8 Arbuscular Mycorrhizal Technology in Sustainable Agriculture: Current Knowledge and Challenges in Agroforestry

Gökhan Boyno, Rizwan Ali Ansari, Emre Demirer Durak, Hasret Güneş, Rojbin Çevik, and Semra Demir

Abstract

In agroecosystems, arbuscular mycorrhizal fungi (AMF) are the most common and ubiquitous. Because of their productive and comprehensive symbiotic connections with plants, AM technology looks to be a viable option for sustainable agriculture and agroforestry. The commercialization of this technology may be utilized in agriculture, horticulture, and agroforestry to improve land use management and reduce the need for synthetic chemicals for plant growth and disease control. Furthermore, while mycorrhiza inoculation of plants is a well-known procedure, developing an inoculum consistently under field circumstances remains a bottleneck for their wide range of applications. Mycorrhizal inoculum generation, on the other hand, is a complicated process that necessitates commercial enterprises having the requisite biotechnological skills and capacity to react to ethical, educational, legal, and commercial needs. The aim of this chapter is to compile the available data on the theme of commercialization of AM technology as a tool and its use in increasing plant growth and yield characters.

Keywords

Sustainable agriculture · Agroforestry · Arbuscular mycorrhizal technology · AM technology · Plant symbionts

G. Boyno · E. D. Durak · H. Güneş · R. Çevik · S. Demir (✉)
Department of Plant Protection, Faculty of Agriculture, Van Yuzuncu Yil University, Van, Turkey
e-mail: semrademir@yyu.edu.tr

R. A. Ansari
Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India

R. A. Ansari et al. (eds.), *Mycorrhizal Symbiosis and Agroecosystem Restoration*,
https://doi.org/10.1007/978-981-99-5030-0_8

8.1 Introduction

Nondestructive methods for achieving low costs and high output can be mutually reinforcing in creating a viable system with low external inputs and long-term farming. This is primarily accomplished through a societal intervention that comprises an increase in crop yield, a reduction in pesticide inputs, and a social assessment of welfare and bioethical elements. The efficient use of soil microorganisms contributes to the long-term viability of agricultural ecosystems (Jeffries et al. 2003; Selosse et al. 2004; Bünemann et al. 2006; Barrios 2007; Vosátka and Albrechtová 2009; Gianinazzi et al. 2010). Growing demand for high-quality food production utilizing these eco-friendly farming techniques has led to the introduction of beneficial microorganism-based fertilizers that do not deplete the natural resource base (Ansari and Mahmood 2017a; Ansari et al. 2017a, b, 2020b). In this case, farmers will be able to utilize bio-fertilizers to boost productivity per unit area. Arbuscular mycorrhizal fungi (AMF) stand out in this group due to several mycorrhizal species colonizing at the same time. AMF species are found in 80–90 percent of all plant species known to science (Rakshit et al. 2002; Rakshit 2015). By replenishing reduced carbon (C) from plant photosynthesis and mineral nutrients like nitrogen (N) and phosphorus (P), this relationship includes a bidirectional movement of matter between symbiotic partners (Ferrol et al. 2002; Demir et al. 2015). AMF has a number of "nonnutritive" impacts on plant physiology, including lowering biotic/abiotic stress, functioning as a biocontrol agent, preventing erosion, stabilizing soil aggregates, and altering plant compatibility and the long-term survival of the entire plant–soil system (Smith and Read 2010; Ansari and Mahmood 2017b, 2019a; Ansari et al. 2020a). Therefore, AMF play a very important role not only as bio-fertilizers but also as bio-protectors and bio-regulators either in solo or in mixture with other potential beneficial microorganisms (Pal et al. 2013, 2015; Parewa et al. 2014; Boyno et al. 2022; Ansari et al. 2019a, b), which are caused by pathogens and pests (Ansari and Khan 2012a, b). This chapter entails AMF distribution, methods of multiplication and application, and commercialization at a large scale. Major prevailing challenges and possible answers have also been put forth to get the readers acquainted.

8.2 AM Technology in Sustainable Agriculture and Agroforestry

Research, commercialization, manufacture, marketing, distribution, and the application of AM inoculum are all activities that fall under the umbrella of AM technology (Benami et al. 2020). In applied mycorrhizal research for sustainable agriculture, the application of combinations of minimal effective propagation to crops, the identification of species, the development of AM technology to produce more effectively, and the assessment of mycorrhizal viability are all priorities (Vosátka et al. 2012; Guo 2019). Aside from these divisions, because of the complexity of these operations, the development and application of AM inoculums have been the

