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ARBUSCULAR MYCORRHIZAL (AM) FUNGI AND PLANT HEALTH

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Abstract

Arbuscular mycorrhizal (AM) fungi are ubiquitous in nature and represent the oldest and most widespread symbiosis with land plants thereby constituting a vital component of terrestrial ecosystems including horticulture and agro based ecosystems. AM fungi prominently facilitate in uptake of nutrients especially phosphorus (P) uptake in plants by the extra-radical mycorrhizal hyphae leading to better plant growth and development, but they can also perform several other functions that are equally beneficial. AM fungi improve nutrient cycling and soil quality by formation of soil aggregates thus controlling soil erosion by a better plant rooting capacity, influence plant biodiversity, help protect against pests and diseases, increase plant establishment and survival at seeding or transplanting, enhance flowering and fruiting, increase crop yield and quality, improve tolerance to drought and soil salinity, and improve the growth of plants in nutrient deficient soils or polluted environments. This study discusses AM fungal inoculum production and multiplication, role and applications of AM fungi in growth of agro-economically important plants including vegetable crops, fruit crop plants and ornamental plants.

Keywords

Mycorrhiza, inoculum, Phosphorus, agro-ecosystem, horticulture.

Introduction

Arbuscular mycorrhizal (AM) fungi (Phylum Glomeromycota) are one of the beneficial soil borne microbial symbionts found in almost all habitats. They associate mutually with plant species by colonizing their roots and developing mycelial network in the rhizosphere to facilitate uptake of nutrients (mostly immobile P) and to provide other benefits to their host plants. As obligate symbionts, the arbuscular mycorrhizal association began more than 400 million years ago with the first land plants and both the partners have coevolved since then by obtaining sustainable net benefits. These benefits can be physiological, nutritional and ecological and therefore exploiting and managing AM fungi has important and sustainable consequences for both agricultural and natural ecosystems.

AM fungi in agriculture

AM fungi form direct link with crop plants and build organic matter. This alone indirectly results in healthier growth of crops (Smith and Read, 1997). AM also bind soil aggregates resulting in increase of soil stability and structure and decrease of erosion. This can result in improved water management and increased plant productivity.

Benefits of AM are the greatest in P-deficient soils, and colonization tends to be minimized with high P concentrations. Because of its nutrient absorption abilities, it is possible that in certain environments and with the proper AM management there would be a substantial reduction of fertilizer usage, which could translate into significant monetary savings and prevent pollutant overload to the system. O' Neill *et al.*, (1991) presented a convincing argument that '*Mycorrhizal Research*' is one such area deserving extensive investigation for sustainable agriculture, primarily because mycorrhizal fungi are a crucial link between roots and soil. Undoubtedly, an improved understanding and management of the symbiosis of the plants with AM fungi in agro-ecosystems ultimately has a large social and environmental impact, particularly in low input sustainable agriculture and in tropical agro-ecosystems (Khade and Rodrigues, 2009).

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

AM fungi are obligate symbionts, growing only in association with a host plant. Current production systems therefore 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 hosts 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:

The search for effective micro-organisms having roles in seed germination, nutrient uptake, growth, productivity and tolerance to abiotic and biotic stresses has led the researchers to explore possibilities of using AM fungi in production of different crop plant species. Research in the past three decades has established the efficiency of AM fungi in crop production (Jeffries, 1987). AM fungi offer a great potential for sustainable agriculture. AM fungi play a fundamental role for the productivity and stability of horticultural and agro-ecosystems.

