Chapter 10
Mycorrhizosphere Interactions for Legume Improvement

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Abstract Legumes, plant species of great agronomical and ecological interest, are known to establish beneficial symbiotic relationships with two types of soil-borne microorganisms: N_2-fixing bacteria and arbuscular mycorrhizal fungi. Additionally, the legume rhizosphere harbors other associative beneficial microorganisms such as plant growth promoting rhizobacteria (PGPR). These microorganisms interact among themselves, and with legume roots, to develop the multifunctional legume mycorrhizosphere, a scenario of diverse activities relevant for legume productivity either in sustainable agriculture or in the maintenance of natural plant communities. This Chapter highlights strategic and applied research conducted so far, which have allowed a comprehensive understanding of the formation and functioning of the legume mycorrhizosphere. Manipulation of the microbial activities allows tailoring efficient mycorrhizosphere systems for improving legume productivity. The technology for the production of efficient rhizobial, free-living PGPR, and AM-fungal inoculants, nowadays commercially available, is likely to support sustainable and environmentally friendly low-input agrotechnological practices. The possibilities to use these bioproducts to help a sustainable development of legumes in either agrosystems or natural ecosystems are discussed.

10.1 Introduction

The production of healthy crops and the self-sustainability of the ecosystems are largely dependent on soil quality (Altieri 1994). And hence, maintaining the quality and fertility of soil is a key issue not only for optimizing the stability and

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productivity of either agro-ecosystems or natural ecosystems but also to prevent erosion and to minimize negative cultural and environmental stresses (Buscot 2005; Chaudhary et al. 2009). It is well known that (1) soil quality/fertility is determined by interactions of the chemical, physical, and biological soil components (2) the variation in soil fertility is based on the diverse genetic and functional groups of extensive soil microbial populations, and (3) the activities of microbial communities affect critical soil functions (Barea et al. 2005b; Mallik and Williams 2008; Avis et al. 2008). Among other functions, soil microorganisms are involved in the biogeochemical cycling of nutrients and matter and the maintenance of plant health and soil quality (Barea et al. 2005a). These activities are particularly relevant at the root–soil interface microhabitats, known as the rhizosphere, where microorganisms interact with plant roots and soil constituents. Formation, development, and significance of the rhizosphere have been widely reviewed (Barea et al. 2002b, 2005b; Richardson et al. 2009; Faure et al. 2009; Jones et al. 2009; Lambers et al. 2009; Hartmann et al. 2009; Dessaux et al. 2010).

Microbial interactions in plant rhizosphere play important roles in the overall development of legumes. Actually, legumes, plant species of great agronomical and ecological interest, are able to establish beneficial symbiotic relationships with two types of soil-borne microorganisms: N_2-fixing bacteria and mycorrhizal fungi. Like most of the major plant families, legume plants also form associations with arbuscular mycorrhizal (AM) fungi (Barea et al. 2004), the most universal mycorrhizal type (Smith and Read 2008). Nodulated and mycorrhizal legumes has to be the normal in sustainable agriculture and in natural ecosystems because both N_2-fixing bacteria and AM fungi naturally protagonize activities fundamental to legume nutrition and health. In turn, legumes impact the fundamental soil properties, including the development/performance of N_2-fixing bacteria and AM fungi (Lupwayi and Kennedy 2007). Since legume–rhizobium symbiosis is discussed by others in this book, emphasis is placed on mycorrhizas in this chapter.

Mycorrhizas are symbiotic, generally mutualistic and balanced, associations established between certain soil fungi and most vascular plants where both partners exchange nutrients and energy (Brundrett 2002). Basically, the host plant receives mineral nutrients via the fungal mycelium (mycotrophism), while the heterotrophic fungus obtains carbon compounds from the host's photosynthates. It is universally accepted that mycorrhizal symbioses, which can be found in almost all ecosystems worldwide, are fundamental to improve plant fitness and soil quality through key ecological processes (Smith and Read 2008). The mycorrhizal fungi colonize the root cortex and develop an extraradical mycelium, which overgrows the soil surrounding plant roots. This hyphal net is a structure specialized for the acquisition of mineral nutrients from the soil, particularly those whose ionic forms have poor mobility or are present in low concentration in the soil solution, as is the case with P (Barea 1991). This mycorrhizal function provides the plant with an adaptive strategy for P acquisition in soils with low P availability, which is an important nutrient for legumes because these species require P for N_2-fixation (Postgate 1998; Vance 2001).

Apart from these microbial symbioses, legumes, like many other plant species, live in association with a great array of soil saprophytic microorganisms inhabiting rhizosphere (Barea et al. 2005b). Both symbionts and saprobes interact in the rhizosphere (Finlay 2008; Jaderlund et al. 2008; Kiers and Denison 2008; Adesemoye and Kloepper 2009), and some of the resultant interactions are fundamental for sustainable legume developments (Barea et al. 2008). Particularly, after mycorrhiza establishment, rhizosphere microorganisms interact with mycorrhizal structures to generate the so-called mycorrhizosphere, a key issue for legume productivity improvement (Barea et al. 2005c), as will be explained in the later section. This chapter critically reviews the related literatures focusing on (1) the types of microorganisms and processes involved in the establishment and functioning of the mycorrhizosphere, (2) the impact of the mycorrhizosphere activities on legume productivity, and (3) the possibilities to tailor an efficient mycorrhizosphere to be used as a biotechnological tool to improve legumes in either agrosystems or natural ecosystems.

10.2 Microorganisms and Processes Involved in the Establishment and Functioning of the Mycorrhizosphere

Many microbial groups live and perform important functions in the ecosystem (Giri et al. 2005; Buée et al. 2009). However, most studies on rhizosphere microbiology, especially those describing cooperative plant–microbial interactions, have focused their attention only on bacteria and fungi (Barea et al. 2004; de Boer et al. 2005). Accordingly, the two types of microorganisms are discussed in the following section.

10.2.1 Beneficial Rhizosphere Bacteria and Fungi in Agro- and Ecosystems

The prokaryotic bacteria and the eukaryotic fungi have a great variety of trophic/living habits whose saprophytic or symbiotic relationship with the plant could be either detrimental (pathogens) or beneficial (mutualists). The beneficial saprophyte microbes promote plant growth and health acting as (1) decomposer of organic substances (detritus), (2) plant growth-promoting rhizobacteria (PGPR), or (3) antagonists of plant pathogens. Beneficial plant mutualistic symbionts include the N_2-fixing bacteria and the multifunctional AM-fungi (Barea et al. 2005c).

10.2.1.1 Saprophytic Beneficial Rhizosphere Bacteria and Fungi

The term rhizobacteria refers to those rhizosphere bacteria that are able to colonize the root environments (Kloepper et al. 1991). Beneficial root colonizing rhizosphere bacteria, the PGPR, however, must have the ability to colonize root, and be able to survive and multiply in microhabitat associated with the root surface, in competition with native microbiota, at least for the time needed to express their beneficial plant activities (Kloepper 1994). Novel techniques to study the colonization pattern, bacterial characterization, and molecular determinants of the root colonization have been described (Gamalero et al. 2004; Richardson et al. 2009; Dessaux et al. 2010). The PGPR are known to participate in many important ecosystem processes, such as the biological control of plant pathogens, nutrient cycling, and/or seedling growth (Adesemoye et al. 2009; Zahir et al. 2004; Lucy et al. 2004; Barea et al. 2004; Lucas-Garcia et al. 2004). Numerous PGPR have been identified as biocontrol agents and used to reduce losses to crops caused by plant pathogens (de Boer et al. 2003; Chin-A-Woeng et al. 2003; Avis et al. 2008). Biological control of soil-borne diseases is known to result from (1) the reduction in the saprophytic growth of the pathogens followed by reduction in the frequency of the root infections through microbial antagonism, and/or (2) the stimulation of “induced systemic resistance (ISR)” in the host-plants (van Loon et al. 1998). Some microorganisms, however, can benefit plants by more than one mechanism. For example, *Trichoderma* species controls fungal pathogens by acting both as antagonist and by inducing localized and systemic responses (Harman et al. 2004). The processes involved in nutrient cycling by PGPR include nitrogen-fixation and phosphate solubilization besides releasing other nutrients in soil (Zaidi et al. 2009; Richardson et al. 2009; Marschner 2008).

