Topic: MASS PRODUCTION OF AM FUNGI

Subject: Botany

M.Sc. (Semester II), Department of Botany
Course: MBOTCC- 5: Biofertilizer technology; Unit – IV

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[i]"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 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.

Production systems for mass multiplication 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 are cultivated in soil or sand based substrate (Fig.1). After the initial production of AM fungal inoculums, these fungi are 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).

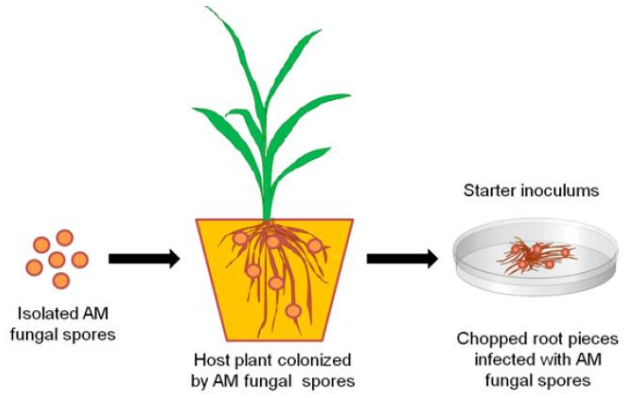

Fig. 1

Substrate based production systems preserves 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, varieties of substrate free cultivation system or nutrient flow techniques are 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 so that roots do not encounter oxygen deprivation. The nutrient flow technique had 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 hydroponic system which 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 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 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. However, 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) developed a system in which the shoot was always outside of petri plate while, the roots and AM fungus were 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, and the root and AM fungus were 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.

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 are also required. However, *in-vitro* plant cultures need regular additions of culture medium which might increase the risks of cross contamination." (Akhtar and Abdullah, 2014)

[ii]"Methods of Application of AM Inoculums

Mycorrhizal spores, pieces of colonized crop roots and viable mycorrhizal hyphae function as active propagules of AM fungi that can be used as inoculums to colonize other plants.

The three methods, which can be adopted to **apply mycorrhiza in the fields** are:
(i) *Adding AM inoculum at the time of sowing*: AMF culture can be added at the time of sowing of seeds with ploughing.

(ii) *Adding AM inoculum at the time of transplanting*: One of the most important factor in the application of AM inoculum is that the inoculums must be placed in the soil, where new roots will grow through it. Colonization will succeed only if the fungi are properly placed and root must be healthy and growing. An ideal AM endophyte should have the ability to infect host plant early in the growth period, efficiently exploit the soil, transfer nutrients readily to the host, multiply rapidly, competes effectively with indigenous AM fungi and infect a wide range of host plant (Mehrotra, 2005).

(iii) *Seed treatment*: Seeds can also be treated with mycorrhizal culture before sowing which will enhance their germination percentage.

Basic concepts of effective strain selection involve efficient production of biomass, universal formulation, long term storage ability and convenient method of application." (Sharma and Sharma, 2017)

Suggested readings

https://iihr.res.in/content/method-mass-production-soil-less-arbuscular-mycorrhizal-fungal-inoculums

[i] Source: Akhtar and Abdullah (2014)
[ii] Source: Sharma and Sharma (2017)