

Mycorrhizal Fungi

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The common mycorrhizal association in most of the plants is the arbuscular type occurring in majority of agricultural crops, most shrubs, most tropical tree species and some temperate tree species. Arbuscular mycorrhizae (AM) are formed by non-septate Glomeromycetous fungi. They belong to the phylum Glomeromycota, which has three classes (Glomeromycetes, Archaeosporomycetes and Paraglomeromycetes) with 5 orders (Glomerales, Diversisporales, Gigasporales, Paraglomerales and Archaeosporales), 14 families and 26 genera. Commonly occurring genera of AM fungi are *Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora* and *Entrophospora*. These fungi are obligate symbionts and have not been cultured on nutrient media. These endophytes are not host specific, although evidence is growing that certain endophytes may form preferential association with certain host plants. Several investigations indicated that even in unsterile soils, plants respond to inoculation with efficient strains of arbuscular mycorrhizae.

The mechanism of improved plant growth caused by mycorrhizal inoculation has been investigated by many workers. Greater soil exploration by mycorrhizal roots as a means of increasing phosphate uptake is well established. They also improve the uptake of other diffusion-limited elements like Zn, Cu, etc. Other beneficial effects include their role in the biological control of root pathogens, biological nitrogen fixation, hormone production and greater ability to withstand water stress. Co-inoculation of AM fungi with other beneficial soil microorganisms is more useful in improving plant growth, thus suggesting the development of suitable “microbial consortia” for inoculating different crop plants. The current day emphasis is on sustainable agriculture, which uses less of chemical inputs having adverse effect on soil health, and environment. Thus, microbial inoculants play an important role in sustainable agriculture.

Key Words: Arbuscular Mycorrhiza; AM fungi and Soil Organisms; Mycorrhizal Dependency; Practical Applications

Introduction

Sustainability refers to productive performance of a system over time. It implies use of natural resources to meet the present needs without jeopardizing the future potential. The Technical Advisory Committee of the Consultative Group on International Agricultural Research has defined sustainability as “successful management of resources for agriculture to satisfy changing human needs while maintaining or enhancing the quality of the environment and conserving natural resources” [1]. Currently, there is

considerable resistance against the use of chemical pesticides and fertilizers because of their hazardous influence on the environment, and on soil, plant, animal, and human health. Hence, use of microbial inoculants, including mycorrhizal fungi is recommended in practical agriculture [2].

The term “mycorrhiza” was coined by Albert Bernhard Frank [3] to describe the symbiotic association of plant roots and fungi. Mycorrhiza literally means “fungus root”. Mycorrhiza results from mutualism between roots of higher plants and

certain fungi. Though the word “mycorrhiza” was coined in 1885, mycorrhizal fungi appear to have coevolved with plants for over 400 million years to become part of the root system as evidenced by fossil mycorrhiza found in carbonaceous deposits [4]. These fungi in soil are ubiquitous throughout the world and form symbiotic relationships with the roots of most terrestrial plants. In natural ecosystems, it is exceptional for a plant not to possess a mycorrhizal root system. Therefore, it could be said that mycorrhizal association is very common or almost universal phenomenon in plant kingdom [5]. Though there are different kinds of mycorrhiza, the most common mycorrhizal association occurring in crops important in agriculture and horticulture is the arbuscular type. Hence, AM fungi is discussed in detail in this review.

Types of Mycorrhizae

There are different types of mycorrhizal associations. The most common are: (i) Ectomycorrhiza, (ii) Ericoid mycorrhiza, (iii) Orchidaceous mycorrhiza and (iv) Arbuscular mycorrhiza

Ectomycorrhiza

Ectomycorrhiza are most common among temperate forest tree species in the families Pinaceae, Salicaceae, Betulaceae, Fagaceae and Tiliaceae, as well as in some members of Rosaceae, Leguminaceae, Myrtaceae and Juglandaceae. Numerous fungi have been identified as forming ectomycorrhiza. Most of them are basidiomycetous fungi belonging to the genera *Boletus*, *Suillus*, *Russula*, *Hebeloma*, *Tricholoma*, *Laccaria*, *Rhizopogon*, *Scleroderma*, *Alpova*, *Pisolithus*, etc. [6]. Some ascomycetous fungi also form ectomycorrhiza such as *Tuber* and *Cenococcum*. Thus, they are mostly fungi forming mushrooms, puffballs or truffles. The mycorrhizal association helps in the uptake of nutrients from soil, protect roots against invasion by pathogens and also decompose organic matter. These fungi can be cultured in the laboratory on suitable media and used for inoculating forest nurseries.

