# MYCORRHIZA NEWS

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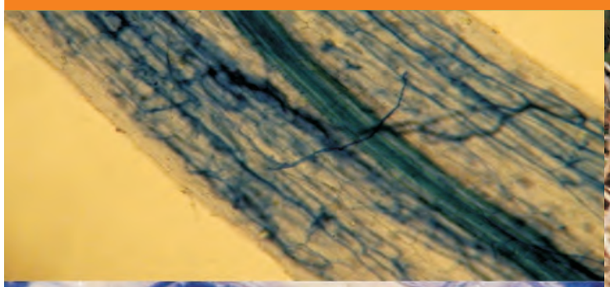
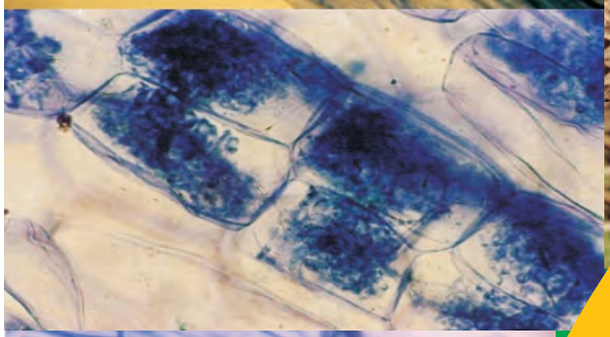
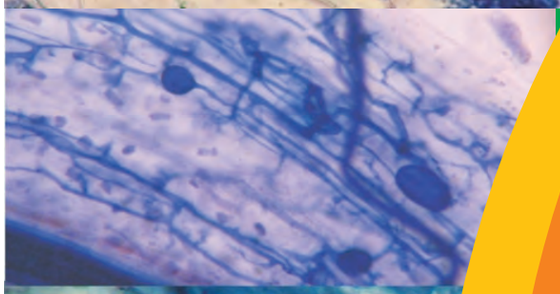
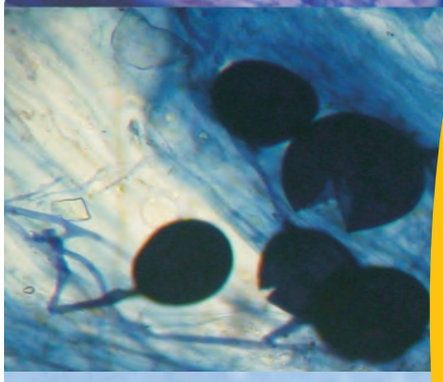
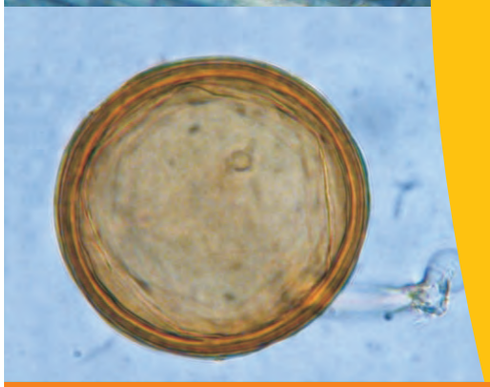










teri

## About TERI

TERI (The Energy and Resources Institute) is a not-for-profit research organization engaged in research on various aspects of energy, environment, and biotechnology, and on documentation and information dissemination. The genesis of these activities lie in TERI's firm belief that the efficient utilization of energy, sustainable use of natural resources, large-scale adoption of renewable energy technologies, and reduction of all forms of waste would move the process of development towards the goal of sustainability.

## TERI's Mycorrhiza Network

Mycorrhiza Network was set up at TERI in 1988 with the objective to strengthen research, encourage cooperation, promote exchange of information and germplasm, and facilitate transfer of technology to the field through the establishment of a mycorrhiza research network. Within the Network, a Mycorrhiza Information Centre (MIC) has been set up with support from the Department of Biotechnology, Government of India, with an objective to function as a specialized centre for collection, compilation, and dissemination of information and resources on mycorrhiza; promote networking and resource sharing.

