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Arbuscular Mycorrhizal (AM) Biotechnology and its Applications

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ABSTRACT

Arbuscular mycorrhizae (AM) are one of the widely distributed types of fungi forming symbiotic associations with almost all land plants. The beneficial association of these Glomalean fungi from the phylum *Glomeromycota* with plant roots dates back to about 460 million years ago, making it the most ancient type of symbiosis. These fungi constitute a crucial functional group of the soil micro-biome by determining the efficacy of agro-ecosystem through formation of a close interface between soil and plant roots. The extra-radical mycelial network associates with plant roots to take up resources from nutrient depletion zones (especially P) in the soil and in turn receive carbohydrates from the host plant, thus influencing plant productivity, diversity and ecosystem sustainability. The symbiosis plays a key role in nutrient cycling (C, N, P), plant tolerance to abiotic and biotic stresses, formation of stable soil aggregates, and various other ecosystem functions, making it important in restoration and conservation of disturbed lands. Advances in the scientific understanding on AM symbiosis have enhanced the potential for implementation of AM biotechnological approaches in different ecosystem processes. However, it is important to support further developments for production of efficient AM inocula and its application in biofertilization of crops to guide sustainable efforts. In this chapter, various strategies for AM inoculum production including pot cultures (soil based) and *in vitro* culture and its application in production of fruit, vegetable and plantation crops and floriculture are discussed.

Keywords: Arbuscular mycorrhizal fungi, agro-ecosystem, inoculum, phosphorus, extra-radical hyphae, biofertilizer

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi represent a group of efficient soil micro biota that can greatly contribute to crop development and productivity, and ecosystem sustainability. AM fungi are able to establish a mutual symbiosis with the root organs of 80% of plant families, they directly influence the plant growth through increased uptake of available soil phosphorus (P) and other essential non-labile soil mineral nutrients, and also have indirect benefits in stabilizing soil aggregates leading to soil formation, in preventing erosion, and in alleviating plant stress caused by biotic and abiotic factors leading to improved agricultural productivity (Smith and Read, 2008; Gianinazzi *et al*., 2010). Moreover, AM fungal diversity can have a direct effect on the ecosystem by driving the structure of plant communities (van der Heijden *et al*., 1998a, 1998b), ameliorating the quality of soil by improving its aggregation and organic carbon content, and finally a positive impact on ecosystem productivity (Oehl *et al*., 2003) thus making them essential for the functioning of terrestrial ecosystems (Bedini *et al*., 2009). The beneficial effects of AM fungi on plant performance and soil health are of interest for the reclamation and re-vegetation of degraded lands and thus of importance for the sustainable management of agricultural ecosystems (Oehl *et al*., 2003; Barrios, 2007).

In the sustainable agricultural systems, the two important components: crop management and soil management play a crucial role in proper functioning of the agro-ecosystem. Crop management is usually essential to sustain the soil fertility with low agricultural inputs. This involves crop rotation and intercropping (Harinikumar *et al*., 1990) which also helps to manage effective and infective AM populations. It is also observed that prior cropping with AM fungal host plant crop can increase the fertility of a soil so that yield benefits are achieved in subsequent crops (Dodd *et al*., 1990). Integrated alternatives for different cropping systems using AM fungi are necessary to maintain the nutrient balance. The profuse use of P fertilizers and chemicals causes pollution problems and health hazards. So the use of AM fungi is being encouraged in agriculture (Dessai, 2013).

The use of AM fungi in agriculture could lead to a considerable decrease in the amount of chemical pollutants in soil and water (Giovannetti, 2001). This clearly indicates the potential of AM fungi for promoting a low chemical input agriculture (Atkinson *et al*., 2002). The recent development of molecular probes able to differentiate AM fungi within roots and soils (Jacquot *et al*., 2000; Jacquot-Plumey *et al*., 2001) opens new biotechnological perspectives for defining their population biology and therefore employ management strategies for their use in agriculture. Modern intensive agricultural practices such as chemical fertilization, pest control, continuous monoculture and tillage affect plant-AM fungal interaction and association. Describing the community of AM fungal diversity at a site is an important step in determining the effects of agricultural treatments on AM fungi and for the formulation of management strategies for these fungi. The beneficial effects of AM fungi on plant growth have led to the development of AM fungi as bioinoculants for agriculture, horticulture and forestry (Mohammad *et al*., 1995). They present valuable opportunities for current agricultural practices with regard to various biotic and abiotic stress conditions making effective utilization of these symbiotic soil fungi indispensable for sustainable agriculture (Sensoy *et al*., 2007).

