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Appraisal of native AM fungi in improving the plant productivity, soil health and sequestering soil carbon in agroecosystems

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ABSTRACT

The soils under tropical and sub-tropical regions are generally nutrient deficient, marginally fertile and growers rely on sub-optimal yields. Among the soil biota, the role of arbuscular mycorrhizal (AM) fungi in sustainable agriculture have been widely recognized. AM inoculation in horticultural and crop plants in the field and during the hardening stage has been shown to improve the plant stand, plant health, saved chemical fertilizer inputs. The crop productivity further improved when AMF is applied with PGPR. AM fungi also improve soil through production of glomalin which contributes to the soil carbon pool. Based on stabilized field studies, the functioning of native AMF enhances crop productivity and soil carbon sequestration under organic and conservation tillage with maize-based crop sequences. These fungi can be produced routinely on host plants in potting substrates, on-farm, and using Ri-plasmid hairy roots under in vitro. Besides employing the conventional microscopic methods (spore density and root colonization), its functioning can be carried out through biochemical methods such as AM-signature 16:1ω5 phospholipid and neutral fatty acids, and glomalin. The production of AM fungi, its quality assurance, and its application in the field for nutrient mobilization and carbon mitigation should be popularized through large-scale field demonstrations at multiple locations.

Keywords : AM signature fatty acids, crop production, farming practices, glomalin, soil carbon sequestration

INTRODUCTION

The United Nations Agenda 2030 of Sustainable Development Goals (SDGs) entails that all the countries have to ensure zero hunger, poverty alleviation, food sufficiency and productivity to achieve a better future for all. Although India's food grain production is somehow being maintained at a conservative pace but at the same time natural resources are being compromised. Over the last century, atmospheric concentrations of carbon dioxide (CO_2) increased from a pre-industrial value of 278ppm to 402ppm in 2019 and may likely go up to 450ppm in next two decades (Behera *et al.,* 2013; IPCC, 2013; Betts *et al.,* 2016). The unilateral approaches *viz.,* intensive use of agro-chemicals, high yielding varieties, resources, monoculture etc., adopted for increasing the production have generated environmental and biological imbalance which are the major challenges to be met for a sustainable agricultural system. The declining trend in carrying capacity of land and other natural resources have drawn the attention of environmental, agricultural and social scientists in the present time to look for an alternative approach for sustaining the resources supporting the ecosystem and human life as a whole. Agricultural practices have begun to integrate emerging technologies like biotechnology and biofertilizers with traditional practices like organic farming. Among biofertilizers, the mycorrhizal fungi particularly arbuscular mycorrhizae (AM), the most common fungal association formed nearly in all cultivated plants whether they are agricultural, horticultural or forestry plant species is gaining importance (Smith and Read, 2008). Mycorrhizal biofertilizer has a broad spectrum benefit to majority of the crop plants. It benefits plants through a multiple action via absorption of nutrients, particularly P, water absorption, disease resistance, heavy metal toxicity, resistance to salt stress, etc. (Bagyaraj *et al.,* 2015; Berruti *et al.,* 2016; Begum *et al.,* 2020; Wijayawardene *et al.,* 2020). Mycorrhizal biofertilizer can also be applied in alleviation of abiotic stresses such as drought (Mathimaran *et al.,* 2017), high temperature (Mathur *et al.,* 2018), etc. Significant yield enhancement through field application of AM fungal inoculum has been recorded in cereals, fruits, ornamentals and timber plants and micropropagated plants (Sharma *et al.,* 1996, 2001; Varshney *et al.,* 2002; Sharma and Adholeya, 2004; Sharma *et al.,* 2011). Apart from growth promoting attributes, AMF also improve soil aggregation through production of a sticky glycoprotein termed as 'glomalin' that stabilizes soil aggregates which contributes to soil carbon pool (Rillig, 2004; Agnihotri *et al.,* 2021a). Further, the efficacy of AMF enhances when co-inoculated with plant growth promoting rhizobacteria (PGPR). For example, co-inoculation of AMF with bradyrhizobia enhances nodulation, growth, yield, and saved fertilizer inputs in soybean (Sharma *et al.,* 2016). The use of native strains of AMF in sustainable plant production systems has been encouraged. The conservation tillage with appropriate crop sequences helps in managing native AMF and sustaining the plant productivity (Sharma *et al.,* 2012a). Conventionally, these AM fungi can be produced on host plants in potting substrates (Agnihotri *et al.,* 2021b) and on-farm raised beds (Douds *et al.,* 2010; Sharma and Adholeya, 2011) and using Ri-plasmid hairy roots under *in vitro* (Ijdo *et al.,* 2011). The efficacy of AMF in mass production, growth promotion and glomalin production can be carried out through microscopic methods *viz.,* spore density and root colonization and biochemical through AM-signature 16:1ω5 phospholipid and neutral fatty acids (Sharma and Buyer, 2015). Glomalin has made a significant contribution to soil organic carbon and hence both glomalin and AM signature fatty acids (16:1ω5 PLFA and NLFA) can be used as potential indicator biomarkers for assessing their potential in sequestering soil carbon (Agnihotri *et al.,* 2021a; Gujre *et al.,* 2021). This article aims to develop understanding on AMF responses in crop plants through (i) responses of