## *Mycorrhiza News*

The *Mycorrhiza News*, a quarterly publication since 1988, provides a forum for dissemination, acquisition, interaction and communication of scientific information on mycorrhizal research activities. It publishes papers/research findings from eminent scientists covering biology, ecology, and other related aspects of mycorrhiza, including biodiversity and conservation of mycorrhizae. Among other components encompassing the newsletter include: notes on important breakthroughs; brief accounts of new approaches and techniques; research activities highlighting the Centre for Mycorrhiza Culture Collection, forthcoming events on Mycorrhiza and related events; important references of research papers published in different national and international journals.

## Contents



# Research finding papers

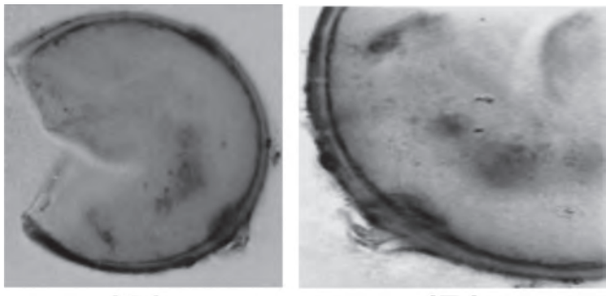
## Synonymies in AM fungi from Goa


*Sharda W Khade* *


### 1. *Glomus claroideum* Schenck and Smith emend. Walker and Vestberg

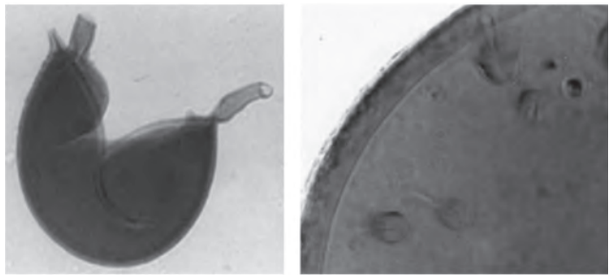
*Annals of Botany* **82**: 601–624, 1998 (Plates 1a, b).
= *Glomus maculosum* Miller and Walker, *Mycotaxon* **25**: 217–227, 1986 (Plates 1c, d).

In my study on *Carica papaya* L. and arbuscular mycorrhiza (AM) fungi in the agro-ecosystem of Goa, a few specimens of *Glomus claroideum* Schenck and Smith emend. Walker and Vestberg (Plates 1a, b) fitted well in the description of *Glomus maculosum* Miller and Walker (Plate 1c) and exhibited the presence of "maculosa" (Plate 1d), which is a characteristic feature of *G. maculosum*. However, such scalloped thickening of the inner membranous wall has been suspected for long as an artifact of parasitism, a view shared by Walker and Vestberg (1998) and they had synonymized *G. maculosum* with *G. claroideum*.



(a) (b)

**Plate 1(a)** Crushed spore of *Glomus claroideum* (×400)
**Plate 1(b)** Portion of a spore of *Glomus claroideum* with recurved hypha (×400)



(c) (d)

**Plate 1(c)** Crushed spore of *Glomus maculosum* (×400)
**Plate 1(d)** Spore surface of *Glomus maculosum* with the presence of scalloped ingrowths called maculosa (×400)

### 2. *Glomus clavisporum* (Trappe) Almeida and Schenck

*Mycologia* **82**:703–714, 1990 (Plate 2a).
= *Sclerocystis microcarpus* Iqbal and Bushra, in Iqbal and Perveen, *Transactions of Mycological Society of Japan* **21**: 57–63, 1980 (Plate 2b).

