# Chapter 5
# Perspectives on the Role of Arbuscular Mycorrhizal Fungi in the In Vivo Vegetative Plant Propagation

**Ravichandran Koshila Ravi and Thangavelu Muthukumar**

**Abstract** Vegetative propagation is an important method for increasing the productivity of economically important agricultural and horticultural plants. Apart from the application of phytohormones, beneficial microorganisms such as arbuscular mycorrhizal (AM) fungi being natural biofertilizers are also widely used in the field of horticultural production systems. The mutualistic association between the AM fungi and plant are not only known for their efficient water and nutrient uptake, less vulnerability to pathogens, and ability to withstand or tolerate abiotic and biotic stresses but are also involved in the production of plant hormones and adventitious root formation in asexual propagation. The inoculation of AM fungi to the rooting substrate could result in similar responses on the cuttings to those obtained through the application of exogenous plant growth regulators. In addition, the combined use of AM fungi along with plant hormones leads to increased root initiation and development of plant parts. The early inoculation of AM fungi onto the rooting medium enhances the plant growth rate of vegetatively propagated plant species after forming a symbiotic relationship with the plant. Moreover, a series of sequential signaling events are known to occur between AM fungi and the host plant during the development of roots. The present chapter focuses on the role of AM fungi in various types of vegetative propagation including cutting, layering, and grafting, the interaction between the plant hormones, and the AM symbiosis. The mechanism involved in the production of plant hormones through AM fungi and thereby the physiological changes occurring in the plant metabolism during propagation is also discussed.

**Keywords** Cuttings · Plant hormones · Grafting · Adventitious roots · Mycorrhizal colonization

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## 5.1 Introduction

Agriculture is the major source of food supply and places important pressure on the environment and the natural resources. Horticulture being the major part of agriculture includes the production of vegetables, ornamentals, fruits, and medicinal plants (Sonah et al. 2011). There has been a significant increase in the productivity as well as the quality of the agricultural crops obtained through several new farming technologies (Edgerton 2009). Nevertheless, there is less progress in the domestication of tree species due to long generation times, irregular production of flowers and fruits, and high prevalence of outbreeding leading to loss of genetic gain in successive generations (Leakey et al. 1994). In addition, farmers often cannot afford high-quality tree transplants, or sometimes seeds may not be available, and some plants or tree species have very low germination rates. In order to overcome these limitations, vegetative propagation method was introduced for rapid production, better quality of horticultural crops and tree species thereby greatly enhancing their yield (Davies et al. 1994; Bisognin 2011).

Plant propagation are of two types, sexual propagation and asexual propagation, of which asexual propagation is considered as an important propagation method in which vegetative parts of plants such as stems, roots, leaves, or other special vegetative structures when detached from the mother plant and placed under suitable conditions develop into novel individuals that are genetically similar to the parent plant. Vegetative propagation is also of great relevance in rapid replication of a plant species under threat with a goal to sustain certain desired characteristics (Hartmann et al. 2002). The propagation of plants involving vegetative parts is advantageous over sexual methods, as the vegetative parts are much larger when compared to seeds and consist of more reserve energy. This enables rapid, constant early growth and facilitates the young plants called clones to establish successfully in spite of extreme competition for light, water, and minerals from already existing vegetation. Therefore, vegetatively propagating perennials can flourish over a wide range of dense plant communities. For example, some grassland weeds like creeping buttercup and stinging nettle invade vigorously through vegetative methods (Forbes and Watson 1992).

The vegetative organs of plants in the wild always prefer to propagate in an environment that is favorable for its growth. Mostly, it circumvents waterlogged or dry soil and heavily compacted area. Hence it is generally site-selective in nature. In contrast, seed dispersal is often a random process in sexual propagation. As the new individual plants or offsprings are produced through purely mitotic cell divisions in vegetative propagation, they are genetically similar to the parent plant, and genetic recombination does not take place (Forbes and Watson 1992). Therefore, the successful plants with genetically identical characteristics suitable to its environment propagate to develop well-adapted offsprings for many generations. Plant propagation through vegetative means is beneficial to agriculturists and horticulturists as they could raise crops and ornamentals that do not produce viable seeds. For instance, one of the initial and major developments in the agricultural system was the production of important crop species such as grapes and figs through the insertion of the base of their woody stems into the ground to develop the adventitious roots and thus regenerating into new plants (Steffens and Rasmussen 2016). Several crop species like strawberries, potatoes, onion, etc. are well developed under natural condition through vegetative propagation method (Megersa 2017).

Besides several advantages, vegetative propagation is not easy or cheap when compared to propagation through seeds. Further, no hybrid or a new variety of plants could be raised by this propagation method (Mckey et al. 2010). The multiplication of vegetative organs could lead to overcrowding of individuals around the parent plant and invariably results in competition for resources like water and nutrients. In natural conditions, vegetatively propagated plants allow only short-range spread. In addition, as there is no genetic variation, plants can lose their vigor easily (Mckey et al. 2010). For example, if a plant is vulnerable to any specific pathogen or disease, all its offsprings produced by the mother plant are also equally vulnerable thus leading to the destruction of the whole plant population in a very short period of time.

The most common method of vegetative propagation includes cutting that is obtained by stem, leaf, or root, layering, grafting or through specialized organs such as tuber, rhizome, or bulbs (Megersa 2017). Of these, propagation by cuttings is the easiest, cheapest, and suitable method for a wide range of herbaceous and woody plant species. When the plant material is scarce or in order to raise a particular plant species rapidly, leaf cuttings or leaf bud cuttings are of great significance. Further, stem cuttings are placed into the growing substrate so as to produce rooting and other vegetative parts and thus developing into a new intact plant. Some of the plants do not root easily by cutting. Such type of plants can be propagated through layering where the propagated plant part is rooted when still remain attached to the mother plant and the sap flow does not get disturbed (Preece 2003). Moreover, forest tree species and other tropical fruits can be propagated through grafting technique in which two parts of the living plant, scion and rootstock, are grafted together that unite and develop into a new plant (Pina and Errea 2005). These different types of propagation techniques have both advantages and disadvantages of their own.