Microbial N_2-fixation is the first step in cycling N to the biosphere from the atmosphere, a key input of N to plant productivity (Vance 2001). It is a well established fact that members of the prokaryotic bacteria are the only organisms able to fix N_2 as they are the only organisms possessing the key enzyme nitrogenase, which specifically reduces atmospheric N to ammonia in the symbiotic root nodules (Leigh 2002; Markmann and Parniske 2009). Furthermore, the PGPR are also known to mediate processes involved in P cycling. In this context, it has been shown that many rhizobacteria (and rhizofungi) are able to solubilize sparingly soluble phosphates (Khan et al. 2007, 2010) largely by releasing chelating organic acids (Farhat et al. 2009; Xiao et al. 2009; Marschner 2008). Phosphate-solubilizing bacteria (PSB) selected from existing PGPR populations have been assayed, but their effectiveness in the soil–plant system is variable (Barea et al. 2007; Zaidi et al. 2009). One of the reasons besides other factors accounting for such variation in the P-solubilizing activity of PSB could be the refixation of P applied exogenously by the soil’s constituents before they reach to the root surface. However, if the phosphate ions, as released by the PSB, are taken up by a mycorrhizal mycelium, this would result in a synergistic microbial interaction that in turn improves P acquisition by the plant. This mycorrhizosphere activity is discussed in Sect. 10.4.

10.2.1.2 Beneficial Mutualistic Symbionts: N_2-Fixing Bacteria and Arbuscular Mycorrhizal Fungi

The bacteria able to fix N_2 in symbiosis with legume plants belonging to diverse genera (Willems 2007) are collectively termed as "rhizobia." How these bacteria interact with legume roots leading to the formation of N_2-fixing nodules, the signaling processes involved, the evolutionary history, and particularly, the molecular aspects determinants of host specificity in the rhizobial–legume symbiosis are described elsewhere in this book and will not be explained here. Other bacteria (actinomycetes), belonging to the genus *Frankia,* form nodules on the root of the so-called "actinorrhizal" species, plants having a great ecological importance (Vessey et al. 2004). The other major groups of mutualistic microbial symbionts are the fungi, which establish the arbuscular mycorrhizal associations with the roots of most plant species (Smith and Read 2008). The AM fungi are obligate microbial symbionts, which are not able to complete their life cycle without colonizing a host plant. They are ubiquitous soil-borne microbial fungi, whose origin and divergence dates back to more than 450 million years (Redecker et al. 2000a). The AM fungi were formerly included in the order Glomales, Zygomycota (Redecker et al. 2000b), but they have recently been moved to a new phylum Glomeromycota (Schüβler et al. 2001), as it is currently accepted (Rosendahl 2008; Helgason and Fitter 2009; Gamper et al. 2010).

Earlier studies on diversity of AM fungal communities were based largely on the morphological characterization of their large multinucleate spores. However, more recently, the ribosomal DNA sequence analysis has been used to determine the diversity of natural AM populations (Santos-González et al. 2007; Hempel et al. 2007; Öpik et al. 2008a,b; Toljander et al. 2008; Alguacil et al. 2009; Rosendahl et al. 2009). A lack of relationship between genetic diversity and functional diversity has been often described (Munkvold et al. 2004; Croll et al. 2008; Ehinger et al. 2009). However, fingerprinting techniques, using gel electrophoresis of PCR-amplified rDNA fragments, are being applied to analyze AM fungal species composition in spore, root, or soil (Cornejo et al. 2004; Santos-González et al. 2007; Hempel et al. 2007; Öpik et al. 2008a,b; Sonjak et al. 2009). Despite the advancement in molecular techniques, the identification approaches employed for AM fungi based on morphological characteristics are still valid and used and are considered complementary to the molecular methods (Oehl et al. 2009; Morton 2009). Recent advances in the genetic and genomics of the AM fungi have been reviewed (Gianinazzi-Pearson et al. 2004; Parniske 2004; Azcón-Aguilar et al. 2009). In this regard, the complete genome of the model AM fungus *Glomus intraradices* has been determined (Martin et al. 2008)

10.2.2 Arbuscular Mycorrhiza

There are two main types of mycorrhiza, ecto- and endomycorrhiza, which have considerable differences in their structure and physiological relationships with symbionts (Smith and Read 2008). In ectomycorrhizas, the fungus develops a sheath or mantle around the feeder roots. The mycelium penetrates the root and develops between the cortical cells forming the so-called "Hartig net" that constitutes the site of nutrient exchange between partners. About 3% of higher plants, mainly forest trees in the Fagaceae, Betulaceae, Pinaceae, *Eucalyptus,* and some woody legumes, form ectomycorrhiza. The fungi involved are mostly Basidiomycetes and Ascomycetes. In endomycorrhizas, the fungi colonize the root cortex both intercellularly and intracellularly. Some endomycorrhizal types are restricted to species in the Ericaceae ("ericoid" mycorrhiza) or Orchidaceae ("orchid" mycorrhiza), while the common arbuscular mycorrhizal (AM) type is widely distributed throughout the plant kingdom. The widespread and ubiquitous AM symbiosis is characterized by the tree-like symbiotic structures, termed "arbuscules," which the fungus develops within the root cortical cells, and where most of the nutrient exchange between the fungus and the plant is thought to occur. An intermediate mycorrhizal type, the ectendomycorrhiza, is formed by plants in families other than the Ericaceae, but in the Ericales, and in the Monotropaceae and Cistaceae. In these mycorrhizal associations, the fungi form both a sheath and intracellular penetrations (Smith and Read 2008). The obligate character of the AM fungi are such that specific methodological approaches are needed to investigate the processes involved in the formation and functioning of the symbiosis (Lambais 2006; Balestrini and Lanfranco 2006; Reinhardt 2007; Martin 2008; Garcia-Garrido et al. 2009; Gianinazzi-Pearson et al. 2009; Facelli et al. 2009; Gryndler et al. 2009; Smith et al. 2009). The AM fungi contribute to nutrient, particularly P, acquisition and supply to plants by linking the geochemical and biotic portions of the soil ecosystem, thereby affecting rates and patterns of nutrient cycling in both agricultural and natural ecosystems (Jeffries and Barea 2001). In addition, the AM fungi are able to tap other nutrients (Barea et al. 2005a), especially N, either from inorganic (Tobar et al. 1994a,b) or organic (Leigh et al. 2009) sources.

