BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2014. Vol. **11**(3), 1199-1204

Mass Production Techniques of Arbuscular Mycorrhizal Fungi: Major Advantages and Disadvantages: A Review

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doi: http://dx.doi.org/10.13005/bbra/1506

(Received: 16 October 2014; accepted: 24 November 2014)

Numerous techniques have been developed in the past few decades for the mass production of arbuscular mycorrhizal (AM) fungi. The main obstacle behind the mass production techniques of AM fungi is the obligate nature of this biotrophic fungus and species level identification of AM fungal species could not be possible at the early stage of development. Currently, *in-vitro* cultivation methods such as hydroponic system and root organ culture has been widely used for the mass production of AM fungi. These methods are not only maintain the quality of AM fungal propagules but they can also be developed as cost effective methods for the mass propagation of AM fungi. The aim of this paper is to highlight the recent and advanced methods used for the mass production of AM fungi.

Key words: Aerophonics, AM fungi, Hydrophonics, Root Organ Culture, Starter inoculum.

Arbuscular mycorrhizal (AM) fungi are ubiquitous in distribution and occur over a wide range of agroclimatic conditions (Akhtar and Siddiqui, 2008; Akhtar and Panwar, 2011). AM fungi form symbiotic associations with the roots of almost 80% of the land plants (Akhtar and Panwar 2011; Smith and Read, 2008). Initially, they have been placed in the phylum Zygomycota, order Glomales (Redecker *et al.*, 2000), but now they have been grouped into the phylum Glomeromycota (Schusler *et al.*, 2001) and currently is comprises of about 200 described species (Brachmann, 2006).

AM fungi are characterized by the presence of their unique extra radical mycelium branched haustoria like structure within the cortical cells, known as arbuscules (Figure 1) (Smith and Read, 2008). The main role of arbuscles is to increase the surface area of roots during nutrient transfer (Akhtar *et al.*, 2011). AM fungi colonize the plant roots and penetrate into surrounding soil, extending the root depletion zone and the root system (Akhtar and Panwar, 2011). Moreover, AM fungi have improved the growth of host plant due to increased nutrient uptake, production of growth promoting substances, increase tolerance to drought, salinity and synergistic interactions with other rhizospheric microbes (Akhtar and Siddiqui, 2008). The aim of this article is to highlight the common methods used for the mass production techniques of AM fungi and the major advantages and disadvantages of the particular AM propagating method.

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Mass production of AM fungi

There are three major well know systems adopted widely in the mass production of AM

fungi. These are substrate based production system, substrate free production system and *in-vitro* production system.

Substrate based production system

This method is also known as the classical method for the productions of AM fungi. In this method first the plants and their associated symbionts were cultivated in soil or sand based substrate (Figure 2). After the initial production of AM fungal inoculums, these fungi were propagated for the mass multiplications by using a single species or a consortium of identified AM fungal species in clay or plastic pots or scaled up to medium-size bags and containers and large raised or grounded beds (Gaur and Adholeya, 2002). The whole system setup is cultivated under controlled or semi-controlled condition in greenhouses or plant growth chambers to easily control the humidity and temperature. The starter inoculums usually consist of a single or a consortium of spores and infected root segments. In order to prepare the after inoculums, the root segments are dried and chopped into fine pieces to obtain the mixed inoculums, while, wet sieving and decanting techniques were used to obtain the single spores.

Mixed inoculums were commonly used for the production of those AM fungal species which may produce intra-radical spores and vesicles (Klironomos and Hart, 2002). The significant work of previous investigators for the production of AM fungi using a substrate based system has been summarized in tabular form (Table 1).

Advantages and disadvantages of substrate based production system

Substrate based production systems preserved the mass production of single or consortia of AM fungal species. In this type of system, the nutrient supplies to the AM fungus and plant could be monitored and regulated properly. This system may provide controlled culture conditions but there might also be a chance for superfluous contaminants.

Substrate free production system

At the present time, variety of substrate free cultivation system or nutrient flow techniques is known. All these available techniques may differ from each other in the mode of aeration and application of the nutrient solution. In the static type of system, the nutrient solution is aerated through an aeration pump to avoid the roots suffering from oxygen deprivation. The pumps must be switched on periodically to minimize the flow of nutrient solutions and stuffed of air bubbles, which might be damage the expansion of the delicate extraradical hyphae (Hawkins and George, 1997; Ijodo *et al.*, 2011). The nutrient flow technique has been initially introduced by Mosse and Thompson (1981) and recently by Lee and George (2005). The nutrient flow technique is an alternative system in which a thin nutrient solution covers the roots and increases the relative area for gas exchange and conquers problems due to insufficient aeration into the inclined channels where the plant roots and AM fungus develop.

