Chapter 21
Rhizosphere *Mycorrhizae* Communities an Input for Organic Agriculture

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Abstract *Mycorrhizae* are an important biotic factor that influences tropical ecological succession and differently affect the woody species belonging to different successional stages. They are key components of the soil microbiota that play an essential role in plant growth, plant protection and soil quality. These fungi are widespread in agriculture systems and are especially relevant for organic farming because they can act as natural biofertilizer and enhance plant yield. The interaction between organic practices and *Mycorrhizae* populations are limited and inconsistent. Here, we explore the various roles they play in organic farming systems with special emphasis on their contribution to crop productivity. Present proceedings highlights that organic low-input systems have a high potential to maintain the *Mycorrhizae*, keeping the soil fertile and productive and point the need to incorporate AM technology in organic farming to stop deterioration of agricultural and forest land and other adverse factors. Symbiotic associations between *Mycorrhizae* and plant roots are widespread in the natural environment and can provide a range of benefits to the host plant. These include improved nutrition, enhanced resistance to soil-borne pests and disease, improved resistance to drought, tolerance of heavy metals and better soil structure. However, many agricultural practices including use of fertilizers and biocides, tillage, monocultures and the growing of non-mycorrhizal crops are detrimental to *Mycorrhizae*. As a result, agro-ecosystems are impoverished in *Mycorrhizae* and may not provide the full range of benefits to the crop without *Mycorrhizae*.

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21.1 Introduction

The dawn of every day poses tough challenges to farming community, producing more food to feed the burgeoning population from shrinking land and less water without eroding the ecological foundation is probably the most uphill task faced by scientists, farmers as well as policy makers. Green Revolution of 1960s gifted the Indian agriculture with fertilizer responsive high yielding varieties, high analysis fertilizers, fast acting pesticides and boosted the production of our nation to greater heights with increased cropping intensity. The use of organic manures as sources of nutrients declined sharply. But miserably in the recent past, the declining trends in productivity are more spectacular in Indian Agriculture. Chemical fertilizers are regularly applied to get maximum yields. But as a result of the chemical reactions these get fixed in the soil resulting only part of it being available over the crop period, necessitating fresh additions. The practice of dependence on inorganic fertilizers is not sustainable because these are produced in ways which can not be continued indefinitely as the resources used in their production are non renewable. Further chemical phosphatic fertilizer production is highly energy intensive process. The excessive use of these fertilizers has deteriorated the soil health and adversely affected its biodiversity. In addition the presence of heavy metals in inorganic fertilizers is well established (Chuck 2008). Eventually these heavy metals can build up to unacceptable levels especially in the vegetable produce. Average annual intake of uranium by adult is estimated to be 0.5 mg from ingestion of food and water and 0.6 μg from the breathing air. Polonium-210 contained in phosphatic fertilizers is also absorbed by the plants and stored in its tissues. This element has been found to cause about 11,700 lung cancer deaths each year world over Scholten and Timmermans (1992). Radioactive Polonium (^{210}PO) is one of the most dangerous carcinogen found in Tobacco leaves. It can be removed from tobacco by various method but risk of cancer will not decrease because there are other carcinogen other than polonium present in tobacco which can cause cancer (Chaudhari 2015). The conventional agricultural practices have caused soil erosion, reduction in water availability, increased salinization, pollution due to fertilizers, herbicides, reduced socio-economic values, degrading effect on the environment, danger to food security, quality and safety, reduced bio-diversity, lack of sustainable agricultural policies for the future generations etc., such concerns and problems posed by modern day agriculture gave birth to organic farming. Organic farming is a system which avoids or largely excludes use of synthetic inputs (fertilizers, pesticides, growth hormones, feed additives etc.) and to the maximum extent feasibly rely upon crop rotation, crop residues, animal manures, off farm organic wastes, mineral grade rock additives and biological system of nutrient mobilization and plant protection.

Organic farming is a holistic production management system which promotes and enhances agro-ecosystem health including biodiversity, biological cycles and soil biological activity, and this is accomplished by using on farm agronomic, biological and mechanical methods in exclusion of all synthetic off farm inputs.

For nutrient management under low input production system many biological inputs are used to support the plant nutrition. Among these mycorrhizae is possessing a unique position by supporting the plant growth and development through its multifaceted role. The establishment of functional symbiosis is achieved with in 4–6 weeks and thereafter the host plants derive nutritional and biological benefits from AM symbiosis.

Soil provides an ecological niche for many of the microorganisms including arbuscular mycorrhizal fungi. German botanist Albert Frank in 1885 introduced the Greek word mycorrhiza which literally means “fungus root”. These fungi form the beneficial symbiotic association with the roots of higher plants and perform the function of root hair. This symbiotic association has been reported to promote plant growth and health by playing the role of biofertilizer and bioprotectant, respectively. Arbuscular mycorrhizal association is found in 80% of the plant species except *Cruciferae, Chenopodiceae, Caryophyllaceae* and *Cyperaceae* (Hirrel et al. 1978). The association also occurs over a broad ecological range from aquatic to desert environment. The mycorrhizal symbiosis has been recognized to play a key role in nutrient cycling in the eco system and to protect plants against environmental and biological stresses. In fact, many high value ornamental and edible crops enter in to some form of mycorrhizal association. Most of the crop plants are mycotrophic (i.e. they have the ability to respond to AMF symbiosis), hence functionally, AMF may benefit the productivity and/or vigour of many crops. In addition the mycorrhizal plants have greater tolerance to toxic heavy metals, root pathogens, drought, high soil temperatures, soil salinity, and adverse soil pH and to transplantation shock. Because of their wide spread occurrence in nature and their numerous benefits to plants the fungi are currently attracting much attention in agricultural, horticultural, forestry research. Though there are different mycorrhizal associations, the most common type occurring in all ecological situations, is the vesicular arbuscular mycorrhiza (Bagyaraj 1989; Barea and Jeffries 1995). Increased plant growth because of AM colonization is well documented (Bagyaraj and Varma 1995). The increased plant growth is attributed to enhanced uptake of diffusion limited nutrients, hormone production, biological nitrogen fixation, drought resistance and suppression of root pathogens. Biological control can be defined as the directed, accurate management common components of ecosystems to protect plants against pathogens. Several workers have reported that AM fungi can act as biocontrol agents for alleviating the severity of disease caused by root pathogenic fungi, bacteria and nematodes.