The vegetative propagation of plants through above-mentioned methods could be improved by the application of plant growth regulators for quick and early regeneration of plant parts (Păcurar et al. 2014; Adekola et al. 2012). Apart from plant growth regulators, some of the beneficial soil microorganisms also play a vital role in upraising plants through vegetative propagation techniques (Du Jardin 2015). Among several soil microbes, arbuscular mycorrhizal (AM) fungi act as an eco-friendly biostimulant that has a significant role in horticulture crops (Rouphael et al. 2015). Apart from numerous positive effects, AM fungi also play a vital role in the formation of adventitious roots when supplemented to the rooting substrate in most of the plant species (Scagel 2004a, b; Fatemeh and Zaynab 2014), thus contributing to the vegetative propagation of plants. Therefore, in the present chapter, we outline the importance and effect of AM fungal application on the regeneration and development of plant species through different methods of vegetative propagation (cutting, grafting, and layering). The interactions between plant hormones and AM fungal symbiosis and the mechanism through which AM fungi enhance the growth of clones raised by vegetative propagation techniques is also discussed.

## 5.2 Arbuscular Mycorrhizal Fungi

Mycorrhizal symbiosis is a mutualistic association between the soil fungi and plant roots. About 80% of the land plant roots forms a symbiotic association with the AM fungi which supports the host plant by providing essential nutrients in exchange for carbohydrates provided by the host plant (Smith and Read 2008). The AM fungal symbiosis is not limited to space within the roots, as the AM fungi produce extraradical mycelium that explores the soil surrounding plant roots. Arbuscular mycorrhizal fungi are characterized by the presence of two important structures: arbuscules and vesicles (Fig. 5.1). The AM fungal hyphae colonize the cortical cells of roots forming a highly branched structure within the cells called arbuscules that function as a site for nutrient exchange (Berruti et al. 2015). The fungal hyphae originating from roots extend into the adjacent soil where they scavenge nutrients especially phosphorus (P) and transfer it to the host plants (Smith and Read 2008). Vesicles are the storage organ developed by the AM fungi in the form of terminal or intercalary hyphal swellings in the root cortical regions consisting of cytoplasm and lipids (Biermann and Linderman 1983). They are inter- or intracellular and are generally initiated after the formation of arbuscules, however, continue to develop even after the formation of arbuscules has ceased. Spores of AM fungi consist of lipids and are covered by multilayered cell wall allowing them to be viable for long duration and thereby are important propagules for initiating new colonization (Brundrett 1991).

Although AM fungal spore can germinate in the absence of the host plant, they fail to form a wide mycelial network and cannot complete their lifecycle without forming an association with the plant host (Porcel et al. 2012). In low fertile soils, AM fungi enhance the crop productivity by improving the uptake of immobile nutrients other than P such as zinc (Zn) and copper (Cu). Mycorrhizal fungi absorb nitrogen (N) from ammonia and transport to the host and enhance the crop productivity in soils of low potassium (K), calcium (Ca), and magnesium (Mg) content (Liu et al. 2002). There is an increasing body of literature exhibiting the beneficial aspects of AM fungi that include improved plant growth, increased acquisition of nutrients and water, tolerance to salinity, drought and metal toxicity, resistance against root pathogens, and maintaining of the soil structure and fertility (Harrier and Watson 2004; Rillig and Mummey 2006; Smith and Smith 2012; Yang et al. 2015).

Further, AM fungi are the important component of rhizosphere soil microbial community and have a positive effect on both soil and plant under natural ecosystem. They promote modifications in the chemical and biological properties of plants under stressed conditions. In addition, AM fungi are widely used as bioinoculants in most of the agricultural crops, thus in turn contributing to sustainable agricultural

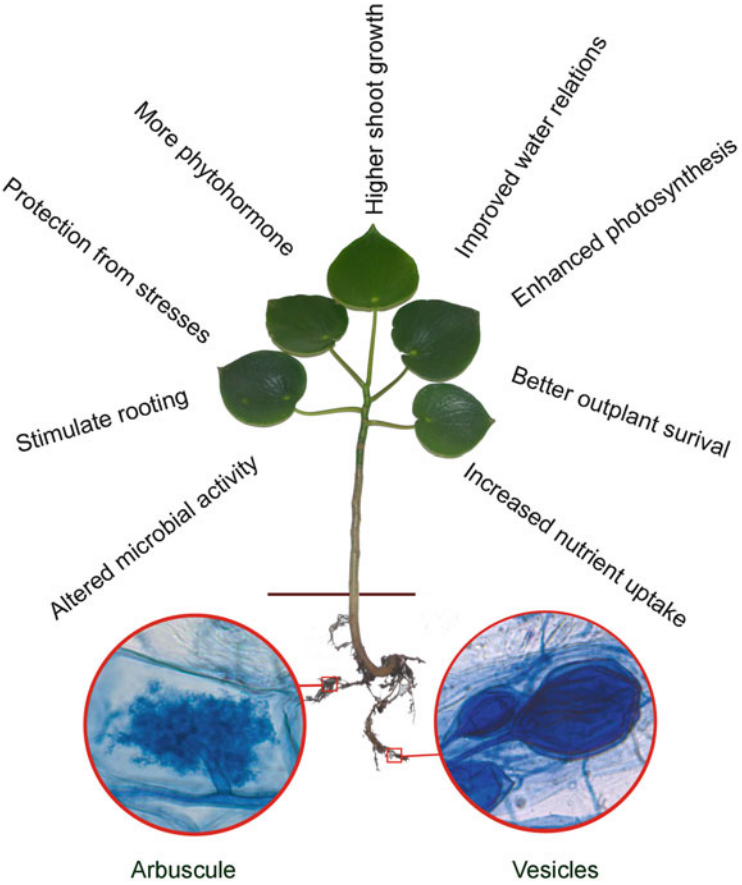

**Fig. 5.1** Various plant benefits in response to arbuscular mycorrhizal (AM) symbiosis in vegetatively propagated clones. The important AM fungal structures, arbuscules, and vesicles are also shown within red circles

practices (Berruti et al. 2015). Apart from these positive effects, AM fungi are of great significance in the field of plant propagation as they stimulate the development of root system, enhance photosynthesis, produce more plant hormones, protect the plants from various stresses, and help in the successful establishment of young plants under natural conditions with improved output survival (Fig. 5.1).