The extraradical mycelium of AM fungi is profusely branched and provides a very efficient nutrient-absorbing system beyond the Pi-depletion zone surrounding the plant roots, thereby reducing the distance that Pi must diffuse through the soil prior to its interception. Actually, the AM fungal mycelium can spread through the soil over considerably longer distances (usually several cm) than root hairs (Finlay 2008). The ability of the AM hyphae to grow beyond the root Pi-depletion zone and deliver the intercepted Pi to the plant is thought to be the reason why AM associations increase Pi accumulation and plant growth in soils with low P availability (Smith and Read 2008). The AM symbiosis not only influence nutrient cycling in soil–plant systems but also improves plant health through increased protection against environmental stresses including biotic (e.g., pathogen attack) or abiotic (e.g., drought, salinity, heavy metals, organic pollutants), and enhancing soil structure through the formation of the aggregates necessary for good soil tilth (Rillig and Mummey 2006; Turnau et al. 2006; Pozo and Azcón-Aguilar 2007; Ruíz-Lozano et al. 2008; Barea et al. 2008; Finlay 2008; Smith and Read 2008; Varma 2008; Ferrol and Pérez-Tienda 2009).

10.2.3 Interactions Between AM and Rhizosphere Microorganisms to Establish a Functional Mycorrhizosphere

Rhizosphere microorganisms can either interfere with or benefit mycorrhiza establishment (Gryndler 2000; Pivato et al. 2009). A particular interest has been about the so-called "mycorrhiza-helper-bacteria" (MHB), a term that was coined by Garbaye (1994) and later updated by Frey-Klett et al. (2007) for those bacteria which stimulate mycorrhizal mycelial growth and/or enhance mycorrhizal formation. This applies both to Ectomycorrhiza (Frey-Klett et al. 2005) and to AM associations (Azcón-Aguilar and Barea 1992; Barea et al. 2004; Sabannavar and Lakshman 2008). Conversely, the establishment of PGPR inoculants in the rhizosphere can also be affected by AM fungal coinoculation (Barea et al. 2005b; Artursson et al. 2006; Jaderlund et al. 2008; Mallik and Williams 2008).

The establishment of the AM fungus in the root cortex is known to change many key aspects of plant physiology. These include the mineral nutrient composition in plant tissues, the hormonal balance and the patterns of C allocation. Therefore, the AM symbiotic status changes the chemical composition of root exudates, while the development of an AM soil mycelium, which can act as a carbon source for microbial communities (Barea et al. 2002a,b). AM-induced changes in plant physiology affect the microbial populations, both quantitatively and qualitatively, in either the rhizosphere and/or the rhizoplane. In addition, there are specific modifications in the environment surrounding the AM mycelium itself, the *mycorrhizosphere* (Linderman 1988; Andrade et al. 1997; Gryndler 2000). Therefore, the rhizosphere of a mycorrhizal plant, generically termed as the mycorrhizosphere, can have features that differ from those of a nonmycorrhizal plant (Finlay 2008). The mycorrhizosphere functions known to improve plant growth and health, and soil quality are depicted in Fig. 10.1.

10.3 Interactions Between AM Fungi and Rhizobial Bacteria to Improve Legume Productivity

Janse (1896) was the first to report the coexistence of endophytic bacteria and fungi colonizing legume roots. The fungal "root infection" was later on recognized as a "mycorrhizal" development (Jones 1924). Thereafter, Asai (1944) concluded that nodulation by rhizobial bacteria appear to be dependent on mycorrhiza formation by the common host legume. Subsequently, both the widespread presence of the AM symbiosis in nodulated legumes and the impact of AM fungi in improving nodulation and N_2-fixation were recognized (Barea and Azcón-Aguilar 1983; Hayman 1986; Mosse 1986; Zaidi et al. 2003). Despite a positive effect of AM fungi on nodule formation and function, some reports on mycorrhiza–legume interaction are contradictory. For example, Franzini et al. (2009) found in an experiment that the tested AM

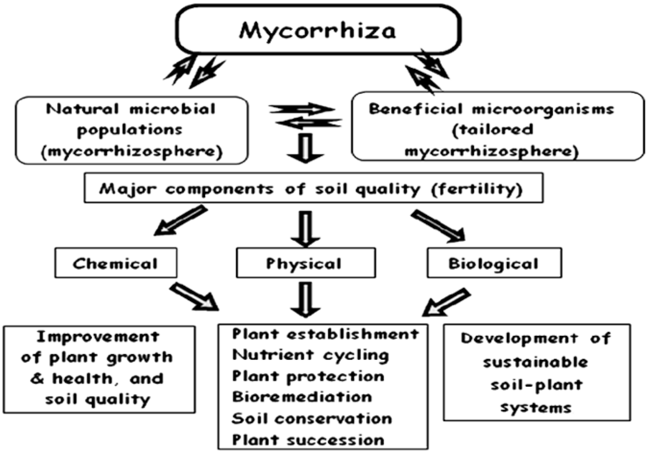

Fig. 10.1 Mycorrhizosphere functions

fungi did not improve nodule formation and function. Between the pioneering work by Asai (1944) and recent publications (Bisht et al. 2009), many papers have reported on the conceptual approaches and experimental developments with regard to the formation and functioning of the tripartite symbiosis. The relevant information will be summarized in this section under the following heads (1) fundamental aspect of the formation and functioning of the tripartite symbiosis in legumes, (2) strategy as to how the performance of legumes could be improved, and (3) strategic studies related to the role of legumes in the revegetation of degraded ecosystems.

10.3.1 Fundamental Aspect of the Formation and Functioning of the Tripartite Symbiosis in Legumes

Rhizobial bacteria and AM fungi are known to interact among themselves and with their common legume host roots, either at the colonization stages or at the symbiotic functional level (Azcón 1987; Barea and Azcón-Aguilar 1983). In this context, numerous studies have been carried out in recent times, which will briefly be summarized under the following heads (1) genetic and molecular relationships of AM fungi and rhizobia, (2) physiological interactions related to the formation and functioning of the tripartite symbiosis, and (3) use of ^{15}N to ascertain the AM role on N_2 fixation by legume–rhizobia associations.

10.3.1.1 Genetic and Molecular Relationships of AM Fungi and Rhizobia

Developmental genetics and evolution timing analysis of microbe–plant symbioses, including both mutualistic, either N_2-fixing or mycorrhizal, and pathogenic associations, have revealed a common developmental program for all of these compatible microbe–plant associations (Markmann et al. 2008; Provorov and Vorobyov 2009b). As the rhizobia–legume symbiosis evolved much later than the AM symbiosis (Douglas 2008; Martínez-Romero 2009; Provorov and Vorobyov 2009a; Zhukov et al. 2009), the cellular and molecular events occurring during legume nodulation may have evolved from those already established in the AM symbiosis (Hirsch and Kapulnik 1998; Parniske 2008; Zhukov et al. 2009). However, the information generated from molecular tools suggests that some plant genes can modulate both types of legume symbiosis (Parniske 2004; Gianinazzi-Pearson et al. 2009). For example, the use of mycorrhiza-defective legume mutants (Myc^{-}) has provided relevant information, which helped in understanding the common cellular and genetic programs involved in the legume root symbioses (Gianinazzi-Pearson and Brechenmacher 2004; Gianinazzi-Pearson et al. 2009). The use of Myc^{-} legumes has also contributed to a better understanding of the signaling processes involved in the formation of microbe–legume symbioses, and hence, it has been suggested that both AM formation and nodulation share a common signal transduction pathway (Parniske 2004).