In my study on *Carica papaya* L. and AM fungi in the agro-ecosystem of Goa, most of the specimens of *Glomus clavisporum* (Trappe) Almeida and Schenck (Plate 2a) resembled the original description of *Sclerocystis microcarpus* Iqbal and Bushra (Plate 2b), while only a few specimens exhibited spores with dimensions exceeding that of *S. microcarpus*. Further,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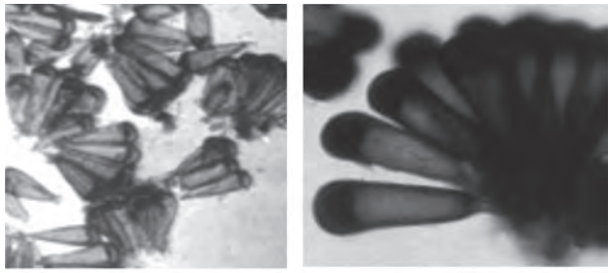


(a) (b)

**Plate 2(a)** Crushed sporocarp of *Glomus clavisporum* showing scattered spores with thickened apex (×400)
**Plate 2(b)** Crushed sporocarp of *Glomus microcarpus* with spores having thickened apex radiating from the central plexus (×400)

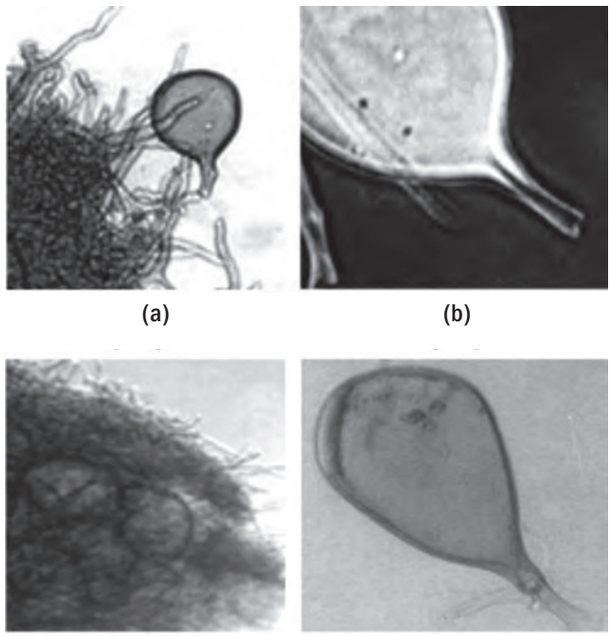
Almeida and Schenck (1990) reported *S. microcarpus* to be a synonym of *S. clavispora* Trappe since the only distinction between the two is the size of the spores and the sporocarps. Wu (1993) had similar opinion. According to Wu (1993), *S. clavispora* produces two distinctly different sizes of spores: one large and the other small. The sporocarps producing smaller spores were formerly identified as *S. microcarpus*. Both the types of spores could be found in the same sporocarp or in different sporocarps isolated from the same rhizosphere.

## 3. *Glomus coremioides* (Berk. and Broome) Redecker and Morton, in Redecker, Morton and Bruns

*Mycologia* **92**: 282–285, 2000 (Plates 3a,b).
= *Sclerocystis dussii* (Pat.) von Hohn., Sitzungsber. Kaiserl. Akad. Wiss. Wein Math. Naturwiss. Kl. Abt. I. **119**: 399, 1910 (Plates 3 c,d).

Until the 1990s, *Glomus* and *Sclerocystis* were separate genera of AM fungi. However, Almeida and Schenck (1990) placed all the species of *Sclerocystis* in *Glomus* based on spore ontogeny and sporocarp formation. Almeida and Schenck (1990) retained only one species in the genus *Sclerocystis* viz. *S. coremioides* Berkeley and Broome. Wu (1993) resisted this change, but his rationale for genus level separation, based on the ontogeny of sporogenesis and sporocarp formation, was not convincing. More recently, Redecker, Morton and Bruns (2000) transferred *S. coremioides* to *Glomus* with the discovery that 18S rDNA sequences placed the species well within the *Glomus* clade.