It is evident that an increased capacity for nutrient acquisition resulting from mycorrhizal association could help the resulting stronger plants to resist stress. However, AM symbiosis may also improve plant health through a more specific increase in protection (improved resistance and or tolerance against biotic and abiotic stresses). Mycorrhizae appear to be extremely advantageous to crops growth in low fertility soils which are characteristic of poorly managed, continuously cropped agricultural lands as well as drastically disturbed landscape and mined reclamation sites. Increases in mineral uptake as the result of mycorrhizal associations are often reflected in increased plant survival, growth and yield as well as nutrition. The improved plant growth is attributed to increased nutrient uptake, especially phosphate, due to the exploration by the external hyphae of the soil beyond the root hair zone. The phosphate uptake is more significant in soils deficient in phosphorus. The increased growth of plants inoculated with AM fungi is not only attributed to improved phosphate uptake but also to better availability of other elements like Zn, Cu, K, S, Al, Mn, Mg, Fe etc. Allen et al. (1982) illustrated that AM affects directly the levels of plant hormones like cytokinins and gibberellin substances. Plants colonized by AM fungi can tolerate a wide range of soil water regimes ad also improve water relations of many plants. Biofertilizer help in increasing crop productivity by way of increased biological nitrogen fixation, increased availability or uptake of nutrients through solubilization or increased absorption stimulation of plant growth through hormonal action orantibiosis, or by decomposition of organic residues. Furthermore, biofertilizer as to replace part of the use of chemical fertilizers reduces amount and cost of chemical fertilizers and thus prevents the environment pollution from extensive application of chemical fertilizers. With using the biological and organic fertilizers, a low input system can be carried out, and it can be helped achieving sustainability of farms (Khosro and Yousef 2012).

Mycorrhiza are the rule in nature, not the exception. In a mycorrhizal association, the fungus may colonize the roots of a host plant, either intracellularly or extracellularly. Mycorrhizae are present in 92% of plant families (80% of species) (Wang and Qiu 2006), with endomycorrhizae or Arbuscular Mycorrhizae (AM) being the ancestral and predominant form and indeed the most prevalent symbiotic association found in all the plant kingdom. AM are formed only by fungi in the division Glomeromycota. AM fungi lives in association with approximately 85% of herbaceous plants and produce microscopic arbuscules within cells of the root. Symbiotic associations between AM fungi and plant roots are widespread in the natural environment and can prove range of benefits to the host plant.

AM fungi play an important role in plant health by improving nutrient (especially inorganic P) and water uptake by their host plant and providing protection against soil-borne pathogens (Kurle and Pfleger 1994; Siddiqui and Mahmood 1996; Ryan and Graham 2002). In return, the fungi receive carbohydrates (sugars) and growth factors from the host plant. Other benefits include: increased resistance to foliar-feeding insects (Gange and West 1994), improved drought resistance (Auge et al. 1994) and increased tolerance of salinity and heavy metals. Increased uptake of macronutrients other than P, including N, K and Mg has also been measured as well as increased uptake of some micronutrients maintaining soil aggregate stability. Many agricultural practices including use of fertilizers and biocides, tillage, monocultures and the growing of non-mycorrhizal crops are detrimental to AM fungus communities (Kabir et al. 1998; Thingstrup et al. 1998). As a result, agroecosystems are impoverished in AMF and may not provide the full range of benefits to the crop. In natural environments, the diversity of AM fungi is a key contributor to the diversity and productivity of plant communities (Van der Heijden et al. 1998). AM fungi are strongly affected by anthropogenic activities (Giovannetti and Gianinazzi-Pearson 1994). A variety of agricultural practices are known to impact on AMF, with fertilizers, cultivation, crop monocultures and non-mycorrhizal crop plants known to reduce inoculum (Kurle and Pfleger 1994; Helgason et al. 1998; Daniell et al. 2001).

Organic farming is the only sustainable farming system that is legally defined. It is a crop production system that avoids the use of synthetic and chemical inputs like fertilizers, pesticides, growth regulators and live stock feed additives. Indiscriminate use of synthetic chemicals and the problems arising from them forced us to think about the alternative means. To the maximum extent feasible, organic farming systems rely on the management of soil organic matter to enhance the chemical, biological and physiological properties of the soil, in order to optimize crop production. Soil management controls the supply of nutrients to crops and subsequently to live-stock and humans (Watson et al. 2002). Organic manures such as farmyard manure, compost, vermicompost, biofertilizers, biopesticides etc. can be used at least as complement, if not a substitute.

Organic systems have longer-term solutions at the systems level. An example of this system is the importance of crop rotation design for nutrient cycling and conservation and weed, pest and disease control (Stockdale et al. 2001). Organic fertilizer sources were shown to have major positive effects on the physical properties of soil. This effect is due to the role of mycorrhiza on soil structure formation (Celik et al. 2004). Soil microbial communities are considered a vital factor for the functioning of agroecosystems and success in organic farming (Gosling et al. 2006). Organic farming systems utilize highly complex and integrated biological systems to achieve their goal and most, if not all, management practices used in this system affect more than one component of the system, for example, cultivation may be beneficial for weed control but may stimulate mineralization of nitrogen when the crop does not require it. Thus, the interaction between soil management practices and different aspects of production and environmental impact will continue to challenge the nature and development of organic farming in the nature.

21.2 Interactions

21.2.1 AMF Interaction with Soil and Crops

Mycorrhizal root systems increase the absorptive area of roots 10–1000 times thereby greatly improving the ability of the plants to utilize the soil resource. AM fungi are able to absorb and transfer all of the 15 major, macro and micro nutrients necessary for plant growth (Lester 2009). This behaviour is particularly evident with soil nutrients that are more immobile such as P, Zn and Cu. The fungal soil network is able to maintain P transport to plant for longer periods (Hodge 2000; Jeffries and Barrea 1994; Lange and Vlek 2000).

