Chapter 13
Tripartite Association Among Plant, Arbuscular Mycorrhizal Fungi and Bacteria

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Abstract Most plant roots are colonized by arbuscular mycorrhizal (AM) fungi and their presence, generally, stimulates plant growth. In addition, AM fungi can interact with different bacterial species establishing a tripartite association and represent a vital component in the plant ecosystem. These interactions may range from loose to endosymbiotic association. In context of AM fungi, interaction with host plant is long been studied, however, information is little on the mechanisms controlling interaction of bacteria with AM fungi and host plant in the mycorrhizosphere. Understanding the interaction between AM fungi and bacteria is essential for describing the soil-plant interface. Although there are several studies concerning interactions between AM fungi and bacteria, the underlying mechanisms behind these associations are in general not very well understood, and their functional properties still require experimental confirmation. Modern tools of molecular biology and genome sequencing have solved the questions about the identity and role of bacteria associated with AM fungi. In this chapter, different aspects of tripartite association among plant, AM fungi and bacteria are discussed with greater emphasis on associated bacterial component.

Keywords Arbuscular mycorrhizal (AM) fungi • Host plant • Mycorrhization helper bacteria (MHB) • Endobacteria

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13.1 Introduction

Mutualistic association between plant and fungi, mycorhhiza (Greek *Mycos*: fungus + *Rhiza*: root), is the most wide spread terrestrial symbiosis. This association is based on the plant component providing carbohydrates and other essential organic compounds to fungi. In return the fungal component, that colonizes both root and the adjacent soil, helps the plant to take up nutrients (those of low mobility; especially P) by extending the reach of its root system. Although the original concept implies an association between fungi and plant root, these associations also includes plants with no true roots for e.g. bryophytes and pteridophytes (Smith and Read 2008).

The commonest mycorrhizal symbiosis is formed by arbuscular mycorrhizal (AM) fungi. They all are members of glomeromycota, a monophyletic group that diverge from the same common ancestor as ascomycota and basidomycota (Schussler et al. 2001). The ecological importance of AM fungi is unquestionable; they certainly have contributed to structuring the plant communities in different ecosystems. The long co-evolution period has rendered AM fungi so dependent on the symbioses that they became obligate symbiont, i.e., they are unable to grow in the absence of living host roots. However, some reports show that AM fungi can grow up to spore production phase in vitro in the absence of plant root and in presence of some spore associated bacteria (Hildbrandt et al. 2002, 2006). In contrast, host plants of AM fungi can survive if deprived of their fungal partner, this condition is virtually unknown in natural ecosystems, in which AM fungi function as true helper microorganisms, improving overall plant fitness.

The plant root-fungus symbiosis is established by inter- and intra-cellular growth of AM fungal hyphae in cortical region of root. Intracellular growth is characterized by formation of highly branched structures 'arbuscules' (site for nutrient exchange between plant and fungus) or hyphal coils (Fig. 13.1). Following root colonization, AM fungi produces runner hyphae forming the extraradical mycelium (ERM). The ERM can explore the soil for resources beyond nutrient depletion zone and is an important mean of translocation of energy rich photoassimilates from plant to soil. The immediate surrounding of the ERM is commonly termed as 'hyphosphere' and the soil compartment influenced by combined activities of root and AM fungi is known as 'mycorrhizosphere'. In the same way as the rhizosphere effect is seen for plant roots, a mycorrhizosphere effect can be seen whereby the soil surrounding fungal hyphae supports distinct bacterial communities compared to the bulk soil (Linderman 1988). Mycorrhizosphere inhabitants may include intra-hyphal bacteria in ectomycorrhizal fungi (Bertaux et al. 2003), and intra-spore bacteria in some AM fungi (Bianiciotto et al. 1996). Some mycorrhizosphere bacteria (mycorrhization helper bacteria; MHB) promote mycorrhiza formation, with a variety of Gram positive and negative strains involved (Garbaye 1994), although the precise mechanisms involved are unclear. The functioning of this ERM network is of key importance in mycorrhizal ecology because it represents not only an uptake point for soil nutrients but also a dispersal mechanism and a complex linkage network among roots within a plant community.