10.3.1.2 Physiological Interactions Related to the Formation and Functioning of the Dual Symbiosis

A great number of reports have focused on the physiological and biochemical basis of AM fungal × rhizobia interactions. The information, reviewed by Barea et al. (1992), established that the main cause of such beneficial interactions is the supply of P by the AM fungi to satisfy the high P demand of nodule formation. The AM fungi has also been shown to have a general influence on plant nutrition, but more localized effects of AM fungi are reported either at the root, nodule, or bacteroid levels. In a study, it was reported that nodules in fact had two to three times more P than the root (Mosse 1986), which was revealed in a time-course experiment that corroborated that the nodules and their rhizobial bacteroids have a “special demand” for P and call first for this nutrient with respect to other plant organs (Asimi et al. 1980). Furthermore, the tripartite symbiosis has been investigated both at physiological and structural levels, with results indicating that the effects depend on the particular endophyte combination (Ruíz-Lozano and Azcón 1993) and/or the legume genotype (Monzón and Azcón 1996). However, during symbiosis under natural conditions, AM fungi and rhizobia do not seem to compete for infection sites and colonize the root almost simultaneously (Bethlenfalvay et al. 1985). Accordingly, when host photosynthesis is limited, AM fungi usually show a competitive advantage for carbohydrates over the rhizobia (Brown and Bethlenfalvay 1988; Ruíz-Lozano and Azcón 1994), but under normal situations, the photosynthetic capacity of plants exceeds the C demand of the tripartite symbiosis (Ha and Gray 2008). The energy cost of the tripartite symbiosis as investigated by Ames and Bethlenfalvay (1987) suggests that the CO_2 fixation rate expressed as g C g^{-1} shoot dry matter h^{-1} increased in symbiotic plants. This is in fact a mechanism that enhances photosynthesis to compensate for the C cost of the symbioses. These results were further corroborated (Mortimer et al. 2008) in experiments, which added new insights into the topic. These authors found that the nodular growth was suppressed during the early development of AM colonization under low P conditions. However, once AM colonization was established, and the efficiency of P nutrition increased, nodule development and host growth were improved, and concomitantly, N_2-fixation enhanced. This indicates that the AM fungi were the dominant symbiont for host C in the tripartite symbiosis, due to its rapid development. The subsequent AM role in supplying P benefited both host legume and nodules performance.

Other effect of AM fungi × rhizobia coinoculation on physiological aspects of symbiotic developments in legumes were investigated with regard to the bio-safety use of genetically modified (GM) rhizobia inoculants in agriculture. Particularly, the effects on AM formation and function of a wild type (WT) *Rhizobium meliloti* strain with those of its GM derivative were compared in time-course greenhouse experiments. It was found that either rhizobial strains coinoculated with the representative AM fungus *Glomus mosseae* increased the number of AM colonization units and the nutrient acquisition ability in AM alfalfa plants (Barea et al. 1996). Indeed, these response to variables were higher in plants inoculated with the GM rhizobial strain than in those inoculated with the WT one (Tobar et al. 1996; Galleguillos et al. 2000). The use of histochemical staining methods as succinate dehidrogenase (SDH) enzyme marker evidenced that both the WT and its GM derivative *R. meliloti* improved the physiological/biochemical activity of the AM fungus *G. mosseae* colonizing alfalfa (*Medicago sativa* L.) roots (Vázquez et al. 2000), and the nitrate reductase activity, protein content, etc. (Vázquez et al. 2002).

10.3.1.3 The Use of ^{15}N to Ascertain the AM Role on N_2-Fixation by Legumes

The addition of a small amount of ^{15}N-enriched inorganic fertilizer and an appropriate "non-fixing" reference crop is the basis for a direct method, which allows us to distinguish the relative contribution of the three N sources for a fixing crop, i.e., soil, atmosphere, and fertilizer (Danso 1988). Consequently, ^{15}N-based methodologies have been used to ascertain and quantify the amount of N that is actually fixed by legume–rhizobia consortia in a particular situation. Particularly, these methods have been applied to measure the contribution of the AM symbiosis to the process in greenhouse and field studies (Azcón et al. 1988, 1991; Barea et al. 2002c). A lower $^{15}N/^{14}N$ ratio in the shoots of rhizobia-inoculated AM plants with respect to those achieved by the same rhizobial strain in nonmycorrhizal plants was found. This indicated an enhancement of the N_2 fixation rates (an increase in ^{14}N from the atmosphere), as induced by the AM activity. The information about the possible role of AM fungi in legume symbiotic performance based on the use of ^{15}N isotope dilution technology has been reviewed (Barea et al. 2005a; Chalk et al. 2006). In addition, the isotopic techniques have been also used to measure N-transfer in mixed cropping where legumes are usually involved (Zapata et al. 1987). Since the AM mycelia can link different plant species growing nearby, and help overlap the pool of available nutrients for the intercropped plant species, the N released into the overlapping mycorrhizospheres by legume root exudation, or by nodule decay, can result in nitrogen available for nonfixing plants (Haystead et al. 1988).

10.3.2 Strategic Studies Related to Legume Performance in Agriculture

Only a few studies involving AM fungi–rhizobia interactions were carried out under field conditions in the past, most of them conducted by us. In one of these studies, Azcón-Aguilar et al. (1979) reported for the first time that the dual inoculation improved the growth and nutrition of *M. sativa* grown in normal cultivation systems using arable soil (Fig. 10.2). Later on, a ^{15}N-based technique was applied to estimate N_2 fixation by the forage legume *Hedysarum coronarium,* and to ascertain the role of AM inoculation in plant N nutrition throughout a growing season under field conditions. The absence of the specific rhizobia for the forage legume in the test soil allowed the use of ^{15}N methodology with the same legume as reference "non-fixing" crop (Barea et al. 1987). The AM fungal inoculation enhanced dry matter yield, N concentration, and total N yield. The use of ^{15}N allowed us to distinguish the effect of AM fungi on N acquisition where mycorrhizal fungi enhanced both the amount of N derived from soil and from fixation, as compared with phosphate-added or control plants. This indicated that AM fungi acted both by a P-mediated mechanism to improve N_2 fixation and by enhancing N uptake by the legume from soil. The isotope ^{15}N was used also to measure both the N_2-fixation by white clover and N-transfer from clover to perennial ryegrass. Pure and mixed stands of these pasture plants were established in a field soil (Barea et al. 1989). The total N, P, and dry matter yields in the grass/clover mixture were greater than in monocultures. A lower ^{15}N enrichment of the grass growing together compared to those growing alone suggested N-transfer. The AM colonization of the grass in the

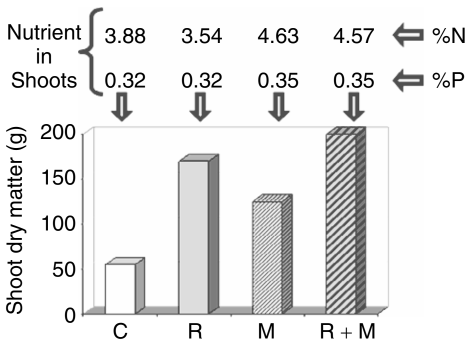

Fig. 10.2 Field inoculation of *Medicago sativa* with *Rhizobium* (R) and Mycorrhizal fungi (M)

mixed grass/clover sward was significantly enhanced, as compared with that of the grass in pure stands. Since the AM hyphae are known to be involved in NH_4^+ uptake, translocation, and transfer to the host plants, this may partly explain the improvement of pasture productivity when grass and mycotrophic legumes are grown together.