Mycorrhizal fungi release powerful chemicals into the soil that dissolve hard to capture nutrients such as P, Fe and other tightly bound soil nutrients (Lester 2009).

This extraction process is particularly important in plant nutrition. AM fungi forms an intricate web that captures and assimilates nutrients conserving the nutrient capital in soils. The same extensive network of fungal filaments important to nutrient uptake are also important in water uptake and storage.

21.2.2 AMF Interaction with Agricultural Practices

Crop management involves a range of practices which have impact on the AM association, both directly, by damaging or killing AMF and indirectly, by creating conditions either favourable or unfavourable to AM fungi. In general, agricultural practices have a negative impact on the AM association and agricultural soils are AMF impoverished, particularly in terms of number of species (Helgason et al. 1998; Menendez et al. 2001). For example, high levels of P fertilization have been found to slow down or inhibit mycorrhizal efficiency in soybean fields (Ezawa et al. 2000). Higher soil infectivity was observed under reduced or no tillage practices (Mozafar et al. 2000) and limited increased mycorrhizal colonization of barley root and soil infectivity (Hamel et al. 1996). Relative to conventional management, there is evidence that organic farming practices can enhance the amounts of AMF inoculum (Bending et al. 2004; Mader et al. 2000).

21.2.3 AMF Interaction with Other Soil Micro-organisms

As well as interacting with disease causing agents, AM fungi also interact with a whole range of causal organisms in soils. AM fungi might provide a means of biocontrol of plant disease in organic systems (Siddiqui et al. 1998; Harrier and Watson 2004; Whipps 2004). Bacterial communities and some strains promote germination of AM fungal spores which will increase the rate and extent of root colonization (Johansson et al. 2004). These interactions suggest that AM might affect plant and soil microbial activity by stimulating the production of root exudates, phytoalexins and phenolic compounds (Morandi 1996; Norman and Hooker 2000).

21.3 Role of AMF Colonization on Plant Nutrition and Growth

The relationship between the development of arbuscular mycorrhizas and increased growth of the host was recognized by Asai (1944) in his studies of AM colonization and nodulation in a large number of legumes. He concluded that colonization was important both in plant growth and in the development of nodules. The C economy of AM plants needs to be considered in the context of the effects of AM colonization on mineral nutrition and the relative costs of fungal C use, in relation to benefits derived from increased nutrient uptake.

AMF plants have two potential pathways of nutrient uptake, directly from the soil or via an AM fungal symbiont. The AM pathway depends on three essential processes: uptake of the nutrients by the fungal mycelium in the soil; translocation for some distance within the hyphae to the intraradical fungal structures (hyphae, arbuscules and coils) within the roots and transfer to the plant cells across the complex interface between the symbionts. The fungal mycelium in soil can absorb nutrients beyond the zone depleted through uptake by the roots themselves, so that they increase the effectiveness with which the soil volume is exploited. Consequently, the effects of AM colonization on P nutrition are often large and may have indirect effects on other aspects of plant metabolism, so that direct effect of the symbiosis on the other nutrients are masked (Smith and Read 2008).

21.4 AM Fungal Carbon Metabolism

AM fungi are completely dependent on an organic C supply from a photosynthetic partner. Between 4 and 20% of net photosynthate is transferred to the fungus and used in production of both vegetative and reproductive structures and in respiration to support growth and maintenance, including nutrient uptake (Smith and Read 2008).

Carbon is deployed in growth of the intra and extra radical mycelium and in respiration to support both growth and maintenance, representing a considerable increase in C flux to the soil. At this stage, there is little indications of the reasons for the variations in the estimates, but they are likely to include species of plant and fungus, fungal biomass and rate of colonization, as well as the metabolic activity of the fungus.

21.5 AM Fungi in Organic Farming Systems

AMF are potential contributors to plant nutrition and pathogen suppression in low input agricultural systems, although individual species of AMF vary widely in their functional attributes. Organic farming has developed from a wide number of disparate movements across the world into a more uniform group of farming systems, which operate broadly within the principles of the International Federation of Organic Agricultural Movements (Stockdale et al. 2001). Though the exact production methods vary considerably, general principles include the exclusion of most synthetic biocides and fertilizers, the management of soils through addition of organic materials and use of crop rotation (IFOAM 1998). The use of readily soluble fertilizers and biocides are severely restricted in organic farming. As a result, organic systems often have lower concentrations of total and available soil P than equivalent conventional systems (Gosling and Shepherd 2005). Biocontrol agents that may used in organic systems to control pathogenic fungi do not appear to damage the AMF association (Ravnskov et al. 2002; Gaur et al. 2004).

In organic plant production, the supply of phosphorus is a bottle neck as P is the only macronutrient that cannot be obtained through biological fixation or weathering of parent rock minerals. Farmers thus rely upon recycling of nutrients from plant residues and manure, or addition of superphosphate to meet plant's demand for phosphorus. The mycorrhizal fungi form hyphae in soil as the extension of the roots, transporting nutrients from the soil to the plant. Regarding organic nutrients, mycorrhiza has been shown to improve the utility of both N and P in plant material, as its wide distribution makes more frequent contact with sites where organic matter is mineralized. This scavenging of the soil is the main mechanism for plant supply of P and some other plant nutrients in agroecosystems where these are not a soluble salts (Joner 1996).

It is widely acknowledged that AM technology can improve soil and crop productivity by allowing farmers to produce their inputs of chemical fertilizers and/or by enhancing plant survival, thus offsetting ecological and environmental concerns. Mycorrhizal fungi have particular value for legumes because of their need for an adequate phosphorus supply, not only for optimum growth but also for nodulation and nitrogen fixation (Azcon-Aguilar et al. 1979; Hayman 1986).

Both improved nitrogen fixation in legumes by *Rhizobium* and increased uptake of phosphorus from AM fungal associations can indirectly reduce the chemical fertilizer requirement and the problems related to water and air pollution by chemicals as residuals to the soil-root zone. The reduction of fertilizer requirements by using efficient isolates of *Rhizobium* and AM fungi with different leguminous agricultural crops grown in Bangladesh is of great value (Mridha and Xu 2001).