The use of AM fungi as 'biofertilizers' in agriculture is becoming a worldwide phenomenon and has been successfully used in places like Taiwan, South Africa and United States (Juang, 2007). Their potential as a biofertilizer lies in their mycorrhizal benefits and plant-soil interactions, hence, their selection as inoculum for management of crops in the field are widely studied (Atkinson *et al*., 2002). The exact definition of biofertilizers remain unclear, however, they are commonly referred to as the use of beneficial soil microorganisms to improve availability and uptake of mineral nutrients required for plant growth (Vessey, 2003). In order to exploit these microbes as biofertilizers, the ecological complexity of these microbes in the mycorrhizosphere needs to be taken into consideration (Khan, 2006).

AM SYMBIOSIS AND AGRICULTURAL ECOSYSTEM SERVICES

Crop nutrition: Enhanced uptake of P is the main benefit obtained by host plants through AM association, and plant P status is often the main controlling factor in the formation of plant-fungal relationship (Graham, 2000). AM fungi can play a significant role in crop P nutrition, increasing the total uptake and in some cases P use efficiency (Koide *et al*., 2000) which is directly associated with increased growth and yield (Gosling *et al*., 2006). When AM fungal colonization is disrupted, uptake of P, plant growth and in some cases yield can be significantly reduced (Gosling *et al*., 2006).

When high concentration of available soil P is present, many crops fail to respond to colonization by native AM fungi (Ryan *et al*., 2002; Gosling *et al*., 2006). Under such conditions, the colonization of roots by AM is often suppressed (Kahiluoto *et al*., 2001). Though P uptake usually dominates the consideration of AM association, it has become increasingly apparent that AM can be important in the uptake of other nutrients by the host plant. Zinc (Zn) nutrition is most commonly reported as being influenced by AM association, though uptake of Cu, Fe, N, K, Ca and Mg have been reported as being enhanced (Smith and Read, 1997; Clark and Zeto, 2000). In some cases, it is the availability of these other nutrients, which control the formation or initiation of AM symbiosis (Ryan and Angus, 2003). AM fungi may also enhance plant uptake of N from organic sources (Hodge *et al*., 2001). AM fungi also interfere with the phytohormone balance of host plants which influences plant development (Rouphael *et al*., 2015).

Crop protection against pathogens: AM fungi also play a role in the suppression of crop pests and diseases, particularly soil-borne fungal diseases (Paulitz and Linderman, 1991; Borowicz, 2001). When plant root cell is colonized by AM fungi, the pathogen is excluded from that cell (Gosling *et al*., 2006). AM fungi also suppress pathogenic nematodes (Talavera *et al*., 2001), above ground fungal diseases (Feldmann and Boyle, 1998) and herbivores (Gange *et al*., 2002). Though the mechanisms involved are complex, changes in nutritional status, resulting in changes to leaf defensive chemicals, are likely to be involved in above ground interactions with herbivores (West, 1995). As with soil fungal pathogens, the most effective control is achieved when colonization by AM fungi takes place before pathogen attack (Sylvia *et al*., 2001).

Crop water relations: AM fungi are able to increase the host plants tolerance to water stress (Augé, 2004) and high salinity (Mohammad *et al*., 2003). Several mechanisms have been proposed to explain the effect, including increased root hydraulic conductivity, improved stomatal regulation, osmotic adjustment of the host and improved contact with soil particles through the binding effect of hyphae, enabling water to be extracted from smaller pores (Augé, 2001, 2004). Often both water and mineral nutrient uptake are higher in drought stressed mycorrhizal plants than in non-mycorrhizal plants (Srivastava *et al*., 2002).

Heavy metal tolerance: AM fungal association helps in alleviation of heavy metal induced stress (Hall, 2002) and the extent of alleviation can vary depending on the heavy metal involved, its concentration in the soil, the fungal symbiosis partner and the conditions of plant growth (Turnau and Mesjasz-Przybylowicz, 2003). AM colonization can have a significant impact on the expression of several plant genes coding for proteins involved in heavy metal tolerance and detoxification (Hildebrandt *et al*., 2007). Enhanced tolerance to specific heavy metals by fungi isolated from soils contaminated with Pb, Zn, Cd or Cu has been observed by González-Guerrero *et al*. (2005). AM fungi tolerant to increased heavy metals application readily colonize host roots despite low spore counts (Jacquot-Plumey *et al*., 2001). Secretion of glycoprotein glomalin by AM fungal hyphae also helps in heavy metal binding to soil (González- Chávez *et al*., 2004). The large surface area explored by AM fungal extra-radical hyphae creates an important sink for soil heavy metal binding since hyphae of heavy metal tolerant AM fungi display a higher affinity to heavy metals than plant cells (Joner *et al*., 2000).