Ericoid Mycorrhiza

Ericoid mycorrhizal fungi usually colonize plants

belonging to the families Ericaceae, Empetraceae and Epacridaceae, which are commonly referred to as heath plants e.g. azalea, rhododendron, blueberry, cranberry etc. These plants occur in temperate regions of the world. The fungus that forms mycorrhizal association is *Hymenoscyphus ericae*, earlier called as *Pezizella ericeae*, which is an apothecium forming ascomycete. Most ericaceous species characteristically grow on nutrient-poor, acidic soil where ammonium predominates over nitrate. Ammonium ions are relatively immobile in soil. Thus, ericoid mycorrhizal fungi help in the uptake of both N and P. In India, azaleas and rhododendrons are grown in Himachal Pradesh and North Eastern regions. The possibility of using the ericoid mycorrhizal fungus for enhancing the productivity of these plants can be an area for future research [6].

Orchid Mycorrhiza

Orchids belong to the family Orchidaceae. This family has nearly 30,000 species. Orchid seeds contain very limited reserves in the form of starch or lipid. At the time of germination, seeds absorb water, swell slightly and the seed coat breaks exposing the epidermal hair and this structure is referred to as the ‘protocorm’. The protocorm has to be infected by the mycorrhizal fungus to develop into a plant. Protocorms wait up to 6 months to be infected by the mycorrhizal fungus. If not infected by the fungus, it dies. Orchids are thus obligatorily dependent on mycorrhizal fungi. The fungi involved were initially identified as *Rhizoctonia solani*, *R. repens* and other *Rhizoctonia* spp. Later, their perfect stage was discovered and they belonged to the genera *Thanatephorus*, *Ceratobasidium*, *Sebacina* and *Tulasnella*. Extensive studies have been made in the orchid mycorrhizal fungus *Tulasnella calospora*. This fungus can be cultured in the laboratory. If the host is not available, it can survive as a saprophyte [7].

Arbuscular Mycorrhiza

Hosts Involved

The common mycorrhizal association in most of the plants is the arbuscular type occurring in the majority of the agricultural crops, most shrubs, most tropical

tree species and some temperate tree species. Hence, this is dealt in detail. It is easier to list plant families that do not form arbuscular mycorrhiza (AM) than to list those that do [8]. Families not forming AM include Pinaceae, Betulaceae, Orchidaceae, Fumariaceae, Commelinaceae, Urticaceae and Ericaceae. Families that rarely form AM include the Brassicaceae, Chenopodiaceae, Polygonaceae and Cyperaceae. Families that form both ectomycorrhizae and AM include Juglandiaceae, Tiliaceae, Myrtaceae, Salicaceae, Fagaceae and Caesalpiniaceae. In addition to the wide spread distribution of AM throughout the plant kingdom, the association is geographically ubiquitous and occurs in plants growing in arctic, temperate and tropical regions. AM occurs over a broad ecological range from aquatic to desert environments [9].

The Fungi

AM fungi belong to the phylum Glomeromycota, which has three classes (Glomeromycetes, Archaeosporomycetes and Paraglomeromycetes) with 5 orders (Glomerales, Diversisporales, Gigasporales, Paraglomerales and Archaeosporales), 14 families and 26 genera [10]. The commonly occurring genera of AM fungi are *Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora* and *Entrophospora*. These fungi are obligate symbionts and have not been cultured on nutrient media. AM fungi are not host specific although evidence is growing that certain endophytes may form preferential association with certain host plants [5, 11].

In the soil, AM fungi produce large thick walled resting spores called extramatricular chlamydospores, which can survive adverse soil conditions and germinate when conditions are favourable. The germ tubes die unless they encounter and successfully penetrate the host root. After forming an appressorium on the root surface, the hypha penetrates the root and ramifies in the root cortex. Branches from the longitudinally running intercellular hyphae enter cortical cells and develop short haustoria-like branched hyphal structures called arbuscules [12, 13]. Vesicles are formed in the cortical cells, which are

thin walled structures of various sizes and shapes containing oil droplets and function as storage organs. The presence of vesicles and arbuscules is the criteria for identifying AM fungus in the roots [14, 15].

Isolation and Maintenance of AM Fungi

A root system colonized by AM fungi does not show any morphological variations from the normal root system and hence cannot be distinguished visually. In some plant species like onions, maize, clover etc. they may appear yellowish. This colour, however, disappears rapidly when exposed. Hence, mycorrhizal status of a root system can only be known through microscopic observation after staining the roots with trypan blue. AM fungi can be isolated from soil by the wet sieving and decantation method [16]. Single spores thus obtained can be surface sterilized by immersing in a solution containing 2% (w/v) chloramine T and 200-ppm streptomycin sulphate for 15 minutes, followed by washing 3 times in sterile water. Individual spores can be picked by a fine capillary pipette, under a dissecting microscope and placed in the collar region of a funnel filled with sterile sand and seeded with any suitable host like sorghum or any other graminaceous host. After 3-4 weeks, the seedlings along with sand from the funnel can be transferred to sand: soil (1:1) mixture in a pot and maintained in the glasshouse as 'pot culture'. Once a pot culture of a single species is achieved, it can be used as inoculum to multiply the fungus for future experiments [17].