primary focus in the mycorrhizal sector. Producing cost-effective mycorrhizal inoculants has been a difficult challenge throughout the company's existence. Mycorrhizal inoculation in agricultural areas, on the other hand, has proven considerable yield advantages in various crop kinds, as recorded in several field studies (Pellegrino et al. 2015; Hijri 2016; Benami et al. 2020). However, it may be argued that the development of next-generation mycorrhizal technology should not be limited to issues about production and inoculation (Rillig et al. 2016). Given various situations in which they assist the plants with which they interact in obtaining nutrients, mycorrhizal fungi have great promise in agriculture. Despite this, their potential impacts on products are almost imperceptible, and mycorrhizae are used in a few sectors (Adholeya 2012). Regardless of potential production gains, the use of mycorrhizae for monetization is not currently on the rise. Forestry, on the other hand, is among the few sectors that fully recognize the importance of mycorrhizae in plant growth. Although mycorrhizal symbiosis is required in exotic woods, AMF are critical in agroforestry (Muleta et al. 2008; Araújo et al. 2019). Mycorrhizal infection is commonly used in a variety of different small businesses. Without mycorrhizal inoculation, orchid seedlings will not germinate in the growth media, making mycorrhizae vital for farmers and small-scale firms. Because it can handle higher levels of heavy metals including aluminium, zinc, nikel, iron, lead, and cadmium, land recovery is one of the most recent areas of commercial expansion for mycorrhiza (Pal et al. 2016).

Many sectors are assumed to be affected by the quickly changing AM technology environment, which is influenced by globalization, resistance, economic burdens, and the progress of new innovations. As the market for organic food grows, especially in developed countries, so does interest in technology (Benami et al. 2020). Instead of utilizing inorganic fertilizers, pesticides, and fungicides, inoculation of soil with mycorrhizae can increase growth and disease resistance. Inoculation of soil with an appropriate fungal isolate can also reduce the need for farmers in impoverished nations to repeat expensive fertilizer treatments that they cannot afford. However, the process of converting this concept into a viable firm is impeded by a lack of knowledge dissemination, prospective consulting services, and a lack of hope (Pal et al. 2016). Sustainable agriculture and agroforestry rely heavily on AM technology (Siddiqui and Mahmood 1996; Akhtar and Siddiqui 2008; Futai et al. 2008; Akhtar et al. 2011). Commercialization of AM based on this technology has accelerated in recent years for the following reasons:

1. Plant development and health benefits, as well as land reclamation, plant breeding, and nutrition and disease control,
2. Growing concern over soil microbes and the adoption of mycorrhizal inoculants as a viable agrochemical substitute, and
3. Giving more importance to sustainable agriculture and forestry by the society.

In essence, the commercialization of AM technology is a lengthy process that necessitates the acquisition of technical competence and compliance with legal, ethical,

educational, and business criteria. However, according to Gianinazzi and Vosátka (2004), future AM technology should address the following requirements:

1. Development of genetic or sensor technology to track AM inoculum in the field;
2. Increasing data gathering on mycorrhizae ecophysiology in stressed environments;
3. Developing a better knowledge of how mycorrhizae interact with the other soil microbes; and
4. Identifying suitable or innovative plant species with improved mycorrhizal characteristics, as well as supplementing mycorrhizae with new symbiotic properties.

8.3 Use of AM Technology in Sustainable Agriculture and Agroforestry

8.3.1 An Overview of the Market and Products

The economic potential of AM technology for agro-plant production in horticulture, agroforestry, bioremediation in degraded regions (Neill et al. 1991; Vural et al. 2018), and other parts of the plant sector has recently grown due to improved scientific knowledge of mycorrhizal symbioses (Tawaraya 2003). Because many important global food crops are highly mycorrhizal-dependent plant species, they can profit from the addition of appropriate AMF inoculums, improving global food output. Successful firms must establish crucial technical competence as well as the ability to conform to legal, ethical, educational, and marketing standards in order to construct these inoculums. Variable volumes of different fungal species, varied percentages of viable spores, and inputs like fertilizers and hydrogels, among other things, are all possibilities. Some inoculums contain just spores from a single species, whereas others have a diverse mix. When selecting commercially manufactured inoculums, it is also necessary to consider the plant's unique requirements and the current soil conditions.

During the recent decade, AMF inoculum manufacturing, related services, and marketing for the wholesaling markets have increased considerably (Singh et al. 2016; Basiru et al. 2021). Commercial producers, as well as governmental and private entities, are among the clients (Tiwari et al. 2002). While exact sales numbers have yet to be gathered, based on the worldwide biofertilizer industry, it can be determined that there is significant development potential. The worldwide biofertilizer market was valued at 787.8 million dollars in 2016 and is expected to grow to 1.65–2.31 billion dollars by 2022 (Market Analysis Report 2018). During the projected year from 2017–2025, global market demand is estimated to grow by 12.9 percent (Transparency Market Research 2018). Increased usage of biofertilizers in soil management operations, expansion of the organic food sector, and rising financial and environmental expenses connected with biofertilizers are all contributing to this tremendous surge in demand (e.g., nutrient inhibitors). Scientific proof of this plant symbionts' beneficial impacts on plant health, compatibility, and production

has fuelled the industry's growth. In addition, when suitable inoculums are created, the economic viability of AM technology becomes increasingly essential. In the present climate-sensitive agrotechnology framework, there has been market awareness that mycorrhizal crops offer a sustainable method for crop production.