Soil stability (soil structure and aggregation): AM fungi have a direct effect on soil structure, which is important in an agricultural context, where cultivations, trafficking and low levels of soil organic matter all tend to result in damaged soil structure (Leifheit *et al*., 2014). The host plant transfers as much as 20% of all fixed C to the fungal partner (Jakobsen and Rosendahl, 1990) and in agricultural soils AM fungi can produce significant biomass (Rillig *et al*., 1999), this in turn has an impact on soil structure. AM fungi bind soil micro-aggregates into larger macro-aggregates through the enmeshing effects of their hyphae (Tisdall, 1991). In addition, AM fungal hyphae secrete glomalin, an extra-cellular fungal glycoprotein. Glomalin accumulation in soil (Rillig *et al*., 2001) exerts a strong influence on soil aggregate stability and soil porosity (Rillig, 2004) there by promoting aeration and water movement, essential for better root growth and development and microbial activity (Amaranthus, 1999). General hyphal exudation and rapid hyphal turnover (Staddon *et al*., 2003) provide C to other soil microorganisms indirectly promoting aggregate stability (Jastrow *et al*., 1998). The overall effect of hyphal enmeshment and C inputs can result in significant enhancement of soil structure and stability (Bethlenfalvay *et al*., 1999).

NUTRITIONAL BENEFITS THROUGH AM FUNGAL ASSOCIATION

Phosphorus: Phosphorus is one of the essential mineral nutrients for plants. The preferred form of P absorbed by plants is ortho-phosphate (Vance, 2003). Due to the fact that P is generally non-labile, narrow depletion zones are formed around P-absorbing roots leading to restricted plant growth (Hinsinger *et al*., 2005). Therefore, plants have developed mechanisms such as beneficial associations with soil biota, to increase their access to soil P. One of the most important symbiotic associations is the formation of mycorrhizae, mutualistic symbiotic relationship between plant roots and specific soil fungi. Arbuscular mycorrhizal association usually increases the growth of the plants by enhancing the uptake of mineral nutrients especially P (Tinker, 1978). It has been reported that the rate of uptake of nutrients by mycorrhizal plants is faster than that by non-mycorrhizal plants (Son and Smith, 1988).

Effective P absorption by the external or extra-radical hyphae is related to a) formation of polyphosphates in the hyphae and thus, maintaining low internal phosphate (Pi) concentrations b) the small hyphal diameter leading to a relatively larger volume delivering P per unit surface area compared to the root surface area (Jungk and Claassen, 1989) and correspondingly 2-6 times higher P influx rate per unit length of hyphae (Jakobsen *et al*., 1992) and production of extracellular acid phosphatases which catalyze the release of P from organic complexes in soil.

Phosphatases represent a broad range of intracellular as well as soil accumulated activities that catalyze the hydrolysis of both esters and anhydrides of phosphoric acid (Speir and Ross, 1978). Phosphatase enzymes are also directly involved in the acquisition of phosphorus by plants. Their importance, however, is not always obvious. The proposition that plants with lower activities of root phosphatases may gain and use phosphorus more readily than plants with higher ones has been put forward by McLachlan (1980) who found that acid phosphatase activity was lower in plants more efficient in P-uptake than grown under P-deficient conditions. Mycorrhizal colonization had been shown to influence root phosphatase activity. Acid phosphatase may be associated with the growth and development of the fungus within the host tissue (Gianinazzi *et al*., 1979) as well as with phosphorus acquisition in the rhizosphere. Alkaline phosphatase activity specific to AM fungi have been reported (Bertheau, 1977). This enzyme activity is closely linked to both the mycorrhizal growth stimulation and the arbuscular phase of the colonization and there is strong evidence that it is of fungal origin (Gianinazzi-Pearson and Gianinazzi, 1978).

