



Review Paper

Harnessing Arbuscular Mycorrhizal Fungi (AMF) for Quality Seedling Production

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Available online at: www.isca.in, www.isca.me
Received 8th May 2015, revised 30th May 2015, accepted 7th June 2015

Abstract

Arbuscular Mycorrhizal Fungi (AMF), a group of obligate biotrophic fungi belonging to the Phylum Glomeromycota are among the oldest fungi in terrestrial systems on earth. Symbiotic associations of AMF 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. AMF is an unexploited potential biofertilizer in forest nurseries which can be utilized for quality tree seedling production. In many forest tree seedlings the inoculation of AMF was found beneficial, resulting in seedlings of higher quality. The high percentage of root colonization in AMF treated seedlings is found to be directly correlated with an improved growth and physiology. Presence of AMF significantly increases root surface area by production of extensive hyphae, increase transpiration, reduce leaf temperature and restrain the decomposition of chlorophyll. The AMF host obtains maximum benefit when the mineral nutrient regime is least favourable for growth. Hyphae work as conduits that transport carbon from plant roots to other soil organisms involved in nutrient cycling processes.

Keywords: Harnessing, arbuscular mycorrhizal fungi (AMF), quality, seedling, production.

Introduction

The contribution of Arbuscular Mycorrhizal Fungi (AMF) in maintenance of plant health and special attention was paid to plant health but not to plant growth which is obviously known that there are many biological and environmental factors that affects plant growth of which plant health is just one of them¹. Comparable increases in shoot mass were obtained by growing mycorrhizal seedlings in the same soil but without the added AMF. It appears, as with sheathing mycorrhizas, that the host obtains maximum benefit when the mineral nutrient regime is least favourable for growth. AMF associations have been of significant help to crop production and soil fertility as reported by many researchers across different agroecological zones in many countries. Root colonization by AMF is a unique area that has justified the potential of AMF as bioprotectant and as biofertilizer providing protection to plants from parasitic fungi and nematodes and also increase plant growth and yield²⁻⁵. The significant amounts of carbon transfer through fungus mycelia connecting different plant species has been measured⁶. Hyphae are conduits that may transport carbon from plant roots to other soil organisms involved in nutrient cycling processes. Harnessing natural biodiversity such as AMF is a biotechnological approach which counter balances the current negative image of genetically modified organisms in conventional production systems⁷.

The obligate biotrophic character of the AMF has always been a challenge in the study of these fungi. The requirement for

establishing a symbiosis on a living plant makes these studies time consuming and limits experimentation. Around 230 morphospecies of these globally important fungi have been identified and described so far⁸, which is a remarkable low number for such an old and widely distributed fungal taxon⁹. Recent introduction of molecular taxonomy has revealed, not unexpectedly, a far greater genetic diversity than morphological characteristics make visible.

Brief Details about AMF

Historical development in AMF research: The naming of organisms and the establishment of their evolutionary relationships are of great importance in any field of science. The name "mycorrhiza" means peculiar association between tree roots and ectomycorrhizal fungi¹⁰. The first time Arbuscular mycorrhizas described in 1842¹¹, but most of Nageli's drawings only remotely resemble the arbuscular mycorrhiza¹²⁻¹³. The distinction between ectotrophic and endotrophic mycorrhizas, which included at the time only ericaceous and orchid mycorrhizas¹⁴. The intramatrical spores "vesicules"¹⁵ and determined that other structures, named "arbuscules"¹⁶ were located in the inner cortex. Thus the name "vesicular-arbuscular mycorrhiza" was established and persisted till Today. The recognition that not all fungi formed vesicles led to the proposal that this symbiosis should be renamed arbuscular mycorrhiza. The problem was largely solved by clearing the roots of cytoplasm by heating in KOH and staining fungal cell walls with trypan blue in lactophenol¹⁷. Quantification of these

structures (hyphae, arbuscules, and vesicles) was standardized by the method proposed¹⁸.

The fungus first isolated by Nicholls¹⁹ from surface-sterilized mycorrhizal onion roots was identified as a strain of *Pythium ultimum* in between 1952 and 1957. This was not to happen until Mosse's first successful "vesicular-arbuscular mycorrhizal infection" of strawberry²⁰ using nonsterile sporocarps of a fungus initially named *Endogone mosseae* in her honor²¹, which later became *Glomus mosseae*. The name for the arbuscular mycorrhizal symbiosis has changed through the years. The symbiosis was once frequently called "phycomycetous endomycorrhiza" to distinguish it from the endomycorrhizal symbioses formed between members of the Ericaceae or Orchidaceae and higher fungi. The name "Phycomycete", however, no longer carries any systematic significance. At the 1974 Leeds meeting²², the name *Endogone* was used by many in attendance to describe the "phycomycetous endomycorrhizal" fungi. Another outdated name for arbuscular mycorrhizal fungi, *Rhizophagus*, was also in use at the time and continued to be used until about 1977.