## 5.3 Effect of AM Fungi on Cuttings

Arbuscular mycorrhizal fungi help in plant's adaptation by promoting the survival and establishment of rooted cuttings (Fatemeh and Zaynab 2014). The inoculation of AM fungi into the rooting medium during propagation by cuttings enhances the rooting ability in different plants (Linderman and Call 1977; Singh 2002; Scagel 2004a, b). The response to AM fungal inoculation by the different plant cultivars propagated through cuttings is presented in Table 5.1. However, the efficiency of the AM symbiosis differs depending upon the AM fungal species and the ability of plant species to form roots (Scagel 2004b). For example, inoculation of *Prunus maritima* Marshall cuttings (hardwood and softwood) with three different AM fungal species, *Funneliformis mosseae* (= *Glomus mosseae*), *Claroideoglomus etunicatum* (= *Glomus etunicatum*), and *Glomus diaphanum*, in sterilized soil induced increased adventitious root growth. Of these, *F. mosseae* was more efficient in adventitious root production (Zai et al. 2007). Nevertheless, the method followed for plant propagation through cuttings does not permit mycorrhizal formation naturally as the rooting medium or substrate is generally sterilized to avoid interference of pathogens or soilless substrates that lack AM fungi are used (Essahibi et al. 2017) (Table 5.1). The quality of cutting, rooting medium, and the environmental condition are important factors for successful rooting of the cuttings. An ideal root medium allows good aeration, avoid water logging, and maintain moisture content and improved and higher root development (Washa et al. 2012).

The application of AM fungi into the rooting medium in the greenhouses could be helpful for the growth of propagating plants in outdoor conditions after transplantation. The early inoculation of cuttings with AM fungi during the formation of adventitious roots benefits the plant growth (Scagel et al. 2003). The response of olive cuttings to inoculation with two AM fungal species *Rhizophagus irregularis* (= *Glomus intraradices*) and *F. mosseae* in the nursery and under field conditions exhibited increased plant growth and yield. Further, pre-inoculation of AM fungi into the field enhanced the plant growth response through the early establishment of symbiosis in clones raised in sterilized substrates (Estaun et al. 2003). Nevertheless, the effect of pre-inoculation treatment reduces over time as the seedlings get colonized with the indigenous AM fungi in the field (Siqueira et al. 1998; Estaun et al. 2003).

Successful establishment of clonal plants in an environment depends on the ability of the clones to produce a large volume of roots, superior root length and clonal vigor (Washa et al. 2012). The mycorrhizal fungal inoculation improves the root growth characteristics of plant species propagated by cuttings. Moreover, Wimalarathne et al. (2014) reported greater root architecture such as root biomass, root length, root volume, and root mean diameter in *Piper nigrum* L. rooted cuttings inoculated with different quantities of *F. mosseae* inoculums in a sterilized rooting medium comprising of top soil, cattle manure, and river sand. Similarly, both runner and orthotropic shoots of *P. nigrum* inoculated with mycorrhizal fungi [*Rhizophagus fasciculatus* (= *Glomus fasiculatum*), *Gigaspora margarita*, and *Acaulospora laevis*] induced higher root growth characteristics when compared to the uninoculated and indole butyric acid (IBA)-treated *P. nigrum* cuttings (Thanuja et al. 2002). Plants of *Origanum vulgare* L., *Origanum onites* L., *Mentha piperita* L., *Mentha spicata* L., and *Mentha viridis* L. raised by stem cuttings when transferred to sterile rooting medium containing *C. etunicatum* propagules had increased the plant growth, nutrients, and production of essential oil (Karagiannidis

**Table 5.1** Response of plant species vegetatively propagated through cuttings to the presence of arbuscular mycorrhizal (AM) fungi

| Plant species | Plant part | Rooting medium | AM fungal species | Observations | References |
|---|---|---|---|---|---|
| *Actinidia deliciosa (A. Chev.) C.F.Liang & A. R.Ferguson* | Stem (Hardwood cuttings) | Steam-sterilized peat-perlite mixture | *Funneliformis mosseae* | Greater mycorrhizal colonization; variations in the morphology of fungal development within the roots | Calvet et al. (1989) |
| *Arctostaphylos uva-ursi* (L.) Spreng. | Stem (softwood cuttings) | Sterilized peat moss/coarse sand | *Rhizophagus intraradices* | Increased rooting | Nelson (1987) |
| *Ceratonia siliqua* L. | Stem | Sterilized peat | *F. mosseae*, *Rhizophagus fasciculatus*, *R. intraradices* | Increased rooting; improved growth and physiology | Essahibi et al. (2017) |
| *Chrysanthemum morifolium* Ramat. | Stem | Sterilized soil | *Glomus* sp. | Increased plant height, leaf area, root length, and fresh and dry weight of shoots, and roots; improved flowering quality and micro- and macronutrient uptake | Sohn et al. (2003) |
| *Coleus aromaticus* Benth. | Stem | Sterilized soil | *R. fasciculatus* | Increased leaf numbers and branches; total biomass, N and P contents | Earanna et al. (2001) |
| *Cyclamen persicum* Mill. var. Rosa mit Auge | Stem | Peat-based medium | *F. mosseae*, *R. intraradices*, *Funneliformis geosporum*, *Funneliformis claroideum* | Decreased plant mortality percentage, increased plant height, produced more leaves and flowers | Dubsky et al. (2002) |
| *Dalbergia melanoxylon* Guill. & Perr. | Stem treated with auxin IBA | Steam-sterilized soil/sand | *Glomus versiforme* | Increased rooting and root parameters of middle and basal cutting positions | Ezekiel Amri (2015) |
| *D. melanoxylon* | Stem/rooted stem | Sterilized soil | Indigenous mycorrhizal species | Improved rooting | Washa et al. (2012) |
| *Euphorbia pulcherrima* Willd. ex Klotzsch | Stem | Peat-based medium | *F. claroideum*, *F. mosseae*, *R. intraradices*, *F. geosporum* | Decreased plant mortality, increased plant height, number of leaves and flowers | Dubsky et al. (2002) |

(continued)