8.3.2 Inoculation Strategies and Application Technology

AMF inoculation to a wide range of crop plants is critical especially in nonirrigated locations or in degraded soils where plants have much turmoil in developing root systems. New and more productive AMF isolates may now be utilized to replace the less successful native AMF isolates that are already present in the soil. When inoculated AMF are left in the soil for a long period, their impact is considered to diminish, although they can still be sporulated (Jansa et al. 2006; Rouphael et al. 2015). In the context of sustainable agriculture, it is also proposed that, while perennial plants in agroforestry areas only require one inoculation, it may also be useful to introduce newly chosen AMF isolates at optimal levels. A single propagule can colonize a root in theory, but it may take a longer period. As a result, starting many infections is the greatest way to speed up the inoculum colonization phase (Sharma et al. 1996; Adholeya et al. 2005). Furthermore, fungal propagules must be adjacent to plant roots for efficient mycorrhizal colonization. The faster the root colonization, the more AM fungal propagules are released into the root zone. The effectiveness of this in practice will, of course, be determined by the product, the setting, the distribution mechanism, and various other edaphic factors. The estimation of AMF propagules per zone or per plant is influenced by various factors: (a) the weight or volume of the packet; (b) the quantity of AMF propagules present; (c) the rate at which the inoculum is applied to seeds or soil; (d) how well the product adheres to the seed; and (e) the planting density per hectare (Adholeya et al. 2005).

Various marketed inoculums that function as natural stimulants of plant growth and development have been launched in recent years (Gousterova et al. 2008; Khan et al. 2009). These inoculums are made up of plant growth-promoting microorganisms (PGPM). A marketed inoculum may contain one or more AMF species, as well as other organisms that help the target plant acquire the required parameters, such as beneficial fungi or bacteria. In addition to AMF, two other PGPMs, plant growth-promoting rhizobacteria (PGPR) and *Trichoderma*, play a role in minimizing plant diseases and increasing plant development (Murphy et al. 2003; Harman 2006; Woo et al. 2006; Grover et al. 2011; Calvo-Polanco et al. 2016; Ilangumaran and Smith 2017). Single and mixed-production PGPMs as marketed inoculums might be a sustainable strategy to boost plant growth while reducing external inputs and increasing biotic/abiotic stress tolerance (Daranas et al. 2018).

Simultaneous inoculation with diverse strains of PGPR, *Trichoderma*, and/or AMF typically resulted in improved yield and growth due to increased nutrient absorption when compared to single inoculation (Belimov et al. 1995; Bashan et al. 2004; Kabdwal et al. 2019). In the case of PGPR (Kloepper 1996; Vassilev et al. 2001a, b; Barea et al. 2002; Akköprü et al. 2005) and N_2-fixing bacteria (Biró et al.

2000; Akköprü and Demir 2005), interactions between bacteria and AMF have positive activities in terms of nutrient absorption.

AMF and several PGPR species, including *Azotobacter, Azospirillum, Pseudomonas*, and *Bacillus* species, have been shown to have a synergistic relationship that benefits plant growth (Barea et al. 2005a). Furthermore, when mycorrhizal fungi were co-inoculated with PGPR, AMF root colonization was improved (Gamalero et al. 2004; Toro et al. 1997). Plants infected with a combination of *G. deserticola* and *Rhizobium trifoli* had four times greater nodule counts than single *R. trifoli*, resulting in grafting and increased mycorrization and nodulation with *R. trifoli* and *Yarrowia lipolytica* coencapsulated (Vassilev et al. 2001a, b). Inoculation with AMF and nodule-inducing rhizobia increased the efficiency of P and N uptake (Xavier and Germida 2003). Mycorrhizal and nodule symbiosis have been shown to have synergistic effects on plant development, mineral nutrition, and infection rate (Barea et al. 2005b). Furthermore, the consortia of AMF + *T. harzianum* (Th43) (Kabdwal et al. 2019), and AMF (*Rhizophagus fasciculatus*) + *T. viride* (talc based) (Doley and Jite 2014) boosted the growth and crop productivity. Co-inoculation of both kind of microorganisms enhanced the absorption of mineral nutrients and growth (Gryndler et al. 2002; Medina et al. 2003). PGPM inoculation with commercial biofertilizers comprising consortia of various microorganisms registered significant improvement in the plant growth and yield characters (Malusà et al. 2001; Malusà et al. 2007; Sas-Paszt et al. 2008).

All of this research shows the usefulness and increased efficiency of biofertilizers including a greater number of species with varying growth-boosting mechanisms. The availability of diverse AMF (Ijdo et al. 2011), PGPR (Lucy et al. 2004), and *Trichoderma* (Kabdwal et al. 2019) strains studied in different crop kinds and field circumstances should enable the development of commercially viable consortia. Indeed, it should not be overlooked that as a result of some consortia created, PGPMs may have a detrimental impact on each other (Boyno et al. 2022).

There are just a few techniques for delivering AMF to crops in the field. Farmers are hesitant to invest in specialist equipment for microbial-based goods. As a result, marketed inoculums should be straightforward to apply using normal agricultural gear and procedures. Therefore, the application of these commercialized inoculums can be divided into five main methods: broadcasting method, in-furrow application method, seed dressing method, root dipping method, and seedling/sapling inoculation method (Muresu et al. 2003; Adholeya et al. 2005; Malusá et al. 2012; Basiru et al. 2021).