native AMF inoculation in relation to improved growth, P. nutrition, yield and productivity of crop plants, (ii) management of native AMF through crop and soil management practices for sustaining the yields, (iii) exploiting the potential of native AMF in soil carbon sequestration and comparing with different farming practices, and (iv) to suggest biochemical parameters such as signature lipids and glomalin in assessing the impact of farming practices to sustain various soil eco-functions and in the assessing the quality of AM inocula produced.

APPLICATION OF AMF IN THE IMPROVED PRODUCTIVITY AND BIOPROTECTION OF PLANTS

The symbiotic association with AMF allows the plant to access phosphorus beyond the depletion zone through the extraradical fungal hyphae, in addition to the root uptake (Pearson and Jakobsen, 1993). AMF have been shown to improve productivity in soils of low fertility (Jeffries, 1987) and also contribute to the uptake by plant of micronutrients, such as zinc (Ryan and Angus, 2003) and the macronutrient nitrogen, both inorganic and possibly also organic (Hawkins *et al.,* 2000; Hodge *et al.,* 2001). Plant responses to AMF vary due to soil nutrient conditions. Plants, which are highly colonized, may not be highly responsive to growth. Wheat cultivars to mycorrhizal responsiveness did not correlate with mycorrhizal colonization and may not be an appropriate indicator of mycorrhizal responsiveness (Hetrick *et al.,* 1993). Hence, selection of most efficacious AMF is an important aspect in plant production studies. Sharma and Adholeya (2015) showed that assessing plant P uptake in the early growth stage (6-8 weeks) of AM-inoculated plants can be used as an indicator to evaluate superior AMF for growth promotion in different plant species. Plant response to AMF differs with soil P which suggested that there is a threshold level of soil P which influences the beneficial AMF colonization (Covacevich *et al.,* 2007). The magnitude of AMF responses varies with applied P doses. The external P requirement of AM strawberry plants was 71 Kg/ha and to get similar yield non-AM plants required 106 Kg/ha and thus, AM application reduced 35 Kg/ha without compromising the fruit yield (Sharma and Adholeya, 2004; **Fig. 1**). These authors suggested that the optimum level of soil P should be considered to manage AMF for getting higher wheat yield. Various studies have been conducted on optimizing AMF responses with P for improving the plant growth, yield and nutrition in forestry, horticultural and agricultural plants (Sharma *et al.,* 1996, 2000a, b, 2001, 2004, 2011, 2012a, b; Sharma *et al.,* 2016). Thus, AMF application response can be optimized at reduced doses of fertilizers. Sharma *et al.* (2016) reported that AMF inoculation responses can be further enhanced with co-inoculation of other PGPR. Irrespective of