Aeroponics is a kind of hydroponics systems involves the dipping of roots of host plant and AM fungal propagules in nutrient solution fog. Spraying of micro-droplets increases the aeration of the medium, and the liquid film surrounding the roots imparts gas exchange. In an interesting experiment, Jarstfer and Sylvia (1995) tested aeroponic devises, atomizing disk, pressurized spray through a microirrigated nozzle, and an ultrasonically generated fog of nutrient solution with droplets and concluded that pump and nozzle spray systems were the most tailored systems for mass production of AM fungi. Similarly, Mohammad *et al.* (2000) compared the atomizing disk with the ultrasonic nebulizer technology and found that the ultrasonic nebulizer method was the finest method for the mass production of AM fungi. The finding of the earlier researchers for the mass production of AM fungi using substrate free cultivation systems has been summarized in the tabular form (Table 2).

Advantages and disadvantages of substrate free production system

The main advantage of this system is the production of substrate free inoculum. The root pieces with a high density of infective propagule could be directly used as inoculum. The liquid nutrient solutions are highly prone to the growth and development of algal contaminants. Moreover, the spore production rates could also be affected by lack of a carrier substrate.

***In-vitro* production system**

In-vitro production system of AM fungi was first established by Mosse and Hepper (1975).

Afterward, the root organ culture system was introduced by Becard and Fortin (1988) using T-DNA transformed root of *Daucus carota*. However, St-Arnaud *et al.* (1996) used split-plate method to facilitate the access to the AM fungus and increase the production of propagules. Mass scale production of AM fungi was achieved by root organ culture in small containers (Tiwari and Adholeya, 2003) in an airlift bioreactor and in a mist bioreactor with perlite as a substrate or in a bioreactor containing solid (Jolicoeur *et al*., 1999; Fortin *et al*., 1996).

Several investigators in the past have used many complicated *in-vitro* systems for the propagation of AM fungi (Voets *et al.,* 2005; de Boulois *et al.*, 2006; Declerck *et al.,* 2009) Voets *et al.* (2005) has developed a system in which the shoot was always outside of Petri plate while, the roots and AM fungus was associated inside the Petri plates filled with a suitable gelled medium. However, in the de Boulois *et al.* (2006) system the shoot was developed in a sterile tube vertically connected to top of a Petri plates, which the root

Table 1. Production of AM fungal propagules in substrate based systems

AM Fungi	Host plants	Substrate used	Method used	Inocula produced	References
Glomus etunicatum, G. mosseae, Gigaspora margarita	Mays	Sand	Pot/drip irrigation	Approximately 64,800-341,000 spores per pot	Millner and Kitt, 1992
G. intraradices	Mays	Perlite/ River sand/ Charcoal/ Coal marl/ Clay brick granules	Pots	Approximately 400-880 propagules per 100 ml substrate	Gaur and Adholeya, 2000
Glomus, Gigaspora, Scutellospora spp.	Mays, Alfalfa Egyptian clover, Oat, Broom-corn	Sandy loam/ Compost	Raised beds	Approximately 100 propagules pe r gram substrate	Gaur and Adholeya, 2002
G. clarioideum	Wild leek Ribleaf Lettuce	Sand/ Cambisol	Pots	Approximately 2,010 spores per plants	Gryndler *et al.,* 2003
G. mosseae, G. etunicatum G. claroideum G. geosporum G. intraradices Gigaspora rosea *G. gigantea*	Bahia grass	Vermiculite /Composts	Raised beds	Approximately 2,150 propagules per cm^3	Douds *et al.,* 2005

Table 2. Mass production AM fungi in substrate free systems

AM Fungi	Host plants	Method used	Inocula produced	References
G. intraradices	Sweet potato	Aeroponic	Approximately 50.7 spores per cm colonized root	Hung and Sylvia, 1988
G. etunicatum	Bahia grass		Approximately 6.5spores per cm colonized root	
Glomus sp.	Onion	Hydroponics	Approximately 3,738 spores per plant	Jarstfer *et al.,* 1988
G. intraradices	Hegari	Aeroponics	Approximately 175,000 propagules per gram dry weight inoculum	Mohammad *et al.,* 2000

