funneliformis* and *Rhizophagus* failed to improve plant N, P, K, Fe, Mn, Zn, and Cu concentrations when grown on an acid Alfisol (Suri et al. 2011).

Conclusions and Future Considerations

Acidic syndrome is a major factor that limits crop production worldwide. Research over the past two decades has contributed immensely to our understanding on the various adaptations plants have evolved to ameliorate the effects of soil acidity. Conventional agricultural practices involve the application of lime, gypsum, and P fertilizer to improve crop growth and yield in acid soils. These amendments though achieved the desired target to certain extent, high input costs, and depleting reserves of raw materials, and their unavailability restricts their widespread and long-term use.

Breeding plant genotypes that are tolerant to acidic soils or genotypes with high nutrient use efficiency may be one possible solution. Nevertheless, available evidence indicates the potential role of AM symbiosis in improving plant growth in acidic soils. Further, studies examining the role of AM fungi on plant growth and yield in acidic soils have been conducted under controlled conditions with a limited number of fungal isolates. Results of such studies though help to elaborate our understanding on AM symbiosis in acid soils; it could substantially differ under field conditions.

Furthermore, there are clear indications that continuous culture of AM fungal genotypes originating from acid soils under normal soil conditions would result in the loss of the acquired characters. Therefore, standardization of culture conditions is essential to retain the acquired characters and exploit these fungi as bioinoculants. An alternative strategy to exploit the symbiosis for the maximum benefit in acid soils would be to understand and manipulate the factors that influence AM symbiosis.

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Chapter 4
Use of Plant Growth-Promoting Rhizobacteria to Alleviate Salinity Stress in Plants

Dilfuza Egamberdieva and Ben Lugtenberg

Introduction

Salinization is recognized as the main threat to environmental resources in many countries and affects almost one billion hectares worldwide (Munns and Tester 2008; FAO Land and Nutrition Management Service 2008). Major factors increasing salinity include irrigation of cultivated lands with saline water, poor cultural practices, and low precipitation. Almost 300 million hectares in the world are irrigated. Irrigated agriculture consumes about 90 % of the total water withdrawal to produce 36 % of the global food (Rengasamy 2006; ICID 2009). It has been estimated that inappropriate irrigation/drainage practices affect approximately fifty percent of the global irrigated areas, with an annual increase of up to 500,000 ha. These facts represent a serious threat to sustainable food production and to our natural resources (Ondrasek et al. 2009).

Natural salinity is the result of long-term natural accumulation of salts in the soil or in surface water. Secondary (anthropogenic) salinity results from irrigation and is widely responsible for increasing the concentration of dissolved salts in the soil profile to a level that impairs plant growth and that will result in abandoning agricultural land (Munns 2005; Egamberdiyeva et al. 2007; Manchanda and Garg 2008). Many of the most cultivated and widely used crops (cereals, horticultural crops, etc.) in human/animal nutrition are susceptible to salt stress (>4 dS m^{-1}), and their productivity is considerably reduced due to improper nutrition of the

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plant (Chinnusamy et al. 2005; Mantri et al. 2012). Salinity and drought also strongly influence many other properties and processes of living organisms (Ondrasek et al. 2009).

Climate change may lead to even more saline landscapes in many non-irrigated regions since it is accompanied by less rainfall and higher temperatures in most agricultural regions. It will result in a change toward again a more arid climate, which is conducive to salt accumulation (Othman et al. 2006). Limiting crop losses due to salinity and drought is a major area of concern to cope with the background of increasing food requirements (Shanker and Venkateswarlu 2011). In a meeting in October 2012, the World Food Security Committee addressed the effects of climate change on food security and invited world leaders (1) to integrate food security and climate change concerns, (2) to increase resilience of food systems to climate change, and (3) to develop agricultural strategies that take into account the need to respond to climate change and to safeguard food security (CFS 2012). Novel agricultural technologies are required to improve food production in saline and dry soils (Wehrheim and Martius 2008). Many scientists have attempted to develop salt-tolerant crops through breeding, but these efforts have met with limited success due to the genetically and physiologically complexity of the salt tolerance trait (Flowers 2004; Araus et al. 2008; Dwivedi et al. 2010).

Promising measures for improving plant health in salinated soils are the use of microbial inoculants, which can ameliorate salt stress, promote plant growth (Lugtenberg et al. 2013a), and control diseases (Lugtenberg and Kamilova 2004; Lugtenberg and Kamilova 2009; Mayak et al. 2004; Lugtenberg et al. 2013b; Egamberdieva et al. 2008; Pliego et al. 2011). The utilization of root-associated bacteria that interact with plants by mitigating stress opens a new advanced technology for combating salinity. Many studies have demonstrated that the use of beneficial microbes can enhance a plant's resistance to adverse environmental stresses, e.g., drought, salinity, nutrient deficiency, and heavy metal contamination. Such inoculants contribute to the development of sustainable agriculture under stressed conditions (Glick et al. 2007; Dodd and Perez-Alfocea 2012; Berg et al. 2013).

The inoculation of seeds of various crop plants, such as tomato, pepper, canola, bean, and lettuce, with PGPR can result in increased root and shoot growth, dry weight, fruit and seed yield and in enhanced tolerance of plants to salt stress (Glick et al. 1997; Mayak et al. 2004; Yildirim and Taylor 2005; Barassi et al. 2006; Egamberdieva et al. 2013a). According to Creus et al. (2004), PGPR may alter plant–water relationships and show enhanced osmotic adjustment.

In the present chapter, we will review the current status of our understanding of the action of PGPR in crop cultivation under conditions of abiotic stress. We will start with studies about the effect of salt stress on plant growth and physiology, followed by the role of plant growth-promoting rhizobacteria in alleviating salt stress in plants and end with the main mechanisms involved in improvement of plant tolerance to salt stress caused by these microbes.

Effects of Soil Salinity on Plant Growth and Physiology

Seed germination and early seedling growth are the most salt-sensitive plant growth stages under environmental stresses, because the seedling root is in direct contact with soil and is affected by many soil changes, including salt stress (Rahman et al. 2000; Jamil et al. 2006). Many studies have demonstrated that salinity inhibits seed germination of various crops such as wheat (Egamberdieva 2009), faba bean (Rabie and Almadini 2005), rice (Xu et al. 2011), maize (Khodarahmpour et al. 2012), and soybean (Essa 2002). Moreover, Jamil et al. (2006) observed significant reductions in germination percentage, in germination rate, and in seedling root and shoot lengths of cabbage, sugar beet, paniculate amaranth, and pak-choi.

In our previous work, we observed that increasing salt content reduced the shoot length (50 %) and root length (7 %) of bean seedling grown in a gnotobiotic sand system in a growth cabinet (Egamberdieva 2011). These observations are in line with earlier reports about ground nut (Mensah et al. 2006), and chickpea (Al-Mutawa 2003), for which was reported that increased salinity leads to decreased root length. A similar result was observed by Demir and Arif (2003), who reported that the root growth of safflower was more inhibited by salinity than shoot growth. Ashraf (2004) and Razmjoo et al. (2008) found that high salt causes a significant reduction in the growth of shoot and root as well as in the essential oil content of *Ammolei majus*, *Hyoscyamus niger*, and *Matricaria chamomile.* Salinity also decreases photosynthesis, stomatal conductance, chlorophyll content, and mineral uptake of basil (*Ocimum basilicum*) (Golpayegani and Tilebeni 2011).

Several explanations for these effects have been proposed, such as disturbance of the hormonal balance (Prakash and Prathapasenan 1990), alteration of protein metabolism (Dantas et al. 2005), inhibition of the activity of enzymes involved in nucleic acid metabolism (Arbona et al. 2005), and the loss of control on nutrient uptake. These effects are assumed to be caused by the osmotic effect (Shirokova et al. 2000) and the ion toxicity of salt (Munns 2002; Tavakkoli et al. 2011).

The inhibition of root growth by salinity may be caused by a reduction in water uptake and an unbalanced nutrient uptake by the seedling (Dolatabadian et al. 2011). In addition, Atak et al. (2006) and Neamatollahi et al. (2009) pointed out that higher saline concentrations may reduce the germination percentage due to increased osmotic pressure. Abundance of Na^+ and Cl^- ions can lead to a reduction in accessibility and uptake of some elements such as N, P, K, and Mg by the plant (Heidari and Jamshid 2010). In another study, Xiong and Zhu (2002) reported that salinity induces inhibition of phytohormone synthesis and maturation of cell walls.

Most legumes are sensitive to salinity. Soil salinity particularly disturbs the symbiotic interaction between legumes and *Rhizobium* bacteria. These bacteria form root nodules in which they fix atmospheric nitrogen through the nitrogenase complex and make it available to the plant (Quispel 1988). Soil salinity reduces N_2 fixation and nitrogenase activity of several legumes such as soybean (*Glycine max*)

(Singleton and Bohlool 1984), common bean (*Phaseolus vulgaris*), and faba bean (*Vicia faba*) (Rabie et al. 2005).

Only a few agronomical legumes can grow in salt-affected soils (Ashraf and McNeilly 2004). *Galega offfcinalis* L (goat's rue, French lilac) might be a good candidate to cultivate in salt-affected soils because they are perennial, deep rooted, and grow fast after initial establishment. We have observed that *G. officinalis* plants inoculated with their rhizobial symbiont *Rhizobium galegae* suffer from retarded growth and impaired nodulation when grown under 75 mM NaCl conditions (Fig. 4.1). Salt stress also decreased the number of *Rhizobium* cells able of colonize *G. officinalis* root tips (Egamberdieva et al. 2013a).

An explanation for the reduction in symbiotic legume growth might be that the salt stress causes a failure of the infection and nodulation process. For example, according to Bouhmouch et al. (2005), salt inhibits the absorption of Ca^{2+} ions, which causes reduction in the growth of roots, root tips, and root hairs, thereby decreasing sites for potential rhizobial infection and further nodule development.

Fig. 4.1 Effect of 50 mM NaCl on growth of goat's rue plants (*Galega officinalis* L.). The effects of the treatment of *G. officinalis* with NaCl solutions were evaluated after plants were grown for eight weeks in lowly fertilized potting soil in the greenhouse. A salt concentration of 50 mM NaCl retarded significantly the growth of shoots and roots, as well as the nodulation of *G. officinalis* plants inoculated with *Rhizobium galegae*

Rhizobacteria in Saline Soils

Soil salinity not only inhibits plant growth and development, but also negatively affects the composition and activities of rhizosphere bacteria (Ofek et al. 2006). Nelson and Mele (2007) reported that sodium chloride affects the rhizosphere microbial community structure through its influence on the quantity and/or quality of root exudates. Also, increasing salinity decreases the diversity of *Pseudomonas* species associated with rice. *Pseudomonas* species found in saline soil include *P. aeruginosa, P. fluorescens*, *P. putida*, *P. stutzeri, P. mendocina, P. mallei,* and *P. diminuta* (Nagarajan et al. 2002). Non-saline soil favors the growth of the fluorescent *Pseudomonas* population, whereas in saline soil the dominant *Pseudomonas* subpopulation consists of *P. alcaligenes* and/or *P. pseudoalcaligenes*.

Loganathan and Nair (2004) isolated salt-tolerant, nitrogen-fixing bacteria from mangrove-associated wild rice and identified them as *Swaminathania salitolerans*. Tripathi et al. (2002) isolated and identified salt-tolerant rhizobacteria from rice roots, including *Serratia marcescens, P. aeruginosa*, *Alcaligenes xylosoxidans*, and *Ochrobactrum anthropi*.

Potential human pathogenic bacteria have been found in saline soils in a surprisingly high frequency. Egamberdieva et al. (2008) have isolated salt-tolerant rhizobacteria with high rhizosphere competence from wheat roots grown in salinated Uzbek desert soils. They observed that many of the root-associated bacteria are potential human pathogens, which were identified as *Alcaligenes faecalis, Acinetobacter* sp., *Enterobacter hormaechei, Pantoea agglomerans, P. aeruginosa*, *Bacillus cereus*, and *Staphylococcus saprophyticus*.

The presence of other human pathogens on plant roots in saline environments, such as *Salinivibrio*, *Halomonas*, *Chromohalobacter*, *Bacillus*, *Salinicoccus, Candida tropicalis, Alcaligenes faecalis, S. marcescens*, and *A. xylosoxidans*, was also reported (Tripathi et al. 2002; Sanchez-Porro et al. 2003; Bastos et al. 2004). Salt-tolerant *Mycobacterium phlei* strains were also found in association with roots of corn planted in saline soils of Uzbekistan (Egamberdieva 2011).

The presence of *P. aeruginosa* in the rhizosphere of wheat has been reported previously (Morales et al. 1996; Germida and Siciliano 2001). The consistent presence of *P. aeruginosa* in saline soils indicates a widespread incidence of this bacterium in the rhizosphere of plants growing in saline soil. Microorganisms compete for nutrients and niches in the plant rhizosphere. Exudates are thought not only to attract beneficial bacteria to colonize the roots, but also human pathogens which apparently have evolved to respond to the same signals (Roberts et al. 2000; Ji and Wilson 2002).

Morales et al. (1996) and Jablasone et al. (2005) reported that the survival and colonization of potentially pathogenic human-associated bacteria in the rhizosphere of plants are poor and that their persistence and colonization on plants are decreased by co-inoculation of pathogens with naturally occurring bacteria. We have also observed that the potential human pathogenic strains *B. cereus, S. saprophyticus, P. aeruginosa*, and *Acinetobacter* sp., isolated from roots of wheat plants growing in salinated soils, showed poor competitive colonization of

the wheat rhizosphere when compared with *P. fluorescens* WCS365, an excellent root tip colonizer (Egamberdieva and Kucharova 2009). Since the potential pathogens were probably derived from manure used for fertilization, it is likely that the root-derived bacteria out compete the potential pathogens derived from humans and animals (Egamberdieva et al. 2011).

Egamberdieva and Kucharova (2009) have selected enhanced root tip colonizing bacteria from wheat grown in saline soil using an enrichment procedure described by Kamilova et al. (2005). The four selected strains were identified as *P. putida, P. extremorientalis, P. chlororaphis,* and *P. aureantiaca*, and since they do not belong to risk group 2 (Anonymous 1998), they are nonpathogenic. Those findings suggest that the screening procedure for the selection of enhanced root-colonizing rhizobacteria can select for environmentally save bacterial strains, which can be applied for plant growth promotion in salinated and stressed soil conditions. Moreover, they are likely to out compete potential pathogens of human and animal origin.

Plant Salt Stress Alleviation Using Plant Growth-Promoting Rhizobacteria

The rhizosphere is colonized more intensively by microorganisms than the other regions of the soil. These microbes can be beneficial, neutral, or pathogenic. Beneficial rhizobacteria can improve seed germination, root and shoot growth, nutrient uptake, and plant stress tolerance. Moreover, they are able to control various diseases. They are often referred to as plant growth-promoting rhizobacteria (PGPR) (Hiltner 1904; Lugtenberg et al. 2001; Compant et al. 2005; Arora et al. 2008; Lugtenberg and Kamilova 2009). A range of salt-tolerant rhizobacteria (e.g., *Rhizobium*, *Azospirillum, Pseudomonas*, *Flavobacterium*, *Arthrobacter*, and *Bacillus*) has so far shown beneficial interactions with plants in stressed environments (Egamberdieva and Islam 2008; Egamberdieva et al. 2011; Adesemoye et al. 2008).

The majority of cultivated plant species, especially widely grown horticultural and cereal crops, are susceptible to excessive concentrations of dissolved ions (e.g., >30 mM or >3.0 dS/m) in the rhizosphere (Ondrasek et al. 2009). For example, the yield of crops such as potato, corn, onion, and bean can be reduced by 50 % when the soil EC is increased to 5.0 dS/m (Horneck et al. 2007).

Earlier reports claim that salinity negatively affects soil bacterial activity by high osmotic strength and toxic effects by salts, but that salt-tolerant bacteria can survive and proliferate in the soil and in the rhizosphere in a harsh environment (Garcia and Hernandez 1996). Diby et al. (2005) observed that the population of the salt-tolerant *P. pseudoalcaligenes* strain MSP-538 did not change considerably with increasing salinity in the soil. Root-associated bacteria are more tolerant to salt stress than soil bacteria, since salinity stress is higher in the rhizosphere due to depletion of water by the plant root, resulting in an increase in both ionic strength and osmolality (Tripathi et al. 1998).

Several PGPR strains, such as *Serratia plymuthica* RR2-5-10, *S. rhizophila* e-p10, *P. fluorescens* SPB2145, *P. chlororaphis* TSAU 13, *P. putida* TSAU1, *P. extremorientalis* TSAU20, *P. fluorescens* PCL1751, and *P. aureofaciens* TSAU22, are salt tolerant up to at least 3 % NaCl and temperature resistant up to 40 °C (Egamberdieva and Kucharova 2009; Egamberdieva et al. 2011). Thus, it is likely that salt-tolerant PGPR strains are able to survive in the rhizosphere of plants due to their persistence and competitiveness under saline arid soil conditions (Mayak et al. 2004; Yasmin et al. 2007).

There are many reports on the improvement of plant growth, development, and nutrient uptake by salt-tolerant bacterial inoculants (Dodd and Perez-Alfocea 2012). An overview of ameliorative effects of PGPR on various plants mentioned in the literature is presented in Table 4.1. For example, Heidari et al. (2011) reported that plant growth, as well as auxin and protein contents of *Ociumum basilicm* inoculated with *Pseudomonas* sp. under conditions of drought stress increased. Golpayegani and Tilebeni (2011) observed that inoculation of basil with *Pseudomonas* sp. and *Bacillus lentus* alleviated the salinity effects on growth, photosynthesis, mineral content, and antioxidant enzymes. Dardanelli et al. (2008) observed that *Azospirillum brasilense* promoted root branching in bean seedling roots and increased secretion of flavonoids and lipochitooligosaccharides.

Inoculation of wheat with the halotolerant *A. brasilense* strain NH improved germination and growth of wheat under saline soil conditions (Nabti et al. 2010). Similar results were obtained by Abbaspoor et al. (2009) who reported increased plant growth, grain yield, and 1,000 grain weight of wheat by inoculation with *P. fluorescens* 153 and *P. putida* 108. In one of our studies, plant treatments with salt-tolerant strains, such as *P. chlororaphis* TSAU13 and *P. extremorientalis* TSAU20, increased shoot growth and dry weight of wheat at 50, 100, and 125 mM NaCl, compared to control plants (Figs. 4.2 and 4.3). The nutrient (N, P, K, and Mg) uptake of wheat was also increased by *Mycobacterium phlei* MbP18 and *Mycoplana bullata* MpB46 (Egamberdieva and Hoflich 2003).

According to Sivritepe et al. (2003), an increase in the potassium content in roots and shoots of plants grown under salt stress can reduce the negative effect of salinity on plant growth. A similar observation, namely that plants with a higher potassium content are more tolerance to salt stress, was reported by Kaya et al. (2003) for pepper and cucumber. *P. chlororaphis* TSAU13 and *P. extremorientalis* TSAU20 are able to stimulate root length (by 47 %) and dry weight (by 50 %) of bean (Egamberdieva 2011). Salinity did not inhibit the plant stimulating properties of salt-tolerant bacterial strains for wheat.