Plant Growth Response to Inoculation and Mechanisms Involved

Earlier experiments conducted in sterilized soil showed that AM inoculation could improve plant growth. Since most of the natural soils usually harbour AM fungi, it was felt that plants may not respond to mycorrhizal inoculation in unsterile soils. But later investigations indicated that even in unsterile soils, plants do respond to inoculation with efficient strains of AM fungi. Now it is proved beyond doubt that AM fungi improve plant growth. The growth increase is favoured in soils with low to moderate fertility, especially phosphorus in limiting concentrations [18].

Several investigators have worked on the mechanism of improved plant growth caused by mycorrhizal inoculation. The improved growth is mainly attributed to uptake of diffusion-limited nutrients like P, Zn, Cu, etc. from soil. The other beneficial effects are their role in the biological control of root pathogens, hormone production, greater ability to withstand water stress and synergistic interaction with nitrogen fixers, P solubilizers and plant growth promoting rhizomicroorganisms (PGPRs) [19]. The role played by these fungi in improving plant growth is much more significant in tropical soils compared to temperate soils. This is mainly because most of the soils of the tropics are of low inherent fertility. They are deficient in phosphorus. In addition to being deficient in phosphorus, they are P-fixing i.e. 75-80% of the phosphatic fertilizers added get fixed in the soil and is not readily available over the crop period, necessitating fresh additions. In acidic soils, they are fixed as iron and aluminum phosphates, while in neutral soils they are fixed as calcium phosphates. Continuous application of P fertilizers will result in increased concentration of total phosphorus in the soil over time, resulting in large reserves of fixed P. According to Ozanne [20], less than 10% of soil P enters the plant-animal cycle.

Experiments with P^{32} labelled phosphorus conclusively proved that AM fungi could not solubilize unavailable inorganic phosphorus sources, but draw extra phosphate only from the labile pool in soil solution [21]. The rate in which plant roots absorb phosphorus from the soil solution is much faster than the rate in which phosphorus moves in soil solution by diffusion. This results in a phosphorus depletion zone around the root. It is here that AM fungi play the most significant role. The external hyphae of AM fungi travel much beyond the P depletion zone, scavenge a large volume of soil and supply P to the plants. Early experiments showed that hyphae could travel 8 cm away from the root system [22].

Anatomical and other physiological studies have brought out that mycorrhizal plants have increased rates of respiration, photosynthesis and

increased amounts of sugars, amino acids, RNA etc. and larger and/ or more numbers of chloroplasts, mitochondria, xylem vessels, motor cells, etc. This suggests that mycorrhizal plants are much healthier with better metabolic activity [23]. AM fungi influencing root exudation and the rhizosphere microflora has also been reported. AM fungi enhancing the population and activities of nitrogen fixing and phosphate-solubilizing microorganisms in the rhizosphere is also well documented [24]. Although interaction studies with plant pathogens brought out that each pathogen-mycorrhizal fungus-plant combination is unique making it difficult to draw generalizations, the possibility of biologically controlling the root pathogens looks promising. All these studies bring out that AM fungi help host plant in more than one way. Whatever be the mechanism of action, it is well established that AM fungal inoculation helps plant growth [25].

Mycorrhizal Dependency and Selection of Efficient AM Fungi for Inoculation

Plants differ greatly in their mycorrhizal dependence. Relative mycorrhizal dependency is defined as the degree to which a plant is dependent on mycorrhizal condition to produce maximum growth or yield at a given level of soil fertility [4]. Based on this, plants can be categorized as highly dependent, moderately dependent, less dependent or not dependent on mycorrhizal fungi. It is always advantageous to categorize plants in a region according to their mycorrhizal dependency and work on those which are highly dependent in order to get better results [26].

It is well known that AM fungi are not host specific. Though a particular AM fungus can infect and colonize many host plants, it has a preferred host, which exhibits maximum symbiotic response when colonized by that particular AM fungus [27]. This led to the concept of 'host preference' in AM fungi and in turn the procedure for screening and selecting an efficient fungus for a particular host. This in turn led to the selection of inoculant AM fungi for many crops important in agriculture, horticulture and forestry [28, 29].

AM fungi are obligate symbionts. Attempts to culture AM fungi on artificial media has met with little or no success. At present time, the only method to produce these fungi is in association with the host plant root. Novel techniques to produce AM inoculum in almost sterile environment through nutrient film technique, circulatory hydroponic culture system, root organ culture and tissue culture are available. However, for large-scale field trials, the only convenient method to produce large quantities of inoculum is by the traditional pot culture technique [29, 30].