**Table 5.1** (continued)

| Plant species | Plant part | Rooting medium | AM fungal species | Observations | References |
|---|---|---|---|---|---|
| *E. pulcherrima* | Stem | Sterilized vermiculate | *Gigaspora margarita* | Stimulated rooting, increased number and weight of roots; amolerated transplant shock under high temperature and low moisture | Barrows and Roncadori (1977) |
| *E. pulcherrima* | Stem | Perlite, peat-based substrate | *R. intraradices* | Promoted adventitious roots formation; accumulated more carbohydrate in leaves and stems | Druege et al. (2006) |
| *Malus pumila* Miller | Rootstock | Mixture of carbonized rice husk and coconut fiber | *Glomus* sp. | Enhanced plant growth and survival | Sbrana et al. (1994) |
| *Mentha piperita* L. | Rooted stem | Autoclaved perlite | *Claroideoglomus etunicatum* | Higher production of essential oil, improved plant growth and nutrient uptake | Karagiannidis et al. (2012) |
| *M. piperita* | Stem | Mixture of carbonized rice husk and coconut fiber | *Rhizophagus clarum*, *C. etunicatum*, *Acaulospora scrobiculata* | Increased aerial biomass and root wet matter of plant (*C. etunicatum*) | Silveira et al. (2006) |
| *Mentha spicata* L. | Rooted stem | Autoclaved perlite | *C. etunicatum* | Increased plant growth, nutrient content and essential oil production | Karagiannidis et al. (2012) |
| *Mentha viridis* L. | Rooted stem | Autoclaved perlite | *C. etunicatum* | Improved plant growth, nutrient uptake in low fertile soil, and higher essential oil production | Karagiannidis et al. (2012) |
| *Olea europaea* L. | Stem | Sterilized soil (sand/clay loam) | *R. intraradices*, *F. mosseae*, *Septoglomus viscosum* | Improved growth; protection against root-knot nematodes (*Meloidogyne incognita* and *Meloidogyne javanica*) | Castillo et al. (2006) |

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|---|---|---|---|---|---|
| *O. europaea* | Rooted stem | Sterilized vermiculate/sand | *R. intraradices* | Enhanced plant growth and improved levels of its major polyphenols | Malik et al. (2017) |
| *O. europaea* | Rooted stem (semi-woody) | Sterilized vermiculate and perlite | *F. mosseae*, *R. intraradices*, *F. claroideum* | Stimulated plant growth during both early and nursery development stages | Porras Piedra et al. (2005) |
| *Olea europaea* sub sp. *Laperrinei* Batt. & Trab | Stem | Autoclaved sand/peat | *Glomus* sp. | Enhanced plant growth of the cuttings | Sidhoum and Fortas (2013) |
| *Origanum onites* L. | Rooted stem | Autoclaved perlite | *C. etunicatum* | Enhanced plant growth, production of essential oils, root colonization and nutrient uptake | Karagiannidis et al. (2012) |
| *Origanum vulgare* L. | Rooted stem | Sterilized perlite | *C. etunicatum* | Increased plant growth, nutrient content and essential oil production | Karagiannidis et al. (2012) |
| *Pedilanthus tithymaloides* L. | Stem | Sterilized soil | *R. fasciculatus*, *F. mosseae*, *G. macrocarpum*, *R. intraradices*, *C. etunicatum*, *Acaulospora laevis*, *G. margarita* | Improved plant growth, biomass and P uptake of cuttings inoculated with *R. fasiculatus* followed by *A. laevis* | Kadam et al. (2011) |
| *Pelargonium zonale* L. | Rooted stem | Peat/biochar | *R. intraradices*, *F. mosseae* | Improved plant growth, lower electrolyte leakage, increased relative water content and chlorophyll content | Conversa et al. (2015) |
| *Piper nigrum* L. | Rooted Stem | Sterilized top soil, cow dung, coir dust and sand | *F. mosseae* | Increased root length, shoot dry weight | Mala et al. (2010) |
| *P. nigrum* | Rooted stem | Sterilized top soil, cattle manure, and river sand | *F. mosseae* | Improved shoot and root development | Wimalarathne et al. (2014) |
| *P. nigrum* | Stem | Sterilized sand | *R. fasciculatus*, *G. margarita*, *A. laevis* | Enhanced rooting and root growth and P content | Thanuja et al. (2002) |

(continued)

**Table 5.1** (continued)

| Plant species | Plant part | Rooting medium | AM fungal species | Observations | References |
|---|---|---|---|---|---|
| *Podocarpus cunninghamii* Colenso | Rooted stem | Heated sand bed | *A. laevis* (indigenous AMF species), *Glomus* sp. (exotic AM species) | Improved the nutrient uptake and showed positive plant growth response (*A. laevis*) | Williams et al. (2013) |
| *Prunus cerasifera* L. | Rootstock | Mixture of carbonized rice husk and coconut fiber | *Glomus* sp. | Induced earlier growth of cuttings | Sbrana et al. (1994) |
| *P. cerasifera* | Rooted microplants | Quartz sand | *F. mosseae*, *R. intraradices* | Improved plant growth parameters, P content in shoots, branching of roots, enhanced soluble proteins in roots and mycorrhizal colonization | Berta et al. (1994) |
| *Prunus maritima* Marshall | Stem | Sterilized vermiculate/sand | *F. mosseae, Rhizophagus diaphanus*, *C. etunicatum* | Enhanced rooting and growth and uptake of macronutrients (*F. mosseae* and *C. etunicatum*) | Zai et al. (2007) |
| *Rosa hybrida* L. cv. Grand Gala | Rootstock | Mixture of perlite-coconut fiber | *F. mosseae*, *R. intraradices* | Improved plant biomass, leaf nutrients, and flower quality | Garmendia and Mangas (2012) |
| *Rosa* L. (Miniature roses) | Rooted stem | Sterilized peat/perlite (8:2) | *R. intraradices* | Enhanced root biomass and number of adventitious roots and increased stem protein content | Scagel (2004a) |
| *Rosmarinus officinalis* L. | Stem (Hardwood, semi-hardwood, soft wood cuttings) | Sterilized sand | *R. intraradices*, *F. mosseae* | Increased rooting percentage, root numbers and total root length in softwood cuttings (*R. intraradices*) | Fatemeh and Zaynab (2014) |
| *Salix purpurea* L. | Rooted stem | Sterilized sand/vermiculate | *Rhizophagus irregularis* | Promoted plant growth under Cu stress, modulated physiological and metabolic responses | Almeida-Rodríguez et al. (2015) |
| *Sciadopitys verticillata* Sieb & Zucc. | Stem | Sphagnum-based potting mix | *R. intraradices* | Increased survival, callus development, and rooting percentage | Douds et al. (1995) |