Hasnain and Sabri (1996) reported that inoculation of wheat with *Pseudomonas* sp. stimulated plant growth by reducing the uptake of toxic ions and increasing the auxin content. In another study, the PGPR strains *P. alcaligenes* PsA15, *P. chlororaphis* TSAU13, *P. extremorientalis* TSAU20, and *B. amyloliquefaciens* BcA12 significantly ($P < 0.05$) increased the length and dry weight of cotton roots and shoots in saline soil in comparison with the uninoculated control plants (Egamberdieva and Jabborova 2013). Similar results were reported by Yue et al. (2007) for *Klebsiella oxytoca* which, upon inoculation, was able to relieve salt

Table 4.1 Summary of the reported ameliorative effects of PGPR on crop plants under stress conditions

PGPR	Crop	Effects of inoculation	References
Achromobacter piechaudii	Tomato (*Lycopersicon esculentum*)	Fresh and dry weight	Mayak et al. (2004)
Pseudomonas fluorescens	Groundnut (*Arachis hypogaea* L.)	Plant growth, yield	Saravanakumar and Samiyappan (2007)
P. fluorescens	Maize (*Zea maize* L.)	Root elongation, fresh weight	Kausar and Shahzad (2006)
Pseudomonas sp.	Pea (*Pisum sativum*)	Plant growth, yield	Arshad et al. (2008)
Azospirillum, Pseudomonas, and *Mezorhizobium*	Chickpea (*Cicer arietinum* L.)	Plant growth, yield	Rokhzadi et al. (2008)
Azospirillum brasilense	Wheat (*Triticum durum* var. *waha*)	Germination, growth, spike length, stem height	Nabti et al. (2007)
Glomus clarum and *A. brasilense*	Bean (*Vicia faba*)	Plant growth, nodule number, protein content, N and P uptake, nitrogenase activity	Rabie and Almadini (2005)
Bacillus pumilus, Exiguobacterium oxidotolerans	Brahmi (*Bacopa monnieri*),	Plant weight, bacoside-A content	Bharti et al. (2013)
Pseudomonas putida, P. fluorescens,	Wheat (*Triticum aestivum*, L.)	Plant growth, grain yield, and 1000 grain weight	Abbaspoor et al. (2009)
Staphylococcus kloosii, Kocuria erythromyxa	Radish (*Raphanus Sativus* l.)	Shoot/root fresh and dry weight, chlorophyll content	Yildirim et al. (2008)
Bacillus megaterium	Maize (*Zea maize* L.)	Root growth, necrotic leaf area, leaf relative water content	Marulanda et al. (2010)
Pseudomonas pseudoalcaligenes, B. pumilus	Rice (*Oryza sativa*)	Shoot biomass, glycine betaine-like quaternary compounds	Jha et al. (2010)
A. brasilense	Bean (*Phaseolus vulgaris*)	Root branching, increased secretion of flavonoid and lipochitooligosaccharide	Dardanelli et al. (2008)
Pseudomonas sp.	Wheat (*Triticum aestivum*, L.)	Root/shoot growth, reducing toxic ions uptake	Hasnain and Sabri (1996)

(continued)

Table 4.1 (continued)

PGPR	Crop	Effects of inoculation	References
Pseudomonas sp., *Bacillus lentus*	Basil (*Ocimum basilicum*)	Improved growth, photosynthesis, mineral content and antioxidant enzymes	Golpayegani and Tilebeni (2011)
Pseudomonas extremorientalis	Milk thistle (*Silybum marianum*)	Root, shoot length and fresh weight	Egamberdieva et al. (2013b)
Pseudomonas sp.	Basil (*Ocimum basilicum*)	Plant growth, auxin and protein contents	Heidari et al. (2011)
Bradyrhizobium japonicum	Soybean (*Glycine max*)	Plant growth, number of nodules, grain yield and protein content	Egamberdieva et al. (2004)
Pseudomonas alcaligenes, P. chlororaphis, Bacillus amyloliquefaciens	Cotton (*Gossypium hirsutum*)	Root/shoot length, dry weight	Egamberdieva and Jabborova (2013)
Klebsiella oxytoca	Cotton (*Gossypium hirsutum*)	Root/shoot length, dry weight	Yue et al. (2007)
P. extremorientalis, P. chlororaphis	Bean (*Vicia faba*)	Root/shoot growth, dry weight	Egamberdieva (2011)
Bacillus megaterium	Maize (*Zea maize* L.)	Higher root hydraulic conductance	Marulanda et al. (2010)
P. mendocina and Mycorrhizal fungi	Lettuce (*Lactuca sativa*)	Plant growth, glomalin-related soil protein (GRSP)	Kohler et al. (2010)
S. plymuthica, S. rhizophila, P. fluorescens	Cucumber (*Cucumis sativus*)	Root shoot length, dry weight, fruit yield	Egamberdieva et al. (2011)
P. extremorientalis, P. trivialis and *Rhizobium galegae*	Goat's rue (*G. officinalis* L.)	Root/shoot length, dry weight, nodule number, N uptake	Egamberdieva et al. (2013a)
Rhizobium tropici and *Paenibacillus polymyxa,*	Common bean (*Phaseolus vulgaris* L.)	Plant growth, nitrogen content, nodule number	Figueiredo et al. (2008)
Serratia sp. and *Rhizobium* sp.	Lettuce (*Lactuca sativa*)	Plant growth, N, P and K uptake, chlorophyll content, antioxidant enzymes	Han and Lee (2005)
A. brasilense and *Glomus clarum*	Faba bean (*Vicia faba*)	Plant growth, N and P uptake, nodule number, protein content and nitrogenase enzymes	Rabie and Almadini (2005)
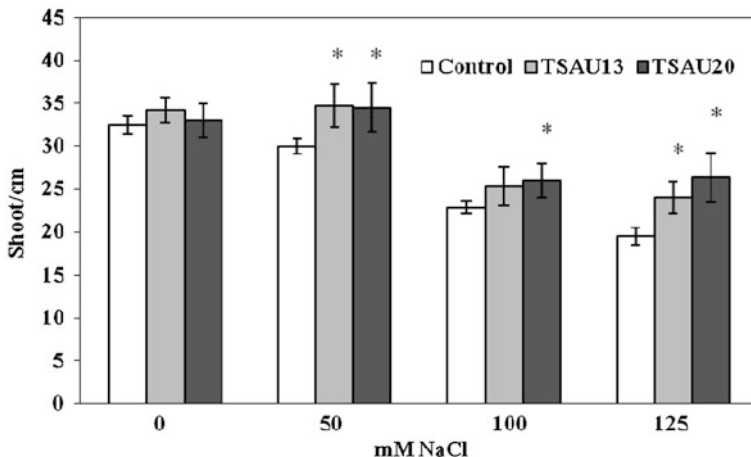
Fig. 4.2 Effect of *P. chlororaphis* TSAU13 and *P. extremorientalis* TSAU20 on shoot growth of wheat under salinated soil. Pot experiments, NaCl concentrations are 50, 100, 125 mM
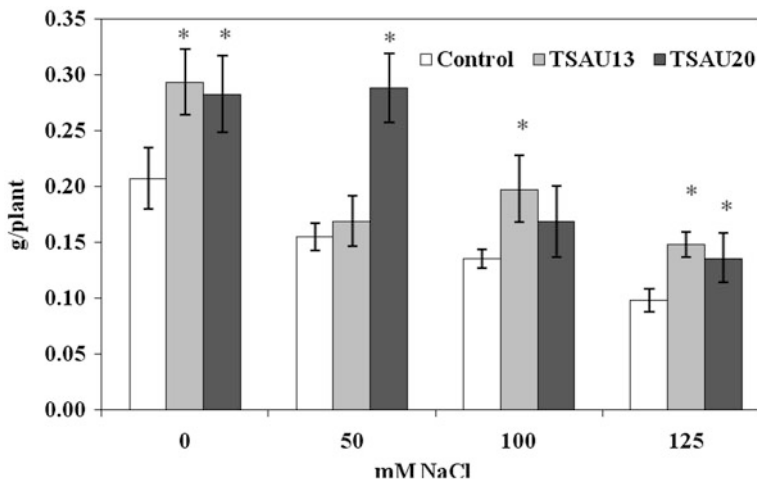
Fig. 4.3 Effect of *P. chlororaphis* TSAU13 and *P. extremorientalis* TSAU20 on dry weight of wheat in salinated soil. Pot experiments, NaCl concentrations are 50, 100, 125 mM

stress and promote the growth of cotton seedlings in salinated soil. Moreover, plant height and dry weight of cotton increased by 14.9 and 26.9 %, respectively.

Rabie and Almadini (2005) reported that inoculation of bean with the AMF (arbuscular mycorrhizal fungus) *Glomus clarum* and the bacterium *Azospirillum brasilense* significantly increased plant growth, nodule number, protein content, and nitrogen and phosphorus uptake in comparison with uninoculated plants and also improved plant stress tolerance. Yildirim et al. (2008) studied the ameliorative

effect of *Staphylococcus kloosii* strain EY37 and *Kocuria erythromyxa* strain EY43 on radish growing in saline soil. They observed that bacterial inoculants significantly increased shoot/root dry weight, leaf number per plant, relative water content of the leaf, and chlorophyll content of radish fruit. Bharti et al. (2013) observed that salt-tolerant *Bacillus pumilus* and *Exiguobacterium oxidotolerans* stimulated plant growth and bacoside-A content of brahmi (*Bacopa monnieri*).

In all reports presented above, the bacterial inoculant strains were isolated from the rhizosphere of plants naturally growing in saline soils. We observed that for the application of bacteria in salinated soils, there is no strict need to isolate these bacteria from plants grown in salinated soil. In our study (Egamberdieva et al. 2011), *S. plymuthica* strain RR2-5-10, *S. rhizophila* strain e-p10, and *P. fluorescens* strain SPB2145, all isolated from regions with a moderate to cold climate and non-saline soil, were able to increase cucumber growth and yield in salinated soil of Uzbekistan. These results are consistent with observations showing that the rhizosphere is characterized by changing osmotic conditions, and that its microbial inhabitants can adapt to increased osmolarity, for example by producing osmoprotective substances (Miller and Wood 1996).

Rhizobium–Legume Symbiosis Improved by PGPR

Under saline conditions, the symbiosis of legumes with *Rhizobium* spp. can be improved by co-inoculation with PGPR (Valverde et al. 2005; Yadegari and Rahmani 2010). Dual inoculation with *Rhizobium* and PGPR result in an increase in the total nodule number of pigeon pea (*Cajanus cajan*) compared to inoculation with *Rhizobium* alone (Tilak et al. 2006).

We have investigated whether the PGPR strains *P. extremorientalis* TSAU20 and *P. trivialis* 3Re27 have the ability to alleviate salinity stress in *G. officinalis* L (goat's rue). In comparison with plants inoculated with *R. galegae* alone, co-inoculation of both unstressed and salt-stressed goat's rue with *Rhizobium galegae* HAMBI 1141 and either *P. trivialis* 3Re27 or *P. extremorientalis* TSAU20 significantly improved root and shoot growth as well as nodulation of the plants. This was the case in both gnotobiotic sand and low-fertilized potting soil. The nitrogen content of the co-inoculated plant roots was significantly increased at both 50 and 75 mM NaCl in potting soil (Fig. 4.4) (Egamberdieva et al. 2013a).

Figueiredo et al. (2008) studied the effect of *Rhizobium tropici,* when co-inoculated with *Paenibacillus polymyxa,* on growth, nitrogen content, and nodulation of the common bean (*Phaseolus vulgaris* L.) under conditions of drought stress. They observed that plants co-inoculated with both *R. tropici* and *P. polymyxa* showed improved plant growth, shoot dry matter, nodule dry matter, and N uptake as well as higher nodule numbers than those inoculated with *R. tropici* alone.

Rokhzadi et al. (2008) showed that the combined inoculation of *Azotobacter*, *Azospirillum*, *Pseudomonas*, and *Mezorhizobium* resulted in promotion of the grain yield and biomass in chickpea. Han and Lee (2005) observed that inoculation of non-legume lettuce with *Serratia* sp. and *Rhizobium* sp. alleviated the negative

H₂O, 50 mM NaCl, 75 mM NaCl   H₂O, 50 mM NaCl, 75 mM NaCl

Rhizobium galegae   *R. galegae + Pseudomonas trivialis* 3re27
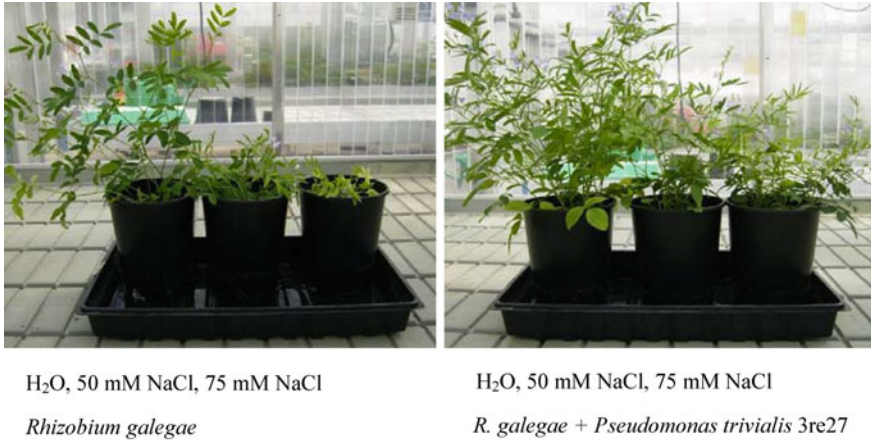
Fig. 4.4 Effect of the salt-tolerant bacterium *Pseudomonas trivialis* 3re27 on the growth of *Galega officinalis* inoculated with *Rhizobium galegae* R1141

effects of salinity on the plant. The inoculation resulted in increased plant growth and N, P, and K uptake. Also, stomatal conductance, chlorophyll content, and the activities of antioxidant enzymes such as ascorbate peroxidase and glutathione reductase increased.

Rabie and Almadini (2005) examined tripartite interactions among a bacterium (*A. brasilens*), an AMF (*G. clarum*), and a legume plant (*Vicia faba*) under increased NaCl levels in pot cultures. Significant positive effects of inoculation were found in the plants with respect to salinity tolerance, mycorrhizal dependence, phosphorus level, phosphatase enzymes, nodule number, nitrogen uptake, protein content, and nitrogenase activity. Based on these findings, the authors suggested that bacterial–AMF–legume tripartite symbioses could be a new approach for increasing the salinity tolerance of legume plants.

The studies mentioned above indicate that PGPR are able to alleviate salt stress in leguminous plants, whereas more nodules might develop into nitrogen-fixing ones, thereby enabling the plant to obtain part of its nitrogen from the atmosphere. Co-inoculation techniques could be a new approach to increase the salt tolerance and yield of legumes used for the food and green manure production in salt-affected soils, providing a supply of biologically fixed N at low cost.

Mechanisms of Action by Which PGPR Alleviate Salt Stress

PGPR can use various mechanisms to stimulate plant growth and development, to protect plants from soilborne diseases, and to increase plant stress tolerance. These mechanisms include (1) the production of phytohormones, antifungal metabolites,

and/or lytic enzymes, (2) increasing the availability of plant nutrients, (3) reduction in stress-induced ethylene production, and (4) induction of systemic resistance (Lugtenberg and Kamilova 2009; Pliego et al. 2011; Egamberdieva et al. 2013a; Penrose et al. 2001; Glick 2005).

Phytohormone Production

Phytohormones have a major role in plant growth development and in stress responses (Shaterian et al. 2005). They may enhance different cellular defence systems for the protection of plants from external adverse conditions (Bianco and Defez 2009). Salinity and drought stresses inhibit the production of auxins, gibberellins, and zeatin in the roots and leaves of plants (Sakhabutdinova et al. 2003; Figueiredo et al. 2008; Perez-Alfocea et al. 2010).

The decrease in hormone levels in the root system of plants results in a reduction in the germination percentage, and of plant growth and development (Werner and Finkelstein 1995; Sakhabutdinova et al. 2003). Salt stress reduces the supply of cytokinin from root to shoot (Naqvi and Ansari 1974) and also the recovery of diffusible auxin from maize coleoptile tips (Itai et al. 1968).

Salinity does not inhibit auxin production of salt-tolerant PGPR. Nabti et al. (2007) isolated the halotolerant *A. brasilense* strain NH which is able to produce auxin at a concentration of 200 mM NaCl. A similar observation was reported in our previous work in which the PGPR strains *S. plymuthica* RR2-5-10, *S. rhizophila* e-p10, *P. fluorescens* SPB2145, and *P. chlororaphis* TSAU13 were shown to produce auxin at 1.5 % NaCl (Egamberdieva et al. 2011; Egamberdieva 2012).

Root-colonizing bacteria which produce auxin under saline condition may supply additional auxin into the rhizosphere, which could help to maintain root growth under stress, and also can contribute to maintaining leaf growth (Albacete et al. 2008). In one of our studies, the inoculation of wheat with the individual auxin-producing bacterial strains *P. aureantiaca* TSAU22, *P. extremorientalis* TSAU6, and *P. extremorientalis* TSAU20 significantly increased seedling root growth up to 40 % and shoot growth up to 52 % at 100 mM NaCl compared to control plants (Egamberdieva 2009). Arkhipova et al. (2007) also observed increased root and shoot growth as well as cytokinin concentrations in plants by treatment with a cytokinin-producing *B. subtilis* strain.

In conclusion, PGPR can have multiple impacts on the phytohormone status, modifying root-to-shoot signalling and shoot hormone concentrations, which may improve growth, development, and physiological processes of plants under salt stress (Dodd et al. 2010).

Osmolites

Plants may protect themselves from drought and salt stress by accumulating compatible solutes such as sugars and amino acids to osmotically adjust

themselves (Serraj and Sinclair 2002; Evelin et al. 2009). Jha et al. (2010) reported that paddy rice (*Oryza sativa* L.) inoculated with *P. pseudoalcaligenes* showed a significantly higher concentration of glycine betaine-like quaternary compounds and a higher shoot biomass under salinity conditions. Bano et al. (2013) observed that *A. lipoferum* increased accumulation of free amino acids and soluble sugars in maize, as compared to the control, under drought stress conditions.

Azospirillum inoculation leads to an increased content of proline (Kandowangko et al. 2009) and free amino acids in maize under drought stress conditions (Sandhya et al. 2010). Verbruggen and Hermans (2008) reported that the accumulation of proline is one of the best-known alterations induced by water and salt stress in plants. Kandowangko et al. (2009) observed that inoculation of corn with *Azospirillum* causes an increase in leaf proline content. Several PGPR strains, such as *Burkholderia* (Barka et al. 2006), *Arthrobacter*, and *Bacillus* (Sziderics et al. 2007), enhance proline synthesis in stressed plants, which helps in maintaining the cell water status, thereby helping the plant to cope with the salinity stress. Proline may enhance the activity of various enzymes, stabilizing the pH within the cell and maintaining antioxidant activity by scavenging reactive oxygen species (Verbruggen and Hermans 2008).

Ashraf (2004) observed that bacterial exopolysaccharides bind the Na^+ ion in the root, through which the plant's Na^+ accumulation decreases. In that way, bacteria may alleviate salt stress in plants. Sandhya et al. (2009) reported that exopolysaccharides produced by PGPR exhibit increased plant resistance to water stress. Kerepesi and Galiba (2000) indicated that the accumulation of sugars in salinity-stressed plants prevents the destruction of soluble proteins. Co-inoculation of *Phaseolus vulgaris* L. with *R. tropici* and the PGPR *Paenibacillus polymyxa* (which produces trehalose) increased plant growth, N content, and nodulation under drought stress (Figueiredo et al. 2008).

ACC Deaminase

The hormone ethylene is involved in the plant developmental cycle, and it may be stimulatory or inhibitory, depending upon its concentration (Penrose et al. 2001). Ethylene has previously been found to be an inhibitor of plant root elongation in several different systems (Glick 2005). The production of ethylene in plants is highly dependent on the endogenous levels of 1-aminocyclopropane-1-carboxylate (ACC). The enzyme ACC deaminase is present in many rhizosphere bacteria (Glick 2010). Such bacteria can take up ACC from the plant root and convert it into a-ketobutyrate and ammonia. This results in the decrease in ACC levels and therefore also in ethylene levels in the plant and in decreased plant stress (Bianco and Defez 2009; Pliego et al. 2011).

