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10. Arbuscular Mycorrhizae in Sustainable Agriculture

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Soil microorganisms play an important role in major processes such as soil formation and nutrient cycling of the agro-ecosystem. They form an essential link between soil nutrient availability and plant productivity as they are directly involved in the cycling of nutrients through the transformation of organic and inorganic forms of nutrients. Arbuscular mycorrhizal (AM) fungi are ubiquitous soilborne mycobionts interacting physically with plants in the rhizosphere through the formation of intra-radical (hyphae, arbuscules, vesicles) and extra-radical (hyphae, spores) structures in the host root and having a positive influence on the host plant growth and development. They are one of the beneficial soilborne microbial symbionts and associate mutually with plant species by colonizing their roots and developing mycelial network in the rhizosphere to facilitate uptake of nutrients and to provide other benefits to their host plants thereby constituting a vital component of terrestrial ecosystems including horticulture and agro-based ecosystems. The benefits obtained through AM symbiosis 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 fungal inoculum has been utilized in agriculture, horticulture, landscape restoration, and site remediation for almost two decades.

AM fungi are capable of significantly improving plant phosphate, nitrogen, and sulfur acquisition and in turn receive plant carbon leading to completion of its life cycle. In addition, they provide numerous other benefits to their host plants including improved soil quality by formation of soil aggregates thus controlling soil erosion by a better plant rooting capacity, influence plant biodiversity, help protect against insect pests and diseases, increase plant establishment and survival at seeding or transplanting, enhance flowering and fruiting, increase crop yield and quality, and improve tolerance to drought and soil salinity.

Various abiotic and biotic factors influence the distribution, growth, and functioning of AM fungi. These include soil chemistry, e.g., pH, nutrient availability, and pesticides, climatic variables, e.g., temperature, light, and precipitation, and soil structure and stability. Biotic factors are primarily linked to the composition of the plant community, which strongly influences the diversity and assembly of AM fungal communities. Other biotic factors that have been shown to influence AM fungi are root predators, plant parasites, and herbivores. Many of these abiotic and biotic factors are interrelated and interact synergistically to influence the habitat and in turn, the composition and functioning of AM fungal communities. In agricultural systems, many of these abiotic and biotic factors are modified by management techniques, which strongly impact AM fungal communities. Studies have shown that practices such as tillage and fallow, monoculture cropping, and fertilization negatively influence the abundance and diversity of AM fungi. On the other hand, AM are stimulated in organic agriculture, which enhances system health (faunal diversity, soil stability, and microbial activity) and crop efficiency. However, agro-ecosystems, in general, have a lower AM fungal diversity compared to natural ecosystems and this loss of diversity appears to be correlated with management intensity.

Host root morphology modification and development of a complex, ramifying mycelial network in soil by AM fungi results in increased plant/soil adherence and soil stability (binding action and improvement of soil structure); increased mineral nutrient and water uptake by plants through AM association promotes plant growth while reducing fertilizer requirement; buffering effect of AM fungi against abiotic stresses results in increased plant resistance to drought, salinity, heavy metals pollution, and mineral nutrient depletion; secretion of 'glomalin', i.e., AM fungal glycoprotein into the soil results in increased soil stability and water retention; protection of the host plant against root pathogens results in increased plant resistance against biotic stresses while reducing phytochemical input (disease reduction and secondary metabolism); and modification of plant metabolism and physiology through AM fungal association results in bio-regulation of plant development and increased plant quality for human health.

Current AM inoculum 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. Non-soil based approaches include in vitro systems involving the use of Ri T-DNA transformed plant root organs (genetically modified with *Agrobacterium rhizogenes*) to grow on media under sterile conditions. These are much cleaner, but have a limited production capacity.

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 knowledge 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. The culture consists of spores, colonized root fragments, and AM hyphae.

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 insect pests and diseases common in the inocula production environment. The plant species used to host AM are too numerous to list (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 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.

A natural genetic transformation of plants by the ubiquitous soil bacterium *Agrobacterium rhizogenes* produces a condition known as hairy roots. This stable transformation produces Ri T-DNA transformed plant tissues that are morphogenetically programed to develop as roots.Their modified hormonal balance makes them particularly vigorous and allows profuse growth on artificial media. 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 Modified Strullu and Romand (MSR) medium for germination after which the germinated propagules are associated with actively growing Ri T-DNA transformed roots for establishment of AM symbiosis.

AM fungal inoculum has a high potential for utilization as bio-fertilizer in agro-ecosystem management. Despite the complexity of the soil ecosystem, it is possible to manage soil AM fungal diversity with proper agricultural practices in order to promote more sustainable and productive agro-ecosystems. AM fungal field inoculation offers cost-effective and non-destructive means to improve the cultivation of crops by boosting their productivity and yield quality, and also reducing fertilizer usage. As global change for promotion of green and sustainable agriculture dictates the need for more efficient cropping systems, the management of beneficial fungi offers many opportunities.