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APPLICATION AND LIMITATIONS OF VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI (VAMF) AS BIOFERTILIZER PRODUCTION: A REVIEW

ALOK TRIPATHI[a1], NAVEEN KRISHNA SRIVASTAVA[b] AND DEEPAK KUMAR SRIVASTAVA[c]

[a]Department of Botany, S.N.P.G. College, Azamgarh, U.P., India
[b]Department of Botany, S.D.J. P.G. College, Chandeshwar, Azamgarh, U.P., India
[c]DPrincipal, Career Convent Girls P.G. College, Lucknow, U.P., India

ABSTRACT

Vesicular Arbuscular Mycorrhizal (VAM) fungi are soil borne microbes belonging to phylum Glomeromycota that form a symbiotic association with roots of higher plants. Hyphae colonize their host roots and from a mycelia network in the rhizosphere soil to facilitate nutrient uptake, especially phosphorus and in turn acquire photosynthate from the host plants. These fungi play an important role in agriculture, forestry and horticulture by increasing crop yield, health and resistance stress by reducing the cost of agrochemical. It has been observed that VAM fungal inoculatia provides beneficial results in plant growth both in controlled and open field conditions. Various further studies have proved that VAM fungi are an effective resource when used as biofertilizers with no adverse environment effect. The present study suggests the application and limitations of VAM fungi as biofertilizer production.

KEYWORDS: Vesicular Arbuscular Mycorrhizal Fungi (VAMF), Crops, Health, Biofertilizer and Production

Mycorrhiza is a mutualistic association between fungi and higher plants (Menge 1983). Frank (185) coined the term mycorrhizae. The term 'Mycorrhiza' in its broadest sense in the non-pathogenic association of fungi and the roots of higher plants. The root-fungus association is symbiotic and the whole association is considered as a "functionally distinct organ" involved in mineral nutrient uptake the soil (Kar, 1993).

Vasicular arbuscular mycorrhizal (VAM) fungi are soil-borne microbes belonging to phylum Glomeromycota that form a symbiotic association with roots of higher plants. Hyphae colonize their host roots and form a mycelia network in the rhizosphere to facilitate nutrient uptake, especially P (Rodrigues and Rodrigues, 2014) and in turn acquire photosynthates from the host plant. Around 90% of vascular plants form VAM association (Smith and Read, 2008). Plant genes and signal molecules enable hyphal entry and development of the fungus in the plant (Parniske, 2008). The extra-radical mycelium extands several centimeters beyond the depletion zone absorbing nutrients that are transported to host roots (Khan *et al.*, 2000). These fungi play an important role in agriculture, forestry, and horticulture by increasing crop yield, health, and resistance to stress by reducing the cost of agrochemicals (Johansson *et al.* 2004). Occurrence of VAM symbiosis is dated back to >460 million years ago (Read eta al. 2000). Based on the spore morphology, approximately 240 VAM fungal taxa belonging to order Glomales have been described (Schubler and Walker 2010; Kruger *et al.* 2012), although molecular analysis data show that the actual number of VAM fungal taxa can be much higher (Vandenkoornhuyse *et al.* 2002).

Various cultivation techniques of VAM fungi inoculum production have been attempted in the last few decade. Sand/soil and substrate based production techniques, substrate free culture techniques (hydroponics and aeroponics), and in vitro cultivation methods have been attempted in the large scale production of VAM fungi. Several parameters must be taken into consideration for the culture of VAM fungi, such as controlled or semi-controlled conditions in greenhouses, VAM fungi species, the host plant, substrate, and amendments.

Conventional production of VAM fungi is commonly achieved by the cultivation of host plants and their symbionts in a soil or sand based substrate (substrate based production system). The inoculum to initiate production consist of dried root fragments or colonized root fragments. VAM spores, sporocarps, and fragments of hyphae. When spores are extracted from the soil and used as inoculum directly they tend to have very low viability or may even be dead or parasitized. To overcome this initially, the rhizosphere soil is used to prepared a 'trap culture' using a suitable host plant. This increases the number of viable spore propagules for further isolation, multiplication and production of monospecific cultures. The pure culture inoculum thus

[1]Corresponding author

produced consist of spores, colonized root fragments and VAM hyphae of a single species.

Selection of host plants is based on numerous criteria, such as plants exhibiting a short life cycle, rapid growth, adaptation to the prevailing growing conditions, and ready colonization by a range of VAM fungal species. A large quantity of roots should also be produced in a relatively short period, the resistance to pest and diseases common to the inocula production environment.

A range of plant species, such as *Zea mays* (Corn), *Allium cepa* (Onion), *Arachis hypogaea* (Peanut), *Paspalum notatum* (Bahia grass), *Peuraria phaseoloides* (Kudzu), *Plectranthus scutellarioides* (Coleus), *Eleusine coracana* (Ragi) etc., have been used as hosts with encouraging results.