8.3.2.1 Mycorrhizal fungi in transplanted crops

Seedlings are cultivated in either sterilized or unsterilized soil containing specific mycorrhizal fungi in a slight nursery beds or containers. They are then transplanted when the mycorrhizal colonization is well established. This approach has proven successful in generating significant and economically viable growth responses in crucial crops like tobacco, tomato, finger millet and chili (Rao et al. 1983; Sreeramulu and Bagyaraj 1986). Additionally, it has demonstrated positive outcomes in horticultural crops like citrus, mango, asters, and marigold (Viyanak and Bagyaraj 1990),

as well as in forest tree species including *Leucaena* spp., *Tamarindus indica, Acacia nilotica,* and *Calliandra calothyrsus* (Reena and Bagyaraj 1990). This methodology holds promise for application in various transplanted crops significant to agriculture, horticulture, and forestry. Further exploration is warranted to investigate the potential introduction of efficient mycorrhizal fungi to cereals through forest tree species in alley cropping system.

8.3.2.2 In-Furrow Application Method

Other methods that are actively used and promoted globally include various types of in-furrow applications (Bashan 1998; Benami et al. 2020). This approach involves placing the inoculum under or besides seeds within a furrows (Owusu-Bennoah and Mosse 1979; Hayman et al. 1981). Soil is applied to the seeds after they have been put on the inoculum. The inoculum layer will colonize the new roots when the seeds germinate. In fact, when the seeds germinate, exudates such as strigolactones, cutin monomers, and chitin-related compounds are secreted, drawing AMF to the plant (Akiyama et al. 2010; Bonfante and Genre 2015). This is important as it will encourage the formation of colonization and increase the amount of sporulation. However, it should not be ignored that some products negatively affect AMF as a result of the exudates they secrete. In particular, it has been reported that there is no symbiotic interaction between AMF and many plant species belonging to the *Brassicaceae*, *Urticaceae*, *Caryophyllaceae,* and *Chenopodiaceae* families (Brundrett 2009; Tushar and Satish 2013; Güneş et al. 2019).

As a result, the in-furrow treatment is quite effective and results in significant mycorrhizal colonization (Adholeya et al. 2005). However, it can be time-consuming when applied to wide areas (Bashan 1998).

8.3.2.3 Application of mycorrhizal fungi as a seed coating

The seed dressing method is a distinct type of inoculation technique. In this method, the inoculum contains an additive that has good adhesion qualities, such as gum acacia. This additive enhances propagule retention on the seed surface and makes seed dressing technology possible. The inoculated seeds are then allowed to dry. For long-term viability, the drying process and keeping product humidity below 5% are critical (Rivera and Fernandez 2006. Seedlings will be quickly colonized with this approach since the inoculum is in direct contact with the seed (Adholeya et al. 2005). It is also a promising approach since it takes less inoculum and little study (Sieverding 1991; Adholeya et al. 2005). In *Sorghum vulgare*, Rivera and Fernandez (2006) reported that seed dressing with marketed mycorrhizal inoculum (EcoMic) at a low dose of 10% of the stated dose resulted in greater root colonization (percent) and an increase in fungal mycelium. Furthermore, Saleh and El-Akshar (2020) demonstrated that seed dressing with AMF inoculum improved rice plant morphological development and yield, as well as resistance to *Bipolaris oryzae* disease. The most straightforward way to inoculate plants with mycorrhizal fungi would be to coat seeds with mycorrhizal inoculum, employing techniques similar to those used for *Rhizobium,* provided it consistently yields effective infection (Bagyaraj 1992). This involves applying an adhesive, such as methyl cellulose, to the seeds, to which

the inoculum is intended to adhere. Regrettably, due to their substantial size, attaching vesicular-arbuscular mycorrhizal propagules in this manner is more challenging than it is for bacteria. Nevertheless, this method has proven effective for large-seeded crops like citrus in field nurseries (Hattingh 1975).

8.3.2.4 Mycorrhizal pellets

Instead of applying vesicular-arbuscular mycorrhizal inoculum onto seeds, a more practical approach for seed inoculation is to create multiseeded pellets. These pellets, approximately 1 cm in diameter, consist of soil or peat inoculum containing vesicular-arbuscular mycorrhizae, stabilized with clay or other binding agents. The inoculum can be produced in a process that involves mixing the soil or peat with mycorrhizal spores, and forming the mixture into pellets. This method has proven to be effective in producing high infection rates of vesicular-arbuscular mycorrhizae on seeds (Hayman et al. 1981). Furthermore, Hall and Kelson (1981) described a system that can produce approximately 5000 of these infected soil pellets per person per day, with seeds attached using gum arabic as an adhesive (Koziol et al. 2017).

8.3.2.5 Fluid drilling in mycorrhiza inoculations

The seed slurry technique for vesicular-arbuscular mycorrhizal inoculation is not only effective, but also presents several advantages over other methods. Firstly, the use of a viscous fluid helps to maintain a uniform mixture of seeds and inoculum, ensuring even distribution and coverage (Hayman et al. 1981) Secondly, the reduction in the bulkiness of the inoculum makes it easier to handle and apply, which can be especially beneficial when working with large areas. Additionally, the ability to combine this technique with rhizobia inoculation provides a more comprehensive approach to promoting healthy crop growth, particularly in leguminous plants. In terms of practical implementation, this method can be scaled up to cover large areas and can be easily integrated into existing seed sowing and soil management practices. Moreover, the benefits of vesicular-arbuscular mycorrhizal associations, such as improved nutrient uptake and stress tolerance, can translate into increased crop yields and reduced inputs, resulting in more sustainable and profitable farming practices.