PGPR containing the enzyme ACC deaminase decrease the ethylene level, enhance the survival of some seedlings, and improve root growth and development in various plants such as tomato, pepper, and bean under stressed conditions (Glick

et al. 1998; Mayak et al. 2004; Nadeem et al. 2009). We have previously reported that PGPR strain *P. trivialis* 3Re27 is able to utilize ACC as its sole N source, indicating that it contains ACC deaminase. This observation suggests that the presence of ACC deaminase leads to an increase in salt tolerance and a stimulation of shoot and root growth of goat's rue in salinated soil (Egamberdieva et al. 2013a).

ACC deaminase-producing *Achromobacter piechaudii* strain ARV8 confers 'induced systemic tolerance' (IST) against drought and salt stress in pepper and tomato (Mayak et al. 2004). Shahzad et al. (2010) observed that rhizobacteria containing ACC deaminase increase the number of lateral roots, lateral root length, and root dry weight of chickpea seedlings and a direct correlation has been found between in vitro bacterial ACC deaminase activity and root growth (Shaharoona et al. 2006). Longer roots may take up relatively more water from deep soil under stress conditions, thus increasing the water use efficiency of the plants (Zahir et al. 2008).

In another study, *P. fluorescens* strain TDK1, which produces ACC deaminase, improved the plant growth parameters and the salt stress resistance of groundnut seedlings under saline condition as compared to plants inoculated with *Pseudomonas* strains lacking ACC deaminase activity (Saravanakumar and Samiyappan 2007). Similar results were observed by Kausar and Shahzad (2006), who reported that *P. fluorescens* containing ACC deaminase stimulated root growth of maize under saline conditions.

It is assumed that ACC exuded from the root will be degraded by ACC deaminase and that the products of hydrolyzed ACC will be used by root-colonizing bacteria. In that way, both plant and bacteria benefit from this process (Glick et al. 1998; Bianco and Defez 2012). In our opinion, a more likely and more efficient explanation is that the ACC deaminase-producing bacterium uses the needle of the type three secretion system to suck up plant sap containing ACC and deliver it in the bacterial cytoplasm where the enzyme ACC deaminase is located.

Root Colonization

Efficient colonization of the plant surface is the only option for bacterial soil inoculants to survive under adverse soil conditions and to compete with the better adapted native microflora in this highly competitive environment (Van Overbeek and Van Elsas 1997; Lugtenberg et al. 2001; Rekha et al. 2007; Lugtenberg and Kamilova 2009). The successful colonization of the rhizosphere by introduced beneficial bacteria also requires that these bacteria are well adapted to the rhizosphere and have some selective advantage over the numerous indigenous bacteria which have the potential to colonize that rhizosphere (Kawaguchi et al. 2002).

In one of our studies, the salt-tolerant bacterial strains *P. cholororaphis* TSAU13 and *P. extremorientalis* TSAU20 were able to colonize the rhizosphere of wheat under saline conditions up to 125 mM NaCl (Table 4.2). The colonization of *P. chlororaphis* TSAU13 was slightly inhibited, from 4.1 to 3.2 [Log (CFU)/cm of root tip], at 125 mM NaCl (Table 4.2). These results show that both bacterial

Table 4.2 Effect of salt stress on the colonization of bacterial strains *P. chlororaphis* TSAU13 and *P. extremorientalis* TSAU20 in the rhizosphere of wheat (Log CFU/cm of root tip), grown in a gnotobiotic sand system for 7 days

Bacteria	NaCl concentrations (mM)			
	0	50	100	125
P. chlororaphis TSAU13	4.1 ± 0.2	4.1 ± 0.3	4.0 ± 0.2	3.2 ± 0.2
P. extremorientalis TSAU20	4.6 ± 0.2	4.6 ± 0.1	4.4 ± 0.1	3.8 ± 0.2

strains are able to survive on the root of wheat under saline soil condition. Similarly, Diby et al. (2005) reported that the population of *P. pseudoalcaligenes* MSP-538 in rice root was not inhibited with increasing salinity. Paul and Nair (2008) also observed that the root colonization potential of the salt-tolerant strain *P. fluorescens* MSP-393 is not hampered by high salinity in the soil.

In our previous study, rifampicin-resistant mutants of the effective biocontrol strains *P. alcaligenes* PsA15, *P. chlororaphis* TSAU13, *P. extremorientalis* TSAU20, and *B. amyloliquefaciens* BcA12 were able to colonize the rhizosphere of cotton and persisted in saline soil (Egamberdieva and Jabborova 2013). Strain *P. extremorientalis* TSAU20, which was isolated as an enhanced wheat root colonizer (Egamberdieva and Kucharova 2009), showed high colonization ability in the rhizosphere of cotton, whereas *B. amyloliquefaciens* BcA12 had lower colonization ability. Bacterial motility could contribute to survival in the soil and the initial phase of colonization, where attachment and movement toward the root surface are important (Turnbull et al. 2001). *Pseudomonas* strains are motile and able to colonize the entire root system, in contrast to *Bacillus* which was unable to effectively colonize the rhizosphere of plants (Fukui et al. 1994).

Conclusion and Future Prospects

The present review indicates that soil salinity decreases germination, plant growth, plant development, and nutrient uptake. PGPR isolates are able to alleviate salt stress in plants, increase germination, shoot/root length, dry matter production, and yield in various agricultural and horticultural plants. Thus, PGPR can contribute significantly to solving the plant production problems caused by high salinity. Elucidation of the mechanisms of alleviation of salt stress and plant growth promotion by PGPR, such as stimulation of root growth by the production of phytohormones, decreasing ethylene levels by the enzyme ACC deaminase, production of osmoprotectants, and competition for nutrient and niches has provided a greater understanding of possible ways to open new doors for strategies which can improve the efficacy of PGPR agents. However, there is still a lot that is not understood regarding the functioning of these organisms under stressed soil conditions and also with respect to their interactions with the host plant. Knowledge of the mechanisms contributing to plant stress tolerance by PGPR as well as

the constraints to their activity under severe conditions can facilitate a more effective use of bacterial inoculants. More detailed studies are needed on the role of abiotic factors in altering the activity of rhizobacteria and managing plant–microbe interactions, with respect to their adaptability to extreme environments. Aspects which have to be included in future research are (1) mechanisms involved in alleviation of salt stress in plants, (2) potential competition between PGPR strains and indigenous soil microflora in the rhizosphere of plants grown in stressed environments, and (3) induction of salt stress tolerance at plant tissue, cell, and molecular level.

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Chapter 5
Drought Stress and Mycorrhizal Plant

Marcela Claudia Pagano

Introduction

Interest in stressful conditions is rising with increasing the recognition that global changes can negatively affect ecosystems (Firbank et al. 2008; Scherr and McNeely 2008). The environment affects organisms in many ways named environmental factors, which can be biotic or abiotic. The effect of abiotic environmental factors (temperature, humidity, light, water supply, nutrients, and CO_2) (see Table 5.1) differs with their intensity as they regulate plant growth (Schulze et al. 2005).

Plant tolerance to abiotic stresses such as drought has been reported for different plant species. For example, Eucalypts species are known for their capacity to tolerate several stresses. Olive trees (Sofo et al. 2008), Agave, and native cactus from Mexico (Monroy-Ata and García-Sánchez 2009) as well as some native trees from semiarid of Brazil (Pagano et al. 2013) are able to survive under soil water conditions. It is worth noting, moreover, that these plant species require symbiotic fungal endophytes for growth under abiotic stress (see below).

Plants are sessile organisms exposed to natural climatic or edaphic stresses (drought, high irradiation, heat, frost, flooding, nutrient differences) and to environmental changes from human activities (air and soil pollution, soil degradation) (Schützendübel and Polle 2002). Nowadays, biotechnological techniques of stress tolerance in plants are increasingly pursued. For example, under stress, arbuscular mycorrhizal fungi (AMF) are able to modify plant physiology in a way so that the plant can subsist with those environmental factors (Miransari et al. 2008). Accordingly, the use of mycorrhizas as plant inoculants is being recommended to help plants to prosper in degraded arid/semiarid areas.

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Table 5.1 Abiotic plant stress factors. Adapted from Schulze et al. (2005)

Type			
Abiotic	Water	Drought	
		Flooding	
	Temperature	Heat	
		Cold	Chilling
			Frost
	Radiation	Light	
		UV	
		Ionizing radiation	
	Chemical stress	Mineral salts	Deficiency, over-supply
			pH, salinity
		Pollutants	Heavy metals
			Pesticides
		Gaseous toxins	
	Mechanical stress	Wind	
		Soil movement	
		Submergence	

Several reports have showed that mycorrhizal symbiosis improves plant health through increased protection against environmental stresses such as drought (Azcón and Barea 2010; Barea et al. 2005a, b). Additionally, recent investigations pointed to the increasing recognition of the occurrence of AMF in dry forests of Brazil (Pagano et al. 2010, 2012, 2013) and northern Ethiopia (Birhane et al. 2010, 2012). Moreover, some plant species need to cope the severe conditions caused by flooding and drought, as in the Netherlands, where riparian edge forests dominated by *Salix* (well adapted to anaerobic soil conditions) associate with only a limited number of mycorrhizal fungi (ectomycorrhizas) (Parádi and Baar 2006). Most of the research is based on limited experiments done in glasshouse or nursery. For example, in India, an important multipurpose fruit tree of arid and semiarid regions (*Ziziphus mauritiana*) showed great dependency on AMF under water stress conditions (Mathur and Vyas 2000).

To finish, there is an increased interest on biochar soil amendment not only to improve soil fertility and plant productivity, but also to alleviate drought stress (Elad et al. 2011). The mechanisms by which biochar increases water retention are scarcely understood; however, it promotes mycorrhizal fungi and modifies soil microbial populations and functions (Elad et al. 2011). The promotion of AMF by biochar is also poorly understood, further studies being needed (Warnock et al. 2007).

This chapter examines the current information on the AM symbioses with respect to the research results on plant growth as affected by drought. Additionally, soil amendments that may have a synergistic influence are discussed.

Plants and Drought Stress

Of severe significance are the effects of global change on soils: increased soil temperatures, increased nutrient availability, increased ground instability in mountainous regions, increased erosion from floods to name just a few (Simard and Austin 2010). It is known that abiotic stresses (Table 5.1), such as drought, adversely affect plant growth, productivity and generate morphological, physiological, biochemical, and molecular changes in plants. However, different plant species can vary in their sensitivity and response to water deficit (Schulze et al. 2005).

Plant reactions to water deficiency (including stress avoidance or tolerance) are complex. Stomata close in response to water deficit; however, it is more related to soil moisture than to leaf water status, involving chemical signals produced by roots (Chaves et al. 2002). Among abiotic stresses, drought and salinity stress are considered to be the most important factors limiting plant growth (Ruiz-Lozano 2003). The symptoms of drought are leaf wilting, reductions in the net photosynthesis rate, stomatal conductance, water use efficiency, relative water content, and gradually diminution in total chlorophyll content.

Plants can react to drought at morphological, physiological, and cellular levels with modifications that allow the plant to avoid the stress or to increase its tolerance (Ruiz-Lozano 2003). These morphological and physiological adaptations can be of vital importance for some plant species, but they are not a general response of all plant species. In contrast, the cellular responses to drought stress seem to be conserved in the plant kingdom. To date, reports including plant tolerance to drought (18,264 documents in SCOPUS from 1984 to June 2013) have increased in the last 10 years (69 % of which were published in the recent decade).

Mycorrhizal Fungi and Drought

It is known that drought can decrease plant growth and production. AMF can improve plant growth and production under different conditions, including various soil stresses (reviewed by Miransari 2010). This was explained in terms of plant allocation of more photosynthate to mycorrhizal hyphae to increase soil resource uptake as nutrient and water limitations increase and can be seen in high latitude and altitude ecosystems (see Simard and Austin 2010).

With regard to ectomycorrhizas, the complex transport of water from deep soil to the mycorrhizal sporocarps has served to understand the dynamic and important complex structural elements of the soil–fungal–plant interface (Allen 2007, 2009). Special attention on trees, e.g., in Europe, showed that oak species (*Quercus robur*, *Quercus petraea*, *Quercus pubescens*) inoculated with ectomycorrhiza (*Cenococcum geophilum*) tolerated strong drought. Moreover, the relative

abundance of ectomycorrhizal species in the community will be manipulated by drought (Herzog et al. 2013).

With regard to AMF, they can promote plant growth increasing plant production under stress due to the establishment of extensive hyphal networks and secretion of glomalin, which enhance water and nutrient uptake meliorating soil structure (Miransari 2010).

Interestingly, biotechnology offers new strategies that can be used to develop transgenic crop plants with improved tolerance to stresses. Moreover, germplasm collected from high-altitude and low-temperature areas, cold-tolerant mutants, and wild species can be exploited for improved tolerant genotypes in other regions.

Earlier studies (Augé et al. 1987; Duan et al. 1996; Subramanian et al. 1995) showed a higher stomatal conductance, transpiration rate, and leaf water potential in mycorrhizal plants under drought. This was attributed to a higher water uptake, which allows plants to maintain higher rates of photosynthesis and higher water contents than non-mycorrhizal plants. The mechanism of modification of host-plant–water relations rests unknown.

However, different hypotheses have been tested with inconclusive results. Among those hypotheses, the following were proposed: (1) an indirect effect of improved P nutrition in mycorrhizal plants (Augé et al. 1986; Fitter 1988), (2) an improvement in water uptake in mycorrhizal roots by the extraradical hyphae (Ruiz-Lozano and Azcón 1995), by increasing effective root hydraulic conductivity or by modifying root architecture, (3) a biochemical modification of water regulation in the host plant through changes in hormonal signaling, (4) stimulation of osmoregulatory responses in mycorrhizal plants (Augé et al. 1986), and (5) changes in soil water retention properties (Morte et al. 2000).

It has been shown that Arbuscular mycorrhizal (AM) symbiosis can modify water relations and drought responses of host plants (Augé 2001). Numerous reports have compared mycorrhizal plants with control plants; however, more suitable comparisons (with different fungal species) are nowadays required (Augé et al. 2003). Among the AM symbiotic characteristics associated with water relations, some authors focused on the extent of extraradical hyphal development in the soil. This was explained in terms of contribution to root water absorption (Ruiz-Lozano and Azcón 1995) or by moisture retention and modification of drainage properties (Augé et al. 2001; Bearden 2001).

Several authors suggested that extraradical hyphal development in mycorrhizal fungi was associated with greater drought resistance of plants growing in those soils or observed a significant occurrence of extraradical hyphae in semiarid ecosystems. To such aim, glasshouse experiments by Augé et al. (2003) showed that soil hyphal colonization (extraradical hyphae) had superior effects on both lethal leaf water potential and soil water potential than did root hyphal colonization, root density, soil aggregation, soil glomalin concentration, and other variables. Moreover, a semiarid mix of mycorrhizal fungi used as inocula was superior to the single inoculation of *Glomus intraradices*. They highlighted the importance of soil hyphae on the water relations of host plants. In semiarid plants of Mexico, Monroy-Ata and García-Sánchez (2009) also showed better water

Table 5.2 Some recent book and reviews* dealing with occurrence of AMF in drought-stressed conditions

Reports	References
Reports on plant–water relations, drought, and AM symbiosis	Augé (2001)*
Reports on molecular studies of Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress	Ruiz-Lozano (2003)*
AMF and soil stresses	Miransari (2010)*
Drought tolerance and AMF in Grassland, Argentina	Busso and Bolletta (2010)
AMF and alleviation of soil stresses	Miransari et al. (2008)
AMF and alleviation of soil stresses	Siddiqui et al. (2008)
AMF and environmental stresses	Smith and Read (2008)

reviews

relations, plant growth, and survival in plants associated with AMF. They tested species of Fabaceae, Cactaceae, and Agavaceae mainly in greenhouse, showing the magnitude of AMF inoculation.

Since the publication of the seminal books of Sieverding (1991), Smith and Read (2008), van der Heijden and Sanders (2003) and Miransari et al. (2008, 2011) and several reports (see Table 5.2), the need for more information on how AMF influence plant drought stress in different plant and crop species was highlighted. However, to increase our ability to optimize AMF research, experiments under field situations are still urgently needed. Most recently, Gholamhoseini et al. (2013) showed that inoculation of AM such as *Glomus mosseae* can be more benefic under drought stress, e.g., for the cultivation of sunflowers under arid and semiarid ecosystems, where water is the most important factor in determining plant yield. Additionally, inoculation of *Glomus* spp. offered a better seedling resistance (improved plant growth and physiological performance) in *Sophora davidii*—spiny, multistemmed, deciduous shrub native to southwestern China, under water stress (Gong et al. 2013). The last plant species has important use for revegetation in the semiarid Loess Plateau and arid valley areas of China.

Mycorrhizal plants under drought conditions increase stomatal conductance, transpiration rate and leaf water potential due to a higher water uptake (Augé 2001) than non-mycorrhizal plants. The mechanism by which mycorrhizas modify host-plant–water relations remains unknown (different hypotheses have been tested with inconclusive results (Morte et al. 2000) and the contribution of AM symbiosis to plant drought tolerance is now seen as the product of accumulative effects (physical, nutritional, physiological, and cellular) (Ruiz-Lozano 2003).

Evidence from different continents indicates that most vegetation types subjected to drought stress present AMF. Monroy-Ata and García-Sánchez (2009) compiled the benefits of AMF in semiarid plants of Mexico. They showed more improved water relations and plant growth in such environments in comparison with uninoculated control plants. In southeastern Spain, Barea et al. (2011) compiled the diversity of mycorrhizas found in semiarid Mediterranean ecosystem. They showed the benefit of mycorrhizal fungi to help plants to establish and deal
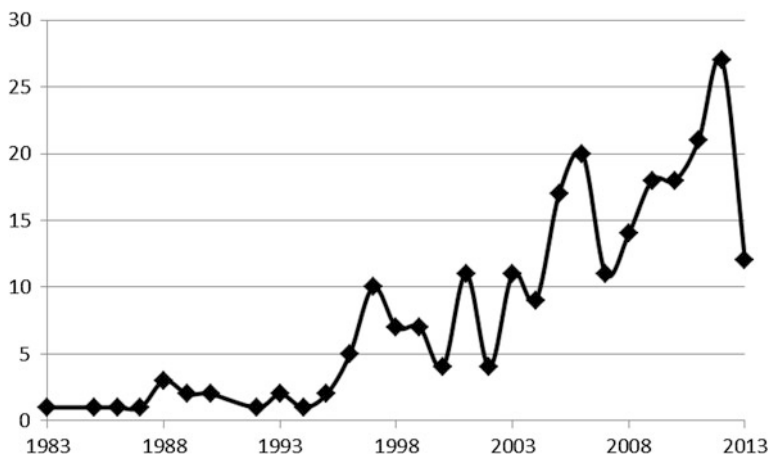
Fig. 5.1 Number of papers on AMF and drought published annually since 1983, included in the SCOPUS. Database survey conducted on June 2013

with nutrient deficiency, drought, soil disturbance, and other environmental stresses characteristically involved in soil degradation.

Modern research (Fig. 5.1) suggests a high diversity of AMF in natural ecosystems, since reports from highland fields as well from deciduous forest (see Pagano and Araújo 2011; Pagano 2012) pointed out a total of ∼28 AM plant species and at least 36 AM species that occurs in those ecosystems (Pagano et al. 2013). Additionally, de Carvalho et al. (2012) reported 49 AMF species in highland fields from Brazil (23 AMF species are in common with the reports cited above). It is worth noting, moreover, that arid and semiarid regions of Argentina present in general xerophytic plants, forming dry forests, open scrublands, shrub steppe, etc. Different vegetal types such as Jarillal and Puna presented 225 AM plant species (Pagano et al. 2012), some of them also associated with dark septate endophytic fungi (DSE) (Lugo and Cabello 2002; Lugo et al. 2008). Moreover, in dry Puna ecosystem (2,000–4,400 m above the see level), ten AMF species were found, and *Glomus* was the predominant genus.