AM fungi and vegetable crop plants

Diversity of AM association in different crops is currently of great interest due to important role played by different crops. Distribution and diversity of AM fungi 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. Many researchers reported the abundance of AM spores in rhizospheres of different crops (Friberg, 2001; Sinegani and Sharifi, 2007; Mathimaran *et al.,* 2007). 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*. Grigera (2007) reported that AM fungi are active during the reproductive growth stages of *Z. mays* and may benefit high productivity of maize crops by facilitating P uptake. Zhao *et al.,* (2010) reported that the AM fungal inoculation can reduce *Citrullus lanatus* replant problems through effectively modifying the soil microbe population and community structure, and increasing the soil enzyme activities

Selected AM fungi have been shown to enhance the growth of numerous plants of economic importance, including vegetables, field crops, and native plants used for revegetation. AM inoculation increased yield of *Capsicum annuum, Solanum lycopersicum, Capsicum* sp. and other vegetables

(Mamatha and Bagyaraj, 2000a, b). Although AM fungi are indigenous to most soils, inoculation with these fungi has increased the yield of numerous field-grown crops, including *S. lycopersicum* (Mohandas, 1987), *Solanum tuberosum* (Duffy and Cassells, 2000), *Allium cepa* (Sharma and Adholeya, 2000) and *Piper nigrum* (Douds and Reider, 2003). 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.

Mycorrhizal fungal symbiotic relationships have many benefits to the plants. These benefits include, improved plant growth and developments, and enhanced plant tolerance to several diseases. El-Shaikh and Mohammed (2009) reported the fresh and seed yield of *A. esculentus* which was enhanced by using AM inoculants. Ban *et al.,* (2011) showed the growth and yield response of *C. lanatus* to in-row plant spacing's and mycorrhiza. AM fungi contribute to the control of plant disease, and the mechanisms by which they do so have been well documented (Whipps, 2004; Ahmed *et al.,* 2009). The presence of AM fungi in roots can reduce development of some soil borne pathogenic bacteria, fungi, and nematodes and can also induce increased tolerance to plant diseases (Liu and Chen, 2007). Mycorrhizal *S. lycopersicum* plants had significantly less infection by *Alternaria solani* than non-mycorrhizal plants (Fritz *et al.,* 2006). Inoculation of *Glomus fasciculatum* 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 on both crops indicating that the AM fungi persists and remains protective against root-knot nematodes over two crop cycles *S. lycopericum* and *Daucus carota* (Affokpon *et al.,* 2011).

Researches in AM fungi widely occur in saline soils (Aliasgharzadeh *et al.,* 2001). In recent years, studies indicated that AM fungi can increase plant growth and uptake of nutrients, decrease yield losses of *S. lycopersicum* under saline conditions and improve salt tolerance (He *et al.,* 2007; Hajiboland *et al.,* 2010). AM fungi may protect plants against salinity by alleviating the salt induced oxidative stress (Abdel Latef and Chaoxing, 2011).