Apart from these pioneering reports, a number of experiments aimed at evaluating the role of AM fungi in improving N_2-fixation, either in controlled or in real field conditions, have been carried out during the last two decades. The accumulating data suggest a beneficial impact of AM fungi on legume symbiotic improvement in agriculture, particularly under low soil P levels (Barea et al. 2005c; Chalk et al. 2006). However, it is important to identify and select the appropriate rhizobial strain/AM fungus combination in order to optimize the benefit of the "tripartite symbiosis" on legume productivity (Azcón et al. 1991; Ruíz-Lozano and Azcón 1993; Ahmad 1995). In any case, the results depend largely on the fertility level of the soils. For instance, recent findings indicate that when the assailable P levels under field conditions were high, the tripartite symbiosis was not effective and hence did not promote N_2 fixation, either by soybean in Canada (Antunes et al. 2006) or *Calliandra calothyrsusen* grown in Senegal (Lesueur and Sarr 2008). In contrast, when the available P and N contents in the test soils were low, the appropriate management of legume by consortia of microbial symbionts improved soil fertility/productivity and consequently the overall performance of legumes. This has also been reported for forage legumes in Spain (Azcón 1993), soybean plants in Nigeria (Babajide et al. 2009), with an agro-forestry system, including tropical tree legumes, in Brazil (Pagano et al. 2008) or with common bean-based production system in Turkey (Uyanoz et al. 2007). Multitrophic interactions involving other microorganism, such as PGPR, have been analyzed in different studies, which describe how these bacteria enhance the beneficial effects of the legume microsymbionts (Azcón 1993; Bisht et al. 2009; Rinu and Pandey 2009). Those multitrophic interactions involving phosphate-solubilizing microorganisms are discussed later in this chapter (Sect. 10.4), and those helping the plant to support environmental/cultural stresses are discussed in Sect. 10.5.

10.3.3 Strategic Studies Related to the Role of Legumes in the Revegetation of Degraded Ecosystems

The information reviewed in this section will highlight the interactions of AM fungi and rhizobia adopted for restoration (by revegetation) of areas suffering an evident disturbance of their plant cover. The analyzed studies describe only field experiments, most of them concerning with Mediterranean desertification-threatened ecosystems. Mycorrhizosphere interactions to improve legume development in soils subjected to environmental/cultural stresses (heavy metal contamination, drought, salinity, pathogen attack, etc.) will be discussed in Sect. 10.5.

As a result of degradation/desertification processes, disturbance of natural plant communities is often accompanied, or preceded by loss of physical, chemical, and biological soil properties, such as soil structure, plant nutrient availability, organic matter content, microbial activity, etc. (Jeffries and Barea 2001). Physicochemical soil properties are fundamental for soil quality in particular with respect to soil structure, especially aggregate stability and organic matter accumulation, being one of the most influential factors (Miller and Jastrow 2000; Buscot 2005). Degradation of physical, chemical, and biological soil properties limits reestablishment of the natural plant cover. In particular, desertification causes disturbance of plant–microbe symbioses, which are a critical ecological factor affecting plant growth in degraded ecosystems (Francis and Thornes 1990). This is the reason why in revegetation programs the recovery of the natural ability of AM fungi and rhizobial bacteria is fundamental to initiate an integral restoration of a degraded area (Jeffries and Barea 2001). In this regard, some experiments aimed at investigating the application of this restoration strategy have been carried out (Barea et al. 2005a). In one of these studies, Herrera et al. (1993) conducted a 4-year field revegetation trial in a semiarid region of Spain, using a number of woody species, commonly used in revegetation programs in Mediterranean ecosystems. These included two native shrubs (*Anthyllis cytisoides* and *Spartium junceum*) and four tree legumes (*Acacia caven, Medicago arborea, Prosopis chilensis,* and *Robinia pseudoacacia*). Plant species and microsymbionts were screened to identify appropriate combinations, and a simple procedure to produce plantlets with an optimized mycorrhizal and nodulated status was developed. Results indicate that (1) only the native shrub legumes were able to establish under the local environmental conditions and (2) inoculation with rhizobia and AMF improved plant survival and biomass development. Since these two shrub legumes are found in the natural plant community, a reclamation strategy was proposed to revegetate desertified areas using these ecoystem-adapted shrub legumes. This strategy, which involves the artificial acceleration of natural revegetation, could be accomplished by replanting randomly spaced groups of these shrubs according to the natural pattern and structure of the undisturbed ecosystem (Francis and Thornes 1990). Particularly, *A. cytisoides*, a drought-tolerant legume species, very dependent on AM to achieve optimal development in natural conditions (López-Sánchez et al. 1992), was selected for these aims.

Using legume, Requena et al. (2001) carried out a long-term field experiment to ascertain the impact of inoculation with indigenous nocrosymbionts as a part of a reclamation strategy. A representative area within a desertified semiarid Mediterranean ecosystem in southeast Spain was chosen. The existing natural vegetation was a degraded shrubland where *A. cytisoides* was the dominant species (Requena et al. 1997). *Anthyllis* seedlings inoculated with an indigenous rhizobial and AM fungal inocula were transplanted to field plots in a 5-year trial. The tailored mycorrhizosphere not only enhanced establishment of the target legume but also increased soil fertility and quality. This included enhanced seedling survival rates, growth, P-acquisition, N-fixation, and N-transfer from N-fixing to associated non-fixing species in the natural succession. The improvement in the physicochemical properties in the soil around the *Anthyllis* plants was shown by the increased levels of N, organic matter, and number of hydrostable soil aggregates. The role of the AM fungi, cooperation with other microbes, in the formation of water-stable soil aggregates (Rillig and Mummey 2006) is relevant here. The glomalin-related proteins, glycoproteins, produced by the external hyphae of AM fungi was found to be involved (Miller and Jastrow 2000; Bedini et al. 2009). The increase in N content in the rhizosphere of the legume can be accounted for by the supply of N-richer root exudates due to an improvement in nodulation and N-fixing capacity resulting from inoculation with both microsymbionts. The dually inoculated shrub legumes were a source of AM fungal inoculum for the surrounding area and in improving N nutrition for non-N-fixing vegetation. Figure 10.3 summarizes the role of AM symbiosis at improving nodulation and N_2-fixation in legumes and helping "N-transfer" from the rhizosphere of a N_2-fixing legume to a nonfixing plant growing nearby.