Carbon: The major fluxes in the AM symbiosis appear to be of C from plant to fungus and of P, and possibly N, from fungus to plant. Reverse C movement from fungus to plant appears only to occur in special cases where the plant has an unusually restricted C supply, most notably in achlorophyllous plants (Bidartondo *et al*., 2002). Carbon supplied from the host to the fungal symbiont is derived from plant sugars and is thought to be transported by passive efflux (Willis *et al*., 2013). The intra-radical hyphae and/or arbuscules take up hexose, a substantial amount of which is used in lipid, trehalose and glycogen synthesis before translocation to extra-radical mycelia (Bago *et al*., 2003). Up to 20% of total photosynthate, always of recent assimilate partitioned to roots, may be supplied to the fungus. Movement of lipid bodies from intra-radical to extra-radical hyphae has been imaged by real-time immunofluorescence technique (Bago *et al*., 2002a). Other work has also shown movement in the opposite direction (Bago *et al*., 2002b). Much of this C is utilized in fungal maintenance and growth and there is evidence that the AM mycelial web releases C into the mycorrhizosphere (Toljander *et al*., 2007), just as roots exude into the rhizosphere soil matrix, influencing biota populations (Jones *et al*., 2009).

Nitrogen: AM fungi also assist in the transfer of N from Organic Matter (OM) and leaf litter to host plants (Leigh *et al*., 2008). As in case with P uptake, AM fungi do not enhance the acquisition of N when present at low levels in soil (Reynolds *et al*., 2005) but can make a significant contribution to plant N requirement (Hodge and Fitter, 2010), particularly in dry soils where mobility to the direct pathway via roots is restricted (Tobar *et al*., 1994). The hyphal pathway converts inorganic N taken up from the labile pool into amino acids, and translocates it principally as arginine from extra-radical to intra-radical hyphae (Govindarajulu *et al*., 2005). Here the N is converted to inorganic N compounds (urea) before passing to the host as NH_4^+ with the resulting C skeletons from arginine breakdown being re-incorporated into the fungal C pools (Govindarajulu *et al*., 2005). AM fungal extra-radical hyphae are thought to contribute indirectly to leguminous plant N status but only in reduced P conditions, supplying essential P and micronutrients to nitrogen-fixing organisms (Smith and Read, 2008).

Uptake of other elements: The symbiosis also contributes in the uptake of other micronutrients by the host plant. Uptake of nutrients such as Na, K, Mg, Ca, B, Fe, Mn, Cu, and Zn is influenced by AM colonization (Cardoso and Kuyper, 2006; Meding and Zasoski, 2008). Mycorrhizal plants may contain higher total quantities of some elements than non-mycorrhizal plants because of their greater biomass. In general nutrient uptake is likely to be affected by P deficiency or whatever is limiting plant growth (Yano-Melo *et al*., 1999).

AM FUNGAL INOCULUM PRODUCTION AND MULTIPLICATION

Inoculum Production: AM fungal inoculum has been utilized in agriculture, horticulture, landscape restoration, and site remediation for almost two decades (Hamel, 1996). In the early 1990s, researchers described multiple ways in which AM species management would be useful for sustainable systems, including agro-systems and restoration (Bethlenfalvay and Linderman, 1992; Pfleger and Linderman, 1994). In a long-term study comparing organic and conventional agriculture, Maeder *et al*. (2002) found that AM were stimulated in organic treatments, which was correlated to enhanced system health (faunal diversity, soil stability, and microbial activity) and to increased crop efficiency.

Sources of AM inoculum: Current production systems rely on soil-based systems (plots or pots), which are not sterile and are often contaminated with other AM species, and other microbes, including pathogens (Gianninazzi and Bosatka, 2004). Non-soil based approaches include *in vitro* systems involving the use of Ri T-DNA transformed plant root organs (genetically modified with *Agrobacterium rhizogens*) to grow on media under sterile conditions. These are much cleaner, but have a limited production capacity (Declerk *et al*., 2005).

Soil based systems or pot cultures: Soil from the root zone of a plant hosting AM can be used as inoculum. Such inoculum is composed of dried root fragments or colonized root fragments, AM spores, sporocarps, and fragments of hyphae. Soil may not be a reliable inoculum unless one has some idea of the abundance, diversity, and activity of the indigenous AM species. Spores can be extracted from the soil and used as inoculum but such spores tend to have very low viability or may be dead or parasitized. In such a case, soil sample can be taken to set up a 'trap culture' using a suitable host plant to boost the number of viable spore propagules for isolation, further multiplication and also to produce pure or monospecific cultures.

Pure cultures or monospecific cultures are obtained after a known isolate of AM and a suitable host are grown together in a medium (sterilized soil/sand) optimized for development of AM association and spore formation. It consists of spores, colonized root fragments, and AM hyphae.