Use of AM Fungi for Plant Growth

AM inoculum of suitably selected strains can be used for inoculation in the nursery bed [31]. Growers only need to incorporate inoculum in the nursery beds or seedling trays at the appropriate rate by hand. Seedlings thus raised will be colonized by the introduced fungus and then can be planted out in the field. There are several reports of increased growth and yield of food, fodder and fuel crops because of inoculation with efficient AM fungi [32, 33, 34]. These studies also brought out that because of inoculation, nearly 50% of phosphate fertilizer application could also be reduced. Some horticultural plants are propagated through cuttings. In such cases, rooting of cuttings is important. Enhanced rooting of cuttings through inoculation with AM fungi has been reported. AM fungi inoculated plants withstanding transplant stock have also been reported in avocado [35]. Later studies showed high percentage of grafting success in cashew.

Soilless media like vermiculite, perlite, potting mixes etc. are used for raising several horticultural crop plants. Such soilless media are usually fumigated or heat sterilized. Inoculation of such soilless media enhancing seedling growth and finally yield has been reported in woody ornamentals and asparagus [36, 37, 38]. Inoculation of micropropagated plantlets with AM fungi after hardening also improved plantlet vigour and growth in coffee, grapevine, apple, avocado, pineapple, kiwi fruit, strawberry, raspberry, asparagus and banana [39, 40].

Interaction Between AM Fungi and Other Beneficial Organisms

Interaction of AM Fungi with Symbiotic Nitrogen Fixers

There are several reports on the interaction between AM fungi and the legume bacterium *Rhizobium* species. These studies suggest that the interaction is synergistic; improving nodulation and AM fungal colonization, with consequential benefit to plant growth. Legumes have repeatedly been shown to require high levels of phosphate for effective nodulation and growth [41]. It is also known that nitrogen fixation has a high phosphate requirement. While the principal effect of mycorrhiza on nodulation is undoubtedly phosphate mediated, mycorrhiza may also have other secondary effects. Such potentially limiting factors include supply of photosynthates, trace elements and plant hormones, which play an important role in nodulation and nitrogen fixation. Colonization by AM fungi has been found to increase the amount of phytoalexins in certain legume roots, which are iso-flavanoid substances. Flavones are known to induce nod gene expression. These findings have paved new way for a line of research in understanding the role of AM fungi in the expression of nodulation gene in rhizobia [42].

Cell-free extracts of *Rhizobium* enhancing the colonization of the host by AM fungi was observed by Spanish workers. It was later attributed to the presence of extra cellular polysaccharides produced by *Rhizobium*, which increased the number of entry points of AM fungi per unit length of root [24, 43]. It has been observed that certain nod factors stimulate mycorrhizal colonization of soybean. Field studies have shown great advantages of dual inoculation by the two symbionts. This has extra importance in the tropics because of the grain-legume programmes introduced to increase protein content of the diet and the fact that tropical soils are highly deficient in phosphorus.

The actinomycete *Frankia* is known to produce nitrogen-fixing nodules on the roots of non-legumes

like *Alnus*, *Casuarina*, *Ceanothus*, *Myrica*, etc. The actinomycete is housed in nodules where atmospheric nitrogen is fixed and made available to the host plant. Dual inoculation with AM fungi and *Frankia* increased the total dry weight of shoots and roots, number of nodules, weight of the nodular tissues, as well as levels of N and P in *Casuarina* [44].

Interaction of AM Fungi with Asymbiotic Nitrogen Fixers

Nearly 25 genera of free-living bacteria can fix atmospheric nitrogen. Species of *Azotobacter*, *Azospirillum*, *Beijerinckia*, *Clostridium* and *Derxia* are well known among these. Bagyaraj and Menge [45] studied the interaction between *Azotobacter chroococcum* and the AM fungus *Glomus fasciculatum* in tomato and found a synergistic effect on plant growth. Mycorrhizal infection increased the *A. chroococcum* population in the rhizosphere, which was maintained at a high level for a longer period and *A. chroococcum* enhanced colonization and spore production by the mycorrhizal fungus. Similar interactions have also been observed between *A. paspali* and AM fungi in paspalum and between *A. chroococcum* and *G. fasciculatum* in tall fescue [46]. Synergistic interaction between AM fungi and associative nitrogen fixing bacteria *Azospirillum* spp. and *Acetobacter diazotrophicus* have been reported [47].

A tripartite interaction study between the free-living nitrogen fixing bacterium *Beijerinckia mobilis*, phosphate solubilizing fungus *Aspergillus niger* and the mycorrhizal fungus *G. fasciculatum* was conducted. A synergistic beneficial effect on the growth of onion with all 3 organisms was observed. These were attributed to hormone production rather than, or in addition to, nitrogen fixation [48]. The hormones produced by these bacteria could exert some synergistic effect on plant growth or mycorrhizal efficiency. The studies conducted so far, reveal a definite positive interaction between free-living nitrogen fixing bacteria and AM fungi in the rhizosphere with consequential improvement in plant growth.

