

Chapter 12

Arbuscular Mycorrhiza: A Tool for Enhancing Crop Production

Suvigya Sharma, Anil Kumar Sharma, Ram Prasad, and Ajit Varma

Abstract Soil microbes play a crucial role in determining many key components such as soil fertility, soil biodiversity and plant health. Due to excessive use of intensive agricultural practices like high inputs of pesticides, insecticides and inorganic fertilizers, the existence of these soil microbes having promising characteristics have become marginalized. However, in today's time introduction of environmental protection programs have created much awareness in many countries, including India, and intensive agricultural pattern is shifting towards low input (sustainable) agricultural regimes. Low input (sustainable) agriculture systems includes minimizing the use of mineral fertilizers, chemical pesticides and other such products and promoting organic and low cost methods into the agricultural system for better yield and protection against diseases. It is therefore, of vital importance for us to understand and manipulate the naturally occurring microorganisms for better crop productivity and establishment of sustainable agroecosystems. Characterization of beneficial soil microbes would be an important step towards understanding such below ground interactions. The focus of this chapter is upon understanding the functioning of Arbuscular mycorrhizae, its ecological significance and possible role in enhancing crop production.

S. Sharma (✉)

Department of Biological Sciences, CBSH, G.B. Pant University of Agriculture & Technology, Pantnagar 263145, India

Amity Institute of Microbial Technology, Amity University Uttar Pradesh, E-3 Fourth Floor, Noida 201313, India

e-mail: suvigya.sharma@gmail.com

A.K. Sharma

Department of Biological Sciences, CBSH, G.B. Pant University of Agriculture & Technology, Pantnagar 263145, India

R. Prasad • A. Varma

Amity Institute of Microbial Technology, Amity University Uttar Pradesh, E-3 Fourth Floor, Noida 201313, India

12.1 Introduction

Crop production all across the globe reached new heights with the onset of green revolution (between 1930s and late 1960s); which encouraged the development of new high-yielding varieties of cereal grains, modernization of irrigation infrastructure, emergence of technology management techniques, availability of hybridized seeds, chemical fertilizers and pesticides to farmers. Out of many beneficial effects of this change in agricultural sector and conventional farming practices, this cannot suffice the human population around the world, as the gap between the crop production and population kept on increasing which led to food shortage and food security issues in near future (Tilman et al. 2011). Heavy inputs of chemical fertilizers and pesticides have led to degradation of soil health and its biodiversity at all levels. Therefore, lower input sustainable agricultural practices are the need of the hour, which includes the replacement of conventional intensive agriculture regimes with cost effective planning and implementation and this can only be achieved by research based strategy of manipulating soil microbial communities and their interactions with in the agri-ecosystems and its rhizosphere. In this chapter Arbuscular Mycorrhiza fungi (AMF) and its role in crop production is discussed.

Decades of research on mycorrhizal fungi have proved that these are the most widespread and beneficial mycosymbionts and their symbiotic association were observed commonly with most of terrestrial plant families. Out of different types of these mycorrhizal fungi, the most commonly found are arbuscular mycorrhiza fungi. It is observed 70–90% of all land plant species form AM symbiosis (Brundrett 2009) AM fungi are obligate, bio-trophic and symbiotic in nature, they colonize the roots of most of the crop plants in natural and agricultural ecosystems (Leal et al. 2009). The infection zone of arbuscular mycorrhiza (AM) is on the surface of the plant root or inside the cortical cells, that is how they got their name—(ancient Greek: $\mu\upsilon\kappa\eta\varsigma$ (mykes) = fungus and $\rho\iota\zeta\alpha$ (rhiza) = root) (Frank and Trappe 2005). AM form arbuscules, which are fine and branched hyphae formed inside the cortical cells, AM hyphae extends outward from the root and helps in transport of inorganic nutrients such as P, N and micronutrients, water from the soil to the plant and collect carbon assimilate from the host plants for their survival (Bucher et al. 2009) also, AMF can strongly enhance the absorption rate of fertilizers, especially Phosphorous based (Tawaraya et al. 2012). AMF produce a polysaccharide compound known as glomalin through their external hyphal structures, which helps in improving the soil structure (Wright and Anderson 2000), also, they increase water availability to their host plant and helps in disease suppression (Graham 2001). AMF have positive impacts on plant growth promotion, soil reclamation and phyto-remediation (Leyval et al. 2002) with all these attributes, AMF can be used as a “Biofertilizer” in a sustainable agro ecosystem.

