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ARBUSCULAR MYCORRHIZAL (AM) FUNGI FOR SUSTAINABLE AGRICULTURE

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Introduction

The term 'Mycorrhiza' was first introduced by Frank in 1885 and comprises of all symbiotic associations of soil-borne fungi with roots or rhizoids of higher plants. Allen (1991) described the fungal-plant interaction from a more neutral or microbially oriented aspect stating that 'Mycorrhiza is a mutualistic symbiosis between plant and fungus localized in a root or root-like structure in which energy moves primarily from plant to fungus and inorganic resources move from fungus to plant'. The group of fungi and plants, which are involved in the interaction, determines the type of mycorrhiza they form (Molina *et al.* 1992).

Soil microorganisms have significant impact on soil fertility and plant health. Microbial symbionts including arbuscular mycorrhizal (AM) fungi form an essential component of the soil microbial community playing a key role in overall plant growth and development. In addition to increasing the absorptive surface area of their host plant root systems, the extra-radical hyphae of AM fungi provide an increased area for interactions with other microorganisms, and an important pathway for the translocation of energy-rich plant assimilates to the soil.

AM symbiosis is the oldest (>460 million years) and most widespread type of mycorrhizal association. It is estimated that 2,50,000 species of plants worldwide are mycorrhizal. The host plants include angiosperms, gymnosperms and pteridophytes (Read *et al.* 2000). Approximately 160 fungal taxa of the order Glomales (Glomeromycota) have been described on the basis of their spore morphology (Schussler *et al.* 2001), although recent molecular analyses indicate that the actual number of AM taxa may be much higher (Vandenkoornhuyse *et al.* 2002; Daniell *et al.* 2001).

During AM symbiosis, the fungal hyphae penetrate the root cortical cell walls by formation of appresoria leading to the development of intra-radical hyphal colonization and formation of arbuscules or coils that interface with the host cytoplasm (Smith and Read 1997). The highly branched arbuscules aid in metabolic exchanges between the plant and the fungus.

AM fungi also produce vesicles, which function as storage organs (Smith and Read 1997). It has been estimated that in natural ecosystems plants colonized with AM fungi may invest 10–20% of the photo-synthetically fixed carbon in their fungal partners (Johnson *et al.* 2002). AM fungi also interface directly with the soil by producing extra-radical hyphae that may extend several centimeters out into the soil thereby helping the host plants in uptake of nutrients especially P (Rhodes and Gerdemann 1975; Read and Perez-Moreno 2003). The extra-radical mycelium of AM fungi can also enhance mobilization of organically bound nitrogen (N) from plant litter (Hodge *et al.* 2001). Hyphae of AM fungi have been shown to play an important role in soil stabilization through formation of soil aggregates by secretion of glomalin (Tisdall and Oades 1979). Glomalin is a glycoprotein produced on hyphae of AM in the soil. The indirect or 'secondary' impacts of glomalin on the formation and stabilization of soil aggregates further improved the efficiency of the symbiotic relationship and the growth environment (Andrade *et al.* 1998b; Rillig and Mummey 2006). In addition, the extra-radical hyphae may interact with other soil organisms either indirectly by changing host plant physiology, including root physiology and patterns of exudation into the mycorrhizosphere, or directly by physically and/or metabolically interacting with other organisms in the mycorrhizosphere. Other microbes, e.g. N fixing bacteria or P solubilising bacteria, may synergistically interact with AM fungi and thereby benefit plant development and growth (Puppi *et al.* 1994). AM symbiosis can also alleviate negative effects of plant pathogens (Niemira *et al.* 1996; Azcon-Aguilar and Barea 1996; Newsham *et al.* 1995; St-Arnaud *et al.* 1997) and toxic levels of metals (Khan *et al.* 2000). AM symbiosis also increases resistance to biotic and abiotic stresses and reduces disease incidence, representing a key component of sustainable agriculture (St-Arnaud and Vujanovic 2007; Subramanian and Charest 1999; Aliasgarzad *et al.* 2006).

AM fungi and Sustainable agriculture

Plant microbe interactions are the interesting events that contribute for the sustainable agriculture. Mycorrhizae are indigenous to soil and plant rhizosphere which makes them as potential tool for sustainable agriculture. The mycorrhizal symbiosis becomes even more important in sustainable agricultural systems where nutrient inputs are low. Under these circumstances AM extra-radical mycelium plays an important role in nutrient mobilization.

Soils generally contain indigenous AM fungal species that colonize plant roots (Covacevich *et al.* 1995). The growth enhancement and P uptake of plants colonized by AM fungi is a well-known process (Pfleger and Linderman 1996; Schweiger and Jakobsen 1999; Jeffries *et al.* 2003). Not all plants are dependent on mycorrhizal associations (Azcón and Ocampo 1981; Trouvelot *et al.* 1982; Hetrick *et al.* 1993). However, in most an increase in yield following inoculation with AM fungi (Jakobsen and Nielsen 1983; Baon *et al.* 1992; Talukdar and Germida 1994; Xavier and Germida 1997; Al-Karaki *et al.* 1998) particularly in low-P soils (Thompson 1990; Rubio *et al.* 2003) is observed. With the current tendency for reduced use of agrochemicals, research is being directed at crop yield improvement and yield sustainability. The efficient use of AM fungi may allow for the attainment of acceptable yield levels with minimum fertilizer dose, while also reducing costs and environmental pollution risk (Covacevich *et al.* 2007). This is a promising approach for obtaining high yields with low fertilizer inputs in order to support sustainable agricultural systems.