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| *Strobilanthes ciliates* Nees. | Stem | Sterilized soil/ farm yard manure | *Rhizophagus aggregatus* | Increased aboveground plant growth parameters and root colonization | Asha Thomas and Rajeshkumar (2014) |
| *Taxus* × *media* Rehder | Stem | Sterilized coarse perlite/peat moss/sand | *R. intraradices* | Stimulated root initiation, increased number of primary roots, root dry weight and growth of adventitious roots | Scagel et al. (2003) |
| *Theobroma cacao* L. | Stem | Sterilized sand | *Scutellospora*, *Glomus* sp. | Increased plant growth, N content and root colonization rates | Chulan and Martin (1992) |
| *Vitis champini* L. | Rootstock | Sterilized soil | *R. fasciculatus* | Increased plant growth and dry matter under salinity | Belew et al. (2010) |
| *Vitis rupestris* Scheele | Rootstock | Sterilized soil | *R. fasciculatus* | Increased plant growth and dry matter under salinity | Belew et al. (2010) |
| *V. riparia* ×<br>*V. rupestris* ×<br>*V. viniferax*,<br>*V. candicans* ×<br>*V. labruska* | Rootstock | Sterilized soil | *R. fasciculatus* | Increased plant growth and dry matter under salinity | Belew et al. (2010) |
| *Vaccinium meridionale* Swartz | Stem (softwood cuttings) | Autoclaved perlite | Mixture of *Glomus*, *Entrophospora*, *Scutellospora*, *Acaulospora* genera | Increased viability of cuttings | Ávila Díaz-Granados et al. (2009) |
| *Viburnum dentatum* L. | Stem | Sterilized perlite/ vermiculate | *R. fasciculatus* | Increased root development and growth | Verkade and Hamilton (1987) |
| *V. champini* | Rootstock | Sterilized soil | *R. intraradices* | Promoted plant growth, higher K and Mg concentration and K/Na ratio in leaf tissue under salinity | Khalil (2013) |
| *Vitis vinifera* L. | Rootstocks | Sterilized sand/ vermiculate | *R. aggregatus* | Higher root development, greater mycorrhizal colonization and plant performance | Aguín et al. (2004) |

et al. 2012). In addition, the uses of AM fungal soil inoculums have been reported to enhance the survival and establishment of *Khaya anthotheca* (Welw.) C. DC. cuttings and also in the restoration of plants in the degraded lands (Dugbley et al. 2015). The colonization of roots by AM fungi promotes the growth rate and nutrient uptake in clones propagated through cuttings (Sohn et al. 2003; Karagiannidis et al. 2012).

The application of indigenous AM fungi is more useful than using exotic AM fungal species for raising plants by cuttings. It has been suggested that the combination of both indigenous and exotic AM fungal species could lead to negative response on plant growth (Klironomos 2003). In support of this statement, Williams et al. (2013) found that addition of indigenous AM fungal species (*A. laevis*) to a slow-growing tree species, *Podocarpus cunninghamii* Colenso rooted cuttings, in pasteurized soil exhibited early and positive growth responses than application of exotic or commercially produced AM fungi (*Glomus* spp.). Different types of cuttings including softwood, semi-hardwood, and hardwood cuttings and also root cuttings of *Dalbergia melanoxylon* Guill. & Perr. tree raised under soil-containing AM fungi exhibited greater rooting traits thereby increasing the plant growth (Washa et al. 2012).

The adventitious root formation in cuttings is a vital process in plants that are widely propagated through vegetative methods. The formation of adventitious root in the tissues of the shoot is a complex developmental process that includes induction, differentiation, dedifferentiation, and growth of roots (Hartmann et al. 2002). It mostly depends on nutrients like carbon (C) and N and is specifically controlled by the interaction of plant hormones (Druege et al. 2004; Kevers et al. 1997). A root-colonizing endophytic fungus, *Piriformospora indica* when inoculated in root substrate with the cuttings of *Pelargonium* and Poinsettia increased the number and length of the adventitious root thereby promoting the formation of adventitious root at the higher rate of seven at the low fungal root colonization rates (Druege et al. 2006). Likewise, the inoculation of hormone-treated miniature rose cuttings with *Rhizophagus intraradices* (= *Glomus intraradices*) enhanced the root biomass and adventitious root formation before the root colonization, which suggests that AM fungi-plant signaling processes could have occurred earlier to rooting (Scagel 2004a).

## 5.4 Influence of AM Fungi on Grafting

Grafting is one of the major methods of vegetative plant propagation that has a crucial role in the development of horticultural crops which involves the production of new plants by inserting the shoot part (scion) onto the rootstock that forms the root system of the scion and generates into a new plant (Lee 1994). The rootstock influences the formation and accomplishment of the union graft. The rapid development of prominent root system is essential for the successful development of the plant, so the rootstock strongly relies on the effective root formation (Yetisir and Sari

2003). As the root system has a pronounced effect on root functions, it is important to know the influence of AM fungi on the performance of rootstock. It is observed that the initial or early inoculation of AM fungi is beneficial for the development of rootstock (Kumar et al. 2008).

Arbuscular mycorrhizal fungi influence the root morphogenesis through metabolites of AM fungi and hormones that are independent of the external supply of nutrients (Hooker et al. 1992). The effect of AM fungal species inoculation on plants through grafting method is presented in Table 5.2. In a study, Kumar et al. (2008) observed that AM fungal inoculation (*G. margarita* and *R. fasciculatus*) increased the rootstock vigor and vegetative and root parameters of mango thus contributing to successful grafting. Likewise, the rootstock of *Syzygium cuminii* L. treated with *R. fasciculatus* and *R. intraradices* when subjected to softwood grafting exhibited higher percentage of graft success and survival when compared to the uninoculated grafted *S. cuminii* (Neeraja Gandhi et al. 2010). The production of growth hormones such as auxins, gibberellins, and vitamins by AM fungi could contribute to the growth enhancement of rootstock. Furthermore, greater root geometry and increased nutrient supply mediated by AM fungi lead to the extramatrical hyphal growth that in turn improves the plant growth. The higher percentage of AM fungal root colonization enlarges the surface area for absorption and nutrient uptake in the rootstocks.