Host plant species: The plant grown to host AM fungi in the inoculum production medium should be carefully selected. It should grow fast, be adapted to the prevailing growing conditions, be readily colonized by AM, and produce a large quantity of roots within a relatively short time (45-60 days). It should be resistant to any pests and diseases common in the inocula production environment.

Gilmore (1968) recommended strawberry (*Fragaria* sp.) for open pot culture propagation of AM fungi. The range of plant species used since then are too numerous to list. Some common temperate host plants included *Zea mays* (corn), *Allium cepa* (onion), and *Arachis hypogaea* (peanut). Widely-used tropical hosts included *Stylosanthes* spp., *Paspalum notatum* (bahia grass) and *Pueraria phaseoloides* (kudzu) (http://invam.wvu.edu/methods/cultures/host-plant-choices).

The host plant should also be fertilized by periodic additions of a nutrient solution such as Hoagland's solution (especially - P) so as to manage the chemical composition of the medium and to regulate the formation of AM association. To ensure that most of the spores in the inoculum are mature, it is essential to grow the host plant for 12-14 weeks. The medium is then allowed to dry slowly by reducing the frequency of watering over a week and then withdrawing water completely. The inoculum can then be further multiplied.

***In vitro* systems or root organ cultures:** Ri-plasmid transformed root cultures were pioneered by Mugnier and Mosse (1987). A natural genetic transformation of plants by the ubiquitous soil bacterium *Agrobacterium rhizogenes* Conn. (Riker *et al*., 1930) produces a condition known as hairy roots. This stable transformation (Tepfer, 1989) produces Ri T-DNA transformed plant tissues that are morphogenetically programmed to develop as roots. Their modified hormonal balance makes them particularly vigorous and allows profuse growth on artificial media (Tepfer, 1989). *Daucus carota* L. (carrot) and *Convolvulus sepium* L. (bindweed) were among the earliest species to be transformed using *A. rhizogenes* Conn. (Tepfer and Tempé, 1981). For *in vitro* culture of AM fungi using Ri T-DNA roots, the disinfected AM fungal propagules (spores and colonized root fragments) are plated on to Modified Strullu Romand (MSR) media for germination after which the germinated propagules are associated with actively growing Ri T-DNA transformed roots for establishment of AM symbiosis (Bécard and Fortin, 1988).

APPLICATIONS

AM fungi and fruit crops: Many fruit tree species are dependent on AM colonization for survival and growth (Schubert and Cammarata, 1986). In *Musa* species, the beneficial effects of AM under *in vitro* conditions (Declerck *et al*., 1995) and field conditions (Sukhada, 1994) are well documented. Mycorrhizal fungi have been shown to increase growth in *Malus* seedlings, both in field and glasshouse conditions (Plenchette *et al*., 1983; Reich, 1988). Improvement of growth and mineral uptake in *Citrus* species is well documented (Menge *et al*., 1978). The response of AM fungi in strawberry (*Fragaria* x *ananassa*) has also been tested (Vestberg *et al*., 2000). Khade and Rodrigues (2008a, 2008b) recorded 18 AM fungal species belonging to four genera *viz., Acaulospora, Glomus, Gigaspora* and *Scutellospora* in mono-culture plantation of *Carica papaya*. Wang *et al*. (2013) identified 18 AM fungal species belonging to 3 different orders, *Archaeosporales* (1 species), *Diversisporales* (7 species) and *Glomerales* (10 species) from rhizosphere soils of *Citrus reticulata* Blanco (red tangerine) rootstock in hillside *Citrus* orchards. In field conditions, grapevine roots normally are colonized by AM fungi (Balestrini *et al*., 2010). Presence and identification of AM fungi has also been detected in different isolated avocado orchards in Michoacan, Mexico (Bárcenas *et al*., 2006). Soares *et al*. (2005) identified 9 native AM species *viz., Rhizophagus clarus, Glomus spurcum, Scutellospora fulgida, G. macrocarpum, G. invermaium, Acaulospora colombiana, S. pellucida, A. appendiculata* and *S. heterogama* from a passion fruit plantation in Brazil with *R. clarus* and *G. spurcum* being the most predominant species. Singh and Prasad (2006) observed maximum colonization and spore population in *Litchi* orchards from Uttar Pradesh and reported colonization by AM species belonging to the genera *viz., Glomus*, *Gigaspora*, *Rhizophagus* and *Acaulospora*. Kachkouch *et al*. (2012) evaluated mycorrhizal status in *Olea europaea* and also conducted the survey of AM species in the olive grove soils of Morocco. They recorded spores belonging to *Glomus, Entrophospora, Gigaspora, Acaulospora* and *Scutellospora*, with *Glomus* being the dominant genus. Sarwade *et al*. (2011) reported the AM association in *Annona squamosa* from Maharashtra, India. They reported the association of *Glomus* and *Acaulospora* with *A. squamosa*. Sukhada (2012) studied the diversity of AM fungi in seven root stocks of mango and found *Glomus* and *Acaulospora* to be the major genera in the rhizosphere with *Rhizophagus fasciculatus* and *Funneliformis mosseae* as the predominant AM species.