The first describe an arbuscular mycorrhiza, which happened to have formed from poplar roots²³. This was considered as a disease and named the fungus *Rhizophagus populinus*²⁴, provisionally placing it within the Chytridiales. The extraction of spores from soil was necessary for their classification. Routine extraction from soil was made possible by wet sieving and decanting, a method commonly used to extract nematodes from soil²⁵⁻²⁶. The fungi divided into two groups of *Endogone*, one forming extrametrical azygospores/zygospores arising from the tip of a swollen hyphal suspensor but producing no intramatrical vesicles²¹. The molecular data established the relationships among arbuscular mycorrhizal fungi and between arbuscular mycorrhizal fungi and other fungi²⁷. The ectomycorrhizal fungi might be beneficial to their hosts²⁸.

Further progress in understanding the effects of arbuscular mycorrhizal fungi on plant growth was made possible by producing large volumes of inoculum initiated from single isolates of fungal species produced in "pot cultures"²⁹⁻³⁰. Thus, there are notable cases of growth depression apparently caused by arbuscular mycorrhizal fungi in "non-host" species³¹ or in host species when phosphate availability is high³²⁻³³ or in other cases³⁴. Mosse did not analyze her apple tissues for phosphorus (P) content³⁵. The suspicion from early on was that the fungi somehow increased nitrogen (N) uptake³⁶. The beneficial mycorrhizal effect was mediated by P uptake. Baylis, who mentored a notable second generation of arbuscular mycorrhiza researchers, studied the growth responses to mycorrhizal infection of five plant species at three levels of added P³⁷⁻³⁸. The transfer of nutrients from fungus to host occurred across functional, intact arbuscules³⁹.

Phosphorus is not the only mineral element taken up and transported to the host by mycorrhizal fungi. The arbuscular

mycorrhizal fungi could increase host Zn content⁴⁰, for Cu⁴¹. The relationships between light and mycorrhization, and it had long been known that starch disappeared from cells with arbuscules⁴². The reduction in light level (and thus presumably photosynthesis) severely decreased mycorrhization⁴³. However, the practicality of inoculating soils that was inherently low in inoculum potential such as sterile citrus nursery beds⁴⁴. Nevertheless, non-nutritional effects of mycorrhizal fungi, such as those on root branching⁴⁵⁻⁴⁶ ethylene production⁴⁷⁻⁴⁸ or protection from pathogens (see below), may still be important. These include nutrient film culture⁴⁹, aeroponics⁵⁰ or expanded clay hydroponics⁵¹.

Relatively early on, researchers noted that different strains of the fungi produced different effects on plant growth⁵²⁻⁵³. Thus, the selection of superior strains of AMF that were notably effective on particular crops was an important activity for a time⁵⁴. Some research focused on the discovery of root exudates, mostly phenolics, which could stimulate growth of the fungus and its entry into the root⁵⁵⁻⁵⁹. One of these phenolics, formononetin, has now been produced commercially and field tests have been performed⁶⁰. The diversity of soils across the United States supported arbuscular mycorrhizal plants⁶¹.

Taxonomical development in AMF research: The history and complexity of the taxonomy and systematics of these obligate biotrophs is addressed by recognizing four periods. First, initial discovery period (1845-1974) which has characterized by description mainly of sporocarp-forming species and the proposal of a classification for these fungi. Second, alpha taxonomy period (1975-1989) which established on solid morphological basis for species identification and classification, resulting in a profuse description of new species and a need to standardize the nomenclature of spore subcellular structures. Third, cladistics period (1990 to 2000) did the first cladistic classification of AMF based on phenotypic characters only. And fourth phylogenetic synthesis period (2001 to present) based on genetic characters using sequences of the multicopy rRNA genes to played a role in defining taxa and elucidating evolutionary relationships within the group.

The discovery period (1845-1974): During this initial period, much of the discovery and description of new species focused on sporocarp-forming species that could be recognized macroscopically⁶². It has characterized by three main events, The description of first species, especially those forming their spores in wellorganized sporocarps., The discovery of the link between large soil-borne spores and sporocarps with the formation of an arbuscular mycorrhizal association, and The first classification of AMF. The time span of 130 years begins with erection of the genus *Glomus*, starting with the description of two species by the Tulasne brothers⁶³ and ending with the classification published.

The alpha taxonomy period (1975-1989): This period contributed to the establishment of a solid morphological basis

for identification and classification of glomeromycotan fungi. It has followed; the proposal of several new genera and families. A profuse description of new species and the proposal for standardization of phenotypic characters of AMF spores to describe new species.

The cladistics period (1990-2000): This period has marked by a new classification and the entry of molecular biology into systematics of glomeromycotan fungi. It has characterized mainly; Proposal of a cladistic classification for AMF based on phenotypic characters. Description of new taxa based on fossil records. Proposal of a spore development model with re-evaluation of terminology for spore subcellular characters, and Use of genetic characters to define taxa and elucidate evolutionary relationships.

The phylogenetic synthesis period (2001 to present): This ultimate period has characterized; the proposal of a new classification based solely on genetic characters (SSU rRNA gene). Description of new taxa based on the fossil record, and the creation of new taxa and a new classification based on a combination of phenotypic and genetic characters.

Only 12 years after the monograph⁶⁴, the number of described glomeromycotan species had jumped to 77⁶⁵, and 6 years later, listed 126 species⁶⁶. In parallel, different keys for AMF species identification developed, such as the synoptic key⁶⁶, the dichotomous key⁶⁷⁻⁶⁸ and keys for groups of species⁶⁹. A significant compiled all summary species descriptions and identification for AMF taxonomy published “Manual for the Identification of VA Mycorrhizal Fungi”⁷⁰. Although this manual has been controversial and is out of print, it is still being used in some laboratories as an aid to identify AMF species.