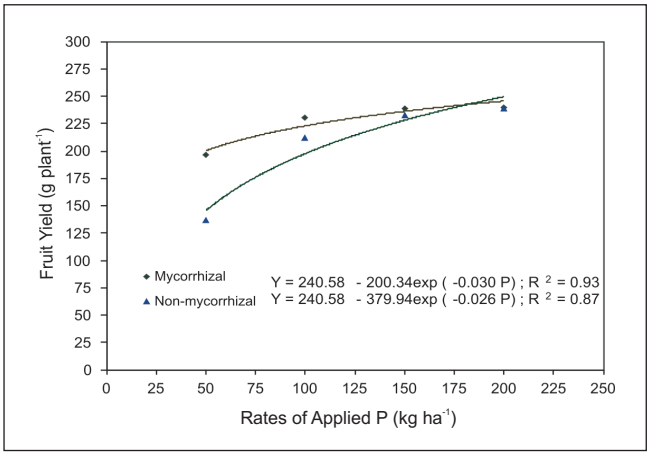


Fig. 1: Fruit yield of mycorrhizal and non mycorrhizal strawberry plants at different doses of P fertilizer applied. External P requirement for mycorrhizal and nonmycorrhizal plants were derived from Mitscherlish equation (Sharma and Adholeya, 2004).

soil conditions, co-inoculation of AMF and *Bradyrhizobium* with or without 50 per cent RDF produced comparatively higher nodulation, N uptake, had higher AM colonization and grain yield than other treatment combinations and was found significantly higher over control plants **(Fig. 2)**. It means

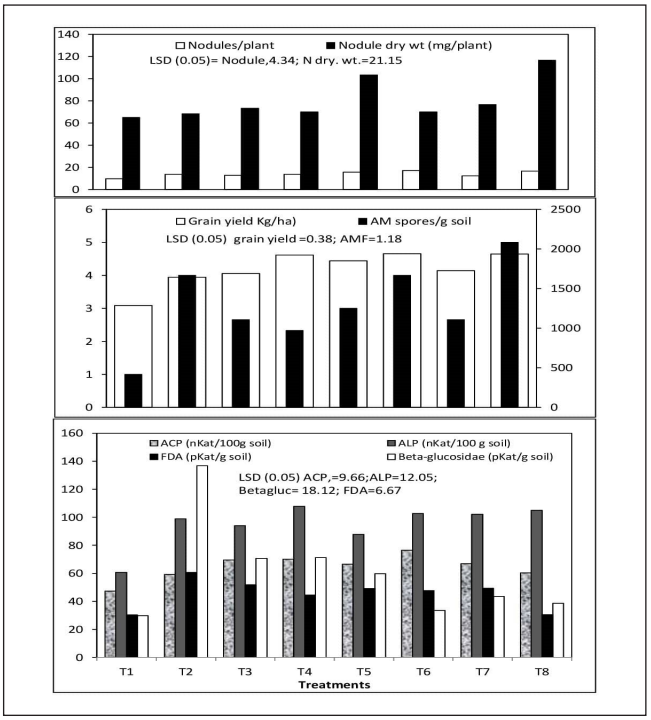


Fig. 2: Co-inoculation of AMF and soybean rhizobia on nodulation on AM formation, grain yield and soil enzyme activities assessed in soybean grown in Vertisols under field; ACP, Acid phosphatases; ALP, Alkaline phosphatases; FDA, fluroscein diacetate; Treatments T^1, Uninoculated/control; T^2, AMF; T^3, *Bradyrhizobium japonicum*, 5a; T^4, Recommended dose of fertilizers (RDF); T^5, T^2+T^3; T^6, 50% of RDF+ T^2; T^7, 50% of RDF+ T^3; T^8, 50% of RDF+ T^2+ T^3 (Sharma *et.al.,* 2016).