12.2 Plant Growth Promotion by Arbuscular Mycorrhizal Fungi

The AM fungi makes most dominant component of the rhizosphere, it provides plants with nutrient uptake and many other beneficial inputs for its plant growth promotion, also, these fungi provide an increased surface area for nutrient absorption as well as to interact and accommodate many other soil organisms through their hyphae, and thus the term rhizosphere is widened to as “mycorrhizosphere” (Giri et al. 2005).

AM are considered as the transporters of various elements from rhizospheric soil to host plant. Elements which are immobile such as phosphorous, copper and zinc are of special significance as their availability towards plants is limited. Plants growing under such conditions where they face deficiency of such immobile elements in soil are greatly benefitted by this plant-fungi association (Chu 1999). AM fungi also, help plants to sustain under drought conditions by providing increased water uptake (Augé 2001), under high salt concentrations in soil (Ruiz-Lozano 2003) and heavy metal contaminated sites such as mine spoils and PHP contaminated sites (Barea et al. 2005; Boer et al. 2005).

Variety of plants which play host to AM fungi can survive in an environment where their AM partners are not present, but in natural ecosystem their association is beneficial to the plant growth promotion by significantly contributing towards nutrient uptake, increased plant biomass, offering the plant improved resistance towards pathogens and stress (Smith and Read 2008). On the other hand, AM fungi are unable to complete their life cycle without making an association with the host plant as they can only absorb the carbon assimilates from inside the plant cells; these fungi are dependent upon their plant partners for their growth and reproduction which makes them obligate biotrophs. These are asexual fungi, the phenomenon of anastomosis is observed between genetically distinct AM strains (Croll et al. 2009; Hijri and Sanders 2005). The hyphae are aseptate and coenocytic in nature, which facilitates the translocation of free flowing nuclei across the cytoplasmic streams within the hyphae (Bago et al. 1999). Due to these unique characteristics, high levels of heterozygosity and absence of a uninucleate stage in their life cycle, classical molecular approaches have failed to provide the basis of their growth and functionality, however, fast evolving tools based on transcriptomics and proteomics provided a partial approach where it is assumed that the plants are the determining factors of the symbiotic association.

12.3 Mechanism of Plant Root Colonization by Arbuscular Mycorrhizal Fungi

12.3.1 Physical

Communication between plant and fungus occurs through morphological changes in the cell structure of both host and AM (Parniske 2008). Physically diameter of mycorrhizal mycelium is relatively much smaller than the plant root or root hair,

hence can explore the soil and soil aggregates much efficiently facilitating the host in many ways (Rillig et al. 2002; Wilson et al. 2009). The process of colonization occurs in a number of morphologically well defined stages. The germinating spore physically approaches the plant root forming a structure called as appressorium, from which hyphal penetration occurs and the fungus accommodates itself by proliferating in the cortical parenchyma (Gianinazzi-Pearson 1996). In spite of accommodating inside the cortical cells of the roots the two partners in this mutualistic relationship remain separated by a zone of demarcation termed as Symbiosome, the bidirectional flow of nutrients take place at this site (Bucher et al. 2009).

12.3.2 Biochemical

The mechanism of AM interaction starts even before the mycelium is physically in contact with the host roots; in response to plant root exudates the resting spore germinates a short mycelium which repetitively extends its branches towards approaching the host roots. The AM symbiosis involves a number of signaling pathways which are partially characterized and are identical to those shared by *Rhizobium*-legume symbiosis (Kuhn et al. 2010). The attachment of pre-symbiotic mycelium to the plant cell is mediated by the host root exudates recognized as Strigolactones (Akiyama et al. 2005; Besserer et al. 2006). Diffusion of these compounds into the soil to short distances leads to the attachment of hyphopodium to the cell surface and formation of PPA (pre-penetration apparatus) in the cytoplasm of epidermal cells and outer cortical cells, the intercellular hyphae profusely branches along the axis forming branched structures called arbuscules which are the site of active nutrient exchange (Bonfante and Genre 2010).