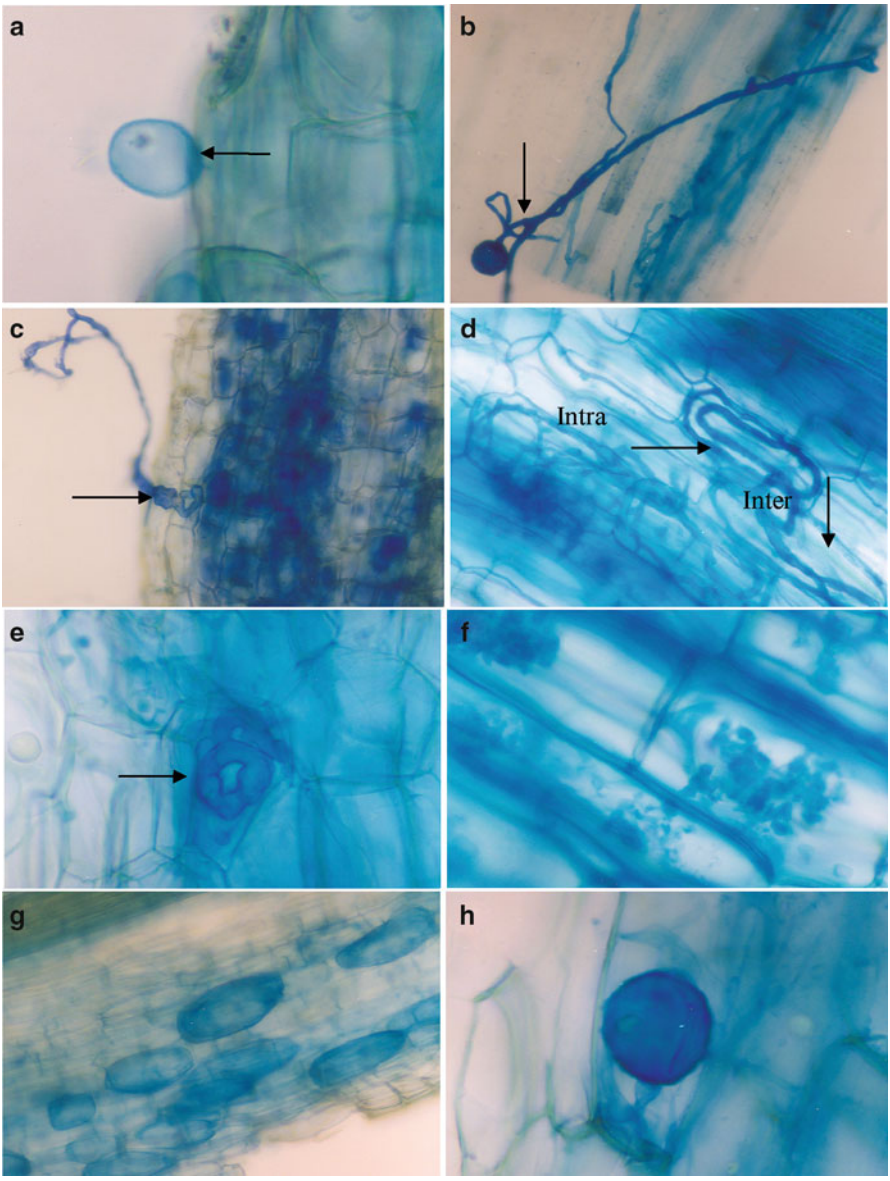

Fig. 13.1 Colonization pattern typical of AM fungi. (**a**) Attachment of spore on the root surface, (**b**) germination of spore outside the root, (**c**) appresorium formation at the root surface just before entry, (**d**) intra and inter cellular growth of hypha, (**e**) intracellular coil, (**f**) formation of arbuscules inside cortical cells, (**g**) formation of vesicles, and (**h**) intraradicle spore formation. *Arrows* show respective individual structures

Despite advancement in our knowledge of molecular basis of plant fungus interactions (Albrecht et al. 1999; Harrison 1999), several aspects of AM fungal biology, particularly their genomes, are still obscure due to their biotrophic nature, their multinuclear condition, and an unexpected level of genetic variability (Honsy et al. 1998; Lanfranco et al. 1999). Furthermore, complexity in the study of AM fungi arises due to the presence of endobacteria, most unculturable, in AM fungal spores. Modern molecular tools in combination with classical morphological approaches have stamped the presence of true bacteria (earlier known as bacteria like organisms 'BLOs') in spores of AM fungi (Bianiciotto et al. 1996).

13.2 Plant-AM Fungi Interaction: Signaling Between Symbionts

13.2.1 Presymbiotic Phase

Several plant-microbe symbioses involve detection or attraction of partners prior to direct contact. However in some instances, a molecular dialog is essential for progression to the physical stages of interaction. Till date *Rhizobium*-legume symbiosis is best characterized for their molecular dialog, in which flavonoids released from the plant signal the biosynthesis of a bacterial signal molecule called nod factor. Perception of nod factor by receptors on the legume roots triggers several initial events required for physical interaction and nodule development (Denarie and Cullimore 1993; Long 1996). Morphological aspects of AM symbiosis are well documented but information is little at the molecular level. The establishment of AM interaction and, in particular, fungus recognition by the host plant are mediated by partially characterized signaling pathway, the so called common symbiosis (SYM) pathway, partly shared with *Rhizobium*-legume symbiosis (Parniske 2008; Oldroyd and Downie 2004).