Analysis of extant species of AMF and the examination of fossil records led to the proposition of new taxa and the transfer of species to other genera. The genus *Glomites* and described

Glomites rhyaniensis from aerial stems and rhizomes of the 400-million-year-old fossil Devonian plant *Aglaophyton major*, based on extraradical and intraradical hyphae, chlamydospore-resembling spores, and arbuscule-resembling structures in the fossil plant⁷¹. The genus *Gigasporites* and the species *Gigasporites myriamycetes* and *Glomites cycestris* from the Triassic plant *Antarcticycas* from a siliceous chert⁷². *Glomites* and *Gigasporites* were hypothesized to be related to the extant genera *Glomus* and *Gigaspora*, respectively. The number of new species described in this “cladistics period” totaled one third of that described in the previous “alpha-taxonomy” period.

Initially there were only six genera and three families (table-1), now by the entry of molecular taxonomy it is increased to 29 genera and 14 families. The rearrangement of species in the genus *Glomus sensu lato* and erected the genera *Simiglomus* and *Septoglomus* in the *Glomeraceae*⁷³, and *Viscospora* in the *Claroideo glomeraceae*, and transferred back to *Glomus* all species of *Sclerocystis* and *Rhizophagus*⁷⁴. Their classification was based on combined genetic (partial sequences of β -tubulin, and SSU and LSU rRNA) and phenotypic (traits associated with subtending hypha, e.g., color, shape and thickness, pore closure) characters, although some of the phenotypic characters used are found across several of their proposed genera. Some of the genera rejected⁷⁵⁻⁷⁶ and were still considered⁷³ and were included in their classification scheme.

Table-1
Comparison of Classification of Phylum Glomeromycota

	Up to 2000	2001-10	Present
Phylum	1	1	1
Class	1	1	3
Order	1	4	5
Family	3	11	14
Genera	6	18	29

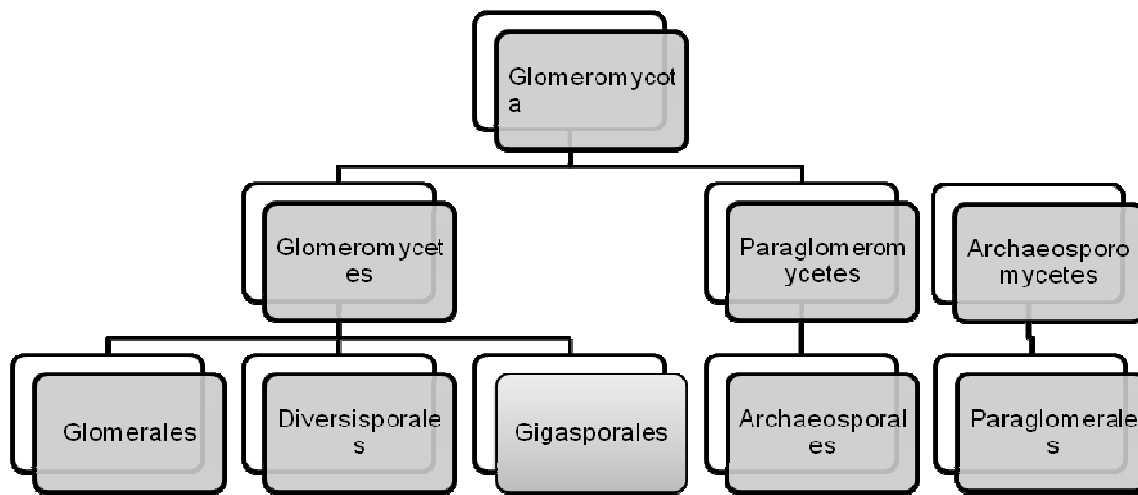


Figure-1
The present classification of Phylum Glomeromycota

New classes and orders have been proposed⁷⁷ at higher levels of the taxonomic hierarchy in the phylum Glomeromycota. These authors erected the classes Archaeosporomycetes and Paraglomeromycetes to contain the orders Archaeosporales and Paraglomerales, respectively. They also proposed the order Gigasporales to be placed within the class Glomeromycetes. In the same year, new genera and families were proposed⁷⁸. *Scutellospora pernambucana* and *S. projecturata* were transferred to the newly erected genus *Orbispora*, hypothesized to be ancestral to species of glomeromycotan fungi forming spores with a bulbous base⁷⁸. In the same period, *Entrophospora* was found to be nonmonophyletic and *E. infrequens* to be closely related to *Claroideo glomus* species, based on ribosomal gene analyses⁷⁹. These authors then transferred the family Entrophosporaceae from the order Diversisporales to the Glomerales, synonymized Entrophosporaceae with Claroideoglomeraceae, and proposed the new genus *Albahypha*. The publication of a large number of taxon names at all levels within the arbuscular mycorrhizal fungi (Glomeromycota) has resulted in conflicting systematic schemes and generated considerable confusion among biologists working with these important plant symbionts⁸⁰.