12.3.3 Molecular

At least seven genes (SYM genes) are required for the symbiosis to establish in both cases but none of them is considered responsible for AM fungi only (Parniske 2008). Plants have membrane proteins that code for receptor like kinases (e.g. *LjSYMRK* in *Lotus japonicas*; *MtDMI2* in *Medicago truncatula*) which plays instrumental role in directly or indirectly recognizing rhizobial and mycorrhizal signals (Markmann et al. 2008), transduction of these signals occurs by phosphorylation of the kinase domain of an unknown substrate into the cytoplasm. All the downstream elements involved in the SYM pathway collectively transduce the signals to the nucleus. However, AM fungi are also known to produce distinct biologically active compounds which in turn can induce different pathways, as it was demonstrated through experiments on Rice (Gutjahr et al. 2008) and *Medicago* (Kuhn et al. 2010) where SYM-independent regulation of AM induced gene

expression was observed. AM releases 'Myc' factors which are similar to the Nod factors released by rhizobia during symbiosis are responsible for the host root colonization (Bucher et al. 2009). It was reported by Kosuta et al. (2003) that expression of *MtENOD11* depends upon the hyphal branching of AM, it implies that the expression of *MtENOD*, a Myc factor requires presence of strigolactones.

12.4 Mycorrhizal Role in Sustainable Nutrient Mobilization and Crop Nutrition

12.4.1 *Role of Extended Hyphal Network in Nutrient Uptake*

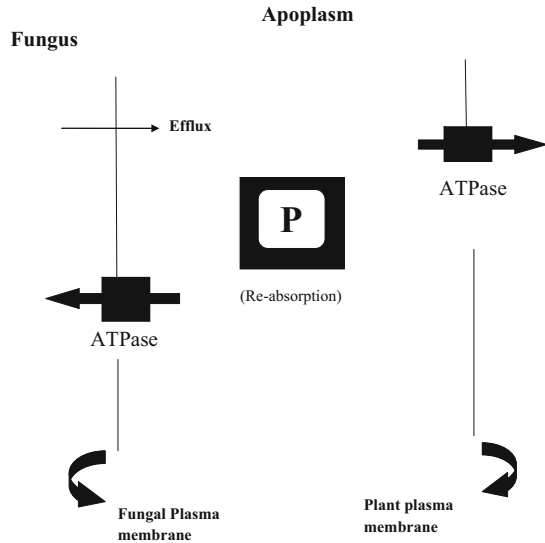
The extraradical mycelium of AM fungi has length that can extend to several centimeters in soil (Rhodes and Gerdemann 1975) and their total surface area can be of many folds greater than that of the host root, the large surface area allows the better acquisition of P and other mineral nutrients (Read and Perez-Moreno 2003). Mobilization of available nitrogen from the litter to the plants is also facilitated by AM extraradical mycelium (Hodge et al. 2001). Counting on these primary characteristics of AM symbiosis, it proves to be an even stronger candidate for low input sustainable agriculture, and through its active network of mycelium it can help in mobilizing nutrients from the crop residues itself.

12.4.2 *Arbuscular Mycorrhizae and P Uptake Kinetics in Plants*

The P uptake kinetics followed by these fungi is very similar to that of plant roots, but there are no kinetic parameters defined for the uptake of phosphorous by extraradical hyphae. The AM hyphae is efficient in transporting P even through a steep concentration gradient and beyond root depletion zones (Harrison and van Buuren 1995). The P-carrier system is driven by a proton-motive gradient created by the ATP-demanding transport of protons out of the fungal cells (Beever and Burns 1980). Sanders and Tinker (1973) evaluated the P flux entering *Allium cepa* roots via the hyphae, based on mean number and size of entry points per cm of the roots; the P flux represented as $\text{mol cm}^{-2} \text{s}^{-1}$ was converted into P flux per unit cross section area of hyphae, this information conveyed a value of $3.8 \times 10^{-8} \text{ mol cm}^{-2} \text{ s}^{-1}$ and indicated the measure of translocated P flux within the mycorrhizal hyphae.

The rate of P absorption per unit area of root measured under standardized conditions of temperature, concentration; pH etc. is considered as the absorption capacity, this feature plays an important and deciding role in P uptake. Cress et al. (1979) reported that mycorrhized roots were more efficient in P absorption by virtue of greater affinity for P carrier system.