Bradyrhizobium inoculation is favoring AMF colonization. It has been shown that AMF has improved P nutrition in infertile and high P-fixing soils (Sharma *et al.,*1996; Dodd, 2000). It was also reported that the inoculation of AM fungi can be further optimized with crop and soil management practices. For example, wheat under raised beds has provided better response to conventional system and could be adopted for achieving higher yield of wheat at reduced fertilizer inputs (Sharma and Adholeya, 2011; **Fig. 3**).

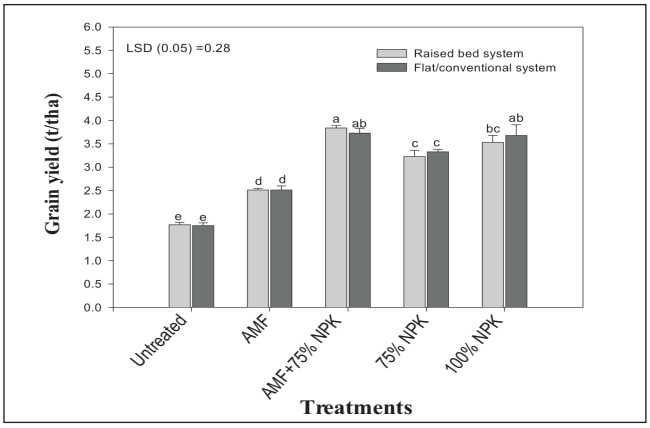


Fig. 3: Influence of AMF and fertilizers on grain yield (t ha-1) of wheat grown under raised bed and conventional/flat system in a sandy loam Alfisol (Sharma *et al.,* 2011)

Under drought conditions the uptake of highly mobile nutrients and hyphal extension enhanced tolerance in AM plants (Mathimaran *et al.,* 2017). In legume plants the importance of AMF symbiosis has been attributed to high P requirements on the nodulation and N2 fixation process which requires enhanced P uptake (Barea and Ázcón-Aguilar, 1983). Mycorrhizal fungi can also improve nodulation in soybean when co-inoculated with soybean rhizobia (Sharma *et al.,* 2016). Another impact of AMF on the plants, including agricultural crops, is their ability to increase their tolerance to drought (Manoharan *et al.,* 2010) and reduce damage caused by pathogens (Cameron *et al.,* 2013). It has been reported that stress induces some specific proteins and metabolites that confer tolerance to microbes to adapt and survive until conditions return to normal (Ocon *et al.,* 2007; Mathimaran *et al.,* 2017; Sharma *et al.,* 2020). A common response of organisms to drought stress is the accumulation of sugars and other osmoprotectants that help in stabilizing biomolecules. One such compound is trehalose, a non-reducing disaccharide, found in a wide variety of organisms including fungi and bacteria and is found to play an important physiological role as drought stress protectant. Trehalose accumulation (as carbohydrate reserve) in an organism protects the cell by stabilizing cell structures and enables protein to maintain their native conformation under drought stress. It has been reported that trehalose may occur in plants colonized by organisms like AMF and rhizobia (Sharma *et al.,* 2020). AMF colonized plants may help in increased levels of trehalose in the plant which eventually enhance the tolerance to cope up with drought conditions (Sharma *et al.,* 2020).

Besides enhanced plant growth and nutrition, AMF also help in disease protection. The use of AMF as biological means paves the way to manage the nematode infestations. Mycorrhizal fungi and plant parasitic nematodes (PPN) commonly found inhabiting the rhizosphere and colonizing the common roots of their host plants. Mycorrhiza-mediated plants brought changes in the host physiology and enhanced P-nutrition, which deters pathogens to attack, and built plants more tolerant to pathogen attack and reduced nematode infestation (Sharma and Bhargava, 1993; Sharma *et al.,* 1994; Bhargava *et al.,* 2008). Numerous reports were published on the suppression of nematode penetration and development following AMF inoculation (Talavera *et al.,* 2001; Diedhiou *et al.,* 2003; Elsen *et al.,* 2003; Schouteden *et al.,* 2015). Harrier and Watson (2004) emphasized the role of AMF in organic and/or sustainable farming systems that rely on biological processes rather than agrochemicals to control plant diseases.