Some of the agronomically important trees, which have AM fungi association include citrus, tea, coffee, rubber and oil-palm where these plants are grown in nurseries, AMF inoculation may greatly facilitate establishment and early growth after transplanting to the field site. Nursery production of ornamental seedlings and cuttings by treating the rooting and growing media with appropriate inocula is another important area where AM can be used.

21.6 Does Organic Farming Favour AM Fungi?

Recent studies have indicated that one important contributor to plant productivity in low input systems, Mycorrhiza (AMF) have very low inoculums in conventional management systems (Mader et al. 2000). In organic farming, a package of actions is applied such as the use of crop rotation, inter and intra cropping and manuring. The soil fertility was enhanced by organic farming and the healthy crops were produced more efficiently with respect to energy and nutrient use. It was found that organically managed soil had greater AMF spore numbers and root colonization potential and therefore higher AMF inoculum potential, than conventionally managed soil (Galvez et al. 2001; Oehl et al. 2003; Ozaki et al. 2004; Shrestha-Vaidya et al. 2008; Khanday et al. 2016), although, low input practices used in such management system do not always allow the level of biodiversity to increase, even after a long time (Franke-Snyder et al. 2001; Bedini et al. 2008). In a number of studies, organic management has been shown to stimulate AM fungi communities, with the effect attributed to reduced soil P under organic management (Ryan et al. 1994; Mader et al. 2000). This suggests that other practices such as the use of fertility building crops, a greater variety of cash crops, non-chemical weed control and non-use of fungicides may be important; all these factors are known to influence AMF populations (Kurle and Pfleger 1994). The relative difference in AMF spore numbers between organic and conventionally managed fields increased with time since conversion. Gryndler et al. (2009) studied that the mycelia of AM fungi are influenced by organic matter decomposition both via compounds released during the decomposition process and also by secondary metabolites produced by micro-organisms involved in organic matter (pure cellulose and alfalfa shoot and root material) decomposition.

21.7 Status Quo and Future Prospects of Mycorrhizal Application

Available summary of publications devoted to the agricultural and environmental benefits of mycorrhiza show their utility in the number cereal crops, fruits and vegetable production. Wheat, barley and paddy amongst cereals have been investigated with reference to effect of AM mycorrhiza on growth, productivity and nutrient uptake.

Attempts have been made to explore the possibility of employing AM technology in improving the production of vegetables, including potato, brinjal, tomato, lady's finger, lettuce, onion, pepper, cucumber, beans, tomato, muskmelon, watermelon, etc. Various fruit crops including citrus, papaya, orange, mulberry, apple and banana have been investigated for their response to inoculation of AM.

Arbuscular mycorrhizal symbiosis must be considered an essential factor for promoting plant health and productivity. Careful selection of compatible host/fungus/substrate combinations, would allow more appropriate management of mycorrhizae in poor soils would allow substantial reduction in the amount of minerals used without losses in productivity, while at the same time permitting a more sustainable production management.

Production of inoculum is expensive due to overhead and labor costs. During research work inoculum is prepared in an amount enough to treat a small research plot. Under field conditions application requires more inoculum than can be produced in pots. However, commercial inoculum production of AM fungi is under the process of improvement for the past decade, although the future prospect of the business is still uncertain. With heightened interest in application of mycorrhizal fungi, due to their potential significance in not only sustainable crop production, but also in environmental conservation, it is likely that large scale production of inoculum would begin in the near future. Also, with the a basic understanding of the biology of AM fungi and refinement of application techniques for inoculum, the future of crop improvement and environmental conservation using mycorrhizal technology shows promise (Abbasi et al. 2015). Environmental stresses are becoming a major problem and productivity is declining at an unprecedented rate. Our dependence on chemical fertilisers and pesticides has encouraged the thriving of industries that are producing life-threatening chemicals and which are not only hazardous for human consumption but can also disturb the ecological balance. Biofertilizers can help solve the problem of feeding an increasing global population at a time when agriculture is facing various environmental stresses. It is important to realize the useful aspects of biofertilizers and implement its application to modern agricultural practices. The new technology developed using the powerful tool of molecular biotechnology can enhance the biological pathways of production of phytohormones. If identified and transferred to the useful PGPRs, these technologies can help provide relief from environmental stresses. However, the lack of awareness regarding improved protocols of biofertiliser applications to the field is one of the few reasons why many useful PGPRs are still beyond the knowledge of ecologists and agriculturists. Nevertheless, the recent progresses in technologies related to microbial science, plant–pathogen interactions and genomics will help to optimize the required protocols. The success of the science related to biofertilizers depends on inventions of innovative strategies related to the functions of PGPRs and their proper application to the field of agriculture. The major challenge in this area of research lies in the fact that along with the identification of various strains of PGPRs and its properties it is essential to dissect the actual mechanism of functioning of PGPRs for their efficacy toward exploitation in sustainable agriculture (Bhardwaj et al. 2014).

21.7.1 Improved Plant Nutrition

21.7.1.1 Phosphorus

Phosphorus is one of the most important nutrients for plant growth. Phosphorus is one of the least available and mobile plant nutrients in the soil (Takahashi and Anwar 2007). Many soils have high reserves of total phosphorus however only 0.1% of it is available to plants (Zou et al. 1992). At present 5, 49.3, 48.8 and 1.9% of Indian soils fall under adequate, low, medium and high categories of available phosphorus status respectively. The soils of Kashmir fall under low category of available phosphorus (Pattanayak et al. 2009). Inorganic fraction is an important form of phosphorus in soils. It is generally categorized into insoluble and readily soluble categories. The insoluble fraction is neither available to growing plants nor to microorganisms and constitutes 94–99% of the total soil phosphorus. This fraction is mostly attached to Fe and Al in acid soils and to calcium in slightly acidic to alkaline soils. Inorganic phosphates when applied to soil get transformed to various reaction products mainly remaining in sparingly soluble orthophosphates of Al, Fe and Ca. Therefore costly phosphatic fertilizers have to be applied to the agricultural fields to maximize production. However, the soluble phosphorus in these fertilizers is easily and rapidly precipitated to insoluble forms with cation such as Ca^{2+}, Fe^{3+}, Al^{3+} or Zn^{2+} or adsorbed to calcium carbonate, aluminum oxide, iron oxide and aluminum silicate so the apparent recovery of applied phosphorus in soils is very less i.e. 15–20%. This transformation decreases the efficiency with which soluble phosphorus can be taken up by the plants and decreases the effectiveness of the fertilizer resulting in the application of increasing amounts of phosphatic fertilizers to the agricultural fields. This unmanaged use of phosphatic fertilizers has increased agricultural costs and instigated a variety of environmental and health hazards as these contain potentially toxic heavy metals (Pb, Cd, As etc). Their excessive use has rendered the fertile soils sick by disturbing the soil microbial biodiversity. Therefore the use of AM in organic agriculture for enhancing host plant phosphorus nutrition is economically and environmentally promising strategy. There are four general assumptions associated with the improved host plant phosphorus nutrition.