Inoculation of the AM fungal species (*A. laevis* and *C. etunicatum*) isolated from the rhizosphere soil of cashew plants from different sites improved the growth performance and the vigor of the cashew rootstock developed through grafting process. The AM fungal inoculation benefitted the grafted plants to withstand the transplant shock and to thrive well under field conditions (Lakshmipathy et al. 2004). Further, some studies have revealed an increased salinity tolerance in response to mycorrhizal inoculation of grafted plants through extension of the mycorrhizal hyphae into the substrate for higher uptake of nutrients and enhancing the root architecture parameters thereby improving the growth performance and fruit yield of grafted plants (Oztekin et al. 2013). The AM fungal root colonization varies among different grafted plant species. For example, Schreiner (2003) investigated the root colonization by AM fungi of ten different rootstocks of grapevines (*Vitis vinifera* L.) and reported only small variations in the mycorrhizal colonization of the rootstock genotype, where root length density of fine roots and AM colonization of fine roots were correlated to vigor and yield of scion. Further, AM fungal mycorrhizal colonization was related to the growth performance of the scion on varied rootstocks (Schreiner 2003).

The scion's quality and yield are gaining more interest in horticulture when compared to the rootstock which is meant for absorption. Some studies have reported that genotypes of scion exert a higher effect on AM fungal communities when compared to rootstock raised in varied types of soil (Song et al. 2015). For instance, Shu et al. (2017) conducted an experiment to find out the influence of Avocado (*Persea americana* Mill.) scions on AM fungi and development of root hairs in rootstocks and observed that scions did not have any impact on AM fungi, but scion influenced both the AM absorption and root directed pathways

**Table 5.2** Influence of arbuscular mycorrhizal (AM) fungi on grafted plants

| Plant species | Scion | Rootstock | AM fungal species | Observations | References |
|---|---|---|---|---|---|
| *Anacardium occidentale* L. | Ullal-3 (Cashew variety) | Ullal-1 | *Acaulospora laevis*, *Claroideoglomus etunicatum* | *A. laevis* increased the plant height, stem girth, and grafting success of the graft; enhanced both shoot and root P and mycorrhizal colonization rates | Lakshmipathy et al. (2004) |
| *Citrullus lanatus (Thunb.) Matsum & Nakai* | Minirossa (*C. lanatus*) | RS841 (*Cucurbita moschata* Duchesne × *Cucurbita maxima* Duchesne | *Glomus* spp. | Increased plant growth, fruit yield, and characteristics; greater grafting vigor and productivity | Miceli et al. (2016) |
| *Citrus lemonia* L. | Seedless lemon | *C. lemonia* | *Funneliformis mosseae*, *A. laevis* | Enhanced the grafting success, grafting survival, thicker stem, and greater sprout length | Barman et al. (2006) |
| *Cucumis sativus* L. | Ekron F1 (*C. sativus*) | Nimbus F1 (*Cucumber maxima* × *Cucumber moschata*) | *Glomus* spp. | Improved growth rate after transplantation and increased yield | Babaj et al. (2014) |
| *Poncirus trifoliata* L. | *P. trifoliata.* | Kumquat *Fortunella hindsii* (L.) Swingle | *Fuscutata heterogama*, *Gigaspora margarita*, *C. etunicatum*, *Acaulospora* sp. | Increased plant growth parameters | Back et al. (2016) |
| *P. trifoliata.* | *P. trifoliata* | citrange ‘Fepagro C37 Reck’ (*P. trifoliata.* × *Citrus sinensis* (L.) Osbeck.] | *F. heterogama*, *G. margarita*, *C. etunicatum*, *Acaulospora* sp. | Increased plant height, stem diameter, leaf area, root and shoot dry biomass | Back et al. (2016) |
| *Solanum lycopersicum* L. | 191 (Gokce) F1 | Maxifort and Beaufort hybrid | *Gloumus* spp. | Enhanced fruit yield, root fresh and dry weights and increased salinity tolerance | Oztekin et al. (2013) |
| *S. lycopersicum* | Milas (*S. lycopersicum*) | Efialto (*Fusarium oxysporum* wilt resistant) | *Rhizophagus intraradices* | Increased plant height and dry biomass; reduced disease incidence | Bolandnazar et al. (2014) |
| *Syzygium cuminii* L. | *S. cuminii* | *S. cuminii* | *Rhizophagus fasciculatus*, *R. intraradices*, *Sclerocystis dussii* | Higher graft take, sprout height, leaf number and stionic ratio | Neeraja Gandhi et al. (2010) |
| *Vitis vinifera* L. | *V. vinifera* | Richter 110 | *R. intraradices* | Improved leaf number, fresh weight, dry weight, plant development and increased root colonization rate | Camprubi et al. (2008) |

systematically. It is believed that the plant hormones and secondary metabolites that are produced by the leaves and shoots and then transferred to the roots are crucial for the development of root hair and AM fungal colonization (Micallef et al. 2009; Shu et al. 2017).

Several studies have highlighted the role of AM fungi in plant protection against phytopathogens. Mora-Romero et al. (2015) conducted a grafting experiment using two varied pathogens, *Sclerotinias slerotiorum* (Lib.) de Bary (fungal pathogen) infected common bean (*Phaseolus vulgaris* L.) and tomato (*Solanum lycopersicum* L.) plant infected with the bacterial pathogen (*Xanthomonas campestris pv. vesicatoria*) and raised the presence and absence of AM fungi. The results of the study showed that for both the plant pathogens, the scions originated from non-mycorrhizal plants had the capacity to exhibit disease protection induced by mycorrhizal fungi through their grafting to rootstocks inoculated with mycorrhizal fungus (*R. irregularis*) (Mora-Romero et al. 2015). Bolandnazar et al. (2014) also reported a decrease in the incidence of *Fusarium* wilt disease in tomato plants through grafting onto resistant rootstocks and mycorrhizal inoculation.