The tree legume *M. arborea* was positively benefited by coinoculation with AM fungi, *R. meliloti* strains, and PGPR (Galleguillos et al. 2000), suggesting that the mixtures of microbial symbionts could serve as a successful biotechnological alternative to aid the recovery of desertified ecosystems in semiarid areas. In follow up studies, a representative legume species from Mediterranean ecoystems, *Retama sphaerocarpa*, was selected as a target species for revegetation programs of degraded land in semiarid areas. Inoculation with native AM fungi improved plant establishment (Caravaca et al. 2003b), enzymatic activities related with C, N, and P cycling, and soil aggregation (Alguacil et al. 2005). In addition, the application of composted urban residues resulted in a complement of the AM effects to benefit such enzymatic activities and aggregate stability in the tailored mycorrhizosphere of transplanted *R. sphaerocarpa* (Caravaca et al. 2003a, c). Similar complementary effects were found by other organic amendments such as sewage sludge (Alguacil et al. 2004; Caravaca et al. 2005a, b) or composted dry olive residues

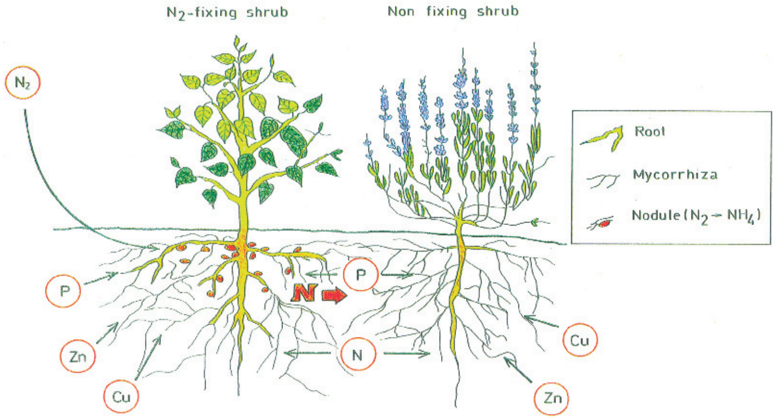

Fig. 10.3 Arbuscular mycorrhiza and nutrient acquisition (and cycling) in plant communities including legumes and nonlegumes plants (drawing by Esperanza Campos)

(Caravaca et al. 2006). The impact of AM inoculation in combination with organic amendments was further tested for *Dorycium pentaphyllum*, an autochthonous legume from semiarid areas in Southeast Spain, which is being used for restoration purposes. The tailored AM-seedlings were transplanted to a degraded semiarid area in Southeast Spain. Several types of *Aspergillus niger*-treated organic amendments and rock phosphate additions were also applied. These treatments, which included either sugar beet residues (Caravaca et al. 2004a) or dry olive cake residues (Medina et al. 2004; Alguacil et al. 2008), produced beneficial effects on physical, chemical, and biological properties of soils in the mycorrhizosphere soil of the transplanted target legume. Apart from the field experiments carried out in Mediterranean ecosystems, Bhatia et al. (1998) demonstrated that dual inoculation with rhizobia and AM fungi helped the establishment and biomass production of the woody legume (*Prosopis* sp.) in wasteland in India. More recently, Siviero et al. (2008) and Schiavo et al. (2009) demonstrated the successful effects of dual inoculation with AM fungi and rhizobia on the performance of representative tree legumes in Brazil. These and other associated data thus suggests that the management of appropriate microsymbionts can help legumes to promote the stabilization of a self-sustaining ecosystem. The mycorrhizal shrub/tree legumes act as a "fertility islands" (Caravaca et al. 2005a), which could serve as sources of symbiont inocula for the surrounding area and to improve N nutrition for the non N_2-fixing vegetation in stressed ecosystems.

10.4 Interactions Between AM Fungi and Phosphate-Solubilizing Microbes to Improve the Use of Natural Phosphate Sources and/or Agro-Industrial Residues by Legumes

The interactions between AM fungi and phosphate-solubilizing-microorganisms (PSM) are important for P acquisition by the legume plants. Therefore, the information generated from several experiments investigating this mycorrhizosphere activity merit some detailed consideration here.

Two general types of microbiologically mediated processes involved in P cycling have been described for increasing the Pi availability in soils (1) solubilization of unavailable P-sources in soils and (2) the uptake of solubilized Pi by plants (Kucey et al. 1989; Richardson 2001). The solubilization/mineralization of unavailable P compounds is carried out by diverse saprophytic bacteria and fungi (Marschner 2008; George and Richardson 2008; Richardson et al. 2009; Khan et al. 2010). Furthermore, since the external mycelium of the AM fungi acts as a bridge between roots and the surrounding soil microhabitats, AM establishment is the main mechanism involved in the uptake of solubilized Pi by plants (Barea 1991). On the other hand, phosphate-solubilizing bacteria (PSB) have also been tested for their ability to improve plant P nutrition (Kucey et al. 1989; Zaidi et al. 2009). The Pi made available by PSB acting on sparingly-soluble P sources, however, may not reach to the root surface due to limited diffusion of this ion in soil solution. However, it was proposed that if the P is solubilized by PSB, AM fungi can tap these phosphatic ions and translocate it to plants suggesting a microbial interaction, which could improve P supply to the host plants synergistically or additively, as reported by Barea (1991). This hypothesis has been tested and found effective later on by several workers (Barea et al. 2005a, 2007; Wani et al. 2007; Zaidi et al. 2003), which involved the application of poorly reactive rock phosphate (RP) and the use of ^{32}P-tracer methodologies. Upon adding a small amount of ^{32}P to label the exchangeable soil P pool, the isotopic composition, or "specific activity" (SA = $^{32}\mathrm{P}/^{31}\mathrm{P}$ quotient), was determined in plant tissues (Zapata and Axmann 1995). These studies found that dual inoculation reduced the SA of the host plant, indicating that these plants acquired P from sources, either endogenous or from added RP, which were not directly available to noninoculated or singly inoculated plants (Toro et al. 1997). Particularly relevant for this Chapter were those experiments using legumes as host plant (Toro et al. 1998). To validate these results, a series of experiments were carried out either in a glasshouse or in the field (Barea et al. 2002c) using rhizobium inoculated legume plants to investigate the interactive effects of PSB and AM-fungi on P capture, cycling, and supply either from naturally existing P-sources or from added RP. A collateral objective was to investigate the agronomic effectiveness of the target microbial interactions on nodulation and legume performance under field conditions. For the greenhouse experiment, the exchangeable soil P pool was labeled with ^{32}P. Both RP addition and microbial inoculation improved biomass production and P accumulation in the test plants, with dual microbial inoculation being the most effective treatment. Independently of RP addition, AM and PSB coinoculated plants showed a lower SA than the noninoculated controls. This confirmed that these tailored legume mycorrhizosphere could acquire soil P from sources unavailable to noninoculated plants. Possibly, the PSB were effective in releasing ^{31}P from sparingly soluble sources, either from the soil components or from the added RP. This release of Pi would constitute a part of the total ^{31}P pool from which the AM mycelium acquired P and transferred it to the plants. Such microbial activities could result in the lower SA in dually inoculated plants. The use of ^{15}N allowed the corroboration of a positive effect of P-supply from the tailored microbial treatments on N_2-fixation by the test inoculated legume. Results from the field trial suggested that interactions between AM fungi, rhizobia, and PSB can have a cooperative fundamental role in P- and N-cycling in a tailored legume mycorrhizosphere.

The use of P-solubilizing fungi in combination with AM-fungi to improve legume mycorrhizosphere performance has been also investigated (Vassilev et al. 2002). For example, the mixtures of agrowastes (sugar beet), P-solubilizing fungus *A. niger*, and RP gives way to a fermentation product that was later investigated in a series of experiments. The host plant (*Trifolium repens*) was inoculated with an AM fungus and grown on a P-deficient soil. It was shown that product improved plant growth and P acquisition and that AM inoculation further favored the effectiveness of the fermentation product. As shown by the isotopic ^{32}P dilution technique, a lowering in the SA was evidenced indicating that plants benefited from P solubilized from RP by the microbial activities. Nodulation and N_2-fixation were also improved by the tailored legume mycorrhizosphere. Recently, Vassilev et al. (2007) reported the effect of four agroindustrial wastes (sugar beet, olive cake, olive mill wastewaters, and dry olive cake) as substrates for microbial (*A. niger*) solubilization of RP. Amendments resulting from all these fermented products improved plant growth and P acquisition, which were further enhanced by AM inoculation. These fermentation products were applied to a degraded and P-limited soil for restoration purposes in combination with AM fungal inoculation (Medina et al. 2005). These biotechnological products improved plant growth, soil structure, and soil biochemical characteristics. The significance of treated agrowastes in interaction with PGPR and AM fungi will be further discussed in relation with the effect of legume mycorrhizosphere in phytoremediation of soils contaminated with heavy metals.