AMF and its importance: AMF developed symbiotic relationship with many of tree species. AMF was inoculated to root surface of the host plant to acquire carbon and help the host plant to take up phosphorous and other nutrients from the soil. Symbiosis is useful for the plant because phosphorous is necessary for plant growth and development, especially under phosphorous deficient conditions⁸¹. The process of root infection by the fungi is made of complex stages including spore germination, hypha differentiation, aprosorium formation, root penetration, intercellular growth, arbuscule formation and nutrient transfer⁸². Arbuscules are branched hypha, found inside root cells from where nutrient exchange takes place between fungi and the host plant⁸³⁻⁸⁵. As roots develop, a condition for inoculation by AMF improves and the carbohydrates are used by AMF for growth (extension of the hypha). AMF may increase plant tolerance to biotic and abiotic stresses⁸⁶⁻⁸⁸. One of the unique characteristics of AMF, to significant increase in surface area due to the production of extensive hypha helping plants grow under relatively harsh conditions, such as drought stress⁸⁹⁻⁹⁰ and nutrient deficiency⁹¹.

Nutrient uptake: The capacity of plants to acquire nutrients has affected by many factors. The formation of AMF, associations between the roots of most terrestrial plant species and a relatively small group of soil fungi, can increase the capacity of plants to acquire nutrients from the soil⁹². The fungi do this by growing beyond the nutrient depletion zones that typically form around roots, and by greatly increasing the absorptive surface of the root system. Their rapid growth and high plasticity enables the fungi to exploit nutrient patches in the soil, and to better respond to the tremendously complex spatio-temporal dynamics of soil nutrients⁹³⁻⁹⁴. AMF are able to take up nutrients in inorganic forms⁹¹. The evidence suggested that AMF may

access nutrients from organic sources⁹⁵⁻⁹⁶, this most likely occurs following the mineralization of nutrients in organic matter⁹⁷. Irrespective of the mechanisms involved, it is likely that AMF will be important in helping plants to acquire nutrients released from compost. Although insights have been gained into how compost addition affects the formation of AMF, relatively few studies have considered impacts on the functioning of AMF⁹⁸⁻¹⁰⁰. AMF has the potential to promote plant nutrition and growth, and reduce nutrient leaching. Enhanced plant phosphorus (P) uptake is generally considered the main benefit of AM to plants¹⁰¹. Effects of P supply on the formation of AMF are especially relevant to farming systems where large amounts of inorganic fertilizer are added to the soil.

Micronutrients uptake: The multifunctionality of AMF with respect to plant nutrition^{91,102-106} has observed differences among AMF are consistent. Variation in plant micronutrients may be also due to differences among AMF. Also, AMF may be important for a wide variety of nutrients and enhance the uptake of nitrogen¹⁰⁵, zinc¹⁰⁷⁻¹⁰⁹, copper^{91,102,110} and iron¹¹¹ among others¹¹². Overall, the effect of AMF on plant micronutrient nutrition has reported to enhanced effects^{105, 113-116}, diminished effects¹¹⁷⁻¹¹⁸ and no effects¹¹⁹⁻¹²⁰.

Disease control: AMF well had known to improvement the plant health and growth¹²¹. It will improve resistance to plant for various stress factors and intimate interrelationship between the mycorrhizal symbiont and the plant, to ensure that it will be highly responsive to management practices¹²². Often, AMF colonized plants are less infected by pathogens and show lower disease incidence than the non-colonized plants¹²³. The prophylactic ability of AMF could be exploited to improve plant growth and health. Several reported evidence of AMF inoculation as a means of biological control against soil-borne diseases¹²⁴⁻¹²⁷, but only few authors have reported the role of AMF against shoot or stem diseases¹²⁸. AMF established symbiosis with host plants, the host plants get benefited from this mutualistic relationship in terms of improved growth and reduced incidence of diseases¹²⁹⁻¹³⁰. This could be attributed to better compensation for the damage caused by the pathogen¹³¹ through increased capacity for nutrient uptake by the AMF and plant association, which may allow host plants to be more vigorous, and consequently more resistant or tolerant of pathogen attacks¹³².

Water uptake: AMF has ability to affected plant water relations¹³³⁻¹³⁵. AMF also contributed to water influx and efflux in host plants, thus affecting tissue water content and leaf physiology¹³⁶. In drought stress, AM soil moisture content (SMC) indicate in root systems symbiosis, stomata conductance and transpiration, with transpiration typically higher and stomata conductance frequently unaffected or greater relative to non-AMF plants. However, it may also result from the adherence of AM hyphae to soil particles, thus improving contact with the soil solution^{121,136}. Enhanced drying by AM plants may also be associated with the access of hyphae to small

pore spaces inaccessible to host roots and root hairs¹³⁷⁻¹³⁸ and the subsequent uptake of water by AM mycelia for the maintenance of physiological activities¹³⁹.

Stress control: AMF has renowned to their exchange for photosynthetic carbon from their host. It improved plant growth through increased nutrient uptake and enhance plant tolerance against abiotic and biotic stress^{92,140-141} such as salinity stress, heavy metal contamination, and desert conditions¹⁴²⁻¹⁴⁶. They have some unique properties to the beneficial host plant under different stresses condition. AMF able to produced very extensive network of hyphae in the soil and colonization of plant roots when in symbiosis with the host plant and formulate some specialized structure, including arbuscules and vesicles which can significantly enhance the absorbing capacity of the root for water and nutrients¹⁴⁷.