Various substrates, such as soil, sand, peat, vermiculite, perlite, calcinated clay, and compost have been used to propagate VAM fungi (Ijdo *et al.* 2011). Addition of different organic amendments also influence VAM fungal colonization. Chitin and humic substances increase colonization levels (Gryndler *et al.* 2003; Gryndler *et al.* 2005). Manipulation of nutrient content has a further impact on VAM fungal propagule production (Douds and Schenck, 1990). The substrate based culture technique is the most widely used method for VAM fungal production as it requires a relatively little less technical support, is cheap, is the least artificial, and a large set of VAM fungal species can be cultured (Ijdo *et al.* 2011). Conversely, the sand/soil based systems have certain disadvantage such as the presence of unwanted contaminants, even with good phytosanitary care, fewer viable spores than in vitro system, and parasitized spores.

Substrate free cultivation systems, such as hydroponic and aeroponic have also been used for the multiplication of VAM fungi where in a continuous flow or mist of nutrient solution is provided for the plant and the symbionts. Although this system offers the advantage of providing inoculum which is free from attached substrate particles, a disadvantage has been that the nutrient solution is prone to microbial contamination and algal growth (Elmes and Mosse 1984).

The first attempt to culture VAM fungi monoxenically dates back to the late 1950s (Mosse 1959). Thereafter, tremendous progress has been made for the mass production VAM fungi using Ri T-DNA transformed roots (Mugnier and Mosse 1987). Different in vitro culture techniques have been derived such as the bicompartment system wherein VAM fungal mycelia and spore are produced free from roots (St-Arnaud *et al.* 1996), and manipulation of culture medium to induce sporulation (Becard and Piche 1992). These developments have enabled studies in spore ontogeny (Pawlowska *et al.* 1999), sporulation dynamics (Declerck *et al.* 2001), response of VAM fungi to cell wall-associated phenolics (Douds *et al.* 1996) and flavonoids (Morandi *et al.* 1992), lipid metabolism (Bago *et al.* 2002), transport of mineral nutrients to roots (Dupre de Boulois *et al.* 2005) and isolation of contaminant-free spores for molecular analysis (Pawlowksa and Taylor 2004). A wide number of VAM fungal species belonging to Glomeraceae and a few Gigasporaceae have been successfully cultured in the root organ culture (ROC) system.

Species, such as *Acaulospora rehmii* (Dalpe and Declerck 2002), *Gigaspora rosea* (Bago *et al.* 1998c), *Gi. margarita* (Miller Wideman and Watrud 1984; Diop *et al.* 1992; Gadkar and Adholeya 2000), *Gi. gigantean* (Gadkar *et al.* 1997), *Gi. decipiens* (Fernandez Bidondo *et al.* 2012), *Glomus etunicatum* (Schreiner and Koide 1993), *G. versiforme* (Diop *et al.* 1994), *G. Deserticola* (Mathur and Vyas 1995), *G. fistulosum* (Nuutia *et al.* 1995; Gryndler *et al.* 1998), *G. clarum* (De-Souza and Berbara-1999; Rodrigues and Rodrigues 2012), *Funneliformis ealedonius* (Hepper 1981; Karandashow *et al.* 2000), *F. geosporus* (Declerck *et al.* 1998), *F. Mosseae* (Douds 1998), *Rhizophagus irregularis* (Chabot *et al.* 1992; St-Arnaud *et al.* 1996), *R. fasciculatus* (Declerck *et al.* 1998), *R. proliferus* (Declerck *et al.* 2000) and *Sclerocystis sinuosa* (Bi *et al.* 2004) have been successfully cultured in vitro.

The most important advantage offered by in vitro cultivation system is the absence of undesirable organisms. Contamination by other undesirable microorganisms can occur, however, during establishment of culture process or during the latter stages of culture maintenance. This type of system can be used for the large-scale production of VAM fungi consisting of high quality inoculum with minimum space. Also, the factors influencing optimum production can be easily detected and controlled, and harvesting time can be determined, The maintenance of a successfully established culture is easily achieved by sub-culture and maintaining the plants in dark condition. As a disadvantage, the in vitro- grown VAM fungal diversity is lower than that under-pot culture system (Rodrigues and Rodrigues 2013). Furthermore, the in vitro production is expensive, requiring skilled technicians and sophisticated laboratory equipment to carry out the whole process in sterile and controlled conditions (Ijdo *et al.* 2011). Further studies are in progress to identify and eliminate contaminants in established cultures.

CONCLUSION

It has been observed that VAM fungal inoculation provides beneficial results in plant growth both in controlled and open-field conditions. VAM fungi have been confirmed to show better performance in terms of plant growth and yield characteristics. This would make the VAM fungal technology more suitable to sustainable cropping systems (Berruti *et al.* 2016). Khan *et al.* (2008) reported that the inoculation of a single or dual VAM fungi increased the growth and nutrient uptake of *Medicago sativa* which results in the increased dry weight of shoot and root. Bhat *et al.* (2010) studied the effect VAM fungi and *Rhizobium* on green gram (*Vigna radiata*) and reported a significant effect on nodulations, yield, crude protein contents, and NPK content in grain. Various further studies have proved that VAM fungi are an effective resource when used as biofertilizers with a no adverse environmental effect.

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