Reverse flows (hydraulic redistribution from plant to fungus) were recognized but we know little about this (they could play a critical role in supporting hyphae through drought). Moreover, the crucial importance of mycorrhizae in plant–water relations is influenced by the drying patterns, the soil pore structure, and the number of hyphal connections extending from the root into the soil (Allen 2007, 2009).

Recently, Li et al. (2013) revealed higher relative water content in colonized roots of maize by *G. intraradices*. The increased expression of two aquaporins genes in both root cortical cells containing arbuscules and extraradical mycelia under drought stress was reported. Moreover, the observed higher hyphal growth can be related to extension of the water absorption area.

Thus, new directions in microbial ecology must include the integration of microbial physiological ecology, population biology, and process ecology as microorganisms have a diversity of evolutionary adaptations and physiological mechanisms to cope with the environmental stress (Schimel et al. 2007).

Drought Stress and Agriculture

Maintenance of soil health has become a serious issue of agriculture, and the sustainable management of agricultural land has gained increasing relevance (Pagano et al. 2011). Moreover, the current intensive farming and agriculture are based on high-yielding cultivars which demand more nutrients, water, and chemicals (Tilman et al. 2002). Additionally, drought has proved to be a usual stress affecting agriculture and forestry, being able to change soil microbial abundances, including mycorrhizas composition. Few projects were based on field experiments (Pagano and Covacevich 2011; Schalamuk and Cabello 2010; Oehl et al. 2010) and showed that AMF occurs in high diversity in the fields (also in soil depth).

The use of different soil amendments in rotation to select AMF in order to benefit a particular crop as well as AMF inoculation is a topic that needs more detailed research and basic knowledge of AMF ecology (Jaison et al. 2011). Mycorrhizal plants can present higher water potential being capable to improve plant growth at a faster rate when irrigation is restored (van der Heijden and Sanders 2003; Miransari et al. 2011).

Little attention has been paid to the soil stresses and their effect on roots. Tillage promotes disruption of the AMF hyphal network and dilution of the propagule-rich topsoil (Schalamuk and Cabello 2010), which disturbs the soil physical and chemical properties, modifying the number, diversity, and activity of the soil microbiota, including both free and symbiotic fungal populations (Pagano 2011).

In this sense, anthropogenic alterations (perturbation stresses) to improve the productivity of crops (e.g., tillage, monoculture, crop rotation, irrigation, amendments and crop protection) result in disruption of the native soil microbial ecosystem. While moderate perturbation will be benefic in the short term, higher levels of stress may result in the degraded soils (Sturz and Christie 2003). The conventional tillage system, still commonly used in some countries, usually consists of moldboard plowing and additional secondary operations to prepare the seedbed. However, field traffic or intensive tillage result in excessive soil compaction and soil water loss. It is recognized that most plant species of agricultural interest associate with AMF (Miransari et al. 2011; Pagano and Covacevich 2011; Miranda 2008).

As tillage reduce AMF spore and hyphal length densities, AM fungi can be strongly decreased by conventional agricultural practices, possibly due to disturbance of AM fungal hyphal networks, changes in soil nutrient content, and altered

microbial activity (Jansa et al. 2003, 2006), which can reduce glomalin content and thus the tolerance to drought.

In Argentina, earlier studies have found less management of AMF in order to increase plant productivity (Covacevich and Echeverría 2009). Soils of the Pampas region present high native AMF that colonize crop plants under different management systems (Covacevich et al. 2006, 2007; Schalamuk et al. 2006); however, they are not yet manipulated. More recently, Schalamuk and Cabello (2010) showed that different types of AM inocula from a field experiment with tilled and no-tilled wheat and from non-disturbed sites (spontaneous vegetation) presented different proportions of AM families, between field and trap cultures. Glomeraceae were higher in the trap cultures, which was attributed to the use of intra- and/or extraradical mycelium, showing advantages in the use of these propagules. Furthermore, those results suggested a huge importance of the selection of AMF species to be included under agricultural practices.

Biochar and Drought Stress

Biochar soil amendment can contribute to improved soil fertility and assumed the potential benefits to the agricultural productivity. However, the mechanisms by which it is effective in enhancing plant growth are scarcely understood, as well as the indirect effects (increased water and nutrient retention, improvements in soil pH, increased soil cation exchange capacity, effects on P and S transformations, neutralization of phytotoxic compounds, improved soil physical properties, and alteration of soil microbiota) (Elad et al. 2011).

In this regard, biochar promotes AMF, but few studies were performed in order to elucidate the "Biochar Effect" (Warnock et al. 2007), indicating the need to more future research to elucidate it (Elad et al. 2011). Recent studies, for example, showed that biochar addition improved AMF colonization of asparagus roots, contributing to the control of diseases (Elmer and Pignatello 2011; Elmer 2012). Nevertheless, the relevance of studies on biochar associated with AMF is still unknown since few studies have been published (13 documents in SCOPUS from 2007 to June 2013).

Reports including biochar and drought are lesser (only 10 documents in SCOPUS from 2009 to June 2013) and have increased in the last four years. Working with maize (Zea mays L.) under field conditions, Liu et al. (2012) demonstrated a synergistic positive effect of compost and biochar on soil fertility and water storage capacity. Working with wheat, Solaiman et al. (2010) suggest improved water supply to reduce drought stress with the addition of AMF. These fungi can prolong crop exploration of water from the wide inter-rows, improving grain yield and survival. Additionally, they tested the residual effect of biochar (after 2 years) and mineral fertilizers in a bioassay showing the improved conditions for root colonization after application of biochar.

Fig. 5.2 Protocol for studying the effect of drought stress and biochar effect on AM plants. Roots of plants are stained for AM colonization (**a**). Determination of infective propagules including spores (**b**) and bioassays against soil samples are required (photos by M. Pagano)
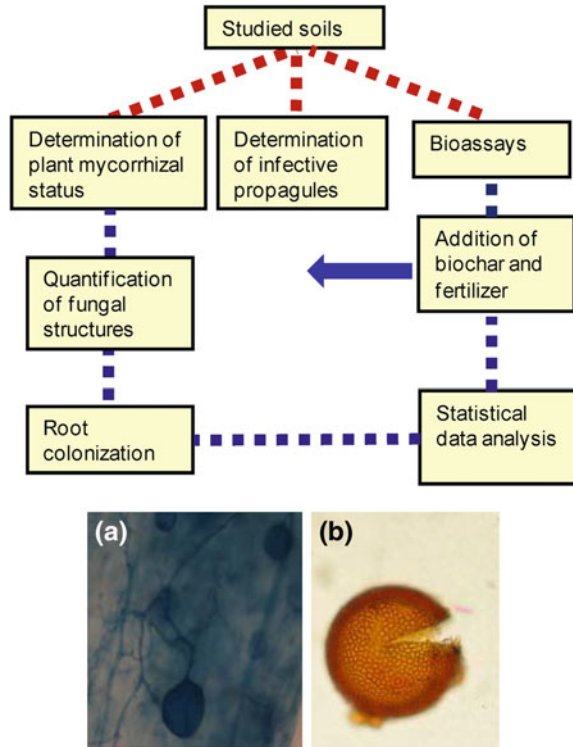
Later, LeCroy et al. (2013) examined the interaction between biochar, AMF (*G. intraradices*), and nitrogen on sorghum seedling growth in greenhouse. They showed that addition of mycorrhizae and low nitrogen caused more oxidation (biotic oxidation) of the biochar surface than the other tested combinations and found a greater fraction of carbon present as carbonyl groups. Moreover, they suggested that the greater oxidation can be related to the AMF behavior with a more activity in their search for nutrients in a nitrogen-limited situation. A protocol for studying the effect of drought stress and biochar effect on AM plants is presented in Fig. 5.2.

It is also known that biochar may help to remove allelopathic effects via adsorption and detoxification (Wardle et al. 1998). However, further studies assessing the types of biochar (depending on original feedstock and pyrolysis conditions) (Downie et al. 2009; Krull et al. 2009) that induce resistance responses in plants against pathogens and parasites including fungi, bacteria, viruses, and nematodes are urgently needed.

Conclusion

In the introduction to this chapter, I briefly described plant stress factors and the benefits that mycorrhizal fungi provide to their plant hosts. Throughout the chapter, I have showed that stress affects soil physical and chemical properties, influencing the population, diversity, and activities of soil microbes, including symbiotic fungal populations. To identify mycorrhizal fungal species, which may contribute to plant growth under stress, the mycotrophic status of plant species is crucial, especially with regard to drought stress, as the fungi mediate the link of the plant to the soil. Additionally, anthropogenic alterations (tillage) were discussed with regard to drought although more detailed studies are lacking. The alleviation of drought stress would have great implication in the manipulation of AMF species able to colonize plants in arid and semiarid soils approving the potential of AMF to be inoculated. This chapter argues that AMF alleviate drought stress, which has great effect on plant growth; however, development of technologies and protocols to cope with drought are crucial. Lastly, the potential benefits to the agricultural productivity of biochar soil amendment and their interactions with mycorrhizal plants under drought were also pointed.

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Chapter 6
PGPR to Alleviate the Stress of Suboptimal Root Zone Temperature on Leguminous Plant Growth

Narjes H. Dashti, Donald L. Smith and Vineetha M. Cherian

Effect of Low RZTs on Legume Nodulation and Nitrogen Fixation

Legumes—soybean, pea, and lentil are medicinally important, health-promoting plants with great nutritive value (Lee 2009). Leguminous plants are capable of meeting much of their nitrogen requirement from symbiotic nitrogen fixation. Certain subtropical legumes, such as soybean, require a temperature in a range from 25 to 30 °C for optimal symbiotic activity. When root zone temperature (RZT) drops below this range, a stress is created, which in turn affects legume nodulation and nitrogen fixation, negatively (Zhang et al. 2002; Madhavi et al. 2007). Lie (1974) noted that all stages of nodule formation and functioning are affected at low RZT. Perhaps one of the reasons for this is that at low temperatures, expression of the nod genes is inhibited, resulting in delayed onset of nodulation (Zhang et al. 2002). Low RZTs have shown to inhibit the biosynthesis and rhizosecretion of plant-to-bacteria signal molecules such as genistein in soybean roots which are necessary for the induction of the *nod* gene of *Bradyrhizobium japonicum* (Zhang et al. 2002; Lee 2009). The disruption of the production/excretion of the *nod* factor at low RZTs in *B. japonicum* has also been reported by Duzan et al. (2004). In a review of the data on environmental effects on the legume–*Rhizobium* symbiosis,

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Gibson (1971) suggested that low RZTs retard root growth infection more than nodule initiation, nodule development, or N assimilation. Studies of the effects of suboptimal RZTs on soybean [*Glycine max* (L.) Merr.] have shown that these conditions decrease N_2 fixation activity by the nitrogenase enzyme complex (Layzell et al. 1984) and suppress and/or delay root infection and nodulation (Walsh and Layzell 1986). The effects of low temperature on the function of N_2-fixing nodules may be, in part, due to changes in nodule O_2 permeability (Sinclair and Weisz 1985; Weisz and Sinclair 1988). Plants such as soybean export the N_2 fixed from the nodule, mainly in the form of ureide. The solubility of ureide is low and decreases sharply as temperature declines. Therefore, low RZTs may also limit the rate of export of fixed N from the nodule. Decreased temperature resulted in progressively less bacteroid tissue (Lie 1974) and a decrease in its formation rate (Fyson and Sprent 1982). The effect of low RZT on temperate zone legumes has been investigated. Low RZT decreases both nodulation and N_2 fixation rates affecting all stages of nodule formation and function (Lee 2009). Lynch and Smith (1993) observed that a RZT of 15 °C severely restricted both infection and nodule development and delayed the onset of nitrogen fixation until approximately 7–8 weeks after inoculation. In Canadian soybean-growing areas, soil temperatures at a depth of 10 cm during the early growing season often range from 10 to 15 °C. Soybean production in eastern Canada is at the most northern limit of North American crop.

Zhang et al. (1995) demonstrated that (1) RZTs less than 17 °C strongly inhibited both infection and nodule development, (2) the early nodule development stages (within 14 days after inoculation) were very sensitive to RZT, (3) an early infection step (within 12 h after inoculation) is most sensitive to low RZTs, and (4) before flowering, inoculated plants at RZTs between 17 and 25 °C fixed some nitrogen, but plants at 15 °C RZT had not began to fix nitrogen.

Root hair infection of *Trifolium subterraneum* is more sensitive to low RZT than nodule development or nitrogen assimilation (Gibson 1971). Lower RZTs decrease nodule growth and total nitrogen fixed per plant by inhibiting infection and nodule initiation (Matthews and Hayes 1982). These RZTs primarily retard root infection (Lindemann and Ham 1979).

Plant Growth-Promoting Rhizobacteria: Benefits and Mechanisms of Growth Promotion

Understanding the rhizosphere biology has progressed with the discovery of a specific group of microorganisms, now called plant growth-promoting rhizobacteria (PGPR), that can colonize plant roots and stimulate plant growth and development (Bianciotto et al. 2000). Most of the identified strains of rhizobacteria occur within gram-negative genera, of which fluorescent pseudomonads are most characterized (Adesemoye and Ugoji 2009). Several gram-positive strains of root-colonizing bacteria were reported such as an *Arthrobacter*-like genus and

Bacillus (Kloepper et al. 2004). Other documented PGPR include *Azotobacter* species, *Azospirillum* species, and *Acetobacter* species (Bashan and Levanony 1990; Tang et al. 1994).

PGPR have many benefits. PGPR's ability to increase crop yields under diverse conditions has been reported (Zehnder et al. 2000; Nelson 2004; Sahran and Nehra 2011; Dashti et al. 2012). PGPR were reported to increase plant yields 10–30 % in non-legume crops such as potato, radish, tomato (Dashti et al. 2007, 2012) and sugar beet.

Studies have shown that simultaneous infection with rhizobia and rhizospheric bacteria increases nodulation and growth in a wide variety of legumes. Such nodule-assisting bacteria may be either epiphytic or endophytic (Dashti et al. 2005; Rajendran et al. 2012).

Specific root-colonizing bacteria can increase seedling emergence. This was first reported with strains that caused increases in emergence rates of soybean and canola seedlings under cold field conditions in Canada (Kloepper et al. 1986). The new class of PGPR strains was termed emergence-promoting rhizobacteria (EPR). Inoculation of conifer seeds with *Bacillus* strains caused increased seedling emergence and biomass (Chanway et al. 1991). Chanway (1995) also reported that seed inoculation with *Bacillus polymyxa* can result in the colonization of western hemlock root systems and increase seedling emergence.

The mechanism by which the PGPR promote plant growth is unknown for many of the bacteria involved; however, a wide range of mechanisms were postulated such as mobilization of insoluble nutrients (e.g., phosphate) and resulting enhancement of uptake by the plant (Nelson 2004), associative nitrogen fixation (Dashti et al. 2007), production of antibiotics toxic to soilborne pathogens (Nelson 2004), production of plant growth regulators that promote plant growth (Joseph et al. 2007), and siderophore production (Dashti et al. 2012). Specific pseudomonad strains have established yield increases, control of soilborne plant pathogens, promotion of seedling emergence, and promotion of legume nodulation by nitrogen-fixing (*Brady*)*rhizobium* spp. under field conditions.

PGPR–Legume Interaction and Symbiosis Establishment Under Suboptimal Root Zone Temperature Conditions

The effects of PGPR under suboptimal root zone temperature conditions have been investigated for leguminous plants, in particular soybean. Soybean is a subtropical legume; RZTs in the 25–30 °C range are optimal for symbiotic activity, compared to 20–24 °C for temperate legumes (Duzan et al. 2004; Subramanian and Smith 2013). Under normal growth conditions, a complex process is involved in the development of symbiotic association between PGPR and the soybean. The first stage of this interaction is the signal exchange between the two. The plant-to-rhizobacteria signals are usually isoflavonoids such as genistein and daidzein, which induce the

nod gene expression. The bacteria-to-plant return signals, the lipo-chitooligosac-charides (LCO) or nod factors, induce nodule formation on the plant roots (Bai et al. 2002). Over the last few decades, the cultivation of soybean has been extended into cool temperate areas where soil temperatures, in comparison with those of its natural habitat, are low during the first part of the growing season. Under such conditions, the poor adaptability of soybean to cool soils is considered the primary factor limiting yield. Another environmental factor affecting yield is the RZT. Studies on the effects of suboptimal RZT (<25 °C) on nitrogen fixation by soybean and other subtropical legume crops have indicated that at low RZT, the production of the plant-to-rhizobacteria isoflavonoid signals is inhibited, which in turn inhibits the subsequent root nodulation process (Bai et al. 2002). In addition to this, low RZTs suppress the bacterial *nod* gene expression. The time between soybean inoculation with certain bacteria such as *B. japonicum* and the beginning of nitrogen fixation increases by 2–2.5 days for every degree between 25 and 17 °C (Zhang et al. 1995). Below 17 °C, the time from inoculation to nitrogen fixation is delayed by a week per degree (Zhang et al. 1994). The greater sensitivity below 17 °C is due to an event that occurs within the first 12 h after inoculation, at 25 °C RZT (Lynch and Smith 1993; Zhang and Smith 1994); the greater inhibition by temperatures below 17 °C is due to an inability of the plant to either synthesize or excrete the plant-to-bacterium isoflavone signal molecule (4′, 5, 7-trihydroxyisoflavone, or genistein) at the beginning of symbiosis establishment (Zhang and Smith 1995). Slow nodule development in cool soils prolongs the period of nitrogen deficiency that occurs between the depletion of cotyledonary nitrogen reserves and the beginning of nitrogen fixation. A period of slow growth early on is reflected in growth throughout the remainder of the season.

PGPR produce many phytohormones and signal molecules, such as genistein, the plant-to-bacteria signal involved in the soybean nodule infection and formation processes. Therefore, inoculation of soybean plants with *B. japonicum* together with a PGPR or genistein may produce better symbiotic relationships at low RZTs as this results in higher relative increases in nitrogen fixation and subsequent soybean growth and yield than *B. japonicum* or PGPR alone (Zhang and Smith 1995). Second, PGPR stimulate overall plant growth, leading to greater nitrogen demand by the developing soybean plants, leading, in turn, to greater nodulation and nitrogen fixation. The addition genistein has shown to partially alleviate the inhibition of the plant-to-rhizobacteria isoflavonoid signals (Bai et al. 2002).

Plant Growth-Promoting Rhizobacteria Accelerate Nodulation and Increase Nitrogen Fixation Activity of Leguminous Plants at Different Root Zone Temperatures

Application of PGPR has been reported to increase nodulation and nitrogen fixation of soybean over a range of RZTs under controlled-environment conditions (Dashti 1997). Co-inoculation of other PGPR with rhizobia is envisaged as an

important practice in the development of sustainable agriculture (Rajendran et al. 2012). Two rhizobacteria, *Serratia proteamaculans* 1–102 and *Serratia liquefaciens* 2–68, co-inoculated with *B. japonicum* 532C were tested, in two separate experiments, for their ability to reduce the negative effects of low RZT on soybean nodulation and nitrogen fixation (Dashti 1997). Three RZTs were tested: 25 (optimal), 17 ± 5 (somewhat inhibitory), and 15 °C (very inhibitory). At each temperature, some PGPR strains increased the number of nodules formed and the amount of fixed nitrogen when co-inoculated with *B. japonicum*, but the stimulatory strains varied with temperatures. The strains that were most stimulatory varied among temperatures and were as follows: 15 °C, *S. proteamaculans* 1–102; 17 ± 5 °C, *S. proteamaculans* 1–102; 25 °C, *S. liquefaciens* 2–68 (Zhang et al. 1996). The total fixed nitrogen, fixed nitrogen as a percentage of total plant nitrogen, and the nitrogen yield also increased due to PGPR application. Interactions existed between PGPR application and soybean cultivars, suggesting that application of the PGPR to cultivars with higher yield potentials was more effective. Inoculation with PGPR only also increased soybean nodulation and nitrogen fixation by native *B. japonicum*. Nodule dry weight per plant was increased, the onset of nitrogen fixation was hastened by *B. japonicum* co-inoculation with PGPR, and total fixed nitrogen and nitrogen yield per plant were increased (Tables 6.1, 6.2).