- The external mycelium of mycorrhizal fungus can take up P in the form of trehelose phosphate more effectively than roots at low concentrations.
- The external mycelium can proliferate for beyond the rhizosphere and increases the soil volume which is exploited for phosphorus uptake. The hyphal transport of phosphorus has been estimated to be 20–90% and likely to fulfill the entire requirement of fertilizer phosphorus.
- Rapid absorption of soluble form of phosphorus by the external hyphae leads to a shift in equilibrium towards the release of bound phosphorus from soil reserves (Smith and Read 1997).
- Mycorrhizal roots of onion increased the acid phosphatase activity by 20–30 times in comparison to non-mycorrhizal roots that catalyze the hydrolysis of complex insoluble phosphorus compounds in the soil and increase the soluble form of phosphorus.

These mechanisms aid in the uptake of phosphorus by the host plants and help in reducing the dependence on inorganic phosphatic fertilizers. Thus mycorrhiza plays a pivotal role in the solubilization, mobilization and uptake of phosphorus and can be exploited in organic farming up to the fullest possible extent.

21.7.1.2 Nitrogen

Nitrogen has the distinction among all the essential nutrients of being called as “Kingpin” nutrient. Its use is indispensible in low as well as conventional production systems. The available nitrogen status in agricultural soils is subjected to various losses through the processes like leaching and volatilization. Under such conditions mycorrhizal fungi play a significant role in improving nitrogen nutrition of plants through acquisition and assimilation mechanisms.

- The external fungal mycelium plays an important role in direct nitrogen acquisition and transport to the root cells thereby contributing to plant nutrition. Studies by Fray and Schuepp (1993) have revealed that the extraradical mycelium in mycorrhizal fungi can derive ^{15}N from the soil. Subramanian and Charest (1999) in a box compartmental experiment have shown that the amount of nitrate (NO_3^-) ions being transported by the external hyphae was about 30–35% under water deficient conditions.
- Mycorrhizal colonization of roots has increased the activities of nitrogen assimilatory enzymes such as nitrogen reductase (NR), glutamine synthetase (GS), and glutamate synthase (GOGAT) in drought stressed maize roots (Subramanian and Charest 1998, 1999).
- Under soil conditions where less mobile ammonium ions are dominant the role of mycorrhizal symbiotic association becomes more important.
- Mycorrhizal fungi enters in tripartiate association (Soybean-*Rhizobium*-*Glomus*) thereby aids in transfer of nitrogen fixed by *Rhizobium* to the non-leguminous neighboring plants.

These evidences suggest that mycorrhizal fungi can successfully be exploited for improvement in nitrogen nutrition of crop plants under organic farming.

21.7.1.3 Micronutrient Nutrition

The external hyphae explores the soil beyond the root hair zone and thereby increasing plant growth by enhancing uptake of diffusion limited nutrients. Mycorrhizal hyphae develop intensively inside the roots and with in the soil forming extensive extraradical which help the plant in exploiting mineral nutrients and water from the soil. In plants particularly those with week/restricted root system, hyphal connections act as a bridge between roots and nutrient sites in soil and facilitate efficient uptake of immobile nutrients by host plant. Depending up on the host plant, colonization by mycorrhizal fungi can increase nutrition of micronutrients especially Zn in addition to Cu, Mn and Fe (Rupam et al. 2008; Khanday et al. 2016). Among the essential nutrients required by crops, zinc is considered the most critical micronutrient causing yield reduction to the tune of 10–50% depending on the severity and stage of occurrence. The magnitude of Zn deficiency is high in almost every type of soil and the major portion of added Zn gets fixed. Further, imbalanced use of fertilizers and non-addition of organic manures are believed to be aggravating the situation. In some cases, zinc deficiency in soil reduces grain yield up to 80% along with reduction in grain Zn content and other nutritional qualities. High dependence on cereal based diets with low levels of Zn brings out malnutrition of human beings and globally, over two billion people are affected by Zn deficiency. Improving food grain production with nutritionally rich grain quality is the need of the hour to sustain grain production and to ensure nutritional security. Despite the fact that importance of Zn nutrition is well known, it is very difficult to ameliorate Zn deficiency in crops due to the extremely low use efficiency of zinc (<1%) by crops and the remaining 99% get fixed in the soil. Indeed, majority of arable lands have high total Zn but the bioavailability is too low, suggesting that there is a need to adopt strategies to transform the unavailable form to available form of Zn. One of the biological means to mitigate Zn deficiency is by exploiting naturally occurring mycorrhizal symbiosis. Arbuscular mycorrhizal (AM) fungi immobile micronutrients such as Zn and Cu.

- Mycorrhizal fungi lower the pH around the rhizosphere that helps in release of Zn from the fixed pool
- The external mycelium of mycorrhizal fungi is very explorative and transport Zn far from the root zone to the tune of 40% contributing for the host plant nutrition.
- Rhizosphere of mycorrhizal roots are biochemically active in term of soil enzymes and release a specific glomalin protein that serves as adsorptive site for Zn which in turn is made available to the host plant.
- Interestingly, mycorrhizal fungi are able to extract Zn from tightly bound residual form of Zn and contribute for the organic bound and water soluble forms of Zn. As the result of these mechanisms and processes, mycorrhizal plants are more efficient in utilizing the Zn from the soil and help the plants to produce higher grain yield by 10–15%. Thus mycorrhizal inoculation is one of the potential strategies to improve Zn use efficiency by crops besides enhancing the yield and quality of grains.