Development of AM symbiosis with plant is accompanied by significant morphological alterations at cellular level in both symbionts to create the novel symbiotic interaction. A hyphal germ tube emerges following germination from spore present in the soil and grows through the soil in search of plant root for a short distance. Upon finding root, AM fungal hyphae are encountered by plant signals present in root exudates. These signals identified as 'strigolactones' induces recursive hyphal branching increasing the probability of direct contact between the symbionts (Akiyama et al. 2005; Besserer et al. 2006). Akiyama et al. (2006) hypothesized that strigolactones are not only involved as primary AM branching signals but also as signals for the directional growth of AM fungal hyphae towards host roots. A number of flavonoids have also been reported to induce hyphal branching effect (Tsai and Phillips 1991; Phillips and Tsai 1992; Scervino et al. 2005a, b, 2006). Since flavonoid induced branching is found only in limited number of plant, their role as general signaling factors for hyphal branching as a prerequisite for a successful AM fungal root colonization is questionable.

AM fungal signals, hypothetically known as 'Myc factors', present in fungal exudates are perceived by Myc factor receptor (MFR) on root surface and thereafter trigger calcium spiking through the activation of 'SYM pathway' (Kosuta et al. 2003; Kuhn et al. 2010). Such calcium spiking is also induced by nod factors in *Rhizobium*-legume symbiosis. However, peak frequency of calcium spike induced by nod factors is regular and an irregular pattern is observed in AM fungi induced calcium spiking (Kosuta et al. 2008; Hazledine et al. 2009). Although 'Myc factors' are still unidentified, these were shown to be less than 3 kDa, partially lipophilic (Navazio et al. 2007), possess a chitin backbone (Bucher et al. 2009) and induce transcriptional activity of symbiosis related genes. Plant responses to 'Myc factors' range from the molecular to organ level and are part of the 'SYM pathway'.

An analysis of calcium spiking in *Lotus japonicum* in response to nod factor revealed that out of the seven SYM genes, five viz. *symrk*, *castor*, *pollux*, *nup*85 and *nup*133 mutants are defective for calcium spiking, whereas *CCaMK* and c*yclops* act downstream (Miwa et al. 2006; Harris et al. 2003). Similar results were obtained with *Medicago truncatula* mutants (Kosuta et al. 2008). Mutants that have common SYM genes do not form infection threads and, with the exception of c*yclops* mutants, do not initiate nodule organogenesis (Szczyglowski et al. 1998; Catoira et al. 2000). These findings suggest that common SYM gene products are involved in the early stages of symbiotic signal transduction, which involves the generation and decoding of calcium oscillations in and around the nucleus and induce early symbiosis related gene expression.

13.2.2 Formation of Prepenetration Apparatus (PPA)

A physical interaction between symbionts (hyphal tip touches the root surface) takes place on signal perception by both fungus and plant, and the plant cell actively prepares the intracellular environment for AM fungal hyphae. Upon finding the appropriate location for penetration on root surface, AM fungal hypha swell, flatten and branched repeatedly to develop hyphopodium also known as appresorium (Genre et al. 2005). Expression of several plant genes changes in the hyphopodium area including *ENOD11* (a gene coding for a putative secreted protein) during early stages of infection (Chabaud et al. 2002; Weidmann et al. 2004). During formation of PPA, new genes also become active including those involved in cell wall remodeling and defense (Siciliano et al. 2007).

Development of PPA takes place by aggregation of cytoplasm at the contact site which turns into thick cytoplasmic bridge across the vacuole of the host cell. Growth direction of PPA is guided by the movement of nucleus. Secretary machinery (abundant endoplasmic reticulum, several golgi bodies and secretary vesicles) is concentrated in PPA. Endoplasmic reticulums that decorate the PPA are ideally positioned for the synthesis of perifungal membrane that marks the appearance of symbiotic interface. This narrow intracellular compartment allows AM fungi to grow inside the plant cell without breaking its integrity (Bonfante 2001). Despite this knowledge, signals that trigger PPA formation are unknown.