The capacity of rhizobia strains for phosphate solubilization (Rivas et al. 2007; Alikhani et al. 2007) was tested for improving legume nutrition in interaction with AM fungi and/or PGPR (Zaidi et al. 2003; Zarei et al. 2006; Matias et al. 2009). Synergistic improvements in legumes were suggested to be due to P-solubilizing activity of the tested bacterial strains.

10.5 Mycorrhizosphere Interactions to Improve Legumes in Soils Suffering from Environmental/Cultural Stresses

The quality and sustainability (stability and productivity) of either agroecosystems or natural ecosystems is usually endangered by different cultural or environmental stresses (Buscot 2005). These stresses are known to affect AM fungal diversity and activity (Chaudhary et al. 2009). However, tailored mycorrhizosphere can help plants to grow better by offsetting the negative impact of stress situations (Barea et al. 2005b). The effect of mycorrhizosphere interactions to help legume performance in soils suffering from environmental/cultural stresses will be considered here for three types of stresses: (1) contamination with heavy metals (HMs), (2) presence of osmotic stresses (drought, salinity, extreme temperatures), and (3) the attack of plant microbial pathogens.

The influence of climatic change on AM formation and function has been the subject of other studies (Staddon et al. 2003; Vargas et al. 2010), but only a few experiments using legumes as test plant will be discussed here. Particularly, the temperature component of the climatic change seems more influential than its CO_2 component (Gavito et al. 2003).

10.5.1 Phytoremediation of Soil Contaminated with Heavy Metals

The use of plants for the remediation (phytoremediation) of soils contaminated with heavy metals, radionuclides, or polycyclic aromatic hydrocarbons has been benefited by the application of AM fungi (Leyval et al. 2002). Depending on the type of pollutant, different AM-assisted strategies of phytoremediation, such as phytostabilization, phytodegradation, and phytoextraction, have been investigated (Leyval et al. 1997). Most phytoremediation assays involving legume mycorrhizosphere concern heavy metals (Turnau et al. 2006; Jasper 2007; Maki et al. 2008; Teng et al. 2008; Azcón et al. 2009a). These studies mostly concentrated on Zn, Cu, Cd, Pb, or Ni (see below for references), but contamination with other metals, like arsenic, have also been attempted (Dong et al. 2008). Most of the reports on this topic concluded that AM colonized plants translocate less HMs to their shoots than the corresponding nonmycorrhizal plants, as shown for herbaceous (Díaz et al. 1996; Redon et al. 2009) or tree (Lin et al. 2007) legumes. These findings suggest that the role of AM-fungi in phytoremediation is mainly based on the immobilization (phytostabilization) of HMs in soil (Leyval et al. 2002; Turnau et al. 2006). Furthermore, both rhizobacteria and AM-fungi have been found to interact synergistically to benefit phytoremediation; however, the selection of HM-adapted microbial components to produce a tailored mycorrhizobacteria is necessary (Biró et al. 1998). This was investigated in a series of phytoremediation experiments using legumes (*T. repens*) as host plants (Vivas et al. 2003a, b, c, d, e, 2005a, b, 2006a, b, c). An agricultural soil from Nagyhörcsök Experimental Station (Hungary), which was contaminated in 1991 with suspensions of 13 microelement salts applied separately (Biró et al. 1998), was the target soil. Microorganisms isolated from this HM-contaminated soil ("autochthonous metal-adapted AM fungi and/or bacteria") were compared to microorganisms in the same taxa from culture collections, which were nonadapted to the tested HMs. The main achievements resulting from these experiments were (1) a number of efficient bacteria and the AM-fungi were isolated and identified by 16S rDNA or 18S rDNA, (2) the target bacteria were able to accumulate large amounts of metals, (3) coinoculation with a HM-adapted autochthonous bacteria and AM fungi increased biomass, N and P content as compared to noninoculated plants, and also enhanced the establishment of symbiotic structures (nodule number and AM colonization), which were negatively affected as the level of HM in soil increased, (4) dual inoculation lowered HM concentrations in *Trifolium* plants, inferring a phytostabilization-based activity; however, as the total HM content in plant shoots was higher in dually inoculated plants, due to the effect on biomass accumulation, a possible phytoextraction activity was suggested, (5) inoculated HM-adapted bacteria increased dehydrogenase, phosphatase and β-gluconase activities, and auxin production, in the mycorrhizosphere, indicating an enhancement of microbial activities related to plant development (Vivas et al. 2005a, b, 2006a, b, c; Azcón et al. 2009a). In yet other study, when agrowasted residues were added to the mycorrhizosphere system, additive/synergistic effects were evidenced (Medina et al. 2005, 2006). Particularly, the antioxidant activities involved in detoxifying the toxicity of heavy metals to plants were found to be increased (Azcón et al. 2009b).

The physiological/biochemical mechanisms by which the tested bacterial isolates enhanced phytoremediation activity in AM plants include: (1) improved rooting, and AM formation and functioning, (2) enhanced microbial activity in the mycorrhizosphere, and (3) accumulation of metals in the root–soil environment, thus avoiding their transfer to the trophic chain, or to aquifers. In conclusion, even though a clear effect of mycorrhizosphere cooperative interactions was obvious on "phytostabilization," a significant effect on "phytoextraction" was also shown. Therefore, whatever be the mechanisms, a tailored mycorrhizosphere, by using selected HM-adapted microorganisms, can apparently be engineered to improve plant tolerance to HMs and to benefit bioremediation of HM-contaminated soils. The molecular mechanisms involved in HM tolerance in AM inoculated plants have been recently discussed (González-Guerrero et al. 2009).

10.5.2 Plant Performance in Soils Exposed to Osmotic Stresses

Since AM colonization can help plants to cope with drought and salinity stresses (Augé 2001; Ruíz-Lozano 2003), the role and the mechanisms involved in the AM symbiosis to help plant performance under osmotic stress conditions has been the subject of many studies (Ruíz-Lozano et al. 2008; Ruíz-Lozano and Aroca 2008). In this context, the pioneering work of Ruíz-Lozano and Azcón (1993) and Azcón et al. (1988) showed that AM inoculation improved nodulation and N_2 fixation at low levels of water potential, and the negative effects of salinity on nodulation and N_2 fixation could be compensated by AM inoculation. More recent experiments have corroborated a positive effect of the interactions between AM fungi and nodulating rhizobia under drought conditions (Goicoechea et al. 2000; Ruiz-Lozano et al. 2001; Echeverria et al. 2008). Particularly, it was found that AM inoculation protected soybeans plants against the detrimental effects of drought to prevent the premature nodule senescence induced by drought stress (Ruiz-Lozano et al. 2001). The alleviation of the oxidative damage exerted by AM inoculation seems to be one of the mechanisms involved in these protective AM-activities (Porcel et al. 2003; Porcel and Ruíz-Lozano 2004). In addition, indigenous drought-tolerant AM fungi are known to improve nutrient acquisition, gas exchange, nitrate reductase (Caravaca et al. 2004a,b,c), water transport, and root development (Marulanda et al. 2006) in the shrub legume *R. sphaerocarpa* under dry conditions. The presence of diverse plasma membrane-localized water channels (PIP aquaporins) in plants, which appears to be involved in water (or other solute) transport (Yamada and Bohnert 2000), has prompted other workers to elucidate further the role of AM fungi under osmotic stress (Ruíz-Lozano et al. 2008). Accordingly, experiments using legume have investigated aquaporin gene expression and its involvement in drought stress tolerance in AM-plants. As an example, research by Porcel et al. (2006) concluded that AM inoculated soybean plants respond to drought stress by downregulating the expression of the PIP genes studies. This could be a mechanism to decrease membrane water permeability to allow cellular water conservation. In any case, PIP gene expression in AM plants (*Phaseolus vulgaris*) depended on the particular conditions of the different stress tested: drought, cold, and salinity (Aroca et al. 2007). The signaling molecule H_2O_2 seems to be involved in the PIP aquaporin regulation in *P. vulgaris* plants (Benabdellah et al. 2009).