In my study, two distinct sporocarps types of *G. coremioides* (Berk. and Broome) Redecker and Morton were recovered. The first type of sporocarp comprised thick-walled hyphae (Plate 3a) enclosing the spore without the presence of septum in the



(a) (b)

(c) (d)

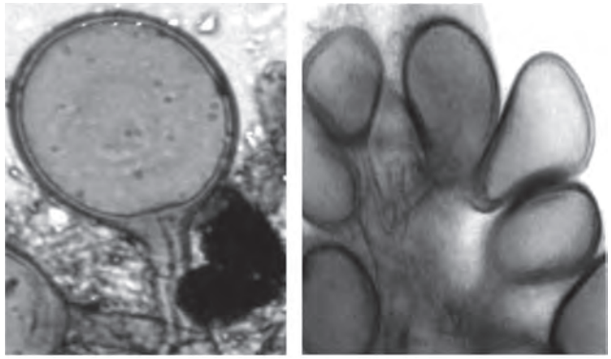
**Plate 3(a)** Crushed sporocarp of *Glomus coremioides* with peridial hypha and chlamydospore (×200)
**Plate 3(b)** A portion of chlamydospore of *Glomus coremioides* without septate hypha (×1000)
**Plate 3(c)** A portion of sporocarp of *Glomus dussi* with filamentous peridial hypha enclosing the chlamydospores (×100)
**Plate 3(d)** Chlamydospore of *Glomus dussi* with basal septum (×1000)

subtending hyphae at the spore base (Plate 3b) resembling the original description of *S. coremioides*. The second type of sporocarp was covered with reddish brown crust, (Plate 3c) enclosing spores with septate subtending hypha at the spore base (Plate 3d) resembling the original description of *S. dussii*. Almeida and Schenck (1990) and Wu (1993) reported *S. dussii* (Pat.) von Hohn to be a synonym of *S. coremioides* since their sporocarps are identical, except a crust or stroma, which covers the sporocarps of *S. dussii*.

## 4. *Glomus rubiforme* (Gerd. and Trappe) Almeida and Schenck

*Mycologia* **82**: 703–714, 1990 (Plate 4a).
= *Sclerocystis pachycaulis* Wu and Chen, *Taiwania* **31**: 65–88, 1986 (Plate 4b).

My study on *Carica papaya* L. and associated AM fungi in agro-based ecosystem of Goa recorded variation in the size and shape of sporocarps, which is in accordance with Almeida and Schenck (1990) who reported *G. rubiforme* (Gerd. and Trappe) Almeida



(a) (b)

**Plate 4(a)** Globose chlamydospore of *Glomus rubiforme* (×400)
**Plate 4(b)** Crushed sporocarp of *Glomus pachycaulis* (×200)

and Schenck (Plate 4a) to be a species with variable characteristics. Most of the specimens recovered during the study also fitted well in the original description of *S. pachycaulis* Wu and Chen (Plate 4b). Furthermore, Almeida and Schenck (1990) and Wu (1993) reported *S. pachycaulis* to be a synonym of *S. rubiformis*. In addition, two features reported by Almeida and Schenck (1990) in *S. rubiformis*, and not mentioned in the original description, are (1) the presence of an evanescent outer spore wall, which is mostly absent in mature specimens and (2) the presence of small pore occluded by wall thickening or, less frequently, by one or two septa of variable thickness in subtending hyphae near the base. The distinguishing feature of *S. pachycaulis*, is that, the wall of subtending hypha is thicker as compared to its spore wall. This same feature was also recorded in *S. rubiformis.*

## References


Almeida R T and Schenck N C. 1990. **A revision of the genus *Sclerocystis* (*Glomaceae*, *Glomales*)**. *Mycologia* **82**: 703–714

Redecker D, Morton J B, and Bruns T D. 2000. **Molecular phylogeny of the arbuscular mycorrhizal fungi *Glomus sinuosum* and *Sclerocystis coremioides***. *Mycologia* **92**: 282–285