8.3.2.6 Pre-cropping

Populations of beneficial mycorrhizal fungi can be significantly upscaled directly within the field condition. Mycorrhizal plants are grown and allow their infected roots and associated spores to remain in the soil and colonize upcoming suitable crops. This method along with the judicious crop rotations that incorporate mycorrhizal plants and organic amendments to encourage native fungal populations, gives a promising tactic to improve the mycorrhizal population and inoculum size within the field (Bagyaraj 1990). This technique is effectively applied to enhance the population of a specific, efficient mycorrhizal fungi.

8.4 Commercialization of AM Technology

The approaches utilized in the commercialization of AM technology were classified into different categories (Siddiqui and Kataoka 2011). Important approaches for obtaining efficient AM fungal propagules have been depicted in Fig. 8.1.

8.4.1 Soil-Based Systems

The isolation of the pure culture strain of AMF using the soil-based approach involves the phases of host plant selection and growth environment optimization (Siddiqui and Kataoka 2011; Fig. 8.1). The host plants and the fungi are cultivated in a solid growth medium such as soil, vermiculite, sand, clay, perlite, or other types of mixed bark in this traditional and extensively used technique (Brundrett et al. 1996; Douds Jr et al. 2010). Traditional sand-based pot culture techniques do not generate enough mycorrhizal inoculum, and it is frequently contaminated by other bacteria. Pesticides such as Captan and Furadan, when used at half the authorized dosage in pot cultures, have been shown to reduce other microbial contaminants leaving no pernicious effect on mycorrhizal fungi (Bagyaraj 1992). It has been proven to be quite useful in creating "clean" mycorrhizal inoculum with great potential in a short amount of time (Bagyaraj 1992; Akhtar and Panwar 2011). Solid

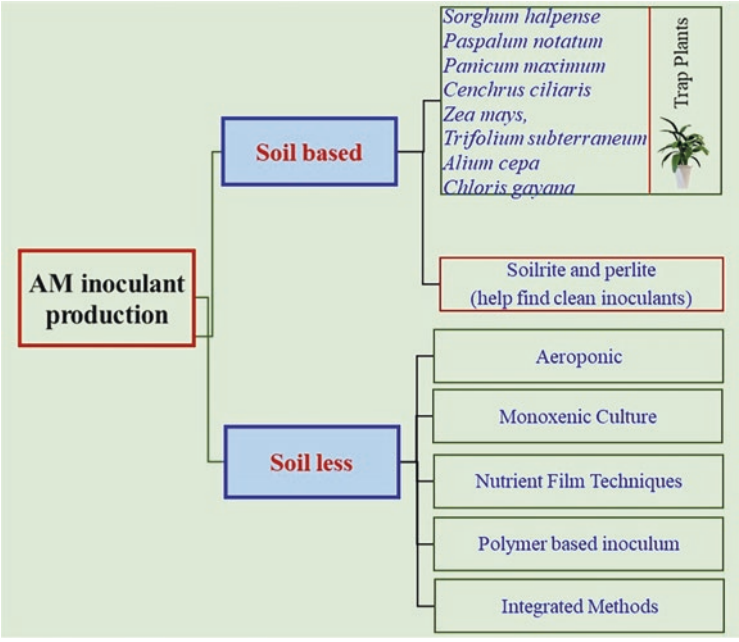

Fig. 8.1 Different methods used for large-scale production of AM fungi. The main logic of the techniques used in commercialization is to obtain a high amount of AM propagules

growth culture inoculum is also heavy, difficult to transport, and too bulky to make it ultra. Inoculum generation is affected by different particle size distributions of substrates. It is also claimed that the best substrate for optimal production has a low nutrient and carbon content (Siddiqui and Kataoka 2011). Phosphorus (P), for example, is rapidly absorbed from soil particles, resulting in Pi-free zones in the plant's rhizosphere soil. Mycorrhizal roots' extraradical hyphae stretch beyond these P-depleted areas, bringing inaccessible Pi to plants and making it available to them (Etesami and Jeong 2021). As a result, in soils low in nutrients, mycorrhizae thrive to reach these nutrients. This aspect is considered an important concept for optimum production.

8.4.2 Aeroponic Culture

It is a soil-free cultivation technique in which plant roots are sprayed with nutritional solutions on a regular or continuous basis (Jarstfer and Sylvia 1995; Mohammad et al. 2000). Several *Glomus* species have been tested through aeroponic cultivation and found promising results (Tiwari et al. 2004, 2020). An inoculum generally takes 12–15 weeks to obtain. The roots are colonized after 9 weeks, and spore production takes 12 weeks (Sylvia and Hubbell 1986; Mohammad et al. 2000). This has several drawbacks, as the system is also susceptible to other undesirable microorganisms. In addition, the nutritional solution and flow must be monitored regularly. Standardization of droplet size is required for successful aeroponic growth because the droplets must adhere to the root system for a significant amount of time. In experiments utilizing it to cultivate Bahia grass (*Paspalum notatum*) and sweet potato (*Ipomoea batatas*), a droplet size of 45 mm is optimum (Hung and Sylvia 1988; Wu et al. 1995). Because the fungus can colonize, and sporulate without a substrate, it is a one of the suitable method for obtaining enough pure AMF-propagules (Abdul-Khaliq et al. 2001).