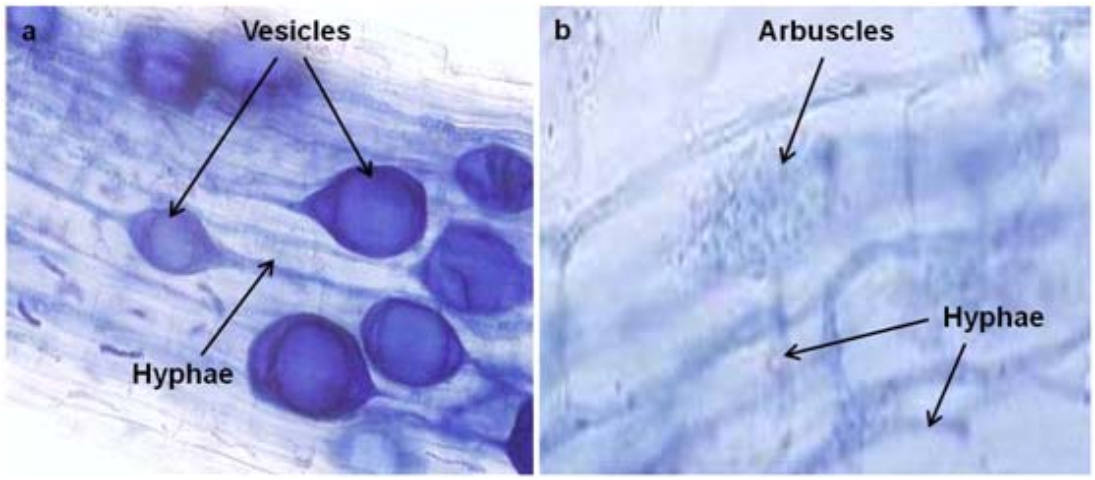

Fig. 1. Microscopic visualization of arbuscular mycorrhizal fungi
(a) showing vesicles and hyphae; (b) showing arbuscules and hyphae in the mays root

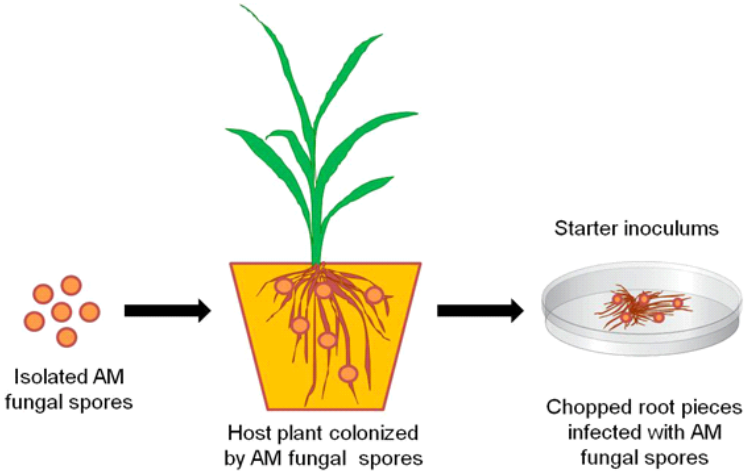

Fig. 2. Schematic presentation of starter inoculums of arbuscular mycorrhizal fungi

and AM fungus was in close association inside the Petri plates. Moreover, the *in-vitro* system developed by Declerck *et al.* (2009) the pre-inoculated plants produced individually introduced AM fungal inoculum in a sterile growth tube in a closed system running with nutrient solution.

Advantages and disadvantages of *in-vitro* production system

The lack of unwanted microorganisms makes this system more appropriate for the mass production of high quality of AM fungal inoculums. In this system there is always requirement for monitoring and regulating the cultures. To make this system cost effective skilled technicians and laboratory equipments were also required. However, *in-vitro* plant cultures need regular additions of culture medium which might be increase the risks of cross contamination.

CONCLUSION

Production of AM fungal propagule is a prerequisite to fundamental research as well as for application purposes. Due to obligate nature of this biotrophs it is quite impossible to propagate these fungi at mass level correct it as in the absence of a suitable host plant. Nowadays, hydroponics, aeroponics, and nutrient flow techniques have been widely used for the mass production of AM fungi. In all the currently used techniques,

aeroponics is most appropriate technique for the propagation of a pure strain of AM fungi. However, nutrient flow technique is frequently used for the propagation of pre-inoculated strains in the host plants, while, nutrient film technique or root organ culture techniques has been mostly used for research purposes.

ACKNOWLEDGEMENTS

The senior author is highly thankful to University of Putra for the award of the Fellow Researcher Grade UDQ9.

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