Interaction of AM Fungi with Phosphate Solubilizers

Many soil bacteria termed as phosphobacteria solubilize unavailable forms of phosphorus and they have been used as bacterial fertilizers on crop plants [47]. Interaction between AM fungi and phosphate solubilizing microorganisms and their effect on plant growth have been studied by several workers. Phosphate solubilizing bacteria (*Agrobacterium* sp. and *Pseudomonas* sp.) inoculated onto the seeds and/or seedlings maintained higher populations for longer duration in the rhizosphere of mycorrhizal than non-mycorrhizal roots of lavender and maize plants. Dual inoculation also resulted in increased plant dry matter and phosphorus uptake in soils. The phosphate solubilizing bacteria also produced plant growth hormones, which enhanced plant growth [15, 49].

The effect of *Glomus fasciculatum* and non-phytohormone producing strain of P solubilizer *Bacillus circulans* on phosphate solubilization, growth of finger millet and phosphorus uptake from ³²P-labelled tricalcium phosphate and superphosphate was studied. The results clearly revealed that AM fungi did not solubilize unavailable form of phosphorus but still enhanced the P uptake, which was attributed to better exploration of soil [50]. Studies conducted with neem and *Pennisetum* grass and many other hosts further confirmed a synergistic interaction between AM fungi and phosphate solubilizing bacteria [47, 51].

Interactions Between AM Fungi and Mycorrhiza Helper Organisms

It has been found that some microorganisms present in soil, can modify the establishment of the mycorrhizal symbiosis. The bacteria associated with the rhizosphere of mycorrhiza, which stimulate the growth of the fungus and mycorrhizal colonization, were called mycorrhiza helper bacteria (MHB) [52]. Later studies showed that certain actinomycetes like *Streptomyces coelicolor* [53] and fungi like *Trichoderma harzianum* could also enhance colonization by AMF. Hence the term “mycorrhiza helper bacteria” was changed to “mycorrhiza helper organisms” to accommodate bacteria, fungi or any

others enhancing mycorrhizal symbiosis [54]. Therefore, MHO selectively helps AM fungal species to form the symbiotic association by increasing its competitive ability against other potential symbionts. It was suggested that it could be due to either the production of enzymes or growth promoting substances, which increase the cell wall plasticity and thereby increase fungal colonization [55, 56].

Several workers have reported that co-inoculation of AM fungi with MHO enhanced mycorrhizal colonization and in turn plant growth and yield. In high-tech agriculture, some crops are raised in disinfected soil/ nursery. Disinfection kills several groups of organisms. In such soils, mycorrhizal inoculation is recommended. If MHO are also killed then colonization by AM fungi will not be proper. It is suggested that milder disinfection treatments, which selectively kill indigenous AM fungi and root pathogens while conserving MHO should be developed. It is probable that, in the near future, commercial mycorrhizal inoculum will contain MHO, improving the efficiency of inoculation in a wider range of conditions and reducing the quantity of inoculum needed.

Recent studies have shown that inoculation with microbial consortia consisting of an efficient AM fungus together with a nitrogen fixer, P solubilizer and PGPR carefully screened and selected for a particular crop plant or forestry species is more beneficial than AM fungus alone in improving the growth, biomass and yield [57].

The fundamental means of assessing the effectiveness of inoculating the nursery seedlings are their survival and growth in the field. Such studies have been done with ectomycorrhizal fungi mostly in the USA and Canada. The success of ectomycorrhizal fungal inoculation in improving seedling growth and survival after out planting is well recognized. However, such studies with AM fungi alone or as microbial consortia are meagre [33]. In a recent study, forest tree seedlings inoculated in the nursery with microbial consortia consisting of selected AM fungi and PGPRs were planted in degraded forest. Inoculated plants survived and grew much better compared to uninoculated plants 52 months after planting (Table 1) [29].

Table 1: Response of *Acacia auriculiformis* and *Tectona grandis* to microbial inoculation in degraded forests at three locations 52 months after planting

Location	Biovolume index [Plant height (cm) x Stem girth (mm)]	
	<i>Acacia auriculiformis</i>	<i>Tectona grandis</i>
Mandya		
Uninoculated	10,390	746
Inoculated	20,479	3,556
Srirangapatna		
Uninoculated	11,661	944
Inoculated	17,889	1,818
Pandavapura		
Uninoculated	7,081	216
Inoculated	11,433	587

Interaction between AM Fungi and Plant Pathogens

Most of the studies on AM fungi-root pathogens interaction suggest that AM fungi decreased or mitigated the disease severity. Consistent reduction of disease symptoms has been described for fungal pathogens, such as *Phytophthora parasitica*, *P. cactorum*, *P. vignae*, *Gaeumannomyces graminis* var. *tritici*, *Fusarium oxysporum*, *Chalara (Thielaviopsis) basicola*, *Rhizoctonia solani*, *R. bataticola*, *Sclerotium rolfsii*, *Pythium ultimum*, *P. splendens*, *Dothiorella gregania*, *Botrytis fabae*, *Ganoderma pseudoferreum* and *Aphanomyces* spp.; bacteria such as *Pseudomonas syringae* and *Ralstonia solanacearum* and nematodes such as *Meloidogyne avenaria*, *M. incognita*, *M. hapla*, *M. javanica*, *Tylenchulus semipenetrans*, *T. vulgaris*, *Pratylenchus brachyurus* and *Radopholus similis* [25, 58, 59].