8.4.3 Root-Organ Culture Technique (Monoxenic Culture)

Researchers have succeeded in obtaining AMF in vitro cultures using various methods (e.g., soil-based systems and aeroponic culture) (Gaur and Adholeya 1994; Aryal 2017) (Fig. 8.1). These culturing procedures result in considerable financial benefits (Aryal 2017). The root organ culture approach enables the successful and large-scale generation of mycorrhizal spores in this context (Ijdo et al. 2011). Samples are obtained from application regions or various rhizosphere soils, and AMF generation is carried out in vivo by trap plants in this approach. The most important of these trap plants is the *Zea mays* plant. Because the roots of *Z. mays* are known to be quite successful in establishing a symbiotic relationship with many AMF (Mathur et al. 2018; Hu et al. 2019), the procedure outlined by Gerdemann and Nicolson (1963) is then used to isolate healthy AMF spores from pot culture

using the wet sieving method. These spores are used to inoculate petri dishes with minimal (M) medium (Bécard and Fortin 1988).

Surface sterilization of AMF spores can be done by combining Chloramine-T with Tween-20 (0.1 percent v/v) for 10 min or washing with various antibiotic solutions. Mycorrhizal spores that have been surface-sterilized can be aseptically transplanted onto fine roots of carrots that have been converted with Ri-T-DNA and put on M medium, also known as white medium (Bécard and Fortin 1988; Adholeya et al. 2005) or Strullu-Romand (MSR) medium (Strullu and Romand 1986) (Fig. 8.1). Doner and Bécard (1991) found that the M medium in the two-compartment petri dish is deficient in sucrose, allowing spores to increase in the absence of roots. Every 15 weeks, clonally subculture the spores and root-containing media produced here in a two-compartment petri plate (St-Arnaud et al. 1996). This subcultured media should be injected with *Agrobacterium rhizogenes* bacteria to boost its growth potential (Bécard and Fortin 1988). According to Kumar and Yadav (2018), roots with 10–50 clusters of mycorrhizal spores are cut and transplanted to new receiver operating characteristic (ROC) medium plates with fresh roots in this arrangement. After 3 months of incubation at 26 °C, the spores generated on ROC plates are cut with a sterile knife and transferred to a falcon tube with 15 mL of citrate buffer. After that, the spores are shaken horizontally at 250 U/min for 60 min at 37 °C. To collect the residue at the bottom of the tube, let the spores be at room temperature for 10 min. The supernatant is then discarded, and the spores are washed with autoclaved Milli Q water, filtered through a sieve, and collected in tubes at −20 °C (Kumar and Yadav 2018).

Several species, including *Rhizophagus intraradices,* have been successfully mass-produced using AM technology. After a 4-month growth period in a single-compartment petri dish, Chabot et al. (1992) developed 750 spores in a 30 ml medium using surface-sterilized spores as starting material. After 3 months of incubation, Diop et al. (1994) got around 890 spores utilizing cut roots as the original inoculum. Jolicoeur et al. (1999) used an innovative airlift bioreactor-based manufacturing method. Cultures of the *R. intraradices* in *Daucus carota* roots were produced from spores obtained from soil, as reported by Chabot et al. (1992). Colonized root sections were transferred to a clean solid M medium in petri plates every ∼3 months for the cultivation of the root-fungus pair (Bécard and Fortin 1988). At 26 ± 1 °C, all petri plates were incubated in the dark. Mycorrhizal roots were removed, chopped into 1 cm sections using a knife, and placed into a bioreactor without the inoculum gel component. Researchers collected 12,400 spores per litre of media at the end of the operation (Jolicoeur et al. 1999). At 3–4 months, St-Arnaud et al. (1996) collected 15,000 spores in a two-compartment petri plate. Douds (2002) created this two-chamber system by periodically changing the distal, medium chamber with the new medium. This technique yielded 65,000 spores on the distal side of the two chambers over 7 months. The infective propagules of AM fungi were recovered by avoiding severe contaminations (Tiwari et al. 2002; Adholeya et al. 2005).

8.4.4 Technique of Nutrient Film (NFT)

NFT is a specialized commercial agricultural production system that recycles enormous volumes of nutrient fluid on a continuous basis on a film that runs over plant roots. MacDonald (1981) created axenic mycorrhizas between *Glomus caledonium* and *Trifolium parviflorum* and others using a small autoclave hydroponic growth system. However, Mosse and Thompson (1984) modified this method for the generation of AMF inoculum. Furthermore, Lee and George (2005) developed a modified NFT enabling large-scale AMF biomass production combining intermittent nutrient supply, optimized P source, and increased aeration with the utilization of glass beads as support materials. In addition, the average number of spores of total AMF (*G. manihotis, G. etunicatum, Glomus sp, Gigaspora margarita*, and *Acaulospora tuberculata*) was determined to be 1783–2023.30 spores/50 g (Karti et al. 2021).