Fig. 12.1 Schematic diagram of membrane transport process involved in movement of nutrients (Smith 1993)



At the site of nutritional interchange between the host plant and fungus, active membranes are present which serves as a tripartite structure and are linked to ATP-ase activities. The intracellular space formed by AM fungi influence the necessary energy gradients required for active transport for both host and fungal partners (Fig. 12.1). AMF possess a number of P transporters such as *GiPT* found in extraradical mycelium of *Glomus intraradices* is found to have expressed in low P situation by the fungi (Maldonado-Mendoza et al. 2001). The process of P uptake through AMF can prove costly to plants some times as the fungal partners can switch their roles from being supportive to neutral (Douglas 2008; Kiers and van der Heijden 2006).

12.4.3 Arbuscular Mycorrhizae and Nitrogen Uptake

Soil consists of volumes of Amino acids that contribute towards the crop nutrition. Glutamate, Glutamine, Aspartate and Alanine are free amino acids generally found in soil, in a range of 1–10 $\mu\text{g g}^{-1}$ of dry soil (Abuarghub and Read 1988). Several reports suggest that AM hyphae plays important role in utilization of inorganic N (Cruz et al. 2007; Hodge et al. 2001). Free amino acids were found in the extraradical hyphae of AM (Finlay 2008) also, maize roots colonized with mycorrhizae showed the presence of free amino acids (Cliquet and Stewart 1993) whereas, (Johansen et al. 1993) reported presence of Asparagine, Aspartate, Alanine, Glutamine and Gutamate in extraradical mycelium of *Glomus intraradices*.

Through these reports it is evident that glutamine and glutamate have a key role in incorporation of inorganic N into organic compounds and precursors for the

synthesis of rest of the amino acids in AM fungi. ^{15}N studies have shown that the N in soil is taken up by the extended extraradical hyphae as NH_4^+ -N (MÄDer et al. 2000) and NO_3^- -N (Tobar et al. 1994) and translocated to plants.

12.4.4 Bacteria-AM-Legume Tripartite Symbiotic Relationship

Bacteria associated with AM also help in nutrient transport. It was observed from the example of bacteria-AM-legume tripartite symbiotic relationship, where diazotrophic bacteria fix N not only for plant but also to AM. The presence of AM fungi increases nodulation and nitrogen fixation in legumes, as AM and legume symbiosis works synergistically to provide plant growth promotion (Amora-Lazcano et al. 1998), as AM provides the plant host and rhizobacteria with P which is essential for the synthesis of enzymes required for establishing legume symbiosis and nitrogen fixation thus promotes mycorrhizal symbiosis. It is reported that rhizobium and AM in dual symbiosis can provide plant growth promotion in many legumes (Zahran 1999).

More research is still required regarding the decomposition processes in soil by mycorrhizosphere organisms as this could be significant in establishing sustainable agro ecosystem and can reduce the inputs of chemical fertilizations into fields.

12.4.5 Micronutrients Provided by Arbuscular Mycorrhizae

These fungi play an instrumental role in the translocation of zinc and copper, the content and uptake is significantly higher in the roots of mycorrhized plants in normal as well as abiotic stress condition (Porrás-Soriano et al. 2009). The efficiency of extraradical hyphae to translocate copper and Zinc is high and it could contribute nearly 50–60% of the total uptake in white clover and about 25% in maize (Kothari et al. 1991; Li et al. 1991). A meta analysis of influence of AMF on Zn uptake showed the mycorrhization has positive influence on Zn concentration of plant (Lehmann et al. 2014).

High levels of Fe were reported by (Caris et al. 1998) in peanut and sorghum. It is suggested that like ectomycorrhizal fungi (Szániszlo et al. 1981). AM also produce Fe chelating compounds, for example siderophores (Cress et al. 1986). In soil natural conditions AM compete for the Fe uptake due to presence of other soil microbes and with the root itself. The contribution towards Fe uptake therefore, is variable and dependent upon the plant-fungus combination as well as physical properties of soil. It's an advantage to AMF host plant, that they get the mineral nutrition through their fungal partner even in stressed environments (Clark and Zeto 2000).