Studies conducted so far suggest that the mechanisms of suppression may be due to morphological, physiological and biological alterations in the host. It has been demonstrated that AM fungal colonization induces remarkable changes in root system morphology, as well as in the meristematic and nuclear activities of root cells. This might affect rhizosphere interactions and particularly

pathogen-infection development. The most frequent consequence of AM fungal colonization is an increase in branching, resulting in a relatively larger proportion of higher order roots in the root system [60]. However, the significance of this finding for plant protection has not yet been sufficiently considered. Thickening of the cell walls through lignification and production of other polysaccharides in mycorrhizal plants preventing penetration and growth of pathogens like *Fusarium oxysporum*, *Phoma terrestris* and *Meloidogyne incognita* have been demonstrated.

A stronger vascular system observed in mycorrhizal plants will increase the flow of nutrients, impart greater mechanical strength and diminish the effect of vascular pathogens. Working with AM-*Phytophthora* interaction it was observed that the pathogen does not penetrate cortical cells containing arbuscule; suggesting that localized competition for infection/colonization site does occur between the pathogen and the AM fungus. Histopathological studies of nematode galls caused by *Meloidogyne* spp. showed that galls in mycorrhizal plants had fewer giant cells or syncytia, which are needed for the development of nematode larvae, compared to non-mycorrhizal plants. The nematodes in mycorrhizal plants were smaller and took a longer time to develop into adults [58].

Colonization of a plant by AM fungi alters the host physiology and in turn the root exudation pattern [61]. Decreased root exudation in mycorrhizal plants, because of increased membrane phospholipid content, possibly helps in reducing the infection by root pathogens. Higher concentration of ortho-dihydroxy phenols present in mycorrhizal plants compared to non-mycorrhizal plants was found to be inhibitory to the root rot pathogen *Sclerotium rolfsii*. Increased phenylalanine and serine in tomato roots due to inoculation with *G. fasciculatum* was found to be inhibitory to root knot nematodes. The activation of specific plant defence mechanisms as a response to AM fungal colonization is an obvious basis for the protective capacity of AM fungi. Among the compounds involved in plant defence studied in relationship to AM formation are phytoalexins,

chitinases, β -1,3-glucanase, peroxidases, pathogenesis related (PR) proteins, hydroxyproline-rich glycoproteins (HRGP) and phenolics [59, 62].

Mycorrhizal plants harbour higher population of microorganisms in the rhizosphere, thus making it difficult for the pathogen to compete and gain access to the root. Microorganisms producing siderophores, which are low molecular weight chelating agents that have high affinity for ferric iron and thus fungistatic to many pathogens, were observed in higher numbers in the rhizosphere of mycorrhizal plants. Mycorrhizal plants harbouring more actinomycetes antagonistic to root pathogens have been reported [63, 64].

Co-inoculation of PGPRs along with AM fungi protecting the plants better than AM fungus alone against root pathogens has been reported by many workers [59, 63]. Wilt of the medicinal plant *Coleus forskohlii* caused by *Fusarium chlamydosporum* is very serious in India. Inoculation with AM fungus plus *Trichoderma viride* was found to increase root yield and root forskolin concentration, and reduce the severity of the disease significantly under field conditions (Table 2) [65].

Most of the AM-root pathogen interaction studies have been conducted in crop plants important

Table 2: Influence of biocontrol agents on the root yield, root forskolin concentration and plant disease index of *Coleus forskohlii*

Treatment	Root dry weight (g/plant)	Root forskolin concentration (%)	Plant disease index
Control	0.03	0.30	85.55
<i>Glomus mosseae</i>	0.12	0.37	68.19
<i>Trichoderma viride</i>	0.17	0.34	65.69
<i>Pseudomonas fluorescens</i>	0.12	0.35	71.52
Emisan	0.10	0.32	79.74
<i>G. mosseae</i> + <i>T. viride</i>	0.27	0.55	33.28
<i>G. mosseae</i> + <i>P. fluorescens</i>	0.18	0.38	56.51
<i>P. fluorescens</i> + <i>T. viride</i>	0.23	0.39	54.79

in agriculture and horticulture. But the information available on forest tree species is scanty. Mycorrhizal technology can thus play an important role in production of low-cost quality seedlings.

Like most instances of biological control, AM fungi cannot offer complete immunity against the infestation by plant pathogens. They could only impart a degree of resistance against soil-borne plant pathogens. However, the possibility of biologically controlling soil-borne plant pathogens looks promising.