13.2.3 Development of Arbuscule: Key Structure of Symbiosis

Arbuscules (Latin *arbusculum*: small tree) are characteristic structures of the symbiosis formed by dicotanomous branching of an intracellular hypha. The exact structure that is formed can vary depending on fungal and/or host genotype (Smith and Read 2008). Mechanisms associated with arbuscule development are largely unknown. However, some genes that affect arbuscule development are recently identified. A marked decrease in epidermal penetration and total block of arbuscule development was observed by RNA interference knockdown of v*apyrin* gene that codes for a cytoplasmic protein with unknown function (Pumplin et al. 2010).

At least two signaling events were suggested by Harrison (2005) in arbuscule development: cell autonomous and non autonomous. The cell autonomous signaling would be responsible for activation of the expression of certain genes (mycorrhiza specific phosphate transporters, a cellulase, a chitinase and aproton ATPase) and occurs exclusively in arbuscule containing cells. This spatial expression pattern suggests that cell autonomous signals activate expression of these genes. Whereas, cell non-autonomous signals are involved in activation of specific genes in cells containing arbuscules and their immediate vicinity (a GST, a chitinase and a β-13 endoglucanase). Reorganization of microtubule cytoskeleton in cortical cells adjacent to arbusculated cells can also be considered as a evidence for this signaling pathway (Blancaflor et al. 2001). Using in situ hybridization, Lambais and Mehdy (1998) showed an induction in the accumulation of transcripts encoding an acidic chitinase in cells containing arbuscules and in their immediate vicinity suggesting systemic signals operating in AM roots containing arbuscules.

The estimated lifespan of arbuscules is of 4–10 days (Sanders et al. 1977); after this short period, AM fungal wall collapse in fine branches following septa formation. Eventually, this senescence extends to trunk of hypha collapsing the whole structure. Consequently, arbuscule disappear and plant cell regains its normal physiology and organization with a large central vacuole (Bonfante 1984).

13.3 Function of AM Fungi: Nutrient Exchange

Mutualistic associations are based on bidirectional nutrient exchange and, as such, are beneficial to both partners (Fig. 13.2). This concept applies to AM symbiosis: fungal hyphae explore the soil substratum to efficiently take up nutrients and water to improve plant nutrition and in return obtain plant carbohydrates for successful completion of the AM fungal life cycle.

13.3.1 Carbon Uptake and Translocation

It is generally assumed that phosphate and carbon transfer occurs at the arbuscule/cortical cell interface, although direct evidence for carbon transfer at this interface

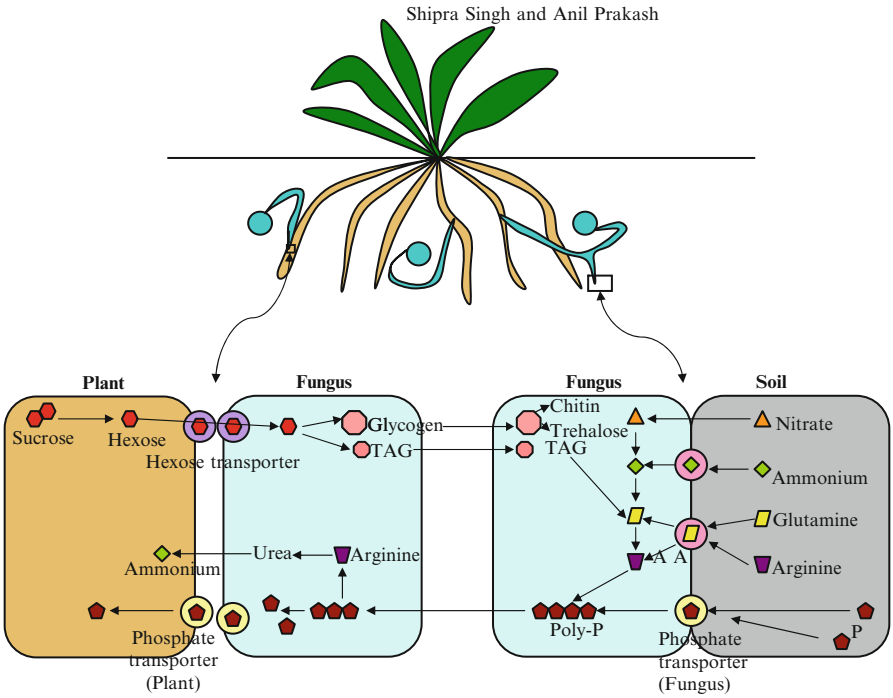

Fig. 13.2 Scheme summarizing nutrient uptake and translocation processes in AM symbiosis