Survival percentage: AMF improves the survival and growth of most plants in natural communities¹⁴⁸. Their ability to increase growth and yield by improving nutrient uptake makes them very important¹⁴⁹. The function of all mycorrhizal systems depends on the ability of the fungal symbiont to absorb inorganic and organic nutrients available in soil⁹¹. AMF allow plants to cope with both biotic and abiotic stresses. They may help to fight off verticillium wilt¹⁵⁰, alleviate certain nutrient deficiencies, improve drought tolerance, overcome the detrimental effects of salinity and enhance tolerance to pollutants¹⁵¹⁻¹⁵³. The extensive activity and survival potential of VA mycorrhizal fungi in most naturally occurring plant populations on undisturbed soil is immediately obvious from an examination of the roots of the vegetation present. Rehabilitation of disturbed sites tends to attract ruderal non-mycotrophic or facultatively mycotrophic plants, which preclude the survival of mycotrophic seedlings and the introduction of mycorrhizal propagules¹⁵⁴. The extensive activity and survival potential of VA mycorrhizal fungi in most naturally occurring plant populations on undisturbed soil is immediately obvious from an examination of the roots of the vegetation present. AMF have not yet been cultured axenically and considered to be obligate symbionts in plants.

Diversity of AMF: The obligate biotrophic fungi belonging to the *Glomeromycota* and oldest fungi in terrestrial systems on earth¹⁵⁵. The symbiotic relationship of the *Glomeromycota* with plants assumed to have played an essential role in the establishment of (pre) vascular plants on the land masses that took place about 460 M years ago in the geologic period Middle Ordovician¹⁵⁶. This assumption is supported by evidence from fossil material. The glomeromycotan fungi develop symbiotic relationships with the majority of vascular plants in almost all habitat types¹⁵⁷.

Ecology of AMF: Fungi significantly play a role in many of microbiological and ecological processes, cycling of minerals and organic matter, decomposition, influencing soil fertility as well as plant health and nutrition. Fungi belongs to heterotrophs,

requiring external sources of carbon for energy and cellular synthesis and they adopted three different trophic strategies to obtain this carbon, occurring as saprotrophs, necrotrophs, and biotrophs¹⁵⁸⁻¹⁵⁹.

Nutrient acquisition: Of all the essential plant nutrients the macronutrient phosphorus is the element that has received most focus in connection to the AMF symbiosis. The acquisition of nitrogen by AMF has long been paid little attention to¹⁶⁰. The uptake of N from organic sources can be substantial, not at least for covering the AMF own need for N¹⁶¹, but also in cases of lack of N mobility under dry conditions the transfer to the plant can be significant¹⁶². Most nitrogen studies are related to N-uptake by AMF.

Soil quality: Arbuscular mycorrhizal fungi have an impact on soil quality, and in turn they are influenced themselves by the properties of soils^{160,163}. A major element in the contribution of AMF to soil quality is their role in aggregate development¹⁶⁴. This is based on the production of the glycoprotein glomalin¹⁶⁵ which has a long lasting stability in the soil¹⁶⁶ and acts as glue for soil particles. The fungal hyphae help to improve the soil particles. The AMF mycelium delivers carbon rich compounds and other bioactive signals further away from the root, thereby stimulating microbial activity in more remote sites¹⁶⁷.

Plant defence interactions: The role of AMF has contributed to plant defence and fungal plant pathogens especially the case for those pathogens with an obligate biotrophic phase^{159,168}. AMF as biocontrol agents have already a long history in academic research¹⁶⁹. Much of the work with biocontrol by AMF, also contemporary literature, has an anecdotic character describing single cases of biocontrol without a clear reference to the mechanisms involved. Few exceptions exist as for example the induction of defence related enzymes following colonization of roots with AMF¹⁷⁰. Besides a direct interaction between pathogen and the mycorrhizal root, indirect effects may occur. This is shown by the stimulus of AMF associated bacteria suppressing pathogenic activity¹⁷¹.

Population behaviour of AMF: Populations of AMF are not fixed; they are dynamic in response to the various forces in the environment. The changes in time and space are both intra and interspecific. AMF have a coenocytic mycelium that harbors many nuclei with probably a certain level of heterokaryosis, although the level of within-fungus polymorphism may be low. In addition, related strains are able to form anastomosis which opens for an exchange of nuclei¹⁷². Spores contain hundreds of nuclei and a high intraindividual genetic diversity may be present in AMF. Over a short time span the genetic assemblage can change¹⁷³⁻¹⁷⁴, and is probably mainly based on changes in allele frequencies¹⁷⁵⁻¹⁷⁶.

AMF-Plant community interactions: The interaction between AMF and their host plants has complicated and still poorly understood. At the plant community level different plant species

share the same mycorrhizal networks. These networks may consist of mycelium from different AMF species and genotypes. The terms of trade for each fungus-host combination seem to vary, and thus influencing the outcome of the symbiosis¹⁷⁷. On the plant community level a correlation was found indeed, and the spatial structure of AMF communities in soil reflects probably the heterogeneity in the vegetation^{160,178-179}. The interaction of weeds-AMF-seedlings receives an increasing focus in forest ecosystems. Here a distinction is made between invasive, ruderal and natural plant species. It is speculated that invasive plant species are hampered in their development by AMF¹⁸⁰⁻¹⁸¹ which may be of advantage regarding management of certain weeds. Although, contrasting results are also obtained¹⁸² showing that native AMF give invasive plant species a competitive advantage. However, most of the experimental data dealing with ecological questions are from isolated pot and microcosm studies. Extrapolation to open field situations is difficult, if not impossible¹⁸³.