12.5 Arbuscular Mycorrhizae Ameliorates Plant Health Through Improved Resistance to Various Abiotic and Biotic Stresses

12.5.1 Soil Salinity

Salt affected soils makes about 10% of the global land surface. Phytotoxic effects of high salinity includes lower water potential in soil solution leading to decreased water uptake, ion toxicity related to increased uptake of Na^+ and Cl^- and reduced mineral uptake and absorption. Several reports have shown the plants have better sustenance against the adverse effects of high salinity when colonized by AMF. Case studies have shown increased salt tolerance in banana plants (Yano-Melo et al. 2003) when infected by *Glomus* consortia, in cotton improved plant growth, biomass accumulation and P –nutrition varied under fungal isolate as well as severity of salt stress. Higher diversity pattern of AM fungi were found in the roots of *Lotus glaber*, a glycophytic perennial legume frequently growing in saline habitats (Mendoza and Pagani 1997; Sannazzaro et al. 2004) showed its dependence on AM colonization under P deficit conditions.

12.5.2 Soil Acidity

Soil acidification is considered to be a natural process in soil under humid environment. As the soil pH lowers to 5 and less, soluble levels of metal cation may cross threshold and become biologically toxic (Goulding 2016). Due to precipitation and adsorption, availability of P gets reduced under acidic conditions in soil. Other factors contributing soil acidification are combustion of fossil fuels, application of ammonium fertilizers, mining practices and other such human activities leads to anthropogenic soil acidification. Many reports suggest most of these soils are found in tropical agro-ecosystems, the role of AM is important as most of the crops in tropics are highly mycorrhiza-dependent in a low P status in acidic soil. The use of commercial AM inoculums can be prompted to reclaim vegetation cover having highly acidic substrate (Arines and Vilariño 1991; Corbett et al. 1996).

12.5.3 Extreme Temperature

Temperature controls the rate of all metabolic reactions; hence, temperature is a very important factor deciding the distribution of microorganism in soil around the earth. Members of Glomales are found in all kinds of temperature zone on earth and with climate their species composition is also influenced (Koske 1987). Due to slower metabolism rate at sub optimal temperature, AMF growth limits.

Extraradical mycelium of certain AM isolates can sustain freezing temperature and can reinitiate the colonization (Gavito et al. 2000, 2003). Varying temperature conditions of extreme cold to extreme high may pose adverse effect on AM and its enzymatic responses (Chen et al. 2014).

12.5.4 Water Stress

Application of AM inoculums to field is one of the research strategies used to curb the water deficiency and sustain the crop yield. During drought conditions the plant undergoes a number of changes such as physiological responses which are regulated by gene expression (Ito et al. 2006). AM symbiosis with plants has shown to improve plant productivity under water stress (Augé 2001; Ruiz-Lozano 2003; Ruiz-Sanchez et al. 2010). The extraradical hyphae of AM serves as extension to plant root and due the thin structure it can explore the soil pores and reach to the water source not available plant root not colonized by AM. Also, the hyphal assistance in water transport to plants was reported by (Khalvati et al. 2005). Some reports also support that in AM fungi the root hydraulic properties (Barzana et al. 2012; Ruiz-Lozano et al. 2009; Sanchez-Romera et al. 2016), stomatal conductance (Auge et al. 2015) and protection against oxidative damage (Ruiz-Lozano 2003). Genes putatively involved in plant response to water stress such as *P5CS*, encoding rate limiting enzyme of Pro synthesis (Porcel et al. 2004), aquaporin genes (Porcel et al. 2006), a gene (*NCED*) plays key role in ABA bio synthesis (Aroca et al. 2008) have been reported for AM colonized plants.

12.5.5 Protection Against Pathogen

A number of AMF-induced resistance was reported against the attack of microbial pathogens (Appoloni et al. 2008). Phytohormones like salicylic acid and jasmonic acid play crucial role in providing ISR (induced Systemic resistance) during pathogen invasion (Bari and Jones 2009); mycorrhization have shown to enhance the levels of these hormones significantly (Meixner et al. 2005). Besides improving uptake of nutrients, AM fungi contribute towards improved resistance to plant against soil borne pathogens. The altered resistance is manifested in physiological responses in plant metabolism through enhanced chitinolytic activity and increased rates of photosynthesis and respiration efficiency. A number of mechanism are now known such as altered exudation of plant roots contribute to change in P nutrition, production of lower molecular weight-phytoalexins, lignification of cell wall and exclusion of pathogen. Mycorrhized plants show a strong vascular system having increased flow of nutrients, higher mechanical strength and decreased effects of vascular pathogen (Huang et al. 2003). The arbuscules formed by AM fungi in plant root gets degraded during digestion of the fungus by the host that increase the

