MASS PRODUCTION OF AM INOCULANTS AND THEIR FIELD APPLICATION

Introduction

The mycorrhizal symbiosis provides soil fertility and soil stability to the degraded (nutrient deficient) soils. From sociological point of view, many research and developmental companies are producing and commercializing AM fungi as a biofertilizer. Mycorrhizal use and application in future have a potentially important role in agriculture, forestry and in socio-economics.

AM fungi are characterized by the presence of their unique extra radical mycelium branched haustoria like structure within the cortical cells, known as arbuscules. The main role of arbuscules is to increase the surface of roots during nutrient transfer. AM fungi colonize the plant roots and penetrate into surrounding soil, extends the root depletion zone and the root system.

When AM fungi come in contact with root of host plant then plant secretes some exudates which have been thought to stimulate the germination of AM spores. Since, isolation and selection of AMF species are effective for growth promotion and raising of pure culture of these species is difficult, a suitable host is required to maintain pure culture of AM inoculum. The most important consideration in inoculum production is the choice of the fungal isolates which are capable of growth promotion of target plant.

Inoculum potential

The suitable inoculum is such which is small in dose and large in reaction. Concentrated inoculum is easy to store, convenient to transport and suitable to apply. The concentrated inoculum can be produced by pot culture and aeroponic technique. AM inoculum should not spread unwanted organisms such as plant pathogens or harmful

bacteria. Unadulterated inoculum is therefore required to maintain purity. A variety of inoculum formulations for AM fungi are known to occur. A mixture of spores, colonized roots, hyphae and soil from pot cultures grown in sterilized soil constitute a form of widely reported inoculum composition. AM fungi vary much in their power to promote plant growth and must be screened for booster strains before they are used for inoculum production. Booster strains are those which quickly enter and colonize the root system and help in nutrient translocation.

The AM fungi should be isolated from rhizosphere of the plant for which the inoculum is to be used. Rhizosphere is the most accessible and most abundant source of inoculum for starter culture. Inoculum is maintained and mass produced in pot cultures on suitable host plants.

The host plant selected should be suitable to agro-climate conditions of the area, having thick root system for sizeable sporulation and infection, annual in growth habit and adaptable to polyhouse conditions. The host plants also may stimulate selectively or limit sporulation of certain AM fungal species suggesting varied affinities between hosts and symbiont. The composition of soil mixture is crucial for ensuring good mycorrhization and sporulation.

Container size has the power to affect AM inoculum. Pot size should match the potential volume of the root system. Light quality and irradiance are very influential for colonization and spore production. Proper irradiance provides good results. Lower light intensities caused reduced sporulation. Unsaturated and unstressed water conditions are best for spore production. A correlation between water content and spore number existed across a natural soil moisture gradient in the field.

Mass Production of AM Fungi

There are three major well known systems adopted widely in the mass

production of AM fungi. These are:

1. Substrate based production system
2. Substrate free production system
3. In-vitro production system.

Substrate based production system

This method is also known as the classical method for the production of AM fungi. In this method first the plants and their associated symbionts are cultivated in soil or sand based substrate. After the initial production of AM fungal inoculums, these fungi are propagated for the mass multiplication 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. The whole system setup is cultivated under controlled or semi-controlled condition in greenhouses or plant growth chambers. The starter inoculum usually consist of a single or a consortium of spores and infected root segments. In order to prepare the after inoculum, the root segments are dried and chopped into fine pieces to obtain the mixed inoculum. While, wet sieving and decanting techniques were used to obtain the single spore. Mixed inoculums were commonly used for the production of those AM fungal species which may produce intra-radical spores and vesicles.

Substrate free production system

There are variety of substrate free cultivation system or nutrient flow techniques is known. 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. 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 extra-radical hyphae.

In-vitro Production System

In-vitro production system of AM fungi was established first, and then the root organ culture system was introduced by using TDNA transformed root of *Daucus carota*. Mass scale production of AM fungi has been achieved by root organ culture in small containers in an airlift bioreactor. The lack of unwanted microorganisms makes this system more appropriate for the mass production of high quality of AM fungal inoculums. However, in-vitro plant cultures need regular additions of culture medium which might increase the risks of cross contamination.

Field application

Mycorrhizal spores, pieces of colonized crop roots and viable mycorrhizal hyphae function as active propagules of AM fungi that can be used as inoculum 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 inoculum must be placed in the soil, where new roots will grow through it. Colonization will succeed only if the AM fungi are properly placed and roots of the host plants 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, compete effectively with indigenous AM fungi and infect a wide range of host plant.

(iii) Seed treatment: Seeds can 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. The number of AM propagules also includes the external hypha growing out from the surface of mycorrhizal roots and colonized root pieces. It has also been reported that organic amendments enhance spore production, extraradical proliferation of hyphae and improve colonization of roots. Organic matter addition to the soil in eroded sites could be an approach to enhance the beneficial effect of AM fungi on soil stabilization and plant establishment.

Conclusion

One of the main tasks for both producers and researchers is to raise awareness in the public about potentials of mycorrhizal technology for sustainable plant production and soil conservation. Supplementing the nutrient requirements of crops through AM inocula for sustaining soil fertility and crop production would spur commercial production and the development of new formulations. Mycorrhiza in combination with other beneficial microorganisms has a promising option and should allow more holistic approaches to rhizosphere health in future and help in a drive towards minimizing excessive chemical usage.

Application of mass production of inoculum, its proper handling and distribution of viable inoculum at the farmer’s level through networking of strong field force will support and give impetus to programme of agro-forestry, wasteland development and energy plantation in rural areas.