21.7.1.4 Plant Protection

Mycorrhizal fungi have been well documented as biocontrol agents and the general conclusion is that they can reduce or even suppress damage caused by soil borne pathogen (Khanday et al. 2016). AMF colonized plants have shown a significant degree of bioprotection against various pathogens like *Fusarium, Pythopthora, Aphanomyces, Verticillium* (Elsen et al. 2001; Azcon and Barea 1996) and nematodes causing respectively root rot, lesions, wilt and galls (Guillemin et al. 1994). Several genes and corresponding protein products involved in plant defense responses have been extensively studied in AMF symbiosis and have been shown to be spatially and temporally expressed (Harrier and Watson 2004). These include callose deposition, phytoalexins, β-1-3 glucanases, chitinases and PR pathogenesis related proteins (Guillon et al. 2002). Cordire et al. (1996) showed that pre-inoculation of tomato with an AM fungus subsequently challenged by *Pythopthora parasitica* resulted in less root damage. In that study the authors used immunogold labelling technique to show that the number of hyphae of the pathogen was greatly reduced in mycorrhizal roots and mycorrhizal root tissues infected by the pathogen. The AMF was able to confer bioprotection against *Phytophthora parasitica via* localized and induced systemic resistance in mycorrhizal and non mycorrhizal roots respectively.

21.7.1.5 Alleviation of Environmental Stresses

Mycorrhization with arbuscular mycorrhizae enable plants to tolerate a wide range of environmental stresses such as drought, toxic metals, saline soil, root pathogens, high soil temperature and adverse pH (Caldwell and Virginia 1989). A well developed mycorrhizal symbiosis may enhance the survival of plants in polluted areas by improving water relations, better nutrient acquisition, pathogenic resistance, amelioration of soil structure, phytohormone production and contribution to soil aggregation thus improving the success of all kinds of bioremedation such as decreased caesium uptake by AMF treated plants and can be used effectively in the establishment of plant cover on radionucleide contaminated soils, thereby reducing environmental risks. Mycorrhization can also be used for attenuation of deleterious soil conditions. They also have the potential to monitor site toxicity or the efficiency of restoration techniques (Weissenhorn et al. 1993). Therefore mycorrhizal fungi enable plants to cope with abiotic stresses by alleviating mineral deficiencies, overcoming the detrimental effects of salinity, improving drought tolerance, enhancing tolerance to pollution and improving the adaptation of sterile micropropagated plantlets to cope up with sudden stress situations arising as a result of change in environmental conditions encountered as a result of their shift from in vitro to in vivo conditions (Barea et al. 1993). Mycorrhizae protects the plants from adverse impact of heavy metals by following mechanisms:

Biosorption by Mycorrhizal Fungi
- Adsorption: Fungal wall (chitin) binds the metals.
- Complexation: Organic acids produced by mycorrhizae forms complex with heavy metals.
- Precipitation: Formation of intra cellular heavy metal phosphates.

Detoxification Mechanism
- Avoidance: Some times mycorrhizal mycellium avoids the absorption of metal ions.
- Solubilization: Dilution of metals.

Arbuscular Mycorrhizal Inoculation
Optimum spore count : 60–100 spores/100 g soil

Rate of Inoculation
Vegetables : 100 g/m^2 nursery
Fruit trees and apple : 100–200 g/tree
Other crops : 10% of seed rate

21.8 Methods of Inoculum Production

The thresh hold point related to the use of AM fungi as plant growth promoters is the development of suitable techniques for the production of large quantities of pure pathogen free inoculum with high infectivity potential. Some of the commonly used methods for mass production of AM spores are listed below:

21.8.1 Soil Based Inocula

21.8.1.1 Pot Culture

It is the most widely used standard and conventional method of maintaining AM fungal cultures around the world. In this method AMF spores are inoculated to the roots of a trap plant raised on sterilized soil. Though the usual substrate used in pot culture is sterilized sand-soil mixture, sometimes inorganic inert material like peat, perlite and vermiculite can be also used as substrate (Abdul Khaliq et al. 2001). The trap plants commonly used for pot culture are *Sorghum halepense, Paspalum notatum, Panicum maxicum, Cenchrus ciliaris, Zea mays, Trifolium subterraneum* and *Allium cepa* (Chellapan et al. 2001). The inoculum so produced, consists of a mixture of soil, spores, hyphal segments and infected root pieces and generally takes around 3–4 months.

21.8.1.2 Inoculum Rich Soil Pellets

A technique of AMF inoculum production, in which soil pellets are enriched with the AMF inoculum was introduced by Hall and Kelson (1981). The pellets had an average dry weight of 1.55 g and measured 12 × 12 × 6 mm. These dry pellets can be glued with seeds by gum Arabic and can easily be broadcasted like other fertilizers and spread during seed sowing or transplantation.

21.8.2 Soil Free Inocula

21.8.2.1 Aeroponic Culture

Apart from soil based pot cultures being the most widely used method for AMF inoculum production. Now a days, for physiological, genetic studies for in vitro mycorrhization, the focus is shifting towards alternative soil less cultures for mass production of clean and pure AMF propagules (Mohammad et al. 2000). In aeroponic cultures, pure and viable spores of a selected fungus are used to inoculate the cultured plants, which are later transferred in to a controlled aeroponic chamber

(Singh and Tilak 2001) where the nutrient solution is provided in the form of a mist. Lack of physical substrate ensures extensive root growth, colonization and sporulation of the fungus and makes it an ideal system for obtaining sufficient amounts of clean AMF propagules (Abdul Khaliq et al. 2001).