Co-inoculation of soybean plants with PGPR strains produced a wide range of effects which varied among PGPR and over RZTs. *S. proteamaculans* 1–102 and *S. liquefaciens* 2–68 were reported to enhance nodulation and nitrogen fixation at suboptimal RZTs (Zhang et al. 1996). Bai et al. (2002) have shown that application of a known isoflavonoid root activator showed the same efficacy in promoting root nodulation in soybeans as that of the PGPR *S. proteamaculans* 1–102. This shows that the PGPR *S. proteamaculans* is capable of producing similar root activator compounds by which they promote nodulation. Moreover, it was capable of activating these compounds even at suboptimal RZTs. At an optimal RZT (25 °C), *S. liquefaciens* 2–68 increased nodule dry weight per plant, nodule size, and ratio of nodule weight to plant weight. The increase in the ratio of nodule weight to plant weight was due to increased nodule size (as indicated by the higher average weight per nodule). At 15 °C RZT, *S. proteamaculans* 1–102 increased nodule number, nodule dry weight per plant, nodule size, and ratio of nodule weight to plant weight, again, confirming the results of Zhang et al. (1996). Other studies on leguminous plants, in which PGPR were co-inoculated with a suitable rhizobacteria at both optimal and suboptimal RZTs, have also reported similar results. Bai et al. 2003 reported three *Bacillus* strains, *B. subtilis* NEB4, *B. subtilis* NEB5, and *B. thuringiensis* NEB17, in order to test their ability to improve soybean nodulation and growth under low RZTs, and these strains were co-inoculated onto soybean plants, with *B. japonicum*, under greenhouse conditions at RZTs of 25, 17, and 15 °C and under field conditions in a short growing season area. All the three *Bacillus* strains enhanced soybean nodulation and growth in greenhouse and field experiments. Co-inoculation of some Pseudomonas and *Bacillus* strains along with effective *Rhizobium* sp. is shown to stimulate chickpea growth, nodulation, and

Table 6.1 Effects of PGPR strain, *B. japonicum* strain, and soybean cultivar on soybean nodule number, nodule weight, and nodule nitrogen at two harvest stages at the unsterilized site (experiment 1)

PGPR	*B. japonicum*	Cultivar	Nodule number	Sampling on June 17				Nodule number	Sampling on August 13	
				Nodule wight		Nodule nitrogen			Nodule wight (mg)	
				(plant⁻¹)	(nodule⁻¹)	(mg g⁻¹)	(mg plant⁻¹)		(plant⁻¹)	(nodule⁻¹)
1–102	USDA110	AC Bravor	13.92	24.60	2.78	42.81	2.75	84.17	519.40	6.18
		Maple Glen	14.00	97.13	7.94	34.14	3.33	68.67	646.55	9.53
	532C	AC Bravor	10.58	42.20	4.40	40.99	1.71	71.00	632.89	9.36
		Maple Glen	15.33	56.82	3.61	39.44	2.29	90.42	804.54	9.61
2–68	USDA110	AC Bravor	13.33	78.86	6.00	40.26	3.17	52.92	502.96	10.49
		Maple Glen	13.30	76.27	6.35	40.27	3.08	103.00	923.00	9.01
	532C	AC Bravor	11.33	52.18	4.90	44.57	2.32	43.33	537.64	12.60
		Maple Glen	14.92	86.43	6.52	39.15	3.35	90.75	742.23	8.49
Control	USDA110	AC Bravor	18.33	11.57	0.62	47.63	0.57	37.83	380.99	10.07
		Maple Glen	19.33	15.73	0.82	32.46	0.51	34.08	410.64	13.03
	532C	AC Bravor	10.50	7.27	0.70	46.38	0.34	46.25	427.32	11.67
		Maple Glen	12.33	6.67	0.53	41.09	0.29	60.38	440.39	10.54
LSD$_{0.05a}$			8.29	19.59	2.78	10.86	1.79	33.32	274.11	4.83
LSD$_{0.05b}$			9.29	20.33	3.33	10.25	1.64	35.86	321.77	6.80
PGPR			NS	***	**	NS	***	**	*	NS
B. japonicum			*	*	NS	NS	NS	NS	NS	NS
Cultivar			NS	***	*	***	NS	**	***	NS
PGPR *B. japonicum*			NS	NS	NS	NS	NS	NS	NS	NS
PGPR*cultivar			NS	**	NS	NS	NS	**	NS	NS

(continued)

Table 6.1 (continued)

PGPR	B. japonicum	Cultivar	Nodule number	Sampling on June 17				Nodule number	Sampling on August 13	
				Nodule wight		Nodule nitrogen			Nodule wight (mg)	
				(plant^{-1})	(nodule^{-1})	(mg g^{-1})	(mg plant^{-1})		(plant^{-1})	(nodule^{-1})
			NS	***	**	NS	NS	NS	NS	NS
PGPR*B. japonicum*cultivar			NS	NS	NS	NS	NS	NS	NS	NS

Values represent the five plants in the ^{15}N microplot (area equal to 0.12 m^2) from each subplot unit. Means within the same column and *B. japonicum* strains or soybean cultivar were analyzed by an ANOVA-protected LSD test. LSD$_{0.05a}$ is for comparison of means within the same main-plot unit, and LSD$_{0.05b}$ is for comparison of means across levels of the main-plot factor. *NS*, *, **, and *** indicated no significant difference or significant differences at the 0.1, 0.05, and 0.01 levels, respectively

Table 6.2 Simple effect means for PGPR strain and soybean cultivar effects on soybean nodule number, nodule weight, and nodule nitrogen at two harvest stages at the sterilized site (experiment 1)

PGPR	Cultivar	Nodule number	Sampling on June 17				Nodule number	Sampling on August 13	
			Nodule weight		Nodule nitrogen			Nodule wight (mg)	
			(plant^{-1})	(nodule^{-1})	(mg g^{-1})	(mg plant^{-1})		(plant^{-1})	(nodule^{-1})
1–102	AC Bravor	11.90	21.69	1.78	34.56	0.79	57.58	180.13	3.35
	Maple Glen	14.17	29.17	2.26	33.20	0.95	78.17	211.59	2.66
2–68	AC Bravor	16.08	22.27	1.21	28.82	0.67	55.08	113.99	2.17
	Maple Glen	21.25	33.78	1.66	35.78	1.19	61.00	108.04	1.90
Control	AC Bravor	14.10	15.23	1.18	28.48	0.44	43.00	97.11	2.19
	Maple Glen	20.75	21.18	1.03	32.53	0.69	59.50	125.93	2.14
LSD$_{0.05a}$		14.33	18.62	0.94	8.93	0.61	21.33	73.77	1.05
LSD$_{0.05b}$		12.09	20.79	0.98	11.34	0.58	25.83	77.14	1.47
PGPR		NS	NS	*	NS	*	*	**	NS
Cultivar		NS	**	NS	NS	***	***	NS	*
PGPR*cultivar		NS	NS	NS	NS	NS	NS	NS	NS

Values represent the five plants in the ^{15}N microplot (area equal to 0.12 m^2) of each subplot unit. Means within the same column and *B. japonicum* strain or soybean cultivar were analyzed by an ANOVA-protected LSD test. LSD$_{0.05a}$ is for comparison of means within the same main-plot unit, and LSD$_{0.05b}$ is for comparison of means across levels of the main-plot factor. *NS*, *, **, and *** indicate no significant difference or significant differences at the 0.1, 0.05, and 0.01 levels, respectively

nitrogen fixation (Parmar and Dadarwal 1999; Rajendran et al. 2012). Stajcovic et al. (2011) showed that co-inoculation of PGPR of the Pseudomonas and Bacilli species along with rhizobacteria *Rhizobium phaseoli* showed increased nitrogen content and nitrogen fixation in the common bean (*Phaseolus vulgaris L.*).

A controlled-environment experiment was also conducted to examine the combined ability of both PGPR and genistein to reduce the negative effects of low RZT on soybean nodulation and nitrogen fixation (Dashti et al. 2000). Genistein, the most important plant-to-bacterial signal in the soybean-*B. japonicum* symbiosis, is a part of the earliest phase of the nodulation process, the release of signal molecules that trigger the coordinated expression of a series of bacterial nodulation (nod) genes in the bacterial symbiont (Bai et al. 2002). The isoflavones daidzein and genistein are the major components of the soybean root extracts responsible for inducing the *nod* genes of *B. japonicum* (Dashti et al. 2000). Genistein and/or related molecules are essential for the development of effective root nodules and responsible for inducing the *nod* genes of *B. japonicum*. Zhang and Smith (1996) have shown that the roots of plants germinated and grown at lower RZTs have lower genistein concentrations and contents than plants grown at higher RZTs. The beneficial effects of genistein increased with decreasing RZT (Zhang and Smith 1995). At suboptimal RZTs (17.5 and 15 °C), the most effective concentrations are in the 15–20 μM range, whereas at an optimal (25 °C) RZT, 5 μM is most effective. PGPR strains, *S. proteamaculans* 1–102 and *S. liquefaciens* 2–68, were co-inoculated with *B. japonicum* USDA110 or 532C preincubated with different concentrations of genistein (0, 15, or 20 μM). The resulting inocula were added to a soybean rooting medium to test their ability to reduce the negative effects of low RZT on soybean growth and development by improving the physiological status of the plants (Tables 6.3, 6.4).

Three RZTs were tested: 25 (optimal), 17.5 (somewhat inhibitory), and 15 °C (very inhibitory). At each temperature, PGPR strains and genistein together increased the number of nodules formed and the amount of fixed nitrogen, but the most stimulatory combination of PGPR, genistein concentration, and *B. japonicum* strain varied with temperature. The combinations that were most stimulatory at each temperature were as follows: at 15 °C—*S. proteamaculans* 1–102, genistein concentration 0 μM, and *B. japonicum* USDA110; at 17.5 °C—*S. proteamaculans* 1–102, genistein concentration 15 μM, and *B. japonicum* USDA110; and at 25 °C—*S. proteamaculans* 1–102, genistein concentration 5 μM, and *B. japonicum* USDA110. In at least some cases, these stimulatory effects can be attributed to additive effects of both PGPR and genistein in enhancing the number of nodules formed and the amount of nitrogen fixed by soybean plants.

The combinations of PGPR and genistein showed additive effects, when compared to PGPR or genistein alone, at higher RZT (25 °C), while they show antagonistic effects at lower RZT (15 °C). Genistein effects increased with decreasing RZT (Zhang and Smith 1995). At suboptimal RZTs (17.5 and 15 °C), the most effective concentrations were in the 15–20 μM range, whereas at an optimal (25 °C) RZT, 5 μM was most effective. At 25 °C, addition of PGPR plus genistein generally resulted in greater, and approximately additive, increases than

Table 6.3 Effects of co-inoculation of *B. japonicum* with PGPR on nitrogen concentrations of different soybean tissues and nitrogen concentration ratios for nodule:shoot, nodule:root, and shoot:root at three temperatures (experiment 1)

PGPR	*B. japonicum*	Genistein (μM)	Nitrogen concentrations (mg g^{-1})			Nitrogen concentrations ratios		
			Nodule	Shoot	Root	Nodule:shoot	Shoot:root	Root:root
15 °C								
1–102	USDA110	0	56.7	24.7	12.6	2.3	4.5	1.4
		20	47.3	19.7	11.7	2.4	4.0	1.7
	532C	0	61.0	17.9	12.9	3.4	4.7	1.4
		20	46.0	21.3	11.3	2.2	4.1	1.9
2 2–68	USDA110	0	53.1	18.0	13.0	2.9	4.1	1.4
		20	49.0	18.9	12.3	2.6	4.0	1.5
	532C	0	37.7	18.1	12.4	2.1	3.0	1.5
Control	USDA110	0	46.0	16.6	11.8c	2.8	3.9	1.4
		20	55.3	22.3	13.2	2.5	4.2	1.7
	532C	0	46.0	17.6	11.4	2.6	4.0	1.5
		20	65.7	17.9	13.0	3.7	5.1	1.4
LSD$_{0.05}$			**4.5**	**3.4**	**1.8**	**0.5**	**0.6**	**0.4**
17 °C								
1–102	USDA110	0	32.6	21.1	14.1	1.5	2.7	1.5
		15	35.4	25.0	14.8	1.4	2.4	1.7
	532C	0	32.3	17.2	14.1	1.9	2.3	1.2
		15	41.0	24.3	13.4	1.7	3.1	1.8
2 2–68	USDA110	0	52.4	21.2	9.6	2.1	5.5	2.5
		15	52.2	20.5	13.7	2.5	3.8	1.5
	532C	0	42.7	23.3	14.8	2.0	2.9	1.4
Control	USDA110	0	35.3	19.3	12.2	1.8	2.9	1.6
		15	48.0	23.7	15.7	2.0	3.1	1.5
	532C	0	37.7	17.0	10.4	2.2	3.6	1.6
		15	50.7	20.4	13.5	2.5	3.8	1.5
LSD$_{0.05}$			**8.2**	**2.8**	**2.6**	**0.5**	**1.1**	**0.4**
25 °C								
1–102	USDA110	0	52.8	35.9	16.1	1.5	3.3	2.2
		5	50.2	38.2	16.1	1.3	3.1	2.4
	532C	0	39.5	28.0	14.3	1.5	2.8	2.0
		5	50.4	34.6	13.5	1.5	3.7	2.6
2 2–68	USDA110	0	49.0	32.3	16.5	1.5	3.0	2.0
		5	50.7	33.8	16.2	1.5	3.1	2.1
	532C	0	51.5	34.8	14.5	1.5	3.6	2.4
Control	USDA110	0	40.9	30.3	13.8	1.3	3.0	2.2
		5	49.7	32.5	16.5	1.5	3.0	2.0
	532C	0	37.7	24.0	13.9	1.6	2.7	1.7
		5	52.8	36.0	14.3	1.5	3.7	2.5
LSD$_{0.05}$			**10.0**	**4.4**	**2.5**	**0.37**	**0.8**	**0.4**

Means the same column an experiment were analyzed by an ANOVA-protected LSD test

Table 6.4 Effects of co-inoculation of *B. japonicum* with PGPR on nitrogen concentrations of different soybean tissues and nitrogen concentration ratios for nodule:shoot, nodule:root, and shoot:root at three temperatures (experiment 2)

PGPR	*B. japonicum*	Genistein (µM)	Nitrogen concentrations (mg g^{-1})			Nitrogen concentrations ratios		
			Nodule	Shoot	Root	Nodule:shoot	Nodule:root	Shoot:root
15 °C								
1–102	USDA110	0	46.2	35.4	13.8	1.3	3.3	2.6
		20	47.1	31.7	12.4	1.5	3.8	2.6
	532C	0	47.0	19.5	12.4	2.4	3.8	1.6
		20	46.8	24.1	12.8	1.9	3.7	1.9
2 2–68	USDA110	0	44.4	21.3	13.9	2.1	3.2	1.5
		20	63.6	20.4	13.1	3.1	4.9	1.6
	532C	0	39.2	21.8	12.0	1.8	3.3	1.8
Control	USDA110	0	43.0	15.7	10.3	2.7	4.2	1.5
		20	54.7	22.3	13.2	2.5	4.1	1.7
	532C	0	39.1	18.9	9.7	2.1	4.0	1.9
		20	52.4	19.9	10.6	2.6	4.9	1.9
LSD$_{0.05}$			**15.2**	**1.4**	**2.1**	**0.6**	**1.4**	**0.3**
17 °C								
1–102	USDA110	0	48.4	25.4	15.4	1.9	3.1	1.6
		20	55.0	25.8	16.8	2.1	3.3	1.5
	532C	0	43.0	30.3	15.8	1.4	2.7	1.9
		20	49.4	28.4	17.7	1.7	2.8	1.6
2 2–68	USDA110	0	44.7	23.2	13.5	1.9	3.3	1.7
		20	46.2	28.8	15.8	1.6	2.9	1.8
	532C	0	41.7	27.3	16.1	1.5	2.6	1.7
Control	USDA110	0	41.7	26.3	15.2	1.6	2.7	1.7
		20	53.7	27.1	17.4	2.0	3.1	1.6
	532C	0	41.0	27.5	16.6	1.5	2.5	1.7
		20	52.0	28.5	17.1	1.8	3.0	1.7
LSD$_{0.05}$			**5.6**	**6.9**	**1.9**	**0.6**	**0.5**	**0.4**
25 °C								
1–102	USDA110	0	49.1	43.6	16.6	1.4	3.0	2.1
		20	46.9	35.0	19.5	1.3	2.4	1.8
	532C	0	42.7	28.5	17.6	1.5	2.4	1.6
		20	45.9	35.2	18.3	1.3	2.5	1.9
2 2–68	USDA110	0	48.1	32.1	17.5	1.5	2.7	1.8
		20	48.0	24.4	17.4	2.0	2.8	1.4
	532C	0	45.3	34.1	16.4	1.3	2.8	2.1
Control	USDA110	0	47.3	31.4	17.0	1.5	2.8	1.8
		20	47.6	35.7	17.7	1.3	2.7	2.0
	532C	0	41.4	29.7	15.4	1.4	2.7	1.9
		20	48.0	34.4	18.6	1.4	2.6	1.8
LSD$_{0.05}$			**3.9**	**1.9**	**2.5**	**0.17**	**0.5**	**0.3**

Means the same column an experiment were analyzed by an ANOVA-protected LSD test

the addition of either alone. However, while co-inoculation of soybean plants with *B. japonicum* USDA110 and PGPR 2–68 increased nodule dry weight per plant (26 %), nodule size (40 %), and the ratio of nodule dry weight to plant dry weight (13 %) over *B. Japonicum* or preincubation of *B. japonicum* USDA110 with 5 μM genistein, which was reported to be most effective at 25 °C (Zhang and Smith 1995), the addition of PGPR 2–68 and genistein resulted in an antagonistic interaction between the PGPR and the genistein.

Nodule dry weight per plant, nodule size, and the ratio of nodule dry weight to plant dry weight were decreased when compared to *B. japonicum* USDA110 co-inoculated with PGPR 2–68. The combination of *B. japonicum* USDA110 and PGPR 2–68 or 5 μM genistein showed the largest proportional increases of any of the possible combinations of *B. japonicum* strains, PGPR strains, and genistein, when compared to *B. japonicum* USDA110 alone, but when genistein and PGPR were added together, an antagonistic effect was observed.

In the same way, the largest proportional increases in nodulation variables and nitrogen fixation (ranging up to 2–3 times), due to the addition of either PGPR or genistein, occurred at 15 °C RZT, and it was at this RZT that antagonistic effects were observed between PGPR and genistein additions. Although the underlying cause for this is unclear, it appears that when the increases are largest, due to the addition of PGPR or genistein alone, the probability of antagonistic interactions between the two is the greatest.

Some PGPR and genistein combinations showed additive effects. The combination of PGPR 1–102, *B. japonicum* USDA110, and 5 μM genistein had an additive effect on the nodule dry weight per plant at 25 °C. At 25 °C RZT, many of the additive effects were approximately complete, with the increases due to genistein addition being nearly the same in the presence or absence of PGPR. On the other hand, the combination of PGPR 2–68, *B. japonicum* USDA110, and 5 μM genistein showed an antagonistic effect on almost all plant variables at 25 °C in contrast with PGPR 2–68, *B. japonicum* USDA110 alone, which was previously reported to increase plant nodule number, nodule weight, nodule size, and nitrogen fixation capacity (Zhang et al. 1996).