MANAGING NATIVE AMF IN AGROECOSYSTEMS

Indian soils are usually deficient in phosphorus, an element that is required more as compared to other major nutrients. For managing AMF in a specific agro-ecosystem, understanding about the occurrence and diversity of indigenous AMF is an essential first step. The community composition of AMF in intensively managed agro-ecosystems of Malwa region is currently not known. Sharma *et al.* (2008) showed that the native AMF species composition is largely influenced by the host plants and not the substrate. The spore morphological identification showed that a total of six different species of *Glomus* were identified from soybean and maize traps. Out of these, three each were from maize and soybean where *Rhizophagus irregularis* was observed to be the dominant AMF colonizing both the host plants and can be exploited for large scale application. Buade *et al.* (2020) reported that AMF species community composition, species distribution and its affinity varied with the growth stages of soybean. They showed that irrespective of growth stages, a total of 40 AMF morphotypes were observed from which species, *viz., Glomus aggregatum, Rhizophagus fasciculatum, Glomus coronatum, G. etunicatum, Glomus* sp (76.92%), were found to be the most abundant. Based on principal component analysis, maximum contribution of all AMF species was observed at pre-sowing. The similar trend followed at harvesting stage which concludes that AMF diversity and colonization pattern are greatly affected with the soybean phenological growth stages. Across all the stages, *Glomus* morphotypes were observed to be more predominant than the *Acaulospora* and *Gigaspora* hence can be selected for further exploitation in soybean.

AMF favourable agricultural practices (such as zero-till, elevated beds, crop rotation and inter-cropping system, etc.) may result in greater hyphal biomass, improved soil texture

and health, increased glomalin with improved crop yields in the soil (Nichols and Wright, 2005; Agnihotri *et al.,* 2021a). Therefore, whole system involving AMF with varied cultivation systems is likely to be important in terms of better functioning of AMF to produce more AMF biomass and sustain crop productivity on long run. Similarly, in our research farm, one of the long term trials having mycorrhizal crops and organic as well as integrated nutrient management also showed higher AMF biomass despite of having no AMF inoculation in long term cropping involving organic practices favouring indigenous AMF population (Agnihotri *et al.,* 2015). The AMF inoculum potential and biomass varied with the crop and soil management practices. A significantly higher AMF population (14.2/g soil and 12.86/g soil) was observed in soybean+maize-wheat rotation taken under conventional-reduced tillage practices. The rhizosphere of both soybean and wheat under conventional-reduced tillage systems in soybean based rotations involving maize showed higher AMF spore density and colonization. The infectivity potential (IP) caused due to resident AMF was observed higher in the plots of soybean-wheat-maize-wheat rotation under conventional-reduced tillage systems. Overall, higher IP was observed in the plots of rotation where maize was included, which also sustained higher grain yield (Sharma *et al.,* 2012a).