is lacking (Javot et al. 2007). The assimilate transfer includes breakdown of sucrose into glucose and fructose, their export across plant plasma membrane and active uptake by hexose transporters across fungal plasma membrane, driven by an increased H^+ATPase activity at the arbuscular membrane (Gianinazzi-Pearson et al. 2000). NMR spectrometry combined with isotopic labelling showed that intraradical hyphae of AM fungi obtain and use hexoses, mainly glucose from plant (Shachar-Hill et al. 1995; Bago et al. 2003). Elevated levels of host extracellular (acid) invertase activity also suggest that hexose is the dominant form of taken up carbon (Denhe 1986). AM fungi convert hexoses into glycogen and lipids for long distance transport to the ERM (Bago et al. 2002, 2003). Although reports of carbon transfer from host plant to AM fungi came in 1960s (Smith and Read 2008), underlying molecular mechanisms are still unclear and requires identification and location of the membrane protein involved. The only hexose transporter of glomeromycota described so far has been reported in a non-arbuscule producing fungus *Geosiphon pyriforme* (Schussler et al. 2006). The major forms of stored carbon in hyphae and spore are glycogen, lipids and trehalose (Pfeffer et al. 1999).

It is stated earlier that there is no carbon transfer from fungus to plant in an AM symbiosis (Pfeffer et al. 2004). However, mycoheterotrophic plants growing in vicinity of plant associated with AM fungi present an exception to this phenomenon and likely to obtain carbon from AM fungi. Bidartondo et al. (2002) showed that

non-photosynthetic plants associate with AM fungi and can display the characteristic specificity of epiparasites. This suggests that AM fungi mediate significant inter-plant translocation of carbon in nature.

13.3.2 Phosphate Uptake

The major benefit of AM symbiosis is improved phosphorus uptake. Growing roots absorb phosphates at much higher rate compared to soil based phosphate diffusion rate resulting in a formation of phosphate depletion zone around the root system. Hyphal network of AM fungi extends beyond this zone of depletion and explore a new pool of available phosphates (Smith and Read 2008). Additionally, hyphal network contributes to the release of phosphates from inorganic complexes of low solubility by influencing directly or indirectly, the physicochemical properties of the soil (Finlay 2008). A breakthrough in understanding of phosphate uptake by AM fungi was provided by Harrison and van Buuren (1995). They identified a cDNA encoding a high affinity transmembrane phosphate transporter (Km = 8 μM) GvPT from *Glomus versiforme*. Later, Maldonado-Mendoza et al. (2001) also reported gene for phosphate transporter GiPT from *Glomus intraradices*. These genes (GvPT and GiPT) are predominantly expressed in the ERM of AM fungi (the site for phosphate uptake from the soil) exposed to micromolar phosphate concentrations. Accumulated phosphates, in the form of polyphosphate, are then rapidly translocated along the aseptate mycelium to the intraradical mycelium (Viereck et al. 2004; Smith and Read 2008). Although arbuscules are known as the site for release of phosphates into plant cells, the mechanism involved is presently unknown. It is predicted that specific carriers, pumps or channels facilitate transfer of phosphate ion through fungal plasma membrane since a concentration gradient is followed by passing phosphate ions inside the root. However, AM fungal inducible plant phosphate transporters involved in phosphate transfer into plant cortex cells have been identified in several plant species (potato: StPT3, StPT4; rice: ORYsa;Pht1;11 and *Medicago truncatula*: MtPT4) using gene expression studies (Rausch et al. 2001; Harrison et al. 2002; Paszkowski et al. 2002). Functions of these transporters were confirmed by functional complementation using yeast mutants.

13.3.3 Nitrogen Uptake and Transfer

Nitrogen uptake and transfer by AM fungi is not well understood. However, some studies highlighted the capacity of the ERM to import nitrogen from organic and inorganic sources (Hodge et al. 2001; Govindarajulu et al. 2005) through protein such as amino acid and ammonium transporters (Lopez-Pedrosa et al. 2006; Siciliano et al. 2007). Taken up nitrogen is probably incorporated into amino acid, mainly

arginine, and translocated to intraradical hyphae, but transferred to plant without carbon (Govindarajulu et al. 2005; Cruz et al. 2007). Although ammonium is considered the form that is released into plant cell, ammonium transporters in the symbiotic interface membranes have not yet been identified.