Sustainable agricultural systems employ natural processes to achieve acceptable levels of productivity and food quality while minimizing adverse usage of fertilizer dose, while also reducing costs and adverse impacts on environment (Harrier and Watson 2004). Sustainable agriculture must, by definition, be ecologically sound, economically viable, and socially responsible. Sustainable agriculture relies on long-term solutions using proactive rather than reactive measures at system levels. Several soil fertility factors contribute to sustainable agriculture through control of soil-borne diseases, including increased soil microbial activity leading to increased competition and parasitism within the rhizosphere (Jawson *et al.* 1993; Knudsen *et al.* 1995). Research and development strategies are presently focused on the search for suitable alternatives to the use of commercial synthetic pesticides. Progress has also been made, however, in exploring the use of microorganisms to improve soil fertility. Greater emphasis is being placed on enhanced exploitation of indigenous soil microbes which contribute to soil fertility, increased plant growth and plant protection.

Interest in AM fungal propagation for sustainable agriculture is increasing due to its role in the promotion of plant health, and improvements in soil fertility and soil aggregate stability. These fungi can be utilized effectively for increasing yields while minimizing use of pesticides and inorganic fertilizers. To improve crop production in infertile soils, chemical fertilizers have been intensively used, organic matter is incorporated and soil management technologies such as fallow or legume cultivation have also been to advance soil conditions, enhance soil biological activity and optimize nutrient cycling to minimize external inputs and maximize the efficiency of their use (Sanchez 1994). This approach has been developed for soil biota management using earthworms and micro-symbionts (Woomer and Swift 1994; Swift 1998). These soil organisms may represent more than 90% of soil biological activity and thus contribute to nutrient cycling, soil fertility and symbiotic processes in the rhizosphere. Soil fungal diversity and activity have not been adequately studied and understood (Hawksworth 1991). Mycorrhizae represent an important group because they have a wide distribution, and may contribute significantly to microbial biomass and to soil nutrient cycling processes in plants (Harley and Smith 1983). Reliance should be on biological processes by adapting germplasm beneficial to plants and thus crop productivity for sustainable agriculture (Gianinazzi-Pearson and Diem 1982; Bethlenfalvay 1992). They improve nutrient uptake, especially P, and also uptake of micronutrients such as zinc or copper; they stimulate the production of growth substances and may reduce stresses, diseases or pest attack (Sylvia and William 1992; Davet 1996; Smith and Read 1997). For appropriate use of this technology, it is necessary to select the best inocula adapted to the specific limiting environmental factors for crop productivity.

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 corn (*Zea mays*), onion (*Allium cepa*), and peanut (*Arachis hypogaea*). 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

O' Neill *et al.* (1991) presented a convincing argument that 'Mycorrhizal Research' is one such area deserving extensive investigation for sustainable agriculture, primarily because AM 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). The use of AM fungi as 'biofertilizers' in agriculture is becoming a worldwide phenomenon and has successfully been in use in places like Taiwan, South Africa and United States. Their potential as a biofertilizer lies in their mycorrhizal benefits and plant-soil interactions. Hence, their selection for inoculum and management in field situations are widely studied (Atkinson *et al.* 2002; Dodd and Thompson 1994).

Significance of AM fungi in sustainable agriculture

Researches in the past three decades have established the efficiency of AM fungi in crop production (Jeffries 1987). AM fungi play a fundamental role for the productivity and stability in horticulture and agro-ecosystems.

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 fungal species 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). 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 fungal symbiotic relationships have many benefits to the plants. These benefits include, improved plant growth and development, and enhanced plant tolerance to several diseases. The AM fungi contribute to the control of plant diseases, and the mechanisms by which they do so have been well documented (Smith *et al.* 1986; Whipps 2004). 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).

Gopal (1991) critically reviewed the role of biofertilizers on the sustainable crop productivity. Saxena and Tilak (1994) critically analyzed the role of biofertilizers on the crop productivity over all influences by AM fungi and *Azospirillum* played a vital role in supplying N and P to onion and found enhanced growth and yield over the untreated control. On-farm production of AM fungal inocula in mixtures of compost and vermiculite (Douds *et al.* 2006) was used as an amendment to horticultural potting media for the production of vegetable seedlings.

Thus there is vast scope for enhancing yield of vegetable crops with AM bioinoculants. In the view of tremendous potential for AM fungi in maintaining sustainability of agro-based ecosystems, ecological and diversity studies warrant attention. Such studies would provide information essential to attempt to use these AM fungi in sustainable agriculture.

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