POTENTIAL OF AMF IN SOIL CARBON SEQUESTRATION THROUGH GLOMALIN PRODUCTION AND CROP AND SOIL MANAGEMENT PRACTICES

Nearly 80% carbon is found in soil and is about 2500 GT which has 1550 GT organic fraction and 950 GT inorganic carbon (Lal, 2004, 2008). AM fungi via their extra radical hyphae help in soil aggregation where carbon gets securely stored inside soil aggregates. The role of glomalin in C sink has been clearly established from the studies conducted by Sara Wright, Rillig and Allen's group in USA that elevated carbon dioxide increases carbon allocation to AMF. Higher assimilation of carbohydrates (due to higher levels of carbon dioxide) and carbon from plant roots is likely to lead to increased exudation of carbon compounds (such as glomalin) to the soil where AMF invest the additional carbon acquired from plants grown in elevated carbon dioxide (Rillig, 2000; Agnihotri *et al.,* 2018). Plant roots associate with extraradical hyphae (ERH) of AMF thereby forming stable soil aggregates which in turn gets supported by glomalin (Wright and Upadhyay, 1996) which is a glycoprotein produced abundantly on AMF hyphae, about 80% of which is present on spores and hyphae and 20% is released into the soil (Driver *et al.,* 2005). AMF has been found to make significant contribution to soil organic carbon (Rillig, 2004). Earlier it was considered that AMF contributes to soil aggregate stability and carbon sequestration, through hyphal mediated entrapment of soil aggregates. But AMF produces an extremely thermostable glomalin which maintains better soil quality and aids in soil carbon sequestration by making stable soil aggregates within which soil carbon gets sequestered (Wright and Upadhyay, 1996; Wright and Upadhyay, 1998). The residence time of glomalin in soil is found to be 6-42 years (Rillig *et al.,* 2001) and helps in sequestering heavy metals (Gujre *et al.,* 2021).

The amount of soil C sequestration depends on land-management practices, edaphic factors, climate and the amount and quality of plant and microbial inputs. While studying different soil niches, the soils of zero-till soils followed by maize grown on raised beds showed significantly higher contents of microbial biomass carbon, soil organic carbon and glomalin than the convention tilled soil (Agnihotri *et al.*, 2021a). The potential of AMF under different crop and soil management practices in soybean-based cropping system was examined in two experiments at ICAR-Indian Institute of Soybean Research, Indore, India. It was observed that AMF inoculation in soybean-maize intercropping system under organic practice enhanced mycorrhizal biomass in soil assessed in the form of signature fatty acids (16:1ω5 PLFA and NLFA) and spore density, improves carbon profile of soil via stable glomalin stocks, and carbon stocks. Apart from this, the higher stocks of soil organic carbon and glomalin also lowered the rates of CO_2 emissions (Agnihotri *et al.,* 2015). In another study dealing with a 15-year long term field experiment managed with organic, inorganic and integrated nutrient management practice having soybean-wheat (S-W) and soybean-chickpea (SC) rotation, the role of native AMF species in soil organic carbon (SOC) sequestration was explored. It was observed that among the soil management practices, higher AMF biomass was observed in plots managed with organic practice, which also maintained highest spore density (5.06 spores/g soil), glomalin stocks (2.10 Mg C/ha/year), C stocks (15.78) and AM-signature lipids with lower CO_2 emission (22.09 mg CO_2/kg soil/day) than the inorganic fertilization practice. Overall, from the both the studies it was concluded that organic practice has promoted the active live-AMF biomass, which consequently enhanced glomalin production. Hence, it is evident that long-term maintenance of rhizosphere under organic nutrient management practices and crop sequences encourage higher build-up of AM-mediated stable carbon pools (e.g., glomalin) thereby help in sequestering higher soil C sequestration **(Fig. 4)**.

Thus, it is concluded that AMF by producing glomalin sequesters excess soil carbon in a form that is stable enough to prevent its release into the atmosphere. This ability is particularly enhanced under organic practice, intercropping and crop rotation systems comprising mycorrhizal crops such as maize and soybean and sustained the productivity of soybean-based cropping system. Recently, Nautiyal *et al.* (2019) showed that the glomalin concentrations and soil aggregation varied across different cropping systems. They observed that glomalin content (total and easily extractable) was positively correlated with soil organic carbon and soil aggregation verifying its contribution in maintaining the soils carbon pool.