AM fungi and fruit crop plants

Many fruit tree species are dependent on AM colonization for survival and growth (Covey *et al*., 1981; Powell and Santhanakrishnan, 1986; Schubert and Cammarata, 1986). Moreover, mycorrhizal fruit trees have enhanced tolerance to biotic and abiotic stresses (Menge *et al*., 1978; Guillemin *et al*., 1994a, 1994b). The importance of AM symbiosis in horticultural crop production is fairly evaluated in many fruit crops like *Citrus* (Menge *et al*., 1978; Onkarayya and Sukhada, 1993), *Malus* (Plenchette *et al*., 1981) and strawberry (*Fragaria* x *ananassa* Duchesne) (Hughes *et al*., 1978). In *Musa* species, the beneficial effects of AM under *in vitro* conditions (Declerck *et al*., 1995) and field conditions (Sukhada, 1994) are well documented. AM fungi have been shown to increase growth in *Malus* seedlings, both in field and glasshouse conditions (Plenchette *et al*., 1981, 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 (Williams *et al*., 1992; Chávez and Ferrara-Cerrato, 1990; Vestberg *et al*., 2000). First evidence of the positive influence of AM symbiosis on fruit crop production was provided by Menge *et al*. (1977), who demonstrated that the AM fungal inoculation is a pre-requisite for the establishment of *Citrus* species in biocide-treated nursery beds. Since then, a number of experiments carried out have been reviewed (Miller *et al*., 1986; Nemec, 1986; Gianinazzi *et al*., 1990 a b; Barea *et al*., 1993; Chang, 1994; Lovato *et al*., 1995 and Varma and Schuepp, 1995). In summary, the information published and recorded in the above review papers refers to vegetable and spice crops *viz.*, lettuce, onion, leek, celery, asparagus, pepper, cucumber, beans, tomato; Temperate fruit crops *viz.*, citrus, strawberry, apple, almond, peach, peach-almond hybrid, olive, grapevine, blackberry, pear, kiwifruit, raspberry, cherry-plum; Tropical plantation crops *viz.*, coffee, rubber, cacao, papaya, banana, oil palm, avocado, pineapple, passion fruit; Floricultural crops *viz.*, rose, lilac, primula, chrysanthemum, begonia, gerbera, marigold. Gracias Flor (2005) assessed the AM fungal diversity in fruit trees from Goa, India and reported a rich diversity of AM species in fruit trees. This study reported the occurrence of 15 AM species belonging to three genera *viz.*, *Acaulospora*, *Glomus* and *Scutellospora* indicating that these fungi play a vital role in the growth and survival of plant species and suggested the need for screening suitable efficient strains of AM for each of the fruit trees studied for better sustenance, increased nutrient uptake and enhanced yield and productivity. Khade and Rodrigues (2008 a, b) recorded 18 AM fungal species belonging to four genera *viz.*, *Acaulospora, Glomus, Gigaspora* and *Scutellospora* in mono-culture plantation of *C. papaya*. *Claroideoglomus claroideum* was the most frequently occurring species and was recovered throughout the study period suggesting AM fungi are well established in *C. papaya* and they exhibit variations depending on edaphic factors and seasonal patterns in the weather. Wang *et al*., (2013) identified 18 AM fungal species belonging to 3 different orders, Archaeosporales (1 spp), Diversisporales (7 spp) and Glomerales (10 spp) from rhizosphere soils of *C. reticulata* Blanco (red tangerine) rootstock in hillside *Citrus* orchards. However, they observed that in all of the surveyed orchards, *Glomus aggregatum*, *F. mosseae* and *Rhizophagus irregularis* were the dominant AM species. AM fungi have an increasingly important role in vineyard production systems, as many vineyards receive little water and are planted on less fertile soils (Schreiner, 2005). AM symbiosis of vines roots can result in increased growth (Linderman and Davis, 2001), enhanced nutrient uptake (Schreiner, 2007) and improved drought tolerance (Schreiner, 2007). Sumorok *et al*., (2011) identified eight AM species *viz., G. aggregatum, F. caledonium, C. claroideum, F. constrictum, R. irregularis, G. macrocarpum, F. mosseae* and *Gi. margarita* in rhizosphere soil of apple variety 'Gold Milenium' from Poland. Khade (1999) reported 17 AM fungal species from 7 varieties of *Musa* species sampled from three sites in North Goa, India and reported *Glomus* (13 spp) as the dominant genus followed by *Acaulospora* (3 spp) and *Gigaspora* (1 sp). Soares *et al*., (2005) identified 9 native AM species *viz., R. clarus, G. spurcum, S. fulgida, G. macrocarpum, G. invermaium, A. 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 four genera *viz., Glomus, Gigaspora, Rhizophagus* and *Acaulospora*. Sarwade *et al*., (2011) reported the AM association in *A. 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 *R. fasciculatus* and *F. mosseae* as the predominant AM species.

AM fungi and ornamental flowering plants

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* L. plants under natural field conditions and found 72% of AM fungal association with the rhizosphere soil harbouring *Acaulospora foveata*, *Entrophospora* sp., *Glomus constrictum*, *G. fasciculatum*, *G. heterosporum*, *G. hoi*, *Sclerocystis pakistanica* and *Scutellospora nigra* among which *G. fasciculatum* was predominant. Muthukumar *et al*., (2006) studied AM fungal status in 15 medicinal and aromatic plants of Western Ghats, Southern India while studying mycorrhizal morphology and dark septate fungal associations. 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) studied root colonization and the diversity of spore populations of AM fungi in rhizosphere soil samples of *Magnolia cylindrica* in Huangshan of Anhui Province, East-Central China and reported the presence of AM fungal colonization with hyphae, hyphal coils and vesicles in all root samples and rich spore density and diversity of AM fungi. Gaur and Adholeya (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 *Impatiens 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.