At 17.5 °C, there was still evidence of additivity, for instance, the combination of PGPR 1–102, *B. japonicum* USDA110, and 15 μM genistein had an additive effect on the nodule number and on nodule size. However, the level of additivity was not complete, with the increases due to the addition of genistein being smaller, or non-existent, in the presence of genistein than in its absence. As at 25 °C RZT, the combination of *B. japonicum* USDA110, PGPR 2–68, and 5 μM genistein resulted in antagonism between the genistein and the PGPR.

At 15 °C, the combination of PGPR 1–102, *B. japonicum* USDA110, and 20 μM genistein had an antagonistic effect on all measured variables. PGPR 1–102 performed better by itself than with genistein at RZT 15 °C.

The additive effects observed at 25 and 17.5 °C RZT could be explained in that genistein and PGPR may work by different mechanisms, stimulating different aspects of soybean plant physiology at optimum RZTs. At a suboptimal RZT (15 °C) or for the combination of *B. japonicum* USDA110, PGPR 2–68, and 5 μM

genistein at 25 °C, the effects are antagonistic. The cause of the antagonistic effects is unclear and requires further study.

The frequent increases in the ratio of nodule weight to plant dry weight demonstrate that plants treated with genistein or PGPR, or both required more nodule mass to achieve each gram of accumulated dry weight. Thus, while the additions of PGPR, genistein, or both increase nodule dry weight per plant and plant nitrogen fixation, it appears that the efficiency with which the additional nodule mass is able to support plant growth is less than for the nodule mass formed without the addition of these materials. These decreases in efficiency could be due to decreased relative efficiency for nitrogenase or greater restrictions of O_2 entry into the nodules (Hunt and Layzell 1993).

The nitrogen distribution data indicated that the applied treatments variably affected nitrogen translocation from root nodules to shoot tissues. The nitrogen concentrations of plant shoots and whole plants grown at 15 °C were lower than those at 17.5 and 25 °C RZT. These results agree with those of Zhang et al. (1996). *B. japonicum* and *S. proteamaculans* 1–102 showed a lower nitrogen concentration ratio for nodule to shoot tissues when compared to plants receiving *B. japonicum* alone. Co-inoculation of *B. japonicum* with *S. proteamaculans* 1–102 was shown to increase nitrogen concentration of plant shoots at 15 °C RZT. In the recent years, Zhang et al. (2002) used UV mutagenesis to generate mutants from *B. japonicum* that were capable of expressing the *nod* genes responsible for nodulation at low temperatures even in the absence of plant-to-bacteria signal molecules such as Genistein. This is a cost-effective alternative to using more genistein to trigger nodulation in leguminous plants at low RZTs.

Application of PGPR to Leguminous Plants Increases Protein and Dry Matter Yield Under Short-Season Conditions

Experiments conducted on the soybean were expanded further to determine the ability of the PGPR to increase the proteins and dry matter in leguminous plants. Plant growth and development were found to be drastically reduced under conditions of suboptimal root zone temperature. Field experiments were conducted on two adjacent sites, one fumigated with methyl bromide and one non-fumigated (Dashti 1997). Two experiments were conducted at each site: one involving combinations of two soybean cultivars and two PGPR strains and the other involving the same factors, but also in combination with two strains *B. japonicum*. Soybean's grain yield and protein yield were measured.

The results of these experiments indicated that co-inoculation of soybean with *B. japonicum* and *S. liquefaciens* 2–68 or *S. proteamaculans* 1–102 increased soybean's grain yield, protein yield, and total plant protein production, compared to the non-treated controls, in an area with low spring soil temperatures.

Interactions existed between PGPR application and soybean cultivar, suggesting that PGPR applied to cultivars with higher yield potentials were more effective. PGPR applied to the rhizosphere without addition of *B. japonicum* only increased plant leaf area and seed number at the fumigated site. Overall, inoculation of soybean plants with PGPR in the presence of *B. japonicum* increased soybean's grain yield, grain protein yield, and total plant protein production under short-season conditions (Tables 6.5, 6.6). *S. liquefaciens* 2–68 performed well at optimal RZT (25 °C), while *S. proteamaculans* 1–102 performed best at suboptimal RZTs ranging from 18 to 15 °C. Co-inoculation with PGPR and *B. japonicum* improved plant growth, development, yield components, and final grain and protein yield in the presence and absence of methyl bromide fumigation.

Inoculation of soybean plants with a mixture of *B. japonicum* and PGPR not only increased plant dry matter accumulation, but also increased grain protein and total protein production at both sites in experiment 1. Zhang et al. (1996) reported that co-inoculation of some PGPR with *B. japoniucm* could reduce the negative effects of low RZT on soybean nodulation and nitrogen fixation. Bai et al. (2003) found that co-inoculation of *Bacillus* strains with *B. japonicum* enhanced the growth of the soybean plants.

Study at McGill University, Montreal, Canada, found that co-inoculation of PGPR and *B. japonicum* accelerated the processes of soybean nodulation and the onset of nitrogen fixation under short-season field conditions. Sprent (1979) postulated that an increase of 10 % in the period of nodule activity of a grain legume, particularly between the onset of nitrogen fixation and the attainment of maximum fixation, could double the seasonal level of nitrogen fixation.

In a controlled-environment experiment, the onset of nitrogen fixation by plants co-inoculated with *B. japonicum* and the most effective PGPR strains began 2–3 days earlier than those receiving only *B. japonicum* (Zhang et al. 1996). Therefore, it is possible that application of PGPR increased grain and total protein yield under field conditions.

The effects of PGPR application without *B. japonicum* addition on soybean growth and development were different in comparison. At the non-fumigated site, although both PGPR *S. liquefaciens* 2–68 and *S. proteamaculans* 1–102 numerically increased plant growth variables such as leaf area and seed numbers, there were no statistically significant differences among treatments. At the fumigated site, both *S. liquefaciens* 2–68 and *S. proteamaculans* 1–102 increased leaf area and seed number.

When each of two PGPR strains, *S. proteamaculans* 1–102 and *S. liquefaciens* 2–68, was applied to *B. japonicum* USDA110 or 532C preincubated with different concentrations of genistein (0, 15, or 20 μM) at three RZTs, 25, 17.5, and 15 °C, respectively, it was found that some combinations of PGPR strains and genistein concentration increased plant growth and development, but the most stimulatory combinations of PGPR strains, genistein concentration, and *B. japonicum* strains varied with temperature (Table 6.7). The combinations that were most stimulatory at each temperature were as follows: at 15 °C—*S. proteamaculans* 1–102, genistein concentration 0 μM, and *B. japonicum* USDA110; at 17.5 °C—*S.*

Table 6.5 Effects of PGPR application, *B. japonicum* strains, and soybean cultivars on soybean growth variables, grain yield, and final protein and grain yield in a non-fumigated field trial (experiment 1)

PGPR	B. japonicum	Cultivar	Leaf (plant⁻¹)		Number (plant⁻¹)		1000 seeds	Yield (t ha⁻¹)			Protein concentration
			Number	Area (cm²)	Pod	Seed	Weight (g)	Grain	Grain protein	Total protein	(mg/g)
1–102	USDA110	AC Bravor	31.3	976.2	29.0	69.0	190.33	5.4	2.2	2.7	401.3
		Maple Glen	19.2	518.3	20.2	50.0	190.33	4.3	1.7	2.0	386.5
	532C	AC Bravor	27.8	977.1	28.7	68.3	161.00	5.0	1.9	2.1	395.4
		Maple Glen	15.2	425.6	19.6	48.3	190.33	3.1	1.3	1.5	365.0
2 2–68	USDA110	AC Bravor	31.3	830.0	28.3	70.3	194.67	5.4	1.9	2.7	391.5
		Maple Glen	26.3	805.4	31.3	75.0	189.00	4.9	1.9	2.3	416.6
	532C	AC Bravor	29.0	1044.2	27.0	45.0	195.00	4.6	1.7	2.1	386.5
		Maple Glen	16.4	628.0	18.3	46.0	169.33	3.3	1.3	1.8	380.5
Control	USDA110	AC Bravor	16.0	516.0	16.0	45.7	180.33	4.4	1.8	2.1	373.8
		Maple Glen	21.4	543.0	24.4	57.3	195.67	3.8	1.4	1.8	365.0
	532C	AC Bravor	21.1	910.0	26.3	59.0	201.67	4.9	1.8	2.0	380.5
		Maple Glen	14.8	533.0	24.1	35.0	196.00	3.1	1.2	2.1	333.5
LSD$_a$			7.6	240.4	7.0	15.4	36.20	0.5	0.3	0.3	32.4
LSD$_b$			6.9	239.5	6.7	16.3	35.14	0.6	0.4	0.3	30.0

(continued)

Table 6.5 (continued)

PGPR	B. japonicum	Cultivar	Leaf (plant^{-1})		Number (plant^{-1})		1000 seeds Weight (g)	Yield (t ha^{-1})		Total protein	Protein concentration (mg/g)
			Number	Area (cm^2)	Pod	Seed		Grain	Grain protein		
PGPR			***	**	**	***	NS	***	***	***	***
B. japonicum			**	NS	NS	***	NS	***	***	***	**
Cultivar			***	***	NS	NS	NS	***	***	***	**
PGPR *B. japonicum			NS	NS	***	**	NS	***	***	***	NS
PGPR*cultivar			**	**	*	NS	NS	**	***	***	*
PGPR*B. japonicum*cultivar			NS	NS	NS	***	NS	NS	***	***	NS

Means of leaf number and leaf area, and seed number represent four plants from each subplot unit, at crop maturity. Means of 1,000 seed weight calculated from the one meter middle row of each subplot unit at harvest maturity. LSD$_{0.05a}$ is for comparison of means within the same main-plot unit, and LSD$_{0.05b}$ is for comparison of means across levels of the same main-plot factor. *NS*, *, **, and *** indicated no significant difference at the 0.1, 0.05, and 0.01 levels, respectively

Table 6.6 Effects of PGPR application and soybean cultivars on soybean growth variables, grain yield, and final protein and grain yield in a fumigated field trial (experiment 1)

PGPR	Cultivar	Leaf (plant^{-1})		Number (plant^{-1})		1,000 seed	Yield (t ha^{-1})			Protein concentration
		Number	Area (cm^2)	Pod	Seed	Weight (g)	Grain	Grain protein	Total protein	(mg/g)
1–102	AC Bravor	52.9	893.3	46.2	80.5	160.5	3.8	1.5	1.8	396.7
	Maple Glen	41.8	473.7	36.0	57.7	152.1	2.1	0.8	1.0	382.6
2–68	AC Bravor	56.2	995.8	38.2	73.1	164.7	4.0	1.6	2.0	396.5
	Maple Glen	23.8	239.0	19.8	47.8	155.8	2.3	1.1	1.3	373.8
Control	AC Bravor	28.2	472.8	24.7	48.7	154.0	3.1	1.0	1.4	371.2
	Maple Glen	16.0	189.7	18.7	47.8	155.2	3.1	1.0	1.4	365.7
LSD$_a$		7.1	165.0	8.2	12.6	11.6	0.6	0.3	0.3	15.4
LSD$_b$		6.0	134.9	9.8	15.4	8.7	0.7	0.3	0.4	12.4
PGPR		***	***	***	**	**	***	***	***	***
Cultivar		***	***	***	***	*	***	***	***	***
PGPR*cultivar		***	***	**	***	*	***	***	***	*

Means of leaf number and leaf area, and seed number represent four plants from each subplot unit, at crop maturity. Means of 1,000 seed weight calculated from the one meter middle row of each subplot unit at harvest maturity. LSD$_{0.05a}$ is for comparison of means within the same main-plot unit, and LSD$_{0.05b}$ is for comparison of means across levels of the same main-plot factor. NS, *, **, and *** indicated no significant difference or significant differences at the 0.1, 0.05, and 0.01 levels, respectively

Table 6.7 Effects of PGPR application, *B. japonicum* strains, and soybean cultivars on soybean growth variables, grain yield, and final protein and grain yield in a non-fumigated field trial (experiment 2)

PGPR	Cultivar	Leaf (plant^{-1})		Number (plant^{-1})		1,000 seeds	Yield (t ha^{-1})		
		Number	Area (cm^2)	Pod	Seed	Weight (g)	Grain	Grain protein	Total protein
1–102	AC Bravor	32.0	754.8	28.3	52.7	177.1	3.2	1.2	1.7
	Maple Glen	23.6	500.8	26.3	73.3	170.9	3.3	1.0	1.4
2–68	AC Bravor	30.3	767.8	20.7	49.7	179.0	3.6	1.2	1.4
	Maple Glen	28.7	866.2	21.7	50.7	177.9	3.2	1.0	1.4
Control	AC Bravor	23.0	740.4	16.0	35.3	151.3	3.4	1.5	1.7
	Maple Glen	22.7	720.3	15.3	35.0	163.1	3.5	1.0	1.6
LSD$_a$		15.6	510.2	14.8	29.7	20.0	0.4	0.4	0.4
LSD$_b$		14.6	445.9	13.9	29.6	18.3	0.4	0.3	0.3
PGPR		NS	NS	NS	NS	NS	NS	NS	NS
Cultivar		NS	NS	NS	NS	NS	NS	NS	NS
PGPR*cultivar		NS	NS	NS	NS	NS	NS	NS	NS

Means of leaf number and leaf area, and seed number represent four plants from each subplot unit, at crop maturity. Means of 1,000 seed weight calculated from the one meter middle row of each subplot unit at harvest maturity. LSD$_{0.05a}$ is for comparison of means within the same main-plot unit, and LSD$_{0.05b}$ is for comparison of means across levels of the same main-plot factor. *NS*, *, **, and *** indicated no significant difference or significant differences at the 0.1, 0.05, and 0.01 levels, respectively

proteamaculans 1–102, genistein concentration 15 μM, and *B. japonicum* USDA110; and at 25 °C—*S. proteamaculans* 1–102, genistein concentration 5 μM, and *B. japonicum* USDA110. In at least some cases, these stimulatory effects can be attributed to the additive effects of both PGPR and genistein in enhancing soybean growth and early development. At 25 °C, some combinations of PGPR strains, genistein concentration, and *B. japonicum* strains have shown an additive effects, while at 15 °C, others have antagonistic effects.

At an optimal (25 °C) RZT, *B. japonicum* USDA110 preincubated with 5 μM genistein increased leaf number, and increased leaf number, leaf area and pod number. At suboptimal RZT (17.5 °C), *B. japonicum* USDA110 preincubated with 15 μM genistein increased leaf number, leaf area, and total plant dry weight in experiment 1 compared to *B. japonicum* USDA110 alone, while in experiment 2, either *B. japonicum* USDA110 or 532C co-inoculated with 15 μM genistein increased leaf number, leaf area, pod number, and total plant dry weight compared to either *B. japonicum* USDA110 or 532C.

At 15 °C, *B. japonicum* USDA110 or 532C preincubated with 20 µM genistein increased leaf and total plant dry weight. *B. japonicum* USDA110 co-inoculated with 20 µM genistein increased leaf number, while *B. japonicum* 532C co-inoculated with 20 µM genistein increased leaf number and total plant dry weight. Zhang and Smith (1995) showed that genistein effects on photosynthesis were only seen after the onset of nitrogen fixation, while the effects of PGPR were seen prior to the onset of nitrogen fixation (Zhang et al. 1996). The changes in photosynthetic rate over time showed that plant photosynthesis was increased by some PGPR strain, genistein, and *B. japonicum* strain combinations over a wide range of plant growth stages. As photosynthesis was increased by stimulatory strain combinations before the onset of nitrogen fixation, the improvements in plant growth, development, and physiological activities must have been through an effect of PGPR on overall plant physiology, followed by a genistein effect on nitrogen fixation. Since PGPR and genistein stimulations appear to take place by different mechanisms, they might reasonably be additive.

Growth, Survival, and Root Colonization of PGPR Under Short-Season Conditions

Root colonization by introduced bacteria is considered as an important step in the interaction of beneficial bacteria with the host plant. A rapid growth rate was suggested to be an important characteristic for successful rhizosphere colonization (Rovira et al. 1983; Schorth and Weinhold 1986). De Weger et al. (1987) suggested that non-motile mutants colonize the roots less efficiently than the corresponding wild types, while others found that non-motile mutants and the corresponding wild types do not differ in their colonizing ability (Scher et al. 1988).

Chemotaxis of bacteria to exudates was reported (Scher et al. 1985), but the direct relationship between chemotaxis and successful colonization remains unclear. Movement along the root was also reported to be very important for successful root colonization (Chao et al. 1986; Schippers et al. 1987). Adherence has also been suggested as an important feature for rhizosphere competence and survival (Schippers et al. 1987; Vesper 1987). Cells of bacteria in the genus *Serratia* are motile (Prescott et al. 1993).

Fluorescent pseudomonads, isolated from the crop rhizosphere, are characterized as a highly rhizosphere competent as they are capable of root colonization. This accounts for their predominance among the PGPR. Several traits of the pseudomonads aid them in seed colonization, such as higher cell division and motility (Arora et al. 1983; Scher et al. 1985). However, these traits may not be directly relative to subsequent root colonization. For example, Howie et al. (1987) found that three non-motile mutants of *Pseudomonas fluorescence* colonized wheat roots as effectively as their motile parents.

Fluorescent pseudomonads are able to establish high population densities in the rhizosphere (Suslow 1982; Bahme and Schorth 1987), an important characteristic for the production of consistent plant growth responses (Kloepper et al. 1980, 1985, 1991; Parke 1991). Van Elsas and Heijnen (1990) reported that lack of consistent effectiveness of the inoculant prevents successful application of PGPR strains into the soil. This always was related to ineffective colonization of the plant, as well as poor survival and/or low activity of the introduced population. Xu and Gross (1986) and Bull et al. (1991) demonstrated a positive relationship between root colonization by a PGPR strain and disease suppression, suggesting that methodologies that improve root colonization may also improve the performance of a PGPR strain in the soil. The extent and amount of root colonization needed by a PGPR strain to increase plant growth rely on numerous interrelated factors. The choice of methods used to try to increase rhizosphere colonization and plant growth has to take these factors into consideration (Stephens 1994a).

Hebbar et al. (1992) reported that the colonization and spread of *Pseudomonas cepacia* (which acts as a biocontrol agent against *Fusarium moniliforme*) on the roots and in the rhizosphere of maize depend on the amount of inoculum on the seed. However, this was not a universal observation. For example, the colonization of introduced pseudomonad strains on maize (Scher et al. 1984) and wheat was shown to be independent from the initial inoculum level. It is obvious that under certain conditions, increasing the level of inoculum could increase the rhizosphere competence of some, but not all bacteria.

Some PGPR strains are able to colonize soybean root plants more efficiently than others, while others proliferated more successfully in the rooting medium. In the research conducted with soybeans, at 15 °C RZT, PGPR *S. proteamaculans* (1–102) had a higher population density associated with the soybean roots, while its population density was reduced in the rooting medium. The same pattern was seen for the PGPR *S. liquefaciens* (2–68) at its more appropriate RZT, 25 °C. These results indicate that the colonization of soybean roots and the rhizosphere by PGPR is altered by temperature.

Zhang et al. (1996, 1996a) have shown that co-inoculation of *B. japonicum* with some PGPR strains increased soybean nodulation and nitrogen fixation and increased soybean growth and development, but the stimulatory effect varied with the RZT. The ability of the PGPR to colonize roots effectively is probably a prerequisite to the stimulation of soybean growth, nodulation, and nitrogen fixation.