Standardization of inoculation dosage: Mycorrhizal develop rapidly, with the high dosed of alginate inoculum used and also proved that the number of fungal propagules in each bead has important factor in the efficiency of the inoculums¹⁸⁴. The positive dose response attributed to a better colonization of the rhizosphere by the introduced microorganism¹⁸⁵, leading to a larger population which produces more of the effective substances either directly, because the cells are more numerous, or indirectly through quorum-sensing mechanisms within high-density micro-colonies¹⁸⁶ increasing the inoculation dose generally increases plant protection^{185,187} and root growth were also observed with high inoculation doses¹⁸⁸⁻¹⁸⁹. A negative response was in bacterial inoculation in different doses, the lowest doses were the most efficient ones¹⁹⁰.

To standardize the critical level of AMF for *Prosopis cineraria* seedling, *Glomus* sp. was used at different spore levels (up to 900 g germinable spores per seedling per polybag). Mycorrhizal inoculation increased plant height, dry matter yield, root length and per cent root infection. Eighty five per cent infections were found to be sufficient for optimum response by *P. cineraria* seedling. The critical level of spores was found to be 400 per polybag (1 kg soil) for *P. cineraria* seedling¹⁹¹. The standardization of inoculum dose in *Tecomella undulata* seedlings found that 100 g rhizosphere soil (500 germinable spores) of AMF was the best dose for better growth¹⁹². Crops for transplantation can be pre-inoculated with AMF in the nursery itself so that the inoculum quantity can be reduced. In chilli among different dose recorded maximum colonization and the economical dose for satisfactory colonization was found to be 850 g m⁻²¹⁹³.

Positive effects of AMF on seedling physiology: The root colonization in AM fungal treated plants has directly correlated with a better nutrient uptake, an increase in the rate of photosynthesis, increased total chlorophyll content and transpiration¹⁹⁴⁻¹⁹⁷, and thereby improved root and shoot growth

were expected¹⁹⁸⁻¹⁹⁹. These results also conformity an increase of total chlorophylls when inoculated with AMF²⁰⁰⁻²⁰¹. The AMF infected plants had a comparatively low transpiration rate and higher water use efficiency (WUE) as compared with non mycorrhizal plants. This reduced transpiration rate was due to increased stomatal resistance provided by the AMF colonization by decreasing stomatal conductance¹⁹⁶. The mycorrhiza could increase the rate of leaf transpiration, reduce leaf temperature and restrain the decomposition of chlorophyll²⁰².

Factors influencing the efficiency of AMF fungi: AMF has established the symbiosis relation with host plants and its range of factors association, both directly, by damaging or killing AMF and indirectly, by creating conditions either favourable or unfavourable to AMF. In general, AMF has interacted of host plant with several other factors such as abiotic and biotic factors.

Abiotic factors: The soil factors exert maximum influence on establishment AMF. The light textured soil supports the AMF sproulate heavily, but their survival was generally more in loamy soils than in sandy soils. The pH optimum of spore germination would probably differ with each AMF species and the environment to which each is indigenous²⁰³. The *G. mosseae* common in alkaline flatland soils germinated well on water or soil extract at pH 6 to 9²⁰⁴. Thus, it appears that pH can influence the germination of AMF spores, but germination seems to occur within a range is still acceptable for plant growth and AMF species have distinct behaviours at different levels of pH²⁰⁵. Moisture to influence below field capacity, germination declined with no germination²⁰⁶. Higher levels of germination could be obtained at low water potential, if spores were incubated longer. Further observed that germ tube length was reduced at low water potential²⁰⁷. The increase in temperatures generally resulted in more root colonization and increased sporulation²⁰⁸⁻²⁰⁹. Increased content of heavy metal pollutants (Cd, Pb, Zn and As) in the soil resulted in a decrease in AMF colonization²¹⁰. Much of the influence of soil fertility on root colonization is plant mediated and the root colonization is inhibited at high phosphorus levels because of the decreased root exudation²¹¹. The increased solar radiation increased percentage colonization^{209,211-214}. The low light intensity can significantly reduce root colonization, but its effect on sporulation may be less pronounced²¹⁴. Seasonal variation in percent root colonization with VAM fungi was noticed and the lowest colonization was during winter and highest during last summer and autumn²¹⁵.

Biotic factors: In addition to abiotic factors the biotic factors like host, genotypic variation among the host, cropping sequence, rhizosphere effect and root exudates exert an equal influence in determining the AMF population in soil. Certain AMF species may be efficient in stimulating the growth of certain plant species, but each AMF is generally able to colonize every AMF host species³². It appeared that the host plant could affect sporulation and possibly survival of AMF²¹⁶⁻²¹⁷. All these

workers point out the necessity of taking into consideration the existence of AMF symbiosis in the selection processes, since greater yields at lowest cost can only be obtained when better fitness of plant species or varieties to this association is exploited. The presence of plant roots causes a rapid and intense stimulation of the microbial population in the rhizosphere region²¹⁸, and AMF symbiosis was initiated at the zone of elongation from where root exudation was greatest²¹⁹.