The nutrient solution in the NFT system must be kept as a thin film (5–10 mm). Mycorrhizal inoculation is also affected by chemical types of nutrients. As a result, it is preferable to employ a well-balanced and appropriate composition. NFT can yield less sporulation than soil-based systems. Contamination issues with undesirable organisms often arise as a result of the nutrient solution utilized. The optimal amounts of various nutritional components vary per mycorrhizal system, based on the plant's size and other characteristics (Sharma et al. 2000). Another factor to consider is the trade-off between growing plants and mycorrhizal colonization, which is impeded by soggy conditions (Tarafdar 1995). The inoculum created by this method, on the other hand, is more concentrated and bulkier than that generated by plants growing in soil or other solid media, and it can be collected more easily (Chellappan et al. 2002; Abdul-Khaliq et al. 2001).

8.4.5 Inoculum Made of Polymers

Polymers are frequently utilized for a variety of applications in biotechnological operations. Gel materials are mostly employed to immobilize live cells, but some are also utilized as components of solid medium for microorganism maintenance.

Hydrogels are the most convenient way to apply polymer materials without having to go through the technical encapsulating process (Vassilev et al. 2005). Many hydrogels were used as transporters of AMF in root-dip and fluid-drill area and greenhouse experiments (Nemec and Ferguson 1985; Johnson and Hummel 1985); however, the pH ranges of the gel substances prevented root colonization and spore germination (Hung et al. 1991; Calvet et al. 1996; Plenchette and Strullu 2003; Jaizme-Vega et al. 2003).

Microbial cells are frequently retained or encapsulated in polymer materials as a strong immobilization technique. The purpose of this method is to keep spores or cells within porous materials created in situ surround biomaterial. Synthetic polymers are not required in mycorrhizal inoculant compositions. The transporter must be reasonably priced and suitable for the materials used in the product's construction. Natural polysaccharides and other hydrophilic hydrogels were utilized as carrier materials. Natural polysaccharides including kappa-carrageenan, agar, and

alginates come in a variety of natural, synthetic, and semi-synthetic polymer combinations. Of the roughly 1350 carrier combinations in use, calcium alginates are the most commonly utilized (Vassilev et al. 2005). Alginate beads provide more flexibility in the encapsulation and inoculation of monoxenically generated AMF (Diop 2003). Flavonoids should be included in these capsules as well (Bécard and Piché 1989; Gianinazzi-Pearson et al. 1996; Siddiqui and Kataoka 2011).

8.4.6 Integrated Method

Mycorrhizal symbiosis should be viewed as more than just a bipartite plant–fungus relationship; it should also include the related organisms (Frey-Klett et al. 2007; Tarkka and Frey-Klett 2008). The "mycorrhizosphere" is the result of these mycorrhiza-associated organisms influencing one other (Frey-Klett and Garbaye 2005). The mycorrhizosphere is made up of mycorrhizas, extramatrical mycelium, and related microorganisms. The interaction of bacterial species with AMF increases propagules (AMF structures such as spores, hyphae) and AMF colonization rates, especially in this mycorizosphere (Barea et al. 2002; Akköprü et al. 2005; Pathak et al. 2017). The use of "mycorrhizal helper bacteria (MHB)" in this context enhances AMF symbiosis in a variety of agricultural plants (Tarkka and Frey-Klett 2008).

Several researchers have examined the function of MHB in the genesis and development of various species of AMF (Siddiqui and Mahmood 1998; Vosatka et al. 1999; Frey-Klett et al. 2007; Tarkka and Frey-Klett 2008). The correct establishment of in vitro-generated plantlets in field circumstances can be achieved by combining and carefully applying AMF and PGPR. PGPR improved mycorrhizal colonization, according to Bhowmik and Singh (2004), and might be used to mass-produce AMF cultures. Silva et al. (2007) found that adding Tris–HCl buffer to the substrate improved AMF sporulation. According to these researchers, large-scale inoculum formation may be accomplished by adding Tris–HCl buffer to the nutritional solution and storing it at +4 °C.

One explanation for improved plant growth is the association of nitrogen fixers and P-solubilizers with AMF (Turk et al. 2006), and these connections are useful in increasing micropropagated plant survival rates (Webster et al. 1995). *Bradyrhizobium, Rhizobium,* and *Frankia* are microorganisms that can aid in mass-produce AMF in vitro by improving soil-binding stability, capacity, and qualities that make the soil favourable to the growth of micro-propagated plantlets like mycorrhizae (Varma and Schuepp 1995).