Agricultural Practices and AM Fungi

The common agricultural practices such as crop rotation, organic amendments, fertilizer and pesticide application have all been reported to influence AM fungal population [38]. An experiment on the effect of mono and mixed cropping with soybean and maize showed that mixed cropping stimulated the proliferation of AM fungi, unlike mono cropping with maize or soybean. One reason for the higher propagule density under mixed cropping may be the more intensively rooted soil in the mixed system [66, 67]. Additionally through higher plant density, nutrients are extracted faster from the soil, thereby stimulating AM fungal reproduction. Leaving the land fallow or growing non-mycorrhizal crop will bring down the population of AM fungi in soil [68]. Studies have shown that less tillage of soil is better for the build-up of mycorrhizal population [69]. Phosphorus has a large negative effect on AM population. Most

pesticides inhibit colonization and development of plants. Fumigation of soil with biocides such as methyl bromide, chloropicrin, etc. kills the AM endophytes. In tropical soils, application of organic matter stimulates proliferation of AM fungi. This is probably because of the low organic matter content in tropical soils [70]. These studies reveal that modern agricultural practices are posing problems to AM fungi. The agricultural intensification declines the AM fungal abundance and effectiveness with respect to good colonization and plant growth promotion. Some soil fauna, like collembolans, devour AM fungi and thus reduce the AM population in soil and can be of potential danger in mass production and maintenance of AM fungi in pot cultures. There are some mycoparasites that parasitize AM fungal spores. These include *Humicola fuscoatra*, *Phlyctochytrium* sp., *Rhizidiomycopsis* sp., *Anguillospora pseudolongissima*, etc. These mycoparasites reduce the survival and competitive ability of AM fungi in soil. Studies related to ecology of AM fungi will provide information on the management of these fungi for improving crop growth [71].

Practical Applications of AM Fungi

The common mycorrhizal association in most of the crop plants is the AM type. Pot experiments conducted earlier in sterilized soil showed that AM inoculation can drastically improve plant growth. Later investigations indicated that even in unsterile soils, plants do respond to inoculation with efficient strains of AM fungi. The increased growth of

Table 3: Effect of different AM fungi and added phosphorus on fruit yield of chilli

Inoculation	Yield of chilli (kg/plot)		
	Addition of P fertilizer		
	No addition	Half the recommended level	Recommended level (75 kg/ha)
Uninoculated	0.27	0.37	0.43
<i>Glomus fasciculatum</i>	0.40	0.52	-
<i>G. albidum</i>	0.38	0.42	-
<i>G. macrocarpum</i>	0.32	0.40	-
<i>G. caledonium</i>	0.37	0.41	-

mycorrhizal plants is favoured in soils with low to moderate fertility [19, 72]. They improve the uptake of diffusion limited nutrients like P, Zn, Cu, etc. The other beneficial effects are their role in the biological control of root pathogens, hormone production and greater ability to withstand water stress. If the beneficial effects of AM fungi observed under pot culture studies could be emulated under field conditions, it would have large economic impact on agriculture.

Recent studies under field conditions have brought out that inoculation with efficient AM fungi increases the growth and yield of plants important in agriculture, horticulture and forestry. For this, it is important to standardize the dose and time of application of AM fungal inoculum apart from the method of application in order to harness the maximum benefit from this association. Inoculation at the time of sowing/planting will result in better colonization of the roots as they emerge and hence help in better establishment of the host. The experiments conducted with micro-propagated banana, syngonium and spathiphyllum have revealed that the best time for inoculation would be just before planting out hardened plantlets.

Methods of application of AM fungi generally include hand placement, placing below the seed material or in case of pot experiments uniformly mixing the inoculum with the substrate. The importance of method of application of inoculum arises with the need to initiate colonization in the early stages of plant growth. Based on the plant parameters, it was concluded that placing AM inoculum 8 cm below the surface of the soil (7 cm below the seed) at a point or as a layer is the best method of inoculum placement for seedlings raised in polybags [73]. For seedlings raised on nursery beds, placing the inoculum 2 cm below the seed was found to be the best approach [74].

Significant increases in plant growth and yield of several plants, important in agriculture, horticulture and forestry, because of AM inoculation in unsterile soils containing less or insufficient indigenous endophytes have been reported by several workers. These studies also brought out that application of

phosphatic fertilizer can be reduced by nearly 50% (Table 3). Further, working with citrus rootstocks, it has been observed that mycorrhizal inoculation resulted in plants ready for budding 4-5 months early compared to uninoculated plants [75]. A similar observation was also made in mango rootstocks, wherein inoculated plants were ready for grafting two months early [76]. Early grafting and faster establishment after mycorrhizal inoculation has been reported in cashew rootstocks as well [77]. Again, inoculation of chrysanthemum with *Glomus mosseae* not only increased the flower yield per plant but also the vase life of cut flowers [78]. In medicinal and aromatic plants, AM fungal inoculation not only increased the crop yield but also that of the active ingredient [79].