Mechanism of AMF infection: The obligate biotrophic character of the AMF has always been a challenge in the study of these fungi. The requirement for establishing a symbiosis on a living plant makes these studies time consuming and limits experimentation. The receptor proteins has modified to fungal plasma membrane and the chemical signals such as flavonoids and strigolactones, together with surface or thigmotropic signals from the rhizodermis exuded by the plant²²⁰ (Figure-2). The signal perception receptor proteins has modified and possibly interacted with downstream components. *Gin1* might be one of the downstream components, located at the plasma membrane where it has covalently modified by plant signals. Through its ATPase activity, *Gin1* might interact or modify other membrane proteins to transmit the signal towards the nucleus. Calcium, released from cellular organelles such as the endoplasmatic reticulum, might act as a second messenger (Figure-3). Due to

activation of mitochondrial respiration and increased ATPase activity caused membrane hyperpolarization occurs after transcriptional induction of the corresponding genes. Some of the fungal genes activated in response to plant signals developed which disable programmed growth arrest and allows the fungus to enter into the symbiotic modus.

A model suggested that events that are mediated by the predicted protein products of cloned common symbiosis receptor kinase (SYM) genes²²¹ (Figure-4). The SYMRK/NORK/DMI2 receptor kinase may be the earliest to act in the AM signalling pathway. It perceives signals emanating from the fungal microsymbiont either directly or indirectly, and transduces the event through its intracellular kinase domain. This, in turn, activates the predicted ion channel, DMI1. The availability of purified bacterial signalling compounds and experimental difficulties arising from the obligate biotrophic nature of the fungus have contributed to a situation in which we know more about early signalling events in root nodule symbiosis than in AM. In particular, we do not know whether the calcium-spiking response that is characteristic of the rhizobial symbiosis also occurs in the mycorrhizal interaction. The DMI3 kinase potentially responds directly to oscillations in calcium-concentration, however, implying that Ca^{2+} is also a messenger in mycorrhizal signalling.

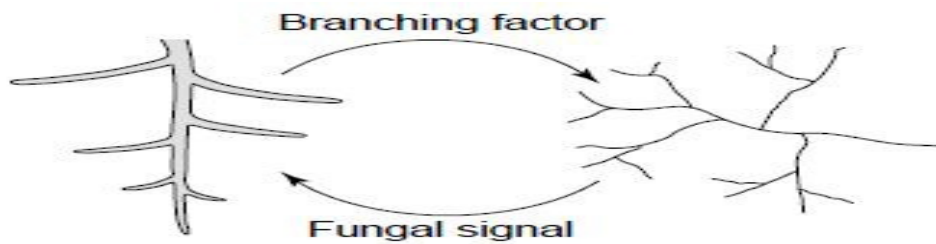


Figure-2
 Signal exchange between the plant root and the hyphae of AMF before infection

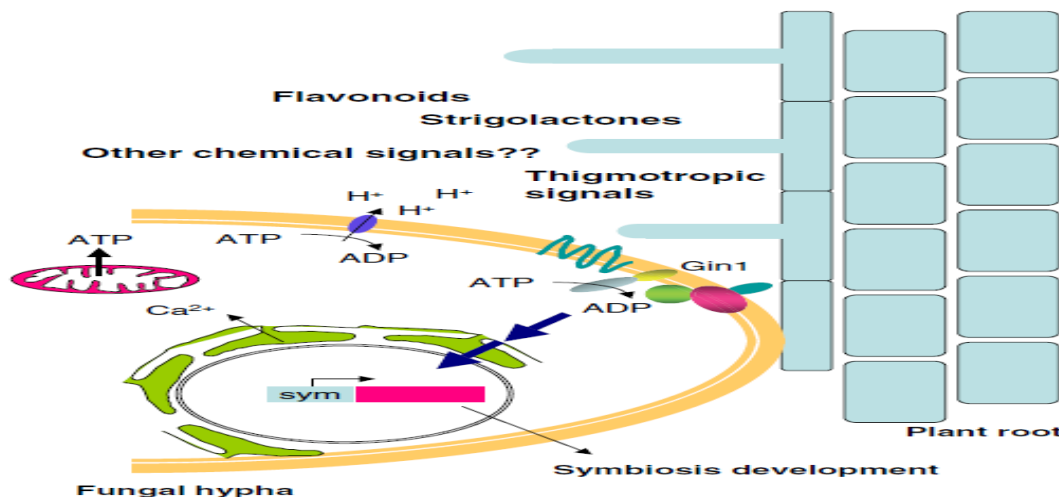


Figure-3
 AMF perception of plant signals during mycorrhiza establishment

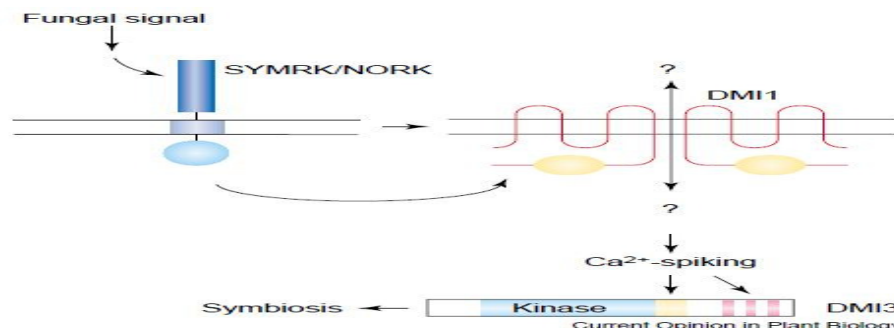


Figure-4

A model of events that are mediated by the predicted protein products of cloned common SYM genes