13.4 AM Fungal Interactions with Bacteria

AM fungi are key component of soil microbiota and obviously interact with other microorganisms in the rhizosphere (Bowen and Rovira 1999; Artursson et al. 2006). In this context, establishment of AM symbiosis changes plant physiology and certain nutritional and chemical properties of the rhizosphere soil. The ERM provide an interface for interaction with other soil microorganisms in the soil through active or passive exudation of plant derived carbon into the surrounding environment. This carbon is utilized by soil microorganisms as a source of energy and, in turn, affects colonization patterns of soil microorganisms in the rhizosphere region by the so called 'mycorrhizosphere effect' (Gryndler 2000). In addition, living or dead hyphae and/or compounds released by AM fungal hyphae may be used as nutrient source or substratum by soil microorganisms. On account of nutrient richness, mycorrhizosphere harbours a great bacterial diversity. Apart from a great and complex bacterial diversity, mycorrhizosphere is a very influential zone for biological system and definitely deserves more scientific attention.

Conversely, bacterial community is known to affect plant and AM fungal formation and functioning, markedly, in various ways. Both saprophytes and symbiotic bacteria interact with AM fungi in mycorrhizosphere and these two groups are potentially consisting of detrimental, neutral and beneficial bacteria (Barea et al. 2002a; Johansson et al. 2004). A number of studies have classified some interactions between populations of bacteria and fungi with AM fungi as parasitism, generating discussion about its consequences at both 'parasite' and host population levels. A review by Purin and Rillig (2008) presented potential consequences of AM fungi parasitism at the population/community level and discussed applied aspects. Deleterious rhizosphere bacteria and mycoparasitic relationships have been found to interfere with development of AM fungi whereas several bacteria (plant growth promoting rhizobacteria; PGPR) can stimulate formation and/or functioning of AM fungi (Gryndler 2000; Barea et al. 2002b). It leads to possibility that the beneficial effects of such PGPR on plant growth are due to stimulatory effect on the growth of AM fungi. For example, PGPR are known to affect the presymbiotic stages of the AM fungal development, such as spore germination and germ tube development (Azcon-Aguilar and Barea 1992; Carpenter-Boggs et al. 1995). In addition, biologically active substances such as amino acids, plant hormones, vitamins, other organic compounds and volatile substances (CO_2), produced by soil microorganisms, can stimulate the growth rates of AM fungi (Becard and Piche 1989).

13.4.1 Mycorrhization Helper Bacteria

Several PGPR possess a variety of direct mechanisms that increase the ability of the root to establish symbiotic interaction with mycorrhizal fungi. For such bacteria, Garbaye (1994) proposed the term 'mycorrhization helper bacteria' (MHB). This concept was initially proposed in the context of ectomycorrhizal fungus; however, several reports have also demonstrated enhanced AM fungal colonization in roots in the presence of PGPR (Meyer and Linderman 1986; Sanchez et al. 2004). The helper effect of these bacteria was suggested to include stimulation of root development, enhanced susceptibility of the root to mycorrhizal fungal colonization, enhancement of the recognition process between root and fungus, production of growth factors that stimulate fungal spore germination, mycelial growth, reduction of soil mediated stress through detoxification of antagonistic substances, and inhibition of competitors and antagonists (Frey-Klett et al. 2007). A classical example of helper effect is presented by rhizobia producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase; the enzyme modulating plant ethylene levels, increasing plant tolerance to environmental stress and stimulating nodulation (Ma et al. 2002). An ACC deaminase producing strain *Pseudomonas putida* UW4 was shown to provide helper effect with AM fungi *Gigaspora rosea* when inoculated into cucumber plants (Gamalero et al. 2008). Reports are also available showing that the helper effect is accompanied with the change in gene expression. In *L. bicolor* S238N, activation of genes potentially involved in recognition process, transcription regulation, and synthesis of primary metabolism protein was observed in the presence of MHB *Pseudomanas flourescence* BBc6R8 (Deveau et al. 2007).

Taken together, these studies suggest the role of active diffusible molecules and physical contact between bacteria and AM fungi for the establishment of their interaction. This can be correlated with signaling events identified in rhizobia-legumes symbiosis and in AM fungi-plant interaction as well. As discussed earlier, both share common SYM pathway and partners release active diffusible molecules that are perceived reciprocally. Ca- mediated responses are also activated whereas a physical contact between fungus and plant is required to elicit several plant responses for successful AM fungal colonization (Genre et al. 2005, 2008; Navazio et al. 2007). On the other hand, volatile organic compounds are also considered to play important role for communication between organisms. Bacterial volatiles are known to affect soil fungi including the mycorrhizal ones (Tarkka and Piechulla 2007). Although poorly understood, they are important determinants for the establishment of symbiosis. MHB are currently the most investigated group of bacteria interacting with mycorrhiza, but there is still much to be investigated about the molecules that lay the foundation of interaction between MHB and their fungal and plant hosts.