In recent years, many high value crops are raised through tissue culture. Micropropagated plantlets cultivated on agar rooting medium are usually delicate and hence hardened before planting in the field. During the process of hardening, micropropagated plantlets get acclimatized to the outside environment. Usually 20-40% mortality is observed during the process. Inoculation of micropropagated plantlets with AM fungi after hardening also improved plantlet vigour and growth in coffee, grapevine, apple, avocado, pineapple, kiwifruit, strawberry, raspberry, asparagus and banana [40, 80, 81]. The information available as to whether perennial plants already established in the field respond to AM fungal inoculation is very meagre. In a study, it was found that ten-year-old mulberry plants and one and half-year-old papaya trees positively responded to mycorrhizal inoculation [82].

Forest seedlings can be raised in small seedbeds or trays in suitable substrate mixed with mycorrhizal inoculum. The seedlings which become mycorrhizal can be transplanted to poly bags and then to field site or directly to the field site. This method has been tried in several experiments which produced healthy, vigorously growing seedlings of *Acacia nilotica*, *Albizia lebbek*, *Azadirachta indica*, *Calliandra calothyrsus*, *Casuarina equisetifolia*, *Dalbergia sissoo*, *D. latifolia*, *Leucaena leucocephala*, *Tamarindus indica*, *Tectona grandis* etc. Further, it

was observed that inoculated seedlings performed better when out planted in the field sites including degraded forests and wastelands [30, 83].

Despite the potential of AM fungi in enhancing nutrient uptake and stress tolerance, the monoculture of these organisms is not often enough to achieve maximum benefit for the plant. It is, therefore, necessary to build up a multi agent inoculum consisting of growth promoting microbial consortia. The various beneficial interactions among plant-mycorrhiza-other growth promoting microorganisms have been well documented.

The method of inoculation is very simple and can easily be followed by farmers. Inoculum production must be given top priority and should be undertaken by private entrepreneurs, to make the inoculum available to growers. The growers should be properly educated through demonstrations and other extension methods so that the inoculation with AM fungi can become a standard nursery practice. In the current era of organic farming, emphasis is given to reduce the use of chemicals and to promote the use of organic manures and pesticides. Thus, use of AM fungi can be considered as an alternate strategy to more rational and sustainable agriculture.

Conclusions

- (i) The role of AM fungi in improving plant growth is now well documented. The beneficial effects include improvement in the uptake of diffusion-limited nutrients, synergistic interaction with beneficial soil microorganisms, production of plant growth promoting substances, and greater ability to withstand water stress and root pathogens. Most of the studies have been carried out under green house conditions. Hence, field evaluation to validate the results of pot culture trials should be carried out.
- (ii) It is important to determine the mycorrhizal dependency of different crop plants grown in a region and to select those which are highly mycorrhiza-dependent for inoculation.
- (iii) Screening is required to determine the most efficient AM fungi for a particular crop plant

that can be used for inoculation.

- (iv) Studies are needed to understand the agricultural practices, which promote the activity of effective indigenous AM fungi, to avoid inoculation with external inocula.
- (v) There are a number of new PGPRs reported in literature. Interaction between these PGPRs and AM fungi should be investigated.
- (vi) Investigations on the biocontrol potential of AM fungi to alleviate soil-borne plant diseases should be intensified.
- (vii) There are some soil microorganisms, which promote the activity of AM fungi, which are referred to as mycorrhiza helper organisms. Screening of several bacteria and fungi available in national repositories can be carried out for their potential as mycorrhiza helper organisms.
- (viii) Research on parasites and predators of AM fungi should be intensified, as it will play an important role in the success or failure of AM inoculation trials.
- (ix) Since many high value crop plants are raised in nurseries, root trainers or polybags, biopriming of nurseries with suitable AM fungi can be taken on a priority basis.
- (x) Recent studies have shown that co-inoculation of AM fungi with other beneficial soil microorganisms is more useful in improving plant growth, thus suggesting the need for development of suitable microbial consortia for inoculating different crop plants.
- (xi) One of the major constraints in the utilization of AM fungi is the poor quality of the commercial products. The recent quality control specifications prescribed by FCO needs re-examination. Adequate number of labs should be established to test the quality control norms.
- (xii) Experiments are needed to track down the introduced AM fungi to study their competitive ability with indigenous AM fungi, using molecular techniques.

- (xiii) There is a need to sensitize stakeholders, particularly the departments of agriculture, horticulture and forestry, on the importance of promoting AM fungi.
- (xiv) Most of the farmers in the country do not have sufficient and clear knowledge on the use of AM fungi. The farmers need to be educated. In

order to educate farmers, education and training extension workers is most important. This can be done through demonstration trials on the cultivator's field, as seeing is believing. Publicity programmes can also be done through media like TV, radio, seminars, exhibitions, write-ups in local papers, etc.

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