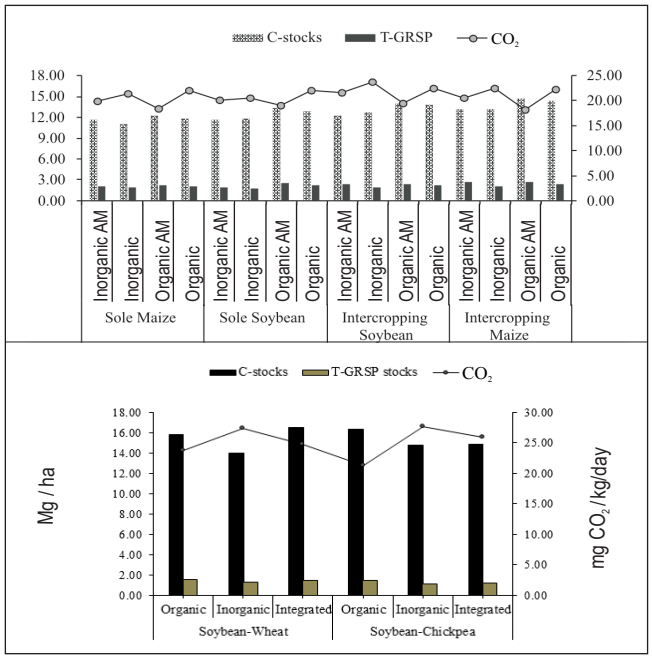


Fig. 4: Impact of crop and soil management practices with and without AMF inoculation on stocks of glomalin and soil carbon and CO_2 respiration assessed in short term (Top) and long-term soybean-based field trials (Bottom) (Agnihotri *et al.,* 2015)

AMF PRODUCTION AND ITS QUALITY ASSESSMENT

Increasing popularity of AMF as potential biofertilizers due to their numerous benefits to plants is making their large-scale production and commercialization indispensable. One of the major bottlenecks for large-scale application of mycorrhiza is the unavailability of adequate inoculum, since AMF is an obligate symbiont and it requires a host to complete its life cycle. There are various methods currently being used to mass-produce these fungi. The root organ culture (ROC) is a most attractive mass multiplication method for providing a pure, viable, rapid and contamination free inoculum using less space and has an advantage over the pot culture multiplication/conventional system (Fortin *et al.,* 2002; Cranenbrouck *et al.,* 2005; Ijdo *et al.,* 2011). But due the lack of technical skills and feasibility of producing the native AMF strains under *in vitro* using root organ culture of AMF is somehow not picking up due to several reasons.

Culturing AMF on living plants growing in disinfected soil has been the most frequently used technique for increasing the number of propagules. These conventional methods involve the use of starter inoculum/material and growing on soil in pots and on-farm on raised beds using suitable trap plants (Douds *et al.,* 2006; Sharma and Sharma, 2008; Sharma and Adholeya, 2011). The use of on-farm system for the production of AMF can reduce the cost of AMF production, enhances its utilization for producing quality seedlings, and wider acceptability to the farmers/growers. The trap plants namely, maize, marigold, sorghum, fenugreek and barseem grown in succession on raised beds amended with vermicompost enhanced the AMF infectious propagules from 1.02 to 16.83 and 12.10 IP/g soil in pots and raised beds, respectively (Sharma and Sharma, 2008). The on-farm production method was found to be more economical and can be used for production of quality seedlings of horticultural plants.

Substrate based pot cultures of AMF use the ability of mycorrhizal trap plants to sustain abundant AMF biomass by the virtue of their higher symbiotic efficiency of such plants. Recently, an improved production technique was optimized where a combination of organic amendments with soybean processing mill waste (rich organic substrate) was optimized. Higher production assessed through AM signature lipids (NLFA and PLFA 16:1ω5 in roots and soil) was obtained in pots amended with a mixture of organically managed soil with vermicompost and soybean hulls. The multiplication profile assessed through microscopic methods was well correlated with AMF-signature lipids (Sharma *et al.,* 2018; Agnihotri *et al.,* 2021b; **Fig. 5**).