13.4.2 Endobacteria

Only a limited number of fungi are reported to host bacteria in their cytoplasm, out of which AM fungi are unique. In 1970s, several reports showed the presence of

BLOs inside the cytoplasm of field collected AM fungi and the number of such reports is continuously increasing (Mosse 1970; MacDonald and Chandler 1981; Scannerini and Bonfante 1991; Bonfante et al. 1994). Further investigation of these BLOs, including their prokaryotic nature, was long hampered due to unculturability of these organisms. However, use of combined morphological (electron and confocal microscopy) and molecular techniques allowed us to identify BLOs as true bacteria and their symbiotic relationship with AM fungi could be demonstrated (Bianciotto et al. 1996).

Family *Gigasporaceae* is most studied for the presence of endosymbiotic bacteria; however, endosymbionts are detected in several other AM fungal species such as *Glomus versiforme*, *Acaulospora laevis* etc. (Mosse 1970; MacDonald and Chandler 1981). These endosymbiotic bacteria were detected through all the steps of fungal life cycle: spores, germ tubes and extra and intraradical hyphae, except arbuscules (Bianciotto et al. 1996). *Gigaspora margarita* isolate BEG 34 is the most extensively studied AM fungus and is currently being used as model system to study AM fungi-endobacteria interaction. *G. margarita* cells were reported to harbour a large number of bacteria (on an average 20,000 bacteria per cell) and these bacteria were initially assigned to genus *Burkholderia* on the basis of their 16S rDNA sequence (Bianciotto et al. 1996). Further studies of the 16S rDNA sequences of endobacteria isolated from *Scutellospora persica*, *S. castanea* and *G. margarita* shown a strongly supported clade located close to genus *Burkhoderia* and to genera *Ralstonia* and *Pandorea* as well. In spite of several attempts, these bacteria could not be cultured and therefore, a new bacterial taxon "*Candidatus* Glomeribacter gigasporarum" was proposed (Bianciotto et al. 2003).

Functional significance of AM fungal endobacteria is still unknown, as is their potential role in the establishment of mycorrhizal symbiosis (Jargeat et al. 2004). Genomic library developed from *G. margarita* spores was shown to also represent the genome of endobacteria and helped us to identify some of such roles (van Buuren et al. 1999). Among the bacterial genes indentified so far, the most interesting are those involved in nutrient uptake: a putative phosphate transporter gene, *pst* (Ruiz-Lozano and Bonfante 1999), in host cell colonization events by enteroinvasive, pathogenic bacteria *Shigella flexneri* and *Escherichia coli*, *vacB* (Ruiz-Lozano and Bonfante 2000), in chemotaxis, *mcpA* (Minerdi et al. 2002b) and *cheY* (Minerdi et al. 2002a), a kinase, *prkA* and a *spoVR* gene involved in coat formation of bacterial endospores (Minerdi et al. 2002a). Three *nif* genes (*nifH, nifD* and *nifK*) were also found (Minerdi et al. 2001) but have not yet been demonstrated to belong to the genome of *Candidatus* Glomeribacter gigasporarum.

The mode of transmission of endobacteria to succeeding generations is not well established. However, two alternatives permanent (remain stable over time) and cyclic endosymbiosis (involve regular reassociation events) have been proposed (Bianciotto et al. 2000). Bianciotto et al. (2004) demonstrated vertical transmission of *Candidatus* Glomeribacter gigasporarum through five fungal vegetative generations of *G. margarita* spores and active bacterial proliferation was demonstrated in fungal mycelium. Transmission of endobacteria from spore to hyphae may be facilitated by the asexual reproduction typical of AM fungi and coenocytic nature of

their hyphae. On the basis of these findings, authors suggested that these bacteria are obligate endocellular component of their AM fungal host.