21.8.2.2 Root Organ Culture

The main obstacle in the study of AMF and AMF symbiosis are the obligate biotrophic and hypogeal nature of the endophyte. Several attempts have been made in the past to overcome these hurdles through the use of *in vitro* root organ culture, because of its potential for research and inoculum production. *Agrobacterium rhizogenes* is a Gram negative soil inhabiting bacteria, which produces a condition called "hairy roots" as a result of the modified hormonal balance of the tissues that makes them vigorous and allows it to grow rapidly on artificial media (Abdul Khaliq et al. 2001). Once the hairy roots are ready, spores are collected either from field or from pot cultures by wet sieving and decanting method (Gerdemenn and Nicolson 1963). Generally two types of fungal inoculum are used for initiating monoxenic cultures; extraradical spores or mycorrhizal root fragments and isolated vesicles of the fungus. In addition to the spores and root fragments, sporocarps of *Glomus mosseae* have also been used by Budi et al. (1999) to establish *in vitro* cultures. After isolating the fungus from the soil, spores are surface sterilized using a suitable surfactant solution. Generally between 20 and S solution containing a strong oxidizing agent chloramine T are used for sterilization of AMF spores (Fortin et al. 2002). Then the spores are subsequently rinsed thoroughly in streptomycin-gentamycin antibiotic solution (Becard and Piche 1992). All steps starting from spore isolation to rinsing should be done on ice, to maintain spore dormancy. The rinsed spores should be stored at 4 °C in distilled water or water agar or on 0.1% $MgSO_4 \cdot 7H_2O$ solidified with gelatin gum, if not used immediately (Fortin et al. 2002).

The final step in raising a successful *in vitro* culture is the selection of the appropriate culture medium for dual cultivation of the partners, the host root and the AMF. The nutrient media should be carefully selected to allow the growth of the host as well as the fungus during the dual culture establishment. Since the root needs rich nutrient medium for its growth and the AMF require normally a relatively poor nutrient medium (Abdul Khaliq et al. 2001). Generally, Murashige and Skoog's (1962) and White's medium are used for establishing the dual culture of the host root and the AMF symbionts.

21.8.2.3 Nutrient Film Technique

The NFT is another technique of soil less inoculum production, pioneered by Cooper (1975). In NFT, the plant roots are provided with a shallow layer of rapidly flowing nutrient solution. As a result of it, root mats are formed and the upper layer above the liquid retains a film of moisture around them. The pre inoculated seedlings are planted in to the NFT unit. The inoculum produced by this method is ideal for the production of easily harvestable solid mats of roots with more concentrated and less bulky form of inoculum than that produced by plants grown in soil based or other solid media (Abdul Khaliq et al. 2001).

21.8.2.4 Polymer Based Inoculum

Encapsulation or entrapment of AMF in polymer materials is frequently used as a powerful means of immobilization. It includes the encasement of AMF spores, vesicles or mycorrhizal roots within a porous structure formed '*in situ*' around the biological material. In polymer based inoculum preparation, the AMF are generally mixed with a compound which is then gelled to form a porous matrix under conditions sufficiently mild, so as not to affect the viability of biological material. Around 1350 combinations of natural, semi synthetic and synthetic polymers exist for entrapment of AMF (Vassilev et al. 2005). But the majority of techniques involving '*in situ*' entrapment make use of natural polysaccharide gels including kappa-carrageeman, agar and alginates. Calcium alginate is the most widely used carrier of choice for encapsulation of AMF. In some cases spores of AMF can be introduced directly in synthetic seeds, which can germinate under suitable conditions and can become complete plantlets.

21.9 Techniques to Observe AM Fungi

Most observations of mycorrhizae are based on the use of Trypan blue (0.05%) to stain fungi in host roots (Phillips and Hayman 1970). In this technique the mycorrhizal roots are treated in hot 10% KOH that first removes the host cytoplasm and then the nuclei. After the roots are neutralized in a weak acid wash, they are stained in Trypan blue. The stain penetrates deeply and usually stains the hyphae but does not deeply stain the plant tissue. This technique generally is satisfactory for agronomic crops and many other species.

Kormanik et al. (1980) described an Acid Fuschin technique in which clearing and staining of many plant root samples for observation can be accomplished. This technique produces more satisfactory results in plants with heavy pigmented roots. Brundrett et al. (1984) developed another technique in which chlorazol black E allowed the detection of the developmental stages of AM fungi in the host roots with more clarity than other techniques. There are problems with all these techniques. All the techniques are destructive to the sample and involve time-consuming procedures. Different taxa are stained with different intensities in the same roots. Many species of *Gigaspora* and *Scutellospora* stain intensely with Trypan blue, regardless of the host species (Morton 1988). *Acaulospora trappei* exhibits intermediate staining in Trypan blue (Abbott 1982). *Glomus dimorphism,* *G. fecundisporum, G. leptoticum, G. maculosum, G. occultum, G. tortuosum, Acaulospora myriocarpa*, and *Entrophospora schenckii* are not stained or are weakly stained in Trypan blue (Morton 1985). The variation in staining may leave regions unstained and cause inaccurate estimations of fungal colonization of a root. Ames et al. (1982) developed a nondestructive approach to estimate fungal metabolic activities in structures within and outside the host roots. This technique depends on using fluorescein diacetate (FDA) as a non-polar molecule that is taken up by the fungus. If the proper enzymes are present, FDA is hydrolyzed, and fluoresce accumulates in the cell. When excited with ultraviolet (UV) light (450–490 nm), becomes fluorescent and emits at 520–560 nm. The problem with this technique is that much of the hyphae, vesicles, and intra radical spores are not visible. A further problem is that suberized or lignified root tissue may occlude the fungal structures and auto fluorescence.

It can be concluded here that AM fungi can be used quite successfully as a nutrient input under low input agriculture production system.

21.10 Benefits

21.10.1 Soil Structure

Arbuscular mycorrhizae are important factors of soil quality through their effects on host plant physiology, soil ecological interactions and their contributions to maintaining soil structure (Rillig 2004). Mycorrhizal filaments produce humic compounds and organic glues (extracellular polysaccharides) that bind soil into aggregates and improves soil porosity. Soil porosity and soil structure positively influence the growth of plants by promoting aeration, water movements into soil, root growth and distribution. In sandy or compacted soils, the ability of mycorrhizal fungi to promote soil structure may be more important than the seeking out of nutrients.