RZT exerts a clear effect on the ability of PGPR to colonize soybean roots, and this probably explains at least part of the differences in the colonization and plant growth-stimulating abilities of PGPR 2–68. Elements of the soil flora and fauna and aspects of the soil chemistry may play a significant role to the differences in performance of the two PGPR tested.

Root and Rhizosphere Colonization of Soybean [*Glycine max* (l.) merr.] by Plant Growth-Promoting Rhizobacteria at Low RZTs

Survival and growth of seven PGPR inoculated on soybean in a sterile rooting medium were studied under low RZTs. Three RZTs were tested: 25, 17.5, and 15 °C. In general, population densities varied with temperature. At each temperature, populations of some PGPR strains increased either on the root or in the rooting medium (rhizosphere). RZT affected the distribution of PGPR populations between the root surface and in the rooting medium (rhizosphere).

The strains with higher population densities on the root, which reflects their ability to colonize the root more rapidly, were as follows: 15 °C PGPR 1–102 *S. proteamaculans*, 17.5 °C G11-32 *Pseudomonas putida*, and 25 °C 2–68 *S. liquefaciens*. These PGPR strains had lower population densities in the rooting medium (rhizosphere) at these temperatures. Other PGPR strains were not able to effectively colonize the roots of the soybean plants, and their population densities remained very high in the rooting medium (rhizosphere). The strains that colonized soybean roots best at 25 and 15 °C were previously shown to be effective at promoting soybean growth at 25 and 15 °C.

Some PGPR are able to grow better and can colonize soybean roots effectively at lower RZTs, while, at the same time, their numbers in the rooting medium decline. Those PGPR that are not able to colonize the root will be present in the rooting medium in relatively high numbers. Other PGPR are able to colonize roots at higher temperatures, and their numbers were higher in the root and lower in the rooting medium at such temperatures. Also, in as much as the PGPR strains that colonized the roots well have been shown to be best at promoting soybean growth at each RZT, some strains that colonized the roots well were shown not to be effective at plant growth promotion (Zhang et al. 1996, 1996a). It seems likely that an ability to effectively colonize plant roots, as affected by PGPR strain, plant type, and environmental conditions, is necessary, but not sufficient condition for the stimulation of plant growth.

In summary, the ability of PGPR strains to grow, multiply, and survive is strain specific and temperature dependent. Some PGPR strains are able to grow and multiply effectively at low RZTs and colonize the roots effectively. Others are able to grow and multiply effectively at higher RZTs and colonize the roots effectively. It was shown that in the optimum RZT range, an effective PGPR would be heavily present on the root, but was relatively less present in the surrounding rooting medium, while outside the optimum RZT range, the reverse was true. Also, the ability of the PGPR to colonize the root effectively could be a prerequisite to the stimulation of growth, nodulation, and nitrogen fixation of soybean plants.

PGPR Growth and Survival Under Field Conditions

Co-inoculation of *B. japonicum* with PGPR has been shown to increase soybean nodulation, nitrogen fixation, and growth, compared to the non-treated controls, in areas with low spring soil temperatures. The survival and growth of rhizosphere populations of two PGPR *S. liquefaciens* 2–68 and *S. proteamaculans* 1–102 inoculated on soybean were examined under short-season conditions. Colonization of soybean plants varied among PGPR strains and soil conditions.

At an unfumigated site, PGPR 2–68 colonized soybean plant roots more efficiently than PGPR 1–102 in the first sampling, while there was no difference by the second sampling, which indicated that PGPR 2–68 was able to grow and colonize the soybean root more effectively, initially, but over time, PGPR 2–68 was able to grow and colonize soybean roots as effectively as PGPR 1–102. PGPR 2–68 was able to proliferate successfully in the soil at both samplings. The population density of both PGPR 68 and PGPR 1–102 with the different combinations of *B. japonicum* strains and soybean cultivars decreased over time except for the combination of PGPR 2–68, *B. japonicum* USDA110, and cultivar AC Bravor where the population density increased over time at the unfumigated site. These observations indicated that PGPR 2–68 can survive and colonize the roots of the soybean plants effectively in the presence of other microflora.

At the fumigated site, where no other microflora was assumed to compete with the PGPR, PGPR 2–68 showed the same pattern as at the unfumigated site. Also, the population density of both PGPR increased over time. These observations suggest that both PGPR were able to survive and increase in number in the absence of other microflora elements.

Studies on rhizosphere colonization have been reviewed by van Elsas and Heijnen (1990), Kloepper and Beauchamp (1992). Lack of consistent effectiveness of the inoculant was found to be the major problem preventing successful application of PGPR strains to the soils (Van Elsas and Heijnen 1990). This has always been caused by ineffective colonization of the plant, as well as poor survival and/or low activity of the introduced population.

Xu and Gross (1986) and Bull et al. (1991) demonstrated a positive relationship between root colonization by a PGPR strain and disease control, suggesting that methods applied that improve root colonization may also improve the establishment of a PGPR strain in the soil. The extent and amount of root colonization required by a PGPR strain to increase plant growth depend on numerous interrelated factors. The choice of methods used to try to increase rhizosphere colonization and plant growth should take these factors into account (Stephens 1994b).

Previous studies have suggested that the fitness of a bacterial strain in the rhizosphere may be dependent on the plant species (van Peer and Schippers 1989; Beauchamp et al. 1993) and even plant cultivar (Weller 1986). One method of increasing rhizosphere colonization by certain PGPR strains may be through maximizing the bacterial inoculum load on the seed. Hebbar et al. (1992) reported that the colonization and spread of *P. cepacia* (which acts as a biocontrol agent

against *F. moniliforme*) on the roots and in the rhizosphere of maize correlated with the amount of inoculum on the seed. However, the dependence of final colonization level on the initial inoculum level has not been a universal observation. For example, the colonization of introduced pseudomonad strains on maize (Scher et al. 1984) and wheat has been shown to be independent of the initial inoculum level. It is apparent that, under certain conditions, increasing the level of inoculum may increase the rhizosphere competence of some, but not all, bacteria.

Beneficial bacteria that are introduced into the rhizosphere are involved in a complex of biological interactions with the host plant. The introduced bacteria are nourished by root exudates and are thus dependent on the host plant. At the same time, the introduced bacteria may affect the host plant by inducing physiological changes (Kloepper et al. 1988). The genetic marking of bacteria with antibiotic resistance for identification purposes allows the study of population dynamics of soil-inhabiting bacteria. Specific PGPR that cause marked increases in plant growth and yield have been marked to follow their populations during the various stages of plant development (Polonenko et al. 1987).

Co-inoculation with PGPR and *B. japonicum* improved plant growth, development, yield components, and final grain and protein yield under field conditions at both fumigated and unfumigated sites. Also, application of PGPR with the *B. japonicum* directly onto the seeds in the furrow at the time of planting also improved plant growth and increased grain and protein yield at the fumigated site. The effects of PGPR *S. liquefaciens* 2–68 and *S. proteamaculans* 1–102 on plant growth, development, and final protein yield were shown to be not different, which was attributed to variations in field soil temperature during the entire soybean-growing season. In addition, co-inoculation of PGPR and *B. japonicum* accelerated soybean nodulation and the onset of nitrogen fixation under short-season conditions. This study indicated that PGPR 2–68 was able to grow and survive better than PGPR 1–102 under short-season conditions. Inoculation with PGPR 2–68 generally increased soybean nodulation, nitrogen fixation, growth, and yield more than PGPR 1–102. These findings suggest that there is a direct relationship between the ability of these PGPR to colonize the roots of the soybean plants and their ability to stimulate soybean nodulation, nitrogen fixation, plant growth, and physiological activities under short-season conditions.

Conclusion

All stages of symbiotic establishment investigated to date, such as root hair curling, infection thread formation and penetration, and nodule development and function, are inhibited by suboptimal RZTs. PGPR are capable of combating these negative effects by increasing plant growth, photosynthesis, amount of fixed N, and number of nodules formed. However, RZT exerts a clear effect on the ability of PGPR to colonize soybean roots and this probably explains at least part of the differences in the colonization and plant growth-stimulating abilities of PGPR. The

ability of PGPR to colonize the root effectively is a prerequisite to their stimu-
latory effects. The addition of genistein to PGPR was found to further alleviate the
effects of RZT. Addition of the PGPR supernatant results in stimulation, which is
strain specific and temperature dependent; each PGPR probably releases a different
growth-stimulating substance. Although given that they have similar effects on
plant growth, they may be similar molecules.

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Chapter 7
Salinity Stress and Arbuscular Mycorrhizal Symbiosis in Plants

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Introduction

Plants being sessile experience various abiotic stresses including salt stress, which limits plant growth and yield and in severe cases leads to cell death. This mainly confers its ionic imbalances, nutritional deficiencies, and also due to changes in the osmotic effects (Zhang et al. 2010; Wu et al. 2010; Zou et al. 2013; Koyro et al. 2012). Various species of plants respond differently to salt stress, such as citrus and many others are salt-sensitive plants. Increasing rate of saline water in agricultural fields leads to a major threat to plant production and hence retards the growth and development of plants (Rabie and Almadini 2005; Pascal et al. 2005; Shokri and Maadi 2009) by affecting various metabolic processes.

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Toxicity of ions results in the disruption of enzyme activity, photosynthesis, respiration, as well as protein synthesis, and damaging of plasma membrane including cell organelles (Feng et al. 2002). Scientists have put an effort to minimize the crop loss due to salt stress by providing salt-tolerant crop plants (Gallagher 1985; Evelin et al. 2009) and also established salt-tolerant crops through breeding (Cuartero and Fernandez-Munoz 1999; Evelin et al. 2009). In addition, different genes have also been employed to enhance the salt tolerance in different plants (Wei-Feng et al. 2008; Tang et al. 2005; Evelin et al. 2009). Leaching of excess accumulated salts in groundwater also provides an alternative means to alleviate the salt stress. But these techniques are very costly and unaffordable to underdeveloped countries.

Among the environmental stress, soil salinity globally results in the greater loss in agricultural productivity and therefore affecting the lives of humans and animals (Aggarwal et al. 2012). Evelin et al. (2009) reported that 50 % loss of cultivated land affected by salinity and also the photosynthesis, protein synthesis, lipid and energy metabolism.

Salinity not only reduces yield of crops but also disrupts the ecological balance of the area (Aggarwal et al. 2012). Several literatures have reported that arbuscular mycorrhizal (AM) fungi act as growth regulator and mitigate the harmful effects of plants exposed to salt stress. Plants grown in fields are surrounded by various microorganisms such as bacteria and fungi that help and improve the plant growth and yield under various stress conditions (Creus et al. 1998). To cope with this stress, AM fungi play a key role in alleviating the toxicity induced by salt stress, thus normalizing the uptake mechanism in plants by supplying the essential nutrients. In this way, the plant recovers the water balance machinery, enhancing their tolerance capacity and thereby enduring the salt stress (Carretero et al. 2008; Porcel et al. 2012).

AM fungi form symbiotic associations with most of the plants and enhance the tolerance capacity to withstand the abiotic stresses including salinity besides increasing the uptake of inorganic nutrients (Hajbagheri and Enteshari 2011; Rabie and Almadani 2005). AM fungi supply mineral nutrients to plants, especially phosphorus, which is precipitated by the ions such as Ca, Mg, Zn (Al-Karaki et al. 2001).

Different mechanisms are required for the efficient growth and yield of plants. Reactive oxygen species (ROS) produced during salinity stress are detoxified by AM fungi as it has the tendency to enhance the production of antioxidant enzymes. Mycorrhizal plants regulate the various gene expressions affecting water balance in their tissues. AM fungi favor plant growth against the salt stress by improving the host plant nutrition, increasing K/Na ratios and efficiently influencing osmo-regulation (i.e., osmotic adjustment) by accumulation of compatible solutes such as proline, glycine betaine, and soluble sugars (Porcel et al. 2012).

Mycorrhizal Plants Under Salt Stress

Mycorrhizae are ubiquitous in most temperate and tropical ecosystems including agricultural systems and form symbiotic relationship with the roots of higher plants (mycorrhizosphere). They act as channel for the exchange of energy and matter between plants and soil (Cardon and Whitbeck 2007). The most important property of AM fungi is to enlarge the surface area of the host plant roots due to an extensive hyphal network that helps to combat against stressed conditions. Mutual benefits between mycorrhizal fungi and the host plants include the exchange of carbon coming from photosynthesis and mineral nutrients, respectively (Mohammadi et al. 2011).

Formation of arbuscules in AM fungi is characterized by an extensive branched haustorium-like structure in the root cortical cells affecting nutrient exchange; however, these arbuscules are considered as the non-living part during the growth of AM fungi (Bonfante and Perotto 1995; Hause and Fester 2005) and are finally decomposed. Once grown inside, i.e., the cortical layer, the tree-like fungal structures called arbuscules are formed within the cortex of root by subsequent division of the fungal hyphae (Smith and Read 1997; Hause and Fester 2005). After the fungal entrance, these differentiated cortical cells simultaneously undergo reorganization by means of skeletal structures (i.e., microtubules and microfilaments).

Besides the exchange of mineral nutrients and phosphates, AM fungi show a positive effect toward stress conditions including osmotic potential (Augé 2001). Colonizing mycorrhizal fungi also play a key role as bioprotector under different pathogen attack (Slezack et al. 2000; Elsen et al. 2001; Strack et al. 2003). Alleviation of salt stress in plants by AM fungi is also mediated by growth hormones (Barker and Tagu 2000). Among these growth hormones, the level of cytokinin is found higher in shoots and roots of mycorrhizal plants as compared to non-mycorrhizal plants (Allen et al. 1980), whereas the amount of Abscisic acid (ABA) has also been found higher in AM roots (Bothe et al. 1994).

Anastomosing or networking hyphae of AM with the roots of plant attributes to an efficient soil texture as well as water relation (Bethlenfalvay and Schuepp 1994). Therefore, these AM fungi provide significant applications in sustainable agriculture (Schreiner and Bethlenfalvay 1995). Colonization of red tangerine (*Citrus reticulata*) by *Glomus mosseae* and *Paraglomus occultum* has shown better growth and increased photosynthetic performance and ionic balance implying the higher tolerance level under salt stress. These positive effects of AM fungi provide a good indicator of bio-amelioration of plants on exposure to salt stress (Zou et al. 2013).

The existence of AM colonization in the roots of halophytic plants has also been reported (Carvalho et al. 2001; Hilderbrandt et al. 2001). Besides this, AM fungi spores have also been obtained abundantly in extremely alkaline soils (Landwehr et al. 2002). It is likely that under increased levels of NaCl stress, mycorrhizal fungi do not affect the growth of the host plants, which is due to the

adverse effects of salinity on the growth and activity of the fungi (Sheng et al. 2008; Juniper and Abbott 2006). Mycorrhizal fungal symbioses have also been reported to enhance tolerance under salt stress in various host plants such as maize, clover, tomato, and lettuce (Feng et al. 2002; Al-Karaki et al. 2001). Mycorrhizal colonization improves not only the yield of plants but also the quality of fruit, for example in water melon (Kaya et al. 2009).

Plant Growth and Salinity

Salinity stress adversely affects plant morphology and physiology. Various studies reveal that AM fungi improve plant growth and yield under salt stress conditions (Al-Karaki et al. 2001; Tsang and Maun 1999). This could be possible by means of adequate supply of mineral nutrients, particularly phosphorus with the help of AM fungi by the host plant (Marschner 1986; Al-Karaki 2000). Published data showed the higher growth of mycorrhizal plants under salt stress (Giri et al. 2003; Sannazzaro et al. 2007; Zuccarini and Okurowska 2008).

Hajbagheri and Enteshari (2011) reported maximum plant growth and biomass under salt stress. Similarly, roots colonization by AM fungi resulted in the enhanced growth of tomato (Al-Karaki 2006), soybean (Sharifi et al. 2007), and citrus (Ying-Ning et al. 2013) on exposure to salt stress. Phosphorus limits plant growth due to its poor mobility in the soil. However, its increased availability due to AM fungi symbiosis with the host plant has been reported to enhance plant growth and biomass.

Application of mycorrhizal plants has proved to significantly increase plant growth as the uptake of phosphorus in chickpea (Azcón-Aguilar et al. 2003). Combination of mycorrhizal fungi with natural rock phosphate based on nutritional content is found to be more effective on *Sesbania* (Mohammadi et al. 2011). AM fungi colonization has found to be effective in several crop plants such as sunflower, maize, soybean, potato, and wheat (Dahlgren et al. 2004; Mohammadi et al. 2011).

Lin et al. (1991) reported phosphorus in double concentrations in the shoots and roots of mycorrhizal *Trifolium repens*, indicating that AM colonization provides higher percentage of phosphorus concentration than non-mycorrhizal plants (Ortas et al. 2011; Mohammadi et al. 2011). In another study, luxuriant growth has been observed in mycorrhizal garlic plants with increased fresh weight under salt stress (Cho et al. 2006; Al-Karaki 2006). Mehdi et al. (2006) also found the increased dry biomass of lentil shoots by mycorrhizal colonization.

In response to salt stress, reduction in root growth of tomato (Latef and Chaoxing 2011) and *Jatropha curcas* (Kumar et al. 2010) has been reported even when the plants were inoculated with the fungi. Similar results are also reported by Hajbagheri and Enteshari (2011). In this study, root dry weight increased due to enhanced salinity and root fresh weight decreased due to reduced osmotic potential of soil and also due to its low water absorption capacity (Hajbagheri and

Enteshari 2011). Similar results were obtained by Ghoulam et al. (2002) in beet root. When inoculated AM fungi were introduced, the fresh and dry weight of root increased because of its increased nutrient and water absorption by the fungal hyphae network. Mycorrhizal fungal fibers entering the plants increase cytokinin content resulting in higher water absorption and formation of extensive root system in plants. Other group of fibers presented outside the root system produces organic acids solubilizing phosphorus like malic acid, thereby enhancing phosphorus absorption and hence plant dry matter. Phosphorus plays a crucial role in cellular division by regulating the activity of growth hormones. Growth and biomass inhibition under salt stress is reported by Siddiqui et al. (2009) and Afroz et al. (2005) due to high accumulation of NaCl salt.

Chlorophyll Content

Chlorophyll content reduces under salt stress due to its enzyme inhibition required for biosynthesis of chlorophyll (Sheng et al. 2008; Murkute et al. 2006) and also by limited uptake of nutrients. Mycorrhizal plants in response to salt stress have been observed to increase the chlorophyll content (Sannazzaro et al. 2006; Colla et al. 2008; Zuccarini 2007), suggesting the less interference of salt with chlorophyll biosynthesis (Giri and Mukerji 2004). Also, the negative effect of Mg on chlorophyll molecules is counterbalanced in the presence of AM fungi under salt-stressed conditions (Giri et al. 2003; Zuccarini 2007). Salt stress causes alterations in the activities of enzyme, affecting the synthesis of chlorophyll, and results in the loss of pigments (Parida and Das 2005). El-Tayeb (2005) found the same in maize and barley plants. Reduction in chlorophyll activity is attributed to diffusional limitations, i.e., stomatal and mesophyll conductance (Paranychianakis and Chartzoulakis 2005).

With increasing the salinity level, photosynthesis is reduced in plants; however, in mycorrhizal plants, the chlorophyll activity is restored due to presence of specific enzymes required for its biosynthesis (Sheng et al. 2008; Hajbagheri and Enteshari 2011). Since mycorrhization increases the absorption of Mg in plants, the synthesis of chlorophyll increases in mycorrhizal plants. Increasing chlorophyll activity in AM-inoculated plants decreases Na level under salt stress. Zhu et al. (2010) found similar results in maize plants inoculated with *Glomus etunicatum*. These results are corroborated with the findings of Kumar et al. (2010). AM symbiosis enhanced the photosynthesis rate under salt stress in garlic plants (Borde et al. 2010). This is in accordance with the result of other studies (Sannazzaro et al. 2006; Sheng et al. 2008; Colla et al. 2008).