The influence of AM fungi varies according to different plant species subjected to grafting technique and the quality of scion and rootstocks. Grafting of mini watermelon (*Melothria scabra* Naudin) onto mycorrhiza inoculated hybrid variety (*Cucurbita moschata* Duchesne × *Cucurbita maxima* Duchesne) rootstocks increased the vigor, production, and quality of mini watermelon fruits. In addition, the vitamin C content in fruit was enhanced due to the increased nutrient uptake, well-developed root system in rootstocks, and production of endogenous hormones on mycorrhization (Miceli et al. 2016). The production of rootstocks of citrus species (citrange 'Fepagro C37 Reck', 'Kumquat') with AM fungal species such as *C. etunicatum*; *Fuscutata heterogama* (= *Scutellospora heterogama*); *G. margarita*; and *Acaulospora* sp. resulted in increased plant growth performance and percentage of AM fungal colonization in citrange 'Fepagro C37 Reck' when compared to the other citrus rootstock which reveals that the effect of AM fungi on vegetative development relies on rootstock species (Back et al. 2016). Moreover, different methods of grafting have also been carried out to determine the successful grafting process. For instance, cucumbers raised using different types of grafting including self-grafted, splice grafted, and root pruned splice graft and inoculated with *Glomus* spp. exhibited higher plant growth and yield. Of these three methods, root pruned splice grafted cucumber produced more yield and superior plant growth response on inoculation with indigenous AM species under greenhouse conditions (Babaj et al. 2014).

In addition to improving plant quality and performance, grafting technique has received great reputation as an important research tool, especially in studies pertaining to the signaling mechanisms between root and shoot (Gaion et al. 2018). In their classical study, Gianinazzi-Pearson and Gianinazzi (1992) showed that intergeneric grafting of lupin scions onto pea root stocks greatly reduced root colonization by *F. mosseae* and *R. intraradices* and totally prevented the development of arbuscules in the root cortical cells. Based on the results, the authors suggested the possible involvement of mobile factors originating in shoots preventing the establishment of mycorrhizal symbiosis in lupines (Gianinazzi-Pearson and Gianinazzi 1992). Foo et al. (2015) based on the intergeneric grafting experiment between lupin and pea showed that AM symbiosis and nodulation are regulated independently of each other probably due to the long-distance signaling. Further, the low strigolactone content in lupin scions grafted pea roots was suggested a possible cause for the suppression of AM symbiosis in lupin-pea graft combination.

In a greenhouse experiment, Kumar et al. (2015) investigated the influence of grafting and *R. intraradices* inoculation on the biochemical, physiological, and metabolite changes as well as gene expression analysis of tomato under two different levels of cadmium (Cd) stress. In this study, there are two graft combinations: self-grafted (*S. lycopersicum* cv. Ikram and *S. lycopersicum* cv. Ikram) and grafted onto interspecific hybrid rootstock Maxifort (*S. lycopersicum* × *S. habrochaites*). The presence of AM fungus was not able to ameliorate the effect of Cd stress and significantly increased the accumulation of Cd in the tomato shoots which subsequently decreased the growth and yield. However, plants of Ikram/Maxifort graft combination accumulated more proline, had higher antioxidant enzyme activity, and reduced lipid peroxidation. Moreover, Ikram-/Maxifort-grafted plants had higher accumulation of P, K, Ca, iron (Fe), manganese (Mn), and Zn and metabolites like fructans, inulins, and phytochelatin PC2 than Ikram/Ikram combination. The increased nutritional status of Ikram-/Maxifort-grafted plants was attributed to the upregulation of LeNRAMP3 gene in leaves (Kumar et al. 2015).

## 5.5 Mycorrhizal Fungi and Layering

Layering is one of the techniques in vegetative propagation in which a branch of the plant produces roots before it is detached from the mother plant. The successful propagation via layering depends on many factors such as moisture availability, season, the position of branching, and quality of rooting substrate and wrapping material (Mishra et al. 2017). Layering is of different types such as simple layering, compound layering, tip layering, and air layering. The combined inoculation of AM fungal species, *Scutellospora* and *Glomus*, in *Theobroma cacao* L. obtained through air layering showed an increase in dry biomass, stem diameter, and P concentration in shoots (Chulan and Martin 1992). Arbuscular mycorrhizal fungi increased the growth of Lychee (*Litchi chinensis* Sonn.) tree propagated by air layering in a soil-free substrate. In addition, AM fungi (indigenous Glomalean fungi) enhanced the copper (Cu) and Fe uptake in the Lychee (Janos et al. 2001). Moreover, the application of AM fungi along with vermicompost and *Azotobacter* as the rooting media improved the root and shoot characteristics and also the survival percentage of air layers of Lychee (Mishra et al. 2017). Furthermore, Sharma et al. (2009) also reported an enhanced total number of roots in *Litchi* air layers combined inoculated with *R. fasciculatus* and *Azotobacter* sp. The betterment in root architecture of air-layered *Litchi* trees was due to enhanced carbohydrates and metabolic activities by the rooting substrate (Mishra et al. 2017). Only very few studies have been carried out through layering propagation using AM fungal species when compared to other types of vegetative propagation. The precise mechanism of AM fungi in propagation through layering is still obscure.

## 5.6 Interaction Between Plant Hormones and AM Fungi

The relationship between the host plant root and AM fungi involves a constant exchange of signals that lead to proper symbiosis development (Gianinazzi-Pearson 1996). Arbuscular mycorrhizal fungi regulate the hormonal balance of the plant by producing growth regulators under stressed conditions (Nadeem et al. 2014). The plant hormones regulate a number of events during the developmental stage of plants and constitute signaling molecules to regulate the establishment of a symbiosis. For example, auxins regulate the shoot and root architecture of plants and also stimulate the early events thereby helping in the formation of lateral roots on the host plant (Kaldorf and Ludwig-Müller 2000). Further, abscisic acid and jasmonates are involved in the formation of arbuscules (Herrera-Medina et al. 2007). However, in the formation of spore and vesicles, no hormones have been specified so far. Thus, these alterations in the fungus development may be induced by autonomous signals of the fungi itself. In addition, phytohormones take part in the temporary defense responses that are essential for establishing a homeostasis between AM fungi and the host plant (Garcia-Garrido and Ocampo 2002). Moreover, they might also stimulate resistance against pathogens to protect the host plant (Pozo et al. 2002).

The application of AM fungal species on cuttings treated with auxins exhibited controversial results. For instance, inoculation of AM fungi and auxin on stem cuttings of *D. melanoxylon* improved the rooting ability in terms of rooting percentage and root parameters (Ezekiel Amri 2015). An increase in the levels of auxins after inoculation of AM fungi in maize and soybean plants has been observed by Kaldorf and Ludwig-Müller (2000); Meixner et al. (2005). Production of indole-3-acetic acid by *R. irregularis* was reported by Ludwig-Müller et al. (1997). Jasmonic acid is known to establish symbiotic association between plant and AM fungus by modifying the endogenous jasmonic acid through repeated wounding of the plant (Landgraf et al. 2012). One of the hormones responsible for inducing AM spore germination is strigolactones, and it acts as a signaling molecule in rhizosphere to form AM symbiosis (García-Garrido et al. 2009).