chitinolytic activity, which are effective enzymes against other fungal pathogens. Many researchers suggested production of PR proteins like chitinases (PR type 3) and β 1-3 glucanases (PR type 2) are result of this increased activity and can even work synergistically during plant-microbe interaction.

12.6 Mycorrhizal Inoculum Production and Management

12.6.1 AMF Mass Multiplication Through Trap and on Farm Production

Mass production of AM fungi is now possible using trap culture techniques using suitable host plants and different substrate such as sand, peat, expanded clay, vermiculite, perlite, soilrite, rock wool, glass beads etc. Through trap culturing the small scale production of spores is possible within a time period of 2–3 months. This can be further used to produce large scale inocula which may involve addition of a carrier material such as charcoal or expanded clay types, where the spore can be embedded into substrate particles and can be used as direct inoculums to crops in field. Another technique of similar basis is trap culturing where the plant is uprooted from a site washed and all the traces of external soil particles are removed and then this plant can be grown in suitable sterile substrate. These types of cultures provide a mixed population which can be purified by subsequent monospore pot culturing.

On site production of inoculum can be an alternative to using commercially available inoculum.

In field experiments, it was reported on farm produced inocula performed better during field trial on tubers and yield was increased to 33–45% as compared to control treatment and commercial inoculums (Douds et al. 2005).

12.6.2 AMF Mass Multiplication Through Aeroponic and Hydroponic System

Aeroponic culturing involves growing plants under a moist environment, closed or semi-closed, where continuous spray of nutrient rich solution is facilitated for roots to profusely proliferate and giving AMF to multiply at a mass level. It is a soilless technique which provides a large number of propagule numbers even higher than the soil based inoculums. Most of all this method allows uniform and higher amount of oxygen in the rhizosphere zone. Aeroponic inoculum can be efficiently stored, transported and applied in different crops like: legume, cereal, grass, vegetable and any other herbaceous or woody plant roots. This method of inoculums production is environmentally and ecologically safe and economically viable (Coelho et al. 2014).

Another technique of AM mass multiplication is growing the fungus in a hydroponic solution. It provides the AM propagation under controlled and soil less condition. Nutrient film cultures allow the flow of large scale of fluid flowing over the plant root. MacDonald reported a system for the production of axenic culture of *Glomus caledonium* and *Trifolium parviflorum*. This system of mass multiplication faces the major challenge of limited proliferation of AM under waterlogged conditions.

12.6.3 Root Organ Cultures

The established root organ cultures were first described by White (1943). The root organ cultures have greatly improved our understanding of AM-root behavior in a compartmented system, as the propagating fungus in a dual culture system with transformed host roots can be easily studied under inverted and compound microscope. This technique can be used as a tool for AMF mass multiplication as it allows only fungus and root to grow and is free from other microbes inhabiting the rhizosphere (Fortin et al. 2002). The fungal propagules produced can be stored at 4 °C and dark for a longer period of time and can be used as inoculums for green house or field level application.

12.7 Conclusion

Enhancing crop production involves identifying factors favorable for the yield of crops and developing strategies for low input environment friendly agricultural practices. Use of biofertilizers is undoubtedly a better option over chemical fertilizers which, if not used in control amounts can, be a hazard to the soil and environment. AMF plays a key role in maintaining the soil health and its use is encouraged in agricultural practices in past many decades. Out of the major nutrients required for crops for better yield like NPK, two (N and P) can be supplemented by co inoculation of AM and rhizobacteria. Commercial inoculums is better option for field level application, higher concentration of inoculums can enhance the effects of decreased disease incidence, hence promoting yield. It is important to select the appropriate combination of plant-fungus and other organism, as this can largely influence the proliferation of extraradical hyphae, which is an important factor in plant growth promotion. An inoculum containing consortia of AM species is more robust and sustaining than inoculums with single species and helps in soil aggregation more consistently.

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