AM fungi and vegetable crop plants: Diversity of AM association in different crops is currently of great interest due to the important role played in different crops. AM fungal distribution and diversity in different plant species of a particular agro-ecological zone are important in order to evaluate the natural status of AM fungi in that region (Dessai, 2013). Many researchers reported the abundance of AM spores in rhizospheres of different crops (Friberg, 2001; Sinegani and Sharifi, 2007; Mathimaran *et al*., 2007). Franke-Snyder *et al*. (2001) compared the composition and structure of the AM fungal communities associated with *Zea mays* and

Glycine max in a conventional and two low-input farming systems for better understanding of the relationship among AM fungi present in different agricultural systems. Akond *et al*. (2008) investigated fifteen vegetable crop plant species in Bangladesh to measure their affinity in harbouring mycorrhizal fungi. Hindumathi and Reddy (2011) reported the occurrence and distribution of AM fungi and microbial flora in the rhizosphere soils of *Vigna radiata* and *Glycine max*. Sinegani and Sharifi (2007) reported that the number of AM spores was significantly higher in the rhizosphere of *Solanum lycopersicum* and *Allium cepa*. Grigera *et al*. (2007) reported that AM fungi are active during the reproductive growth stages of maize and may benefit high productivity of maize crops by facilitating P uptake. Zhao *et al*. (2010) reported that the AM fungal inoculation can reduce watermelon replant problems through effectively modifying the soil microbe population and community structure, and increasing the soil enzyme activities. Manjunath *et al*. (1981) studied the effects of AM inoculum on growth of onion. AM inoculation increased yield of chillies, tomato, capsicum and other vegetables (Mamatha and Bagyaraj, 2000). Douds *et al*. (2007) reported an increase in potato yield in a high P soil due to AM inoculation. Sheng *et al*. (2012) studied the inoculation of AM fungi and reported that intercropping with pepper improves soil quality and watermelon crop performance in a system previously managed by monoculture. AM fungus *Rhizophagus fasciculatus* enhanced fruit growth and quality of *Capsicum annuum* plants exposed to drought (Mena-Violante *et al*., 2006). Eight different pepper genotypes inoculated by two AM fungi (*R. intraradices* and *Gi. margarita*) showed greater dry weights compared to non-inoculated plants (Sensoy *et al*., 2007). Castillo *et al*. (2009) studied the effect of AM fungi on an ecological crop of *Capsicum annuum* and explained that the inoculation with native fungi decreased transplantation stress thus accelerating the maturation stage of plants and resulting in higher and better yield quality. El-Shaikh and Mohammed (2009) reported enhancement of yield of okra through AM inoculation. Lingua *et al*. (2002) studied the mycorrhizal-induced differential response to a yellow disease in tomato where symptoms induced by the phytoplasma were less severe when the plant was colonized by AM fungi which showed improved morphological parameters and reduced nuclear senescence. Mycorrhizal tomato plants had significantly less infection by *Alternaria solani* than non-mycorrhizal plants (Fritz *et al*., 2006). Inoculation of *R. fasciculatus* significantly reduced nematode population, number of galls and root knot index besides increasing the growth, plant biomass, P uptake and yield of tomato plant (Shreenivasa *et al*., 2007). Akhtar and Siddiqui (2010) studied the effect of AM fungi on the plant growth and root-rot disease of chickpea. Application of AM fungi mostly resulted in significant suppression of nematode multiplication and root galling damage in tomato and carrot over two crop cycles indicating that the AM fungi persist and remain protective against root-knot nematodes (Liu *et al*., 2012). AM fungi increase the plant growth and nutrient uptake, decrease yield losses of tomato under saline conditions and improve salt tolerance of tomato (Al-Karaki *et al*., 2001; Hajiboland *et al*., 2010). Dessai and Rodrigues (2012) conducted a survey of different vegetable crop plants cultivated in Goa to assess the associated AM fungal diversity and recorded a high spore density in *Zea mays* (95.33 spores $100g^{-1}$ soil) with *Acaulospora scrobiculata* being the dominant AM species. Dessai (2013) evaluated AM fungal diversity in vegetable crop plants of Goa, India and reported a rich diversity of AM fungal species associated with vegetable crops. Fifty one AM fungal species belonging to eleven genera *viz., Acaulospora, Glomus, Gigaspora, Funneliformis, Dentiscutata, Rhizophagus, Claroideoglomus, Racocetra, Simiglomus, Ambispora* and *Scutellospora* were recovered from the rhizosphere soil. The study also revealed a significant increase in growth and nutrient uptake in *C. annuum* and *Abelmoschus esculentus* upon inoculation with dominant indigenous AM fungal species in all the treatments compared to un-inoculated control plants.