10.5.3 Soil-Borne Pathogen Infested Soils

The establishment of AM fungi in plant roots has been shown to reduce damage caused by soil-borne plant pathogens leading to an enhancement in plant resistance/tolerance in mycorrhizal plants. In any case, the effectiveness of AM in biocontrol depends on the AM fungus involved, as well as the substrate and the host plant (Barea et al. 2005b; Pozo and Azcón-Aguilar 2007; Pozo et al. 2009). Different mechanisms have been suggested for the biocontrol activity of AM-fungi (Barea et al. 2005b). One mechanism involves microbial changes that results as the mycorrhizosphere develops, which is based on the shifts and resulting microbial equilibria that could help plant health. Activation of plant defense mechanisms, which can develop systemic resistance reactions, including protection against foliar pathogens, have been also reported (Pozo and Azcón-Aguilar 2007; Pozo et al. 2009).

10.6 Biotechnological Developments for Integrated Management of Legume Improvement

An increasing demand for low-input agriculture has resulted in greater interest in the manipulation and use of some soil microorganisms because of their beneficial impacts on plant growth and health and soil quality. It is expected that an appropriate management of beneficial soil microbes can reduce the use of chemicals and energy in agriculture leading to a more economical and sustainable production, while minimizing environmental degradation. These strategies are becoming more attractive as the use of chemicals for fumigation and disease control is progressively discouraged and fertilizers have become more and more expensive (Atkinson 2009). Consequently, some biotechnological inputs have been proposed concerning mycorrhizosphere technology, a fact of special interest for legume growers. These agrobiotechnological approaches include the use of microbial inoculants. For legumes, the target microbes are obviously the AM-fungi and rhizobia, but other selected PGPR can also be included and tested under pot/field conditions (Barea et al. 2002c; Zaidi and Khan 2007; Wani et al. 2007), as summarized in Table 10.1. While the technology for the production of inexpensive rhizobial and free-living PGPR is commercially available, the production of inocula and the development of inoculation techniques have limited the manipulation of AM-fungi. The difficulty to culture AM fungi in absence of

Table 10.1 Interactions of arbuscular mycorrhiza with soil microorganisms in a tailored mycorrhizosphere

Types of microorganisms	Interaction effects	References
N_2-fixing rhizobia	Increased N availability	Barea et al. (2005c)
Phosphate solubilizers	Increased P availability	Barea et al. (2007), Zaidi and Khan (2007)
Plant hormone producers	Rooting and establishment of seedlings	Artursson et al. (2006)
Antagonists	Control of plant pathogens	Barea et al. (2005b)
Specific rhizobacteria	Remediation of contaminated soil	Turnau et al. (2006)
Microorganisms involved in soil aggregation	Improvement of physical soil quality	Barea et al. (2005a)

their host plant, obligate symbionts, is a major obstacle to produce AM inoculants (Baar 2008). Despite these problems, several companies worldwide are producing plant-based AM inoculum products, which are now commercially available (Gianinazzi and Vosátka 2004; Vosátka et al. 2008). Selection of the appropriate AM fungi is however a key step (Estaún et al. 2002), while specific procedures are required to multiply AM-fungi and to produce high quality inocula (von Alten et al. 2002). Recent developments in AM-inoculum production systems rang from nursery plots (Koltai et al. 2008; Cuenca et al. 2008) to in vitro monoxenic root organ cultures (Bago and Cano 2005). The resulting materials (spores, hyphae, root fragments, etc.) are added to different carriers, resulting in a wide range of formulations, including encapsulation, to be applied at an agronomical scale using different application methods (Cuenca et al. 2008; Vosátka et al. 2008), including hydroseeding (Estaún et al. 2007). It is indeed a matter of discussion whether "generic products" containing several AM fungi, potentially suitable for a range of applications, are more appropriate for the market than those with precise formulations and AM fungi specifically tuned to particular end-uses (Smith and Read 2008).

Inoculation at broad scale in highly developed farming systems have, however, many constrains. To overcome this, management of indigenous populations is currently a viable option (Brito et al. 2008). However, at a relatively small-scale (nursery production), AM inoculation is feasible and advantageous. Inoculation of seedlings is potentially a good method for establishing selected fungi in roots before potting on or planting-out into the field. Inoculation is appropriate where transplanting is part of the normal production system, as is the case with horticulture, including plantation crops. Management strategies for inoculum build-up include the use of pastures, sequential cropping, or intercropping. Biodynamic and organic farm management results in higher per cent colonization of roots of pasture and annual crops than conventional management. In particular, the use of AM fungi in horticulture, in association with other beneficial microorganisms, appears as an effective way for improving fertilizer use and for minimizing losses due to disease (Baar 2008; Brito et al. 2008).

10.7 Conclusion and Future Perspective

Legumes are plant species of great agricultural/environmental importance known to establish beneficial symbiotic relationships with N_2-fixing bacteria and AM fungi. Associated to this tripartite symbiosis live many saprophytic microorganisms, which develop a peculiar mycorrhizosphere systems. Managing the microbial symbiont and saprobe, including PGPR, involved in legume mycorrhizosphere will have a great relevance to improve legume productivity either in sustainable agriculture or in the maintenance of natural plant communities. Current developments in the ecology, physiology, biochemistry, molecular biology, and biotechnology of the microbe–plant relationships have given new insights into the understanding of the formation and functioning of legume mycorrhizosphere. However, further research is still needed to better understand the tripartite symbiosis and consequently rationalized agricultural/forestry applications. While the technology for the production of rhizobia and free-living PGPR is commercially available, the production of inocula and the development of inoculation techniques have restricted the manipulation of AM fungi. However, biotechnological approaches now available, allows the production of efficient AM-fungal inoculants. Therefore, an appropriate management of selected AM fungi, rhizobia and PGPR is currently a viable alternative for agriculture, horticulture, and revegetation of degraded ecosystems. The application of selected microbial inoculants are likely to become even more important in future due to the agroecological threats of agrochemicals, which urgently requires to be reduced, and even avoided, to increase food quality, sustainable food production and environmental protection. Therefore, popularizing and improving the use of tailored mycorrhizospheres in legume plants is a major challenge for both the scientists and industry. To achieve such objective, it is important to explore the natural diversity of rhizobial, PGPR, and AM fungal populations in the mycorrhizosphere of legumes, and to exploit selected microbes as a source of inocula for providing sustained and balanced nutrients to legumes. This is though difficult, but particularly relevant for developing countries.

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