8.5 Challenges to Commercial Use

Even though mycorrhizal research has just achieved a critical mass, it is essential to identify the obstacles in their commercialization. The inability to develop AMF in pure culture in particular is a significant disadvantage (Sharma et al. 2017). It can only be cultivated with plants by adding inoculum under certain conditions,

according to the available knowledge, and it cannot be easily mass-produced in laboratory conditions (Sharma et al. 2017; Kadian et al. 2018). Currently, the mycorrhizal inoculum is created as another non-sterile substrate, including a non-sterile medium, soil, and propagule (spores, hyphae, and colonized root fragments) in the majority of the samples. Counterfeit bio-products are another issue in commercializing AM technology. Increased sales of counterfeit bio-products, a dearth of live quality control procedures, and fewer propagule numbers than advertised in many products all hurt AM technology (Nagpal et al. 2021). Counterfeit mycorrhizal products have a major impact on the natural resource driven products. In addition, the composition of the carrier medium and the quantity of active spores per unit weight/volume varies considerably among commercial suppliers. The fact that these fungi grow slower than other microbes, limits their use in large-scale farming. One of the challenges that mycorrhizal inoculum manufacturers confront is finding consumers in the agricultural and agroforestry sectors. In fact, in both established and emerging areas, the "organic" sector is regarded to be one of the most profitable segments in which mycorrhizal technologies may penetrate. Organic agricultural sectors are anticipated to have the largest value and profit margins, at least in industrialized countries, because marketed mycorrhizal inoculums can supplement or even replace conventional and chemical-based fertilizers (Vosátka et al. 2008). However, the market's progress is limited by a lack of awareness in prospective emerging nations, poor infrastructure, money, and a lack of knowledge of critical mycorrhizal characteristics. Plant mycorrhization in agroforestry and sustainable agriculture has drawn a lot of attention in recent years because of its role as a biofertilizer to boost host development. However, further effort is needed to identify acceptable local AM fungal strains for high-quality crop production and educate farmers in developing countries about the function of mycorrhiza in agroforestry and sustainable agricultural systems (Dobo et al. 2018). Also, due to shelf life or unclear storage stability, production constraints and technological challenges, as well as the time and labour needed to cultivate appropriate numbers of propagules, mycorrhizal markets are not very convincing (Benami et al. 2020).

8.6 Formulation of AM Technology

Today, commercialized AM technology is available in several forms. Some businesses sell a single mycorrhiza strain along with a carrier. However, most businesses sell microorganisms in the form of mixtures using different substrates.

Formulation methods account for possible negative environmental impacts as well as ingredients that might render the inoculum ineffective. To create a substance that can be efficiently transported to the intended application, a combination of microbial propagules with a variety of transporters or excipients is utilized. There have been several different mycorrhizal inoculum compositions proposed. Glass beads (Redecker et al. 1995) and expanded clay (Plenchette et al. 1983; Adholeya et al. 2005) have been utilized in research laboratories and the commercial sector, respectively. These formulations benefit from permitting the spontaneous retention

of mycorrhizal roots and spores during the growth period in greenhouse settings. Mycorrhizal invaginations can settle in the porous structure of the beads, which has many air gaps. Inoculum can also be mixed with carriers like air-dried sand, vermiculite, and soil (Millner and Kitt 1992). Liquid and powder inoculum, granules or tablets/pellets, granules and gel beads are all examples of mycorrhizal inoculum. *Glomus* spp. intraradical vesicles/spores can likewise be preserved and utilized as such in alginate beads (Redecker et al. 1995). Under controlled settings, intraradical elements isolated in such beads have been found to regenerate and colonize new roots (Strullu and Plenchette 1991). Trapping monoxenically generated spores in alginate particles has also been demonstrated to be successful (Declerck et al. 1996).

8.7 Conclusions and Future Prospects

Mycorrhizal fungi can help restore economic efficiency and environmental safety by increasing natural and managed ecosystems without depleting natural resources. They can also help lower fertilizer prices and energy demands, restoring economic efficiency and environmental protection. Appropriate mycorrhizal inoculums, on the other hand, improve biocontrol potential in a wide range of agricultural and soil characteristics in both academic and commercial settings worldwide. Under traditional agroecology or agroforestry, the main challenges in commercializing AM technology are a lack of large-scale field testing and appropriate finance. Manufacturers and distributors of mycorrhizal inoculum also confront similar problems across the world. To satisfy the needs of a broad client base, these constraints involve the need to modify products, boost market knowledge, and develop more effective distribution tactics. Concerning its commercialization plan, AM technology must be competent, efficient, and enlightening to succeed. Another requirement is to foster an entrepreneurial culture within the company, supported by excellent research infrastructure, networking, and financing. Mycorrhizal bio-fertilizers are expected to become a trustworthy partner with chemical inputs in the upcoming years, benefiting from agricultural, economic, and social perspectives. Carrier cost is a significant factor in commercial process development since the cost of the completed product grows with each stage of the manufacturing process. A suitable formulation carrier should be cheap (preferably from locally available nontoxic waste) and have no negative impacts on mycorrhizal symbiosis. It should also be simple to use and apply so that maximum dispersion is achieved. In potted plants, the formulation should allow for early breakdown or dissemination (for pellets, granules, and tablets). Because the roots and mycorrhizal propagules may not make contact if the transporter is too firmly adherent and does not disintegrate after watering, the impact may be reduced. Growth conditions should be strictly controlled, with specific care devoted to retaining the inoculum's potency. Even a minor error might cause the organism to lose viability, discouraging the end user from using these techniques in agriculture. Growth conditions should be strictly controlled, with specific care devoted to retaining the inoculum's potency. Even a little inaccuracy might result in the organism losing viability, deterring farmers from employing these approaches.

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