21.10.2 Plant Growth Hormones

Certain AMF spores or seeds of the fungus have been selected for their establishment and growth-enhancing abilities. Mycorrhizal inoculants can be sprinkled onto roots during transplanting, worked into sed beds, blended into potting soil, watered in via existing irrigation systems, applied as a root dip gel or probed into the root zone of existing plants. AM fungi also increase the production of plant growth hormones such as cytokinins and gibberellins.

21.10.3 Plant Roots

AM fungi increase overall absorption capacity of roots due to morphological and physiological changes in the plant. There is increased absorption surface area, greater longevity of absorbing roots, better utilization of low-availability nutrients and better retention/storage of nutrients, thus reducing reaction with soil colloids or leaching losses. Nodulation and atmospheric nitrogen fixation capacity in legumes were also increased by AM fungi.

21.10.4 Crop Yield

Mycorrhizal fungi improve crop yields (Siddiqui and Mahmood 2001), especially in infertile soil (Hayman 1982). Many crops are grown in acid soil, where their establishment is frequently limited by low availability of phosphorus. In this case, appropriate mycorrhizal fungi can greatly improve crop yields by increasing the phosphorus uptake by plants (Howeler et al. 1987). When the availability in soil is low, non-mycorrhizal root systems may be unable to absorb P effectively and the plants become P deficient and grow poorly. AM colonization and P uptake lead to relief of this nutrient stress and, in consequence, plant growth is increased. This is the well-known mycorrhizal growth response (the big and little plant effect) which has been demonstrated for an enormous number of species mainly in pot experiments.

21.10.5 Nutrient Uptake

It is now established that the fungal partner can make a considerable contribution to nutrient uptake. AM fungi can mediate inter-plant transfer of phosphorus (Francis et al. 1986; Newman and Ritz 1986), carbon (Newman 1988; Read et al. 1985) and nitrogen (Read et al. 1985; Kessel et al. 1985; Haystead et al. 1988; Barea et al. 1988; McNeil and Wood 1990). The largest effect of AM formation is on P nutrition. In addition to phosphorus uptake, AM fungi can also enhance the uptake of relatively immobile micro nutrients, particularly zinc and copper (Killham and Firestone 1983; Lambert et al. 1979; Gnekow and Marschner 1989; Gildon and Tinker 1983;Pacovsky 1986).

21.10.6 Disease and Pathogen

AM fungi are recognized as high potential agents in plant protection and pest management (Quarles 1999; Sharma and Dohroo 1996; St-Arnaud et al. 1995).

Mycorrhizal roots have a mantle that acts as a physical barrier against the invasion of root diseases AMF secretes antibiotics that competes or antagonizes pathogens, thus aiding in disease suppression. AM fungi can decrease the severity of diseases caused by root pathogenic fungi, bacteria and nematodes (Jalali and Chand 1988; Siddiqui and Mahmood 1995a, b; Bhat and Mahmood 2000; Shafi et al. 2002). In several cases direct biocontrol potential has been demonstrated, especially for plant diseases caused by *Phytophthora*, *Rhizoctonia* and *Fusarium* pathogens (Siddiqui and Mahmood 1996; Abdelaziz et al. 1996; St-Arnaud et al. 1997; Siddiqui et al. 1998; Dalpe and Monreal 2004).

21.10.7 Weed Control

Mycorrhizal fungi can contribute to weed control also. They suppress the competitive ability of weeds relative to sunflower (Van der Heijden et al. 2008). AM fungi have the potential to be a much more environmentally sound method of *Poa annua* (weed of temperate zone golf) control in sports turf than the currently used chemicals (Gange et al. 1999). Btehlenfalvay et al. (1996) studied that mycorrhizal fungi enhance weed control and crop growth in a soybean-cocklebur association treated with herbicide bentazon.

21.10.8 Land Rehabilitation

The effective role of AM fungi in land rehabilitation has been well documented (Allen and Allen 1988; Sylvia and Will 1988; White et al. 1989). The AM fungi, by maintaining the uptake of slowly diffusing nutrients under water stress conditions, can help plants resist drought stress (Azcon et al. 1988). AM fungi can help plants become established in saline soils (Hirrel and Gerdemann 1980; Pond et al. 1984) and in nutrient deficient soil or degraded (eroded) habitats, in coal wastes, eroded desert and disturbed soils (Hall and Armstrong 1979; Khan 1981). Mycorrhizal fungi appear to have beneficial effects on soil aggregation and may be an important means of controlling soil erosion. Extramatrical mycelia of AM fungi have been reported to bind soil grains in sandy soils and dunes and many sand dune plants are known to be mycorrhizal.

21.11 Conclusion

Biofertilizer help in increasing crop productivity by way of increased biological nitrogen fixation, increased availability or uptake of nutrients through solubilization or increased absorption stimulation of plant growth through hormonal action or antibiosis, or by decomposition of organic residues. Furthermore, biofertilizer as to replace part of the use of chemical fertilizers reduces amount and cost of chemical fertilizers and thus prevents the environment pollution from extensive application of chemical fertilizers. With using the biological and organic fertilizers, a low input system can be carried out, and it can be helped achieving sustainability of farms. Worldwide, considerable progress has been achieved in the area of mycorrhizal technology. Mycorrhizal fungi are one of the more important groups of soil organisms and play a critical role in nutrient cycling, mediating plant stress and protection against pathogens. They are also cornerstones in the ability of plants to survive transplant shock. Plants have co-evolved mutualistic relationships with symbiotic mycorrhizal fungi such that their survival and fitness depends upon the healthy functioning of these fungi and *vice-versa*. The evidence suggests that the organic farming system leads to increase the inoculum levels of AMF with greater crop colonization that resulted in enhanced nutrient uptake and therefore mycorrhiza may be used as a substitute to reduced fertilizers.

It has been demonstrated and proved that mycorrhizae have great potential for field application to improve productivity of cereal, fruit and vegetable crops and suppress nematode and fungal infestations. The public demand to reduce environmental problems associated with excessive pesticide usage has prompted research on reduction or elimination of pesticides and increasing consumer demands for organic or sustainably-produced food requires the incorporation of microorganisms, such as arbuscular mycorrhizal (AM) fungi. There is also an urgent need to strengthen further the regional collaboration so that benefits of technology advancements could reach those presently left behind.

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