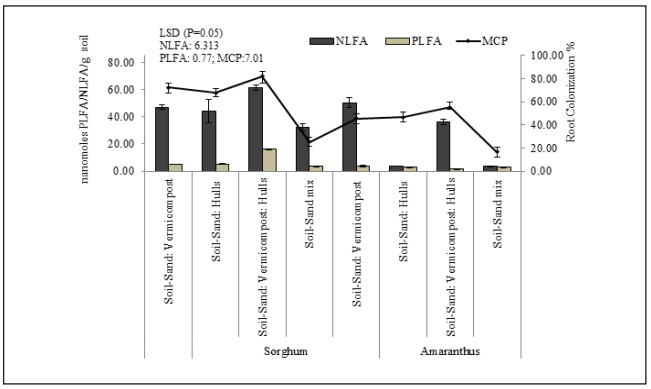


Fig. 5: Mycorrhizal colonization percentage with 16:1ω5 phospholipid and neutral lipid fatty acid assessed in AMF pot cultures (Sharma *et al.,* 2018)

Recently, in Gazette 2010 by Ministry of Agriculture, Govt. of India, AMF have been notified as AMF biofertilizers. The quantification of AMF population is the prerequisite for maintaining its quality and protection from spurious AMF products. The AMF propagules are routinely examined microscopically by quantification of spore density and root colonization. The most probable number (MPN) method (Porter, 1979) and infection unit method (Liu and Luo, 1994; Sharma *et al.,* 1996) are also being commonly used. But these methods of AMF biomass quantification via conventional microscopic methods rely on utilization of specific stains to visualize the AM-roots to differentiate between living and dead biomass. Moreover, the quantification is based on person's skills in microscopic observations and often lacks reproducibility of quantified data by different observers. Some time back molecular techniques have also been developed to determine AMF

based on gene copy number using taxa specific primers and probes (Thoner *et al.,* 2012). Other biochemical techniques involving analysis of signature lipid biomarkers mainly NLFA and PLFA 16:1ω5 in roots and soil (Olsson, 1999; Sharma and Buyer, 2015) and sterols (Fontaine *et al.,* 2004) in roots are becoming reliable and useful tools to quantify the live biomass AMF in inoculum and colonized roots/root zone soil samples. However, these methods need to be re-visited with a known AMF species with a known count to identify the most suitable analytical method with high reproducibility. Molecular and biochemical methods seem to be more promising than microscopic methods as unlike microscopic methods the reproducibility of such method is not much affected by person to person skill variation.

FUTURE DIRECTION OF RESEARCH

The potential of AMF in sustaining the agro-ecosystems has widely been studied. Since Indian soils are P deficient and high P-fixation is a common problem in achieving potential yields. AMF are largely site-specific and responses are generally more pronounced in P limiting marginal soils. Therefore, long-term studies are needed across the zones to optimize the crop response to AMF at different P doses on variety of crops. The utilization of AMF in micropropagated industries particularly during hardening and field transplantation should be encouraged. The large-scale production of resident AMF is still yet to go on a long way. Globally, conventional pot culture based inoculum production technique is being practiced where further advancements is needed through optimizing the organic substrates and host plants. Although AMF production through root organ culture (ROC)/*in vitro* technology is commercially available, but it still needs optimization on producing well-adapted native strains. Besides production of AMF, their mode of application with increased efficacy under field conditions is questionable which needs rigorous evaluation and for that large-scale mutilocation trials across the different zones should be attempted. Finally, to promote AMF as commercial formulation and fulfilling regulatory requirements and quality assurance, the potential of AMF in crop production and C cycling and C-sequestration should be carried out in holistic manner through:

- Understanding the relationship of AMF-associated glomalin with C_3 and C_4 plants under controlled environmental conditions (FACE, OTP chambers) should be investigated to quantify the contribution of AMF in soil C sequestration and carbon mitigation.
- NMR studies to identify glomalin protein.
- Third party evaluation of AMF application at multilocations across the different zones.
- Mass production and quality assay of AMF inoculum need to be revisited.

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