Yang et al. (2010) also reported the blockage of water absorbance by cucumber roots, thereby influencing stomatal opening and hence decreased biochemical reactions. According to Evelin et al. (2009), a tremendous loss in chlorophyll content and nutrient imbalances is among the adverse effects of salinity on the growth of plants.

Unavailability of carbon dioxide leads to increased stomatal closure due to its reduced consumption of NADPH produced by Calvin cycle (Ruiz-Lozano et al. 2012). Microorganisms such as bacteria and fungi increase plant growth and yield under adverse environmental conditions as they have the tendency to resist the damage and hence develop resistance against harmful effects of salinity stress.

The increased photosynthetic pigments by mycorrhizal colonization in plants is due to the inhibition of Na transport, which leads to better functioning of photosynthetic machinery (Borde et al. 2010; García-Garrido and Ocampo 2002). Production of proline by the application of mycorrhizal fungi demonstrates the high tolerance capacity in wheat plants by stabilizing the osmotic balance and scavenging the toxic radicals (García-Garrido and Ocampo 2002).

Under salt stress, AM fungi increase the rate of chlorophyll contents that is attributed to higher translocation of photosynthase by the fungi (Lösel and Cooper 1979). Levy and Krikun (1980) reported the same in mycorrhizal citrus plants related to water uptake as affected by stomatal regulation. Similar results were observed in grass by Allen and Allen (1981). This improvement with AM fungi is also due to enhancement in the cytokinin concentrations (Allen et al. 1980). Salinity stress adversely affects all different parameters, i.e., chlorophyll, growth, biomass, water status, nutrient uptake; however, inoculation with mycorrhizal fungal may simultaneously improve these parameters (Yohannes 2006).

Water Status

Kumar et al. (2010) have demonstrated normal levels of water in leaves of mycorrhizal *J. curcas* under salt stress. This symbiosis results in efficient water conductance in roots and simultaneously increases stomatal conductance and hence transpiration (Colla et al. 2008; Jahromi et al. 2008). AM inoculation helps the host plant to acquire nutrients and thereby improves the photosynthetic rate as well as water osmotic homeostasis (Porras-Soriano et al. 2009; Sheng et al. 2008; Zuccarini 2007).

Water status is disrupted by salt stress; however, mycorrhizal colonization prevents the host plant from dehydration and thereby increases the root hydraulic conductivity at low water potential (Aroca et al. 2007). These inoculated plants allow fixing carbon dioxide freely relative to the non-colonized plants (Querejeta et al. 2007). Increased transpiration rate by AM symbiosis is related to the changes of ABA:cytokinin ratio (Gorcoechea et al. 1997; Porcel et al. 2012). Mycorrhizal fungal colonization enables the host plants to absorb higher water through their hyphal network, and hence, water status (Khalvati et al. 2005; Bolandnazar et al. 2007; Porcel et al. 2012) and the intercellular carbon dioxide concentration are maintained in plant. Lower water saturation deficit and higher turgor potential in mycorrhizal plants efficiently regulate plant water status (Sheng et al. 2008).

Relative Cellular Permeability

Mycorrhizal plants improve the stability as well as the integrity of membrane proteins by maintaining higher relative permeability of the cell (Kaya et al. 2009; Garg and Manchanda 2008). This results in increased phosphorus uptake as well as antioxidant enzymes production (Feng et al. 2002). *Cajanus cajan* shows higher relative permeability when treated with AM fungi (Kaya et al. 2009). Also electrical conductivity of mycorrhizal plants was found higher in certain plant roots (Garg and Manchanda 2008). Mycorrhizal pigeon pea showed similar results as exposed to different levels of salt stress; this has been attributed to the higher electrolyte permeability of root plasma membrane (Feng et al. 2002), which is a result of higher phosphorus uptake and enhanced production of antioxidant enzymes. Proper combinations of mycorrhizal fungal species and the host plant result in the alleviation of the salt stress and make the cultivation of plants even more likely under stress.

Betaines

Betaines belong to *N*-methylated derivatives of amino acids and provide an effective indicator of salt stress like proline (Duke et al. 1986; Evelin et al. 2009). In addition, it has an osmotic regulating mechanism, protecting and stabilizing the integrity of cell membrane structure against the negative effects of excess salt accumulation. Mycorrhizal plants have found to be more effective during accumulation of betaines under salt stress (Al-Garni 2006; Evelin et al. 2009). In higher plants, proline is catalyzed by pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR). P5CS over-expressed gene in transgenic tobacco leads to enhanced production of proline under salinity (Kishor et al. 1995; Porcel et al. 2012). Glycine betaine protects the plants against adverse effects of salinity stress. Plants treated with mycorrhizal fungi accumulate betaine under stress and thus prevents plants from any stress damage. Various reports have shown that AM-treated plants enhanced the production of betaines that contribute to the osmotic adjustment of plants and hence results in a more efficient photosynthesis process (Sheng et al. 2011).

Proline

Proline accumulation is one of the natural means to adapt to environmental stress conditions. Proline is a non-toxic and good osmolyte and maintains the osmoregulation under salt stress (Ahmad and Jhon 2005; Ahmad and Sharma 2008; Ahmad 2010; Ahmad et al. 2010b, 2011, 2012a; Katare et al. 2012; Rasool et al. 2013a, b).

Plants when colonized by AM fungi show high degree of protection by accumulating more and more solute as it has been indicated in mung bean (Jindal et al. 1993; Evelin et al. 2009). Such solutes have been found more in roots than shoots as roots are the primary sites of water absorption. Proline accumulation is not only due to salinity stress but also by mycorrhizal colonization. In some plants, proline accumulation is observed due to salt stress and not by mycorrhizal colonization, and hence, it is required to clarify such finding to assess the mechanisms of salt tolerance in various plants. Proline also acts as energy storage (i.e., C and N) during salt stress (Goas et al. 1982; Aggarwal et al. 2012). Enhanced proline accumulation can be linked with increased N-fixing ability of plants as demonstrated by Evelin et al. (2009) in pigeon pea.

Symbiotic plants, under salt stress, are thought to prevent nodule destruction by avoiding the protein denaturation (Irigoyen et al. 1992). Maximum proline synthesis has been found in salt-stressed plants in the presence of bacteria *Burkholderia* (Barka et al. 2006), *Arthrobacter*, and *Bacillus* (Sziderics et al. 2007). The introduction of proBA gene extracted from *Bacillus subtilis* into *Arabidopsis thaliana* enhances proline accumulation and increased salt tolerance in transgenic plants (Chen et al. 2007). Proline accumulation was found to increase tremendously when the host plant gets stimulated by colonization under salt stress.

Carbohydrates

Carbohydrates lower the water potential of plants and provide defensive mechanism against salt stress (Thanna and Nawar 1994; Ahmad and Jhon 2005; Koyro et al. 2012). Increased carbohydrate content due to salinity stress has been observed in *Phragmites australis* and corresponds to mycorrhizal plants (*Glomus fasciculatum*) (Al-Garni 2006; Thomson et al. 1990). Similar results have been observed in soybean roots colonized by *Glomus intraradices* (Porcel and Ruiz-Lozano 2004). Enhanced level of soluble sugar in the host plants is resulted by mycorrhizal symbiosis (Evelin et al. 2009).

Trehalose among the non-reducing sugar is the main storage part of carbohydrate in extra-radical mycelium as well as in spores of AM fungi and plays a key role in maintaining the integrity of biological membranes against salt stress. Trehalose accumulation has been exploited as a stress protector and has the potential to adapt to hyperosmotic conditions of symbiotic bacteria and in turn provides a powerful tool in the response of AM fungi under salt stress (Lopez et al. 2008).

This forms the close association to withstand the capacity to endure salt stress (Borde et al. 2010). Conversely, some scientists have shown negative effects regarding the mycorrhizal association and sugar accumulation in host plants during salt stress.

Furthermore, carbohydrate accumulation is associated with the transport and supply of food to different parts of plants necessary for plant adaptation, growth,

photosynthesis, and biomass allocation (Balibrea et al. 2000). Eventually, the accumulation of carbohydrate in the sink associated with salt stress represents the first limiting step for salt tolerance and can be restored and enhanced in mycorrhizal plants (Perez-Alfocea et al. 2010; Dodd and Perez-Alfocea 2012). High sugar content in maize plants due to AM symbiosis was observed under salt stress by Feng et al. (2002). This result can lead to improved plant water level, efficient chlorophyll synthesis, and increased tolerance level (Sheng et al. 2008).

Polyamines

Polyamines play a significant role in response to various abiotic stresses including salinity (Krishnamurthy and Bhagwat 1989; Ahmad et al. 2012b) and high osmotic potential (Besford et al. 1993) as they act as a defense strategy (Kurepa et al. 1998). They also play an important role in the architecture of roots under salt stress (Couée et al. 2004). Salinity decreases the level of polyamines; however, in mycorrhizal plants, the activity of polyamines is improved (Sannazzaro et al. 2007).

Various species of salt-tolerant mycorrhizae have been observed to enhance the adaptability to salinity stress of *Lotus glaber* (Sannazzaro et al. 2007). Spermine and spermidine are formed from methionine and ornithine, whereas putrescine is produced from arginine. The initial step undergoes the loss of carbon dioxide catalyzed by ornithine decarboxylase (Ahmad et al. 2012b; Evelin et al. 2009). Nevertheless, associated enzymes linked with polyamines are increased under salinity (Lefevre and Lutts 2000). This might lead to an extensive enhancement of polyamines in plants when inoculated by mycorrhizal fungi. Polyamine also stimulates various protein biosyntheses via nucleic acid interaction and thereby stabilizes the biomembranes (Evelin et al. 2009).

Antioxidants

ROS generated under salt stress become a major devastating effect in plants. The radicals are leaked during the aerobic respiration in chloroplast and mitochondria (Møller 2001; Asada 1999). These in turn damage the photosynthetic machinery of the cell. ROS negatively affects biomolecules such as proteins, carbohydrates, nucleic acids, and membrane lipids. To combat the stressful environment, plants possess several antioxidant enzymes to protect them from such harmful effects of ROS. Therefore, antioxidative enzymes play a key role as a defense mechanism in various plant species and hence salt tolerance level (Yamane et al. 2004; Jiang and Zhang 2002; Evelin et al. 2009; John et al. 2007; Ahmad 2010; Ahmad et al. 2008a, b, 2009, 2010a, b, 2011, 2012a, c, 2013; Ahmad and Umar 2011; Koyro et al. 2012; Ahmad and Prasad 2012a, b; Rasool et al. 2013a, b).

Besides antioxidant enzymes, several non-enzymatic compounds such as carotenoids, glutathione, tocopherols, and ascorbic acid are also responsible to scavenge the oxygen radicals (Alguacil et al. 2003; Wu et al. 2006; John et al. 2007; Ahmad 2010; Ahmad et al. 2008a, b, 2009, 2010a, b, 2011, 2012a, c, 2013; Ahmad and Umar 2011; Koyro et al. 2012; Ahmad and Prasad 2012a, b; Rasool et al. 2013a, b). Incorporation of AM symbiosis helps to endure the salt stress and increases the antioxidant enzymes (Ocon et al. 2007; Harinasut et al. 2003).

Enhanced antioxidant enzymes associated with AM plants have been demonstrated by many scientists. Catalase (CAT), ascorbate peroxidase (APOX), and superoxide dismutase (SOD) have shown increased activity in *Olea europaea* and *Retana splaerocarpa* (Alguacil et al. 2003). Smirnoff (1993) reported detoxification of superoxide to hydrogen peroxide by enhanced SOD. This produced hydrogen peroxide is in turn scavenged by CAT and peroxidase and APOX (Lopez et al. 1996; Benavides et al. 2000). Mycorrhizal plants enhance the production of antioxidant enzymes as affected by the micronutrients available to the enzymes such as CAT, POX, and SOD (Alguacil et al. 2003). Deficiencies and excess of micronutrients alter the expressions of metalloenzymes, e.g., Fe increases the CAT and APX activities in *Nicotiana plumbaginifolia* (Kamfenkel et al. 1995). Accumulation of ROS depends upon the balance between ROS production and ROS scavenging (Miller et al. 2010). There are many reports that showed mycorrhizal plants provide higher accumulation of antioxidative enzymes and thereby improve the whole plant growth under stress (Miller et al. 2010; Scheibe and Beck 2011).

Ascorbate plays a crucial role to protect the chlorophyll activity during salt stress (Shao et al. 2008; Noctor and Foyer 1998). Türkan and Demiral (2009) have reported the tremendous link between antioxidant capacity and salinity tolerance. Studies reveal that mycorrhizal symbiosis enables the host plant to survive under salt or water deficit stresses by enhancing the production of various antioxidant enzymes (Zhong Qun et al. 2007; Ruíz-Sánchez et al. 2010; Talaat and Shawky 2011).

Manchanda and Garg (2011) also reported that POX and CAT activity enhances salt tolerance in *C. cajan* (Mehdy 1994). Soybean plants colonized with AM fungi indicate the increased antioxidant capacity with the potential to adapt to the various salt stress conditions (Ghorbanli et al. 2004). Increased level of antioxidant enzymes might also result in the efficient colonization of mycorrhizal fungi under salt stress (Alguacil et al. 2003). Similar results were obtained with *Gmeline arbarea* inoculated with *Glomus fasciculatum* (Dudhane et al. 2010; Aggarwal et al. 2012).

Root colonization by mycorrhizal fungi induces accumulation of proline and thereby facilitates osmotic adjustment (Sheng et al. 2011; Ruiz-Lozano and Azcon 1995). Proline is an indicator of salt and other stresses that scavenge the free radicals and stabilize the water balance mechanisms in plants (Yang et al. 2009; Dodd and Perez-Alfocea 2012). Under salt stress, levels of antioxidants enzymes vary depending on the species, metabolic state of plant, and also the intensity of stress (Reddy et al. 2004). Enhancement of antioxidant enzymes is

also associated with increased potential to withstand the stress indicating the tolerance of mycorrhizal garlic plants to salt stress (Borde et al. 2010). Evidences show the greater and increased growth of AM-treated plants on exposure to different levels of salt concentrations.

Abscisic Acid

AM fungi have the capacity to alter the levels of ABA and thereby adapt to different environmental stresses including salinity (Estrada-Luna and Davies 2003). ABA levels are found higher in *L. glaber* colonized by AM fungi (Sannazzaro et al. 2007). Spermine content in mycorrhizal plant tends to regulate the ABA activity in the shoot. Nevertheless, some authors have reported less accumulation of ABA in association with mycorrhizal fungi under salt stress (Evelin et al. 2009). ABA is one of the growth hormones responsible to protect the plant against salt stress (Miransari et al. 2013).

Nodulation and Nitrogen Fixation

During the symbiosis process, nitrogen-fixing bacteria form nodules on the roots, especially in leguminous plants. The number of nodules decreases under salt stress as the process of nitrogen fixation is adversely affected by the stress due to the inhibition of leg-hemoglobin production, thereby reducing the nitrogenase activity (Garg and Manchanda 2008; Rabie and Almadini 2005; Harinasut et al. 2003). Reduction in nodulation and nodule activity has also been observed by Serraj et al. (2001), Tejera et al. (2005), Bolanos et al. (2006), and Garg and Manchanda (2008) in different plants.

Under salt stress, mycorrhizal plants improve their productivity due to their adequate leg-hemoglobin content and nitrogenase activity. Therefore, nodulation seems to enhance at low salt concentration (Johansson et al. 2004; Rabie and Almadini 2005; Garg and Manchanda 2008). AM fungi possess the ability to alleviate the harmful effects of salinity during the process of nitrogen fixation and nodulation in legumes as AM fungi increase the number of nodules (Garg and Manchanda 2008; Giri and Mukerji 2004; Ruiz-Lozano et al. 2001; Porcel et al. 2003). Exogenous application of AM fungi improved the pink color of leg-hemoglobin, indicating the higher pigment content and hence higher nitrogenase activity and nitrogen fixation in mycorrhizal plants. This is also attributed to the free availability of phosphorus required for nitrogenase enzyme of bacterial symbionts and also uptake of essential micronutrients, leading to the enhanced growth and yield of plants (Founoune et al. 2002; Evelin et al. 2009). Therefore, to prevent from such harmful effects of abiotic stress, association of AM fungi under salinity stress can alter various changes and make the plants to adapt to different

types of stress including salt stress. Mycorrhiza-treated plants enhanced nodule formation, photosynthesis, and water status in *S. helvola* under salt stress (Tsang and Maun 1999).

Under extreme conditions of salinity stress, the AM fungi have been found to alleviate these stresses and create a strong association with their host plants (Dodel and Ruíz-Lozano 2012; Wilde et al. 2009; García-Garrido and Ocampo 2002). Several studies have reported a tremendous yield loss under salt stress (Al-Karaki et al. 2001; Cantrell and Linderman 2001; Hajiboland et al. 2010).

Nutrient Uptake

Nutrients are essential for the proper functioning of plants and any deficiency hamper plant growth and yield production. All essential nutrients seem to be adversely affected by salt stress. Accordingly, to combat the poor supply of nutrients from the soil, mycorrhizal fungi help their host plant to restore the uptake of mineral nutrients and hence plant growth (Giri and Mukerji 2004; Sharifi et al. 2007).

Phosphorus is essential for plant growth and is not readily available as the phosphate precipitates with some of the cations such as Ca, Mg, and Zn. However, AM fungal symbiosis plays a key role in supplying the poor mobility nutrients like phosphorus to the host plant by the roots and hence suppress the negative effects of salt (Feng et al. 2002; Al-Karaki and Clark 1998). This is attributed to the extensive network of AM fungal hyphae that explore higher volume of soil (Ruiz-Lozano and Azcón 2000). In fact, depleted areas around the plant roots can also become fertile due to the presence of mycorrhizal hyphae, which acquire nutrients from the soil under the salinity stress.

During salt stress, plants absorb Na more than K (Rus et al. 2001), thereby providing the competition for K within the same binding site. Potassium has its peculiar functions such as participating in the activities of various enzymes, regulating the stomatal movement, and also involving in the synthesis of proteins (Blaha et al. 2000). Salinity cause imbalance in K^+/Na^+ ratio adversely affecting the plant growth. Since mycorrhizal plants possess higher Na^+/K^+ (higher K^+ uptake in shoots), they are able to mitigate the salt stress by the dilution effect (Juniper and Abbott 1993). Similar results in the concentration of K have been demonstrated by Ojala et al. (1983) and Mohammad et al. (2003) who showed higher K^+ accumulation and hence higher K/Na ratio by mycorrhizal plants, favorably affecting the enzymatic processes as well as protein synthesis under salt stress (Audet and Charest 2006). Calcium act as a second messenger to transducer signals. Calcium ions have tendency to raise K uptake, thereby adapting various changes under salt stress. Therefore, Ca accumulation under salt stress has been found to enhance the colonization as well as sporulation of mycorrhizal fungi (Jarstfer et al. 1998).

Conclusion and Future Prospects

Salt stress has been shown to adversely affect plant growth and physiology; however, association with AM fungi seems to effectively enhance plant growth under stress through the accumulation of different solutes and higher uptake of water and nutrients. Investigations have been carried out to find the depth of mycorrhizal symbiosis and activity under stress. Enhanced production of antioxidative enzymes in mycorrhizal plants needs to be further evaluated to reveal the ultrastructure aspects of AM fungi. These in turn would open new avenues for the alternative way of increasing tolerance by AM symbiosis in order to overcome the adverse effects of salt stress. AM symbiosis plays a crucial role in plant growth promotion and prevents the plants from the adverse effects of various stresses including salinity. Genetic techniques and molecular approaches may indicate new insight in the alleviating role of mycorrhizal symbiosis under stress.

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