The production of abscisic acid by the AM fungal hyphae of *R. irregularis* was revealed by Esch et al. (1994). This could give rise to early signal to enhance the production of indole-3-butyric acid to increase the lateral root numbers in the young roots and thus constituting a path for the fungal entry (Kaldorf and Ludwig-Müller 2000) as the production of indole-3-butyric acid was stimulated by abscisic acid (Ludwig-Müller et al. 1995). This might be a good example which indicates that hormonal signal formed by the symbiont can affect synthesis of hormones in plants. Deficiency of abscisic acid leads to increased level of ethylene that adversely regulates mycorrhizal fungal colonization. Moreover, abscisic acid deficiency seems to downregulate the formation of arbuscules directly (Martín-Rodríguez et al. 2011).

## 5.7 Mechanism of AM Fungi in Plant Propagation

The primary mechanism accountable for plant growth is the improvement in the uptake of nutrients especially P induced by AM fungi. The production of plant hormones through these mutualistic fungi may also contribute to plant metabolic processes. Both the physiological and morphological alterations that microbial plant hormones could stimulate in the plant may help in the AM fungal symbiosis establishment and its activity, thereby resulting in the increased acquisition of nutrients by the host plants. In addition, gibberellins enhance the leaf area and lateral root formation, cytokinins play an important role in the fundamental processes of plant growth such as enhancement of photosynthetic rate, and auxins regulate the formation of roots and improve cell wall elasticity (Barea and Azcón-Aguilar 1982). Moreover, increased levels of cytokinin are reported with the association of plant roots with AM fungi thereby maintaining the chlorophyll levels and influencing the iron transport (Khade and Rodrigues 2009). The AM fungal colonization enhances the internal cytokinin levels in the colonized tissue and increases the fluxes of cytokinin to other plant parts, independent of the nutrient status of the host plant (Hirsch et al. 1997).

A series of sequential signaling events take place during various stages of plant-AM fungi interactions; however, there is no accurate information available about these signaling molecules (Roussel et al. 2001). The functioning of these molecules is examined in root-AM fungi interactions, but not between the stem and AM fungi (Scagel 2004a). In the propagation of plants obtained through cuttings, AM fungi benefit the plants when inoculation is done during the formation of the adventitious root (Fatemeh and Zaynab 2014). Moreover, the presence of precolonization signal among propagules of AM fungi and cutting is alike to those prevailing in the existence of host plant roots (Scagel 2001). This signal is activated in the cuttings of basal ends due to the release of carbon dioxide or other metabolites that was able to stimulate AM fungi propagule (Tamasloukht et al. 2003). The exudates released by the AM fungi might cause alterations in the metabolism of cuttings, thus increasing initiation of the adventitious root, thus improving the rooting ability on the cuttings on inoculation with AM fungi (Scagel 2004a). Furthermore, AM fungi induce new root formation after colonizing the root by enhancing the phenolic compound accumulation that is involved in tolerance against soilborne pathogens and also increases the water and nutrient uptake through the extraradical mycelia (Larose et al. 2002).

Arbuscular mycorrhizal symbiosis improves the ability of roots to uptake soil elements that are of low mobility through their mycelial network, thus enhancing plant growth. Inoculation of AM fungi in the soilless rooting substrate decrease the mortality percentage during transplantation and enhance the productivity of several ornamental plants through vegetative propagation (Scagel 2004a). Mostly, another mechanism behind the rooting of cuttings is ascribed to the alterations in the N, amino acid, protein, and carbohydrate metabolism occurring during the development of adventitious roots. For example, miniature roses inoculated with AM fungi showed changes in the protein and amino acid contents in the cuttings (Scagel 2004a).

The beneficial aspect of AM fungi is more noticeable in the adaptation of rooted cuttings. As already mentioned, AM fungi improved the survival of the clones through the hardening stage and protected them from transplantation shocks (Yadav et al. 2013). Arbuscular mycorrhizal fungi improve the nutrient contents and stomatal conductivity of rooted cuttings. Mycorrhization positively influences the plant's gas exchange through enhancing the stomatal conductance (Sánchez-Blanco et al. 2004), subsequently supplying a large amount of carbon dioxide assimilation to the plant and hence increasing photosynthetic process in cuttings (Essahibi et al. 2017). Arbuscular mycorrhizal fungi increase the production of secondary metabolites (Sangwan et al. 2001). The increased synthesis of secondary metabolites in AM-inoculated plants could be ascribed to the stimulation of the aromatic biosynthesis pathway. The age and developmental stages of the plant are also important during secondary metabolite production. The AM symbiosis results in increased secondary metabolism due to the higher content of chlorophyll, amino acids, and proteins (Tejavathi et al. 2011).

## 5.8 Conclusion

The application of AM fungi in raising horticulturally important crops and tree plantations through vegetative propagation techniques is of great importance. The mycorrhizal inoculation increased the viability, rooting ability, survival, and overall plant growth of the vegetatively propagated plants. It has been suggested that production of hormones by AM fungi is responsible for the stimulation of plant growth in addition to the formation of adventitious roots and improved nutrient uptake. A number of signaling events take place during the interaction between the host plant and AM fungi during root formation on cuttings (Scagel 2004a, b). Although hormone production has been recognized as the potential mechanism responsible for plant growth promotion, the exact mechanism still remains unclear. Further, the role of AM fungi in plant propagation through layering is not explored largely as for plants obtained through cuttings and grafting methods. Therefore, studies related to AM fungi and layering method could be useful in understanding their effects on plants. The use of indigenous or native AM fungal species might be considered to be beneficial than inoculation with exotic AM species, thereby improving the growth performance of plants under field conditions. Though mycorrhizal fungi enhance the plant growth through plant propagation methods, the combined application of plant hormones and other beneficial microbes such as plant growth-promoting rhizobacteria can increase the rooting of cuttings more efficiently. The application of beneficial microbes like AM fungi over chemical treatments could reduce the propagation costs in the nursery and defend against soil pathogens.

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