AM fungi and plantation crops: AM fungi are widespread in agricultural systems. Numerous studies have reported the natural diversity of AM fungi in the soils of coffee orchards, as well as the presence of AM colonization in coffee roots (Theodoro *et al*., 2003; Muleta *et al*., 2007). Lopes *et al*. (1983) identified 22 AM species in coffee rhizosphere soil from a Brazilian coffee production region; with *Acaulospora* and *Glomus* as the most frequently occurring genera. These were also the predominant genera found in other coffee-cultivated soils in Venezuela, Colombia and Mexico (Riess and Sanvito, 1985; Toro-Garcia, 1987; Cruz, 1989). Other AM genera, such as *Scutellospora, Gigaspora* and *Sclerocystis*, have also been described in different coffee orchard soils (Colozzi-Filho and Cardoso, 2000). Recently, in a native forest in Ethiopia, where coffee coexists with other trees in its original ecosystem, high AM species richness has been reported, with representatives of five genera of AM. *Glomus* was the dominant genera, followed by *Gigaspora, Acaulospora, Entrophospora* and *Scutellospora* (Muleta *et al*., 2007). Sanchez *et al*. (2005) found a positive effect of AM inoculation on coffee P concentrations, which resulted in higher growth when compared with non-inoculated plants. Arbuscular mycorrhizal associations have been well reported in palms, such as *Cocos nucifera* (Thomas *et al*., 1993), arecanut (Bopaiah, 1991), and, more recently in some tropical palms (John, 1988) under natural conditions. In India, a number of fungi belonging to four genera viz., *Glomus, Gigaspora, Sclerocystis*, and *Acaulospora* have been found to form mycorrhizal associations with coconut (Harikumar and Thomas, 1991). The occurrence of a mixed population of AM has been commonly recorded from the coconut rhizosphere soils. Research on genotypic dependency of AM in coconut revealed higher colonization rate in tall varieties compared to dwarf ones (Thomas and Ghai, 1987). The quantitative and qualitative distribution of AM also varied in response to a single crop or a combination of intercrops (Ramesh, 1984). Ananthakrishnan *et al*. (2004) studied mycorrhizal association in cashew (*Anacardium occidentale*) grown in different plantation areas of south India and recorded AM species belonging to the genera *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora* in the rhizosphere soils. The species of *Glomus*, *G. aggregatum*, *G. fasciculatum* and *G. mosseae* were the most abundant in the majority of the experimental sites and hence were selected and used for experimental studies. Of the three AM species *G. fasciculatum* significantly increased shoot length, internode number and length, leaf number, stem diameter, root length and number. Karthikeyan *et al*. (2005) studied the response of tea (*Camellia sinensis*) to inoculation with six species of indigenous AM fungi, *Acaulospora scrobiculata*, *Glomus aggregatum*, *G. fasciculatum*, *G. geosporum*, *G. intraradices* and *Scutellospora calospora* under plantation nursery conditions. AM inoculated tea seedlings showed an increased growth and nutritional status over un-inoculated seedlings. The extent of growth and nutritional status enhanced by AM fungi and the mycorrhizal dependency of the host varied with the species of AM fungi. However, AM association decreased nutrient use efficiencies. Seedlings inoculated with *S. calospora* had greater biomass and seedling quality than other mycorrhizal seedlings. Given the unusual morphology of the oil palm root systems and the results from experimental studies it appears that oil palms are strongly mycorrhizal dependent (Phosri *et al*., 2010). Well-established field-grown oil palm roots are naturally heavily colonized by AM fungi (Blal and Gianinazzi-Pearson, 1990). Both the African and American oil palms (*Elaeis oleifera*) produce thick cylindrical adventitious roots and do not produce root hairs (Corner, 1966). Root hairs are used by most plants for efficient water and nutrient uptake and, therefore, oil palms are probably functionally dependent on AM fungi to obtain their nutrition (Corley and Tinker, 2003).