Ranganayaki and Manoharachary (2001) studied the impact of AM fungi on the growth of *Tagetes erecta* with native AM inoculum and *Gl. fasciculatum* was also studied and reported positive effect by both the AM treatments on plant height, root length, early flowering, number of flower heads, flower head diameter and, shoot and root dry weights and plant tissue N, P, K levels in AM treated plants over non-AM plants. Scagel (2003a) studied the effect of AM fungal inoculation on flower and corm production in *Freesia* spp. grown in sterilized or non-sterilized soil. They observed AM fungi had no influence on flower opening in the first growth cycle, but inoculated plants flowered approximately 20 days earlier than non-inoculated plants in the second growth cycle. When grown in non-sterilized soil, inoculated plants produced more leaves, flowers, inflorescences and flowers per inflorescence than non-inoculated plants. Mycorrhizal plants produced heavier daughter corms with increased number of cormlets than non-inoculated plants. In *Zephyranthes* species soil pasteurization and inoculation with *Glomus intraradices* altered flower production and bulb composition (Scagel, 2003b). Gange and Smith (2005) studied three species of annual plants *viz., Centaurea cyanus, Tagetes erecta* and *T. patula* to evaluate the effect of AM inoculation and showed that inoculation with AM fungi influence visitation rates of pollinating insects to these plants due to increase in total plant size, flower number and size and, amount of pollen produced over un-inoculated control. Gaur and Adholeya (2005) studied the response of five ornamental plant species *viz. Petunia hybrida, Tagetes erecta, Callistephus chinensis, Papaver rhoeas* and *Dianthus caryophyllus* to mixed indigenous and single isolate AM inocula in marginal soils amended with organic matter and observed that AM inoculation increased flowering only in *C. chinensis*, whereas in *P. hybrida* and *T. erecta* fewer flowers were recorded in AM inoculated plants. Scagel and Schreiner (2006) demonstrated the effect of AM inoculation on plant development, reproduction and tuber quality in *Zantedeschia* sp. by growing plants with or without AM inoculum at different rates of P supply in order to separate P mediated effects from any non P mediated effects of the mycosymbiont. It was observed that AM inoculation had organ specific effects on tuber and flower quality and productivity. Long *et al.*, (2010) evaluated effects of AM fungi on *Zinnia elegans* and the difference in colonization between *Gigaspora* and *Glomus*. They showed that mixed inoculations are not much effective in the growth promotion than the corresponding inoculation with *Glomus* alone. Asar and Elhindi (2011) studied the effect *Glomus constrictum* on growth, pigments and P content of *T. erecta* plant grown under different levels of drought stress and observed that AM inoculation positively stimulated all growth parameters such as plant growth, pigments, P content and flower quality compared to un-inoculated plants. 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 ornamental plant species of commercial importance, *Chrysanthemum morifolium* Ramat. and *Tagetes erecta* L. The treatments included uninoculated control and plants inoculated with pure cultures of AM species

(*Acaulospora laevis*, *A. scrobiculata*, *Glomus coremioides*, *G. intraradices, G. fasciculatum, G. mannihotis* and *Gigaspora albida*). Results showed that inoculation of AM species had a significant effect on plant growth and flower quantity and quality. Increased flower number in *C. morifolium* (11–22%) and *T. erecta* (13–66%) was observed upon AM inoculation compared with the uninoculated control. *Glomus intraradices* proved to be the most efficient AM fungal bioinoculant, increasing 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

AM fungi play an essential role in plant growth and plant protection. Mycorrhizal association in many cases stimulates plant growth and development while substantially reducing the use of chemical fertilizers. Plants associated with AM fungi are better resistant to environmental stresses such as drought, chilling, salinity and have improved capacity to resist and survive pathogen attacks. AM fungi also improve soil quality and texture through better plant rooting capacity. AM symbiosis is associated with a range of benefits for the plant, making it significantly important in natural and agricultural ecosystems. AM fungi are the keystone for the development of sustainable agriculture and as such there is a necessity to accelerate their incorporation as biofertilizers in agricultural production systems.

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