13.5 Economical and Ecological Significance

Natural activities of soil microorganisms may contribute to the maintenance of crop and production health by improved nutritional status and biological control of plant pathogens in low input agriculture systems. Therefore, understanding of these microbial interactions and the mechanisms involved is essential for the progress of sustainable agriculture. A breakthrough was reported by van der Heijden et al. (1998); they showed that the below ground biodiversity of AM fungi is a major factor contributing to the maintenance of plant diversity and ecosystem functioning. They have emphasized the need to protect and consider the role of these fungi in the management of diverse ecosystems. Read (1998) has further pointed out that conservation of the fungal gene pool is likely to be a prerequisite for maintenance of floristic diversity in terrestrial ecosystems, wherein the "mycorrhizal web" is known to influence natural resources. Changes in AM fungal communities may be observed with both plant community succession (Janos 1980; Johnson et al. 1991) and with changing land use intensity (Oehl et al. 2003). The more diverse assortment of host plants may support more diverse community of AM fungi (Johnson et al. 2004). In a study by Singh et al. (2008a), higher number of AM fungi was recorded in natural tea site (35 morphospecies) compared to cultivated tea site (27 morphospecies). The most obvious difference between the two AM fungal communities lied in the single dominant AM fungus at the cultivated site and the six AM fungi at the natural site, being either less frequently detected or absent in the cultivated site. Oehl et al. (2003) have noted that some AM fungi present in the natural ecosystems get strongly depressed under conventional high-input farming practices, indicating loss of at least some ecosystem function in the latter. In mixed *Araucaria* forest ecosystems, higher diversity index was recorded in native stand without any anthropogenic interference as compared to planted stand (Moreira et al. 2007). In long term field trials, Madar et al. (2000) found that root colonization by AM fungi in organic farming systems was 30–60% higher than in conventional systems. Overall higher biomass and biodiversity of soil organisms, and higher microbial activity were recorded in organic farming systems (Mader et al. 2002). However, crop yields were lower in the organic system when inputs of fertilizers, energy and pesticides were reduced. They concluded that enhanced soil fertility and higher biodiversity found in organic plots may make these systems less dependent on external inputs. Consequently, monetary loss in production may be compensated for by a reduced need of chemical fertilizers and pesticides.

The high significance of AM associations lies in the supply of mineral nutrients, in particular phosphorus, to their host plants (Miller 2000; Nielsen et al. 2002; Tiwari et al. 2004; Singh et al. 2008b). In this context, possible role of extracellular phosphatases of AM fungi in mineralization of organic phosphorus pools in soil has

attracted attention (Feng et al. 2003). Improved growth of crop plants associated with AM fungi is well documented (Tanu et al. 2004; Prakash et al. 2004) but reports are scanty for the effect of AM fungi on active principles of economically important plants. Analysis of tea plants following inoculation with AM fungi revealed that these inoculations may be effective for improved quality of tea (Singh et al. 2010).

Biofertilizer properties of some AM fungi associated bacteria have been documented and they have been found to synergistically affect nitrogen fixation and mycorrhizal development. Toro et al. (1997) demonstrated that both *Enterobacter* sp and *Bacillus subtilis* promoted the establishment of *Glomus intraradices* and increased pant biomass as well as tissue nitrogen and phosphorus contents. In addition to general plant nutrition (Sahgal et al. 2004; Sharma et al. 2009), microbial interactions may have implications for biological control of phytopathogens (Choudhary et al. 2009). A number of studies have indicated that some AM fungi and associated bacteria as well (Barea et al. 1998; Budi et al. 1999) exhibit biocontrol properties against root pathogens. However, practical use of AM fungi in single and co-culture with bacteria possessing biocontrol properties remains to be explored.

13.6 Conclusions and Future Perspectives

Use of genomics and functional genomics shed light on the advances in the understanding of the mechanisms controlling AM development and symbiosis with plants. Evidences are available that the plant and AM fungi perceive signal molecules prior to their physical interaction. Identities of several molecules of the signaling pathway such as 'Myc factor' are unknown and expected in near future. AM fungi use 'common SYM pathway' for colonization similar to rhizobia-legume symbiosis. Apparently, the signature of Ca^{2+} spikingin the cytoplasm is one of the events discriminating rhizobia from AM fungal signals, even though there is an alternative transduction pathway still to be detailed. Similarly, some transporters involved in nutrient translocation at symbiotic interface are still to be identified.

Mycorrhizal technology seems to be an unavoidable tool for developing new plant management systems in agriculture in order to ensure adequate levels of food production with satisfactory reduction in chemical fertilizers and pesticides. In the context of current global challenges, AM fungi may be crucial in several fields such as environmental change, ecosystem conservation and food safety issues. AM fungi mobilize P and N, and are an important C sink in the soil, having therefore considerable impact on the cycling of these elements. As biofertilizers, they may provide an effective alternate to the synthetic chemicals thus promote sustainable agriculture and protection of the environment. In this frame, insights on the contribution of AM fungi to the nutritional quality of the edible plant parts become a priority. Another long term issue is to identify or design crop-AM fungus combinations with optimized AM fungal performance leading to reduced fertilizer and energy inputs.

Another interesting field is the study of roles of bacteria associated with AM fungi as third component of symbiosis. Their cultivation and determination of potential in terms of improved health/growth of AM fungi and/or plants will be helpful in sustainable agriculture. Answers of these basic questions will make us able to exploit tripartite interaction among plant-AM fungi- and bacterial community for benefiting plants and humans.

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