AM fungi and Floriculture: There are fewer studies on the association and diversity of AM fungi in ornamental flowering plants. Ranganayaki and Manoharachary (2001) studied AM colonization in *Tagetes erecta* plants under natural field conditions and found the rhizosphere soil harbouring *Acaulospora foveata, Entrophosphora* sp., *Funneliformis constrictum, R. fasciculatus, G. heterosporum, G. hoi, Sclerocystis pakistanika* and *Scutellospora nigra* among which *R. fasciculatus* was predominant. Muthukumar *et al*. (2006) studied mycorrhizal morphology and dark septate fungal associations in medicinal and aromatic plants of Western Ghats, Southern India. Radhika and Rodrigues (2010) found *Glomus maculosum, G. glomerulatum* and *Acaulospora scrobiculata* associated with *Hibiscus rosa-sinensis* while carrying out survey of AM fungal diversity in some commonly occurring medicinal plants of Western Ghats, Goa region. Yang *et al*. (2011) reported AM colonization in *Magnolia cylindrica*. Johnson *et al*. (1982) studied the effect of flower bud development in *Chrysanthemum* on AM formation and showed that carbon and nutrient allocation patterns between mycorrhizal and non-mycorrhizal plants can influence flowering. Gaur *et al*. (2000) evaluated effects of mixed AM inocula and chemical fertilizers in a soil with low P fertility on growth and flowering in *Petunia hybrida, Callistephus chinensis* and *Impateins balsamina*. An increase in P and K concentration in shoots of AM inoculated plants along with an improvement in both flower number and vegetative phase of plants was reported. Sohn *et al*. (2003) evaluated the effect of different timing of AM inoculation on rooting rate, colonization percentage and early plantlet growth at transplanting stage and successive plant growth, nutrient uptake and flower quality of *Chrysanthemum morifolium.* A significant difference in plant growth, nutrient uptake and flower quality was observed in AM inoculated plants compared to non-inoculated plants. Soil pasteurization and inoculation with AM fungi can alter plant characteristics that affect the quality and composition of corms and cut flower production in *Brodiaea laxa* (Scagel, 2004). Gange and Smith (2005) evaluated the effect of AM inoculation in three species of annual plants viz., *Centaurea cyanus, Tagetes erecta* and *T. patula* and reported that AM inoculation influenced visitation rates of pollinating insects to these plants due to increase in total plant size, flower number and size and, amount of pollen produced. Long *et al*. (2010) evaluated effects of *Gigaspora* and *Glomus* on *Zinnia elegans* and showed that mixed inoculum is not much effective in promoting growth as compared to inoculation with *Glomus* alone. Asrar and Elhindi (2011) studied the effect of *F. constrictum* on growth, pigments and P content of *Tagetes erecta* plant grown under different levels of drought stress and observed that AM inoculation positively stimulated all growth parameters. Vaingankar and Rodrigues (2012) conducted a study to screen the most efficient AM fungal bioinoculant to evaluate its possible effects on growth, yield and flower fresh weight loss in two commercially ornamental plant species *viz., Chrysanthemum morifolium* Ramat. and *Tagetes erecta* L. The study revealed that *Glomus intraradices* was the most efficient AM fungal bioinoculant that increased flower number in both the plant species. This was attributed to its ability to colonize and multiply at a faster rate than the other AM fungal species used in the study.

CONCLUSION

The search for an effective microorganism having role in seed germination, nutrient acquisition, vegetative growth, productivity and tolerance to environmental stress factors has led the researchers to explore possibilities of using AM fungi in agro-ecosystems making these myco-symbionts a pivotal factor for improvement and management of the plant production systems. Mycorrhizal fungi also play an important role in reducing the fertilizer usage. The benefits provided by AM fungi can vary depending on the AM species or strains used for inoculation and in turn can affect the crop productivity. Screening of AM fungal diversity in the field or natural environment where a native community is present is necessary so as to obtain maximum benefits through AM association.

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