Seedling Quality Improvement: The goal of forest tree nursery practices is to produce high quality seedlings with useful characters²²¹. Evolving appropriate nursery management strategies to enhance productivity and thereby reducing the long nursery period has been the basic challenge. Among the variable silvicultural options, early tree nutrition practices have bagged considerable attention in the recent times in view of their long standing effect on tree growth and productivity. However, the species is still poorly studied in relation to its management in plantations and its physiological responses²²² to AMF applications. Artificial inoculation with mycorrhizal fungi in the nursery can be used to increase seedling performance consistently positive results have been obtained with *Acacia nilotica* and *Albizia lebbeck*²²³, *Acacia mangium*²²⁴, *Acacia mearnsii*²²⁵, *Acacia nilotica*²²⁶, *Acacia tortilis*²²⁷, Apple²²⁸, Apricot²⁰¹, *Calliandra calothyrsus*²²⁹, Cashew²³⁰, Chineses fir²³¹, Citrus^{232,233}, *Dalbergia sissoo*²³⁴, Douglas-fir¹⁸⁴, *Eucalyptus tereticornis*²³⁵, *Leucaena leucocephala*²³⁶, Olive²³⁷, *Pistacia vera*²³⁸, *Pongamia pinnata*²³⁹, *Prosopis juliflora*²⁴⁰, *Santalum album*²⁴¹ and *Tectona grandis*²⁴².

Negative growth: Generally, AMF associate with the host plant and helps to uptake of nutrients and enhance the multiple benefits to the host plant it may not be obviously mutualistic at every time but it has possible under some conditions that the AMF may cheated their host plant for supply of nutrients such as decline in growth²⁴³ and its very difficult process²⁴⁴ because of the wide range of benefits to the host, which may only in under certain environmental conditions or stresses. The few researchers reported that disturbance the colonisation of AMF may be significantly reduced the yield²⁴⁵⁻²⁴⁷. The apparently contradictory evidence regarding the effect of AMF on plant nutrient absorption may be connected to the increasing realisation that degree of selectivity between the host and the fungi and that different AMF have varying effects on different plant species, from strongly positive increases in nutrient uptake and or growth to strongly negative²⁴⁸⁻²⁵² though the range of results, from positive, to neutral and negative suggests dependency on the host/fungal combination²⁵³. Use of other readily soluble fertilisers, particularly N fertilisers, has also been reported to have a negative impact on AM colonisation and/or

diversity in some cases²⁵⁴⁻²⁵⁷, though not in others²⁵⁸⁻²⁵⁹. However, overuse of organic amendments, especially those high in P, such as chicken manure, may impact negatively on AMF and the precise effect of organic amendments has been shown to be unpredictable on any given soil or with any particular amendment²⁶⁰⁻²⁶². Other types of biocide can have negative, neutral or positive effects on the AM association²⁶³⁻²⁶⁴.

Though increasing crop diversity is generally beneficial to AMF, adding a non-mycorrhizal host crop can have a strongly negative impact on AM colonisation, nutrient uptake and yield of subsequent AMF reliant crops^{261, 265-268}. Inoculation experiments have shown that different AMF species produced a wide range of growth responses in the host plant, from significantly positive to significantly negative. Often the concentration of soil P influences the effectiveness of inoculation^{80,269-271}.

Conclusion

Harnessing natural biodiversity such as AMF is a biotechnological approach which counters balances the current negative image of genetically modified organisms in conventional production systems. Mycorrhizal colonisation of plants can offer considerable benefits in terms of growth, nutrient uptake and yield. The real significance of AMF connects the primary producers of ecosystems, plants, to the heterogeneously distributed nutrients required for their growth, enabling the flow of energy rich compounds required for nutrient mobilization whilst simultaneously providing conduits for the translocation of mobilized products back to their hosts. Inoculation increases biomass production, rate of transpiration, rate of photosynthesis, reduce leaf temperature and restrain the decomposition of chlorophyll. The effect of AMF on plant nutrient absorption may be connected to the increase the degree of selectivity between the host and the fungi. New molecular tools have enabled identification of AMF symbiont genes with a higher degree of resolution of SYMRK/NORK/DMI2 signal perception. However, there is a big gap in understanding of AMF and its standardization of optimum level of AMF species for physiologically sound quality tree seedling production in

tropics region especially in India. The limited studied has carried related to molecular mechanism of symbiosis and diseases /stress tolerance of AMF inoculated tree seedlings because of obligate biotrophic character of the AMF. If we will be achieve efficient use and manipulation of AMF for long-term quality improvement and productivity in forest tree nursery, our understanding of their physiology and function and their interactions with seedlings and environmental conditions needs to be improved.

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