Walker C and Vestberg M. 1998. **Synonymy amongst the arbuscular mycorrhizal fungi: *Glomus claroideum*, *G. maculosum*, *G. multisubstensum,* and *G. fistulosum***. *Annals of Botany* **82**: 601–624

Wu C. 1993. ***Glomales* of Taiwan: III. A comparative study of spore ontogeny in *Sclerocystis* (*Glomaceae, Glomales*)**. *Mycotaxon* **47**: 25–39


## Bibliography


Khade S W. 2003. **Studies on arbuscular mycorrhizal status of *Carica papaya* L. in agrobased ecosystem of Goa**. PhD, Goa University, Goa, India. 235 pp.

# Status of AM fungi associated with some medicinal plants of Mysore

*Rajkumar H Garampalli* and C P Sunilkumar*

## Introduction

Arbuscular mycorrhiza (AM) is one of the most common symbioses worldwide and about 80% of known plant species form AM (Smith and Read 1997). Distribution and diversity of AM fungi (AMF) in different medicinal plants found in a particular region are important to evaluate the natural status of AMF in that region. The beneficial effect of indigenous AMF is most important in stressed environments and circumstances, termed ecological crunches (Allen and Allen 1986). It is now widely accepted that climatic and edaphic factors can substantially influence AM association and its population. Recently, AMF species were assumed to be functionally similar, so there was little focus on their diversity in natural habitats. However, the work of van der Heijden, Klironomos, Ursie, *et al.* (1998) has shown that plant diversity and ecosystem variability and productivity (Hart and Klironomos 2002) are directly influenced by AMF diversity, making an accurate assessment of species richness and community composition crucial to understanding their role in ecosystem functioning. Previous studies on AMF diversity in medicinal plants indicate that the mean colonization and diversity patterns were dependent on edaphic factors and type of vegetation (Sundar, Palavesam, and Parthipan 2010). AM association increases the uptake of immobile nutrients, especially phosphorus and micronutrients (Douds and Millner 1999). Cultivation of medicinal and herbal plants has assumed greater importance in recent years due to their tremendous potential in modern and traditional medicine. Occurrence of microorganisms, especially vesicular-arbuscular mycorrhiza (VAM) fungi, on medicinal plants has been reported earlier (Jaleel, Gopi, Gomathinayagam, *et al.* 2009). AMF have been shown to improve the status of both water and nutrients (Auge 2001) in host plants. It has been noted that the diversity of spores in soil does not correlate with the mycorrhization rate (López-Sánchez and Honrubia 1992). VAM fungi association not only enhances the growth of medicinal plants but also improves the productivity of secondary metabolites. Many of these chemicals are of commercial importance as these are utilized in making medicines, scents, dyes, pesticides, and so on. Hence, there is a need for research to improve the quality and quantity of drugs produced from native medicinal plants by using AMF. Thus it is clear that mycorrhizal fungi are important components of natural ecosystems, and they can have strong influence on plant community composition and ecosystem functioning. The objective of this study is to evaluate the status of AM fungi in some medicinal plants growing in the wild. Assessing the presence and status of AM fungi in some important medicinal plants will help to carry out further research in this field.

## Materials and methods

### *Survey area*

This study was carried out in the Mysore district (12.30°N, 76.65°E), which is situated in the southern region of Karnataka, India. It lies about 146 km south-west of Bengaluru, is spread across an area of 128.42 km$^2$, and has an average altitude of 770 m (above mean sea level). The highest recorded temperature was 40°C, and the average annual rainfall was 798.2 mm. The district is divided into seven subdivisions (taluks). The study samples were collected throughout these subdivisions.

### *Collection of soil and root samples*

Rhizospheric soil and root samples were collected from 25 species of medicinal plants throughout the Mysore district. The samples were taken from a particular plant block covering an area of 100 m$^2$. These were collected randomly from a depth of 10–30 cm. Soil particles attached to fine roots were removed by generous shaking (root samples stored in standard FAA solution). The soil samples were combined to make the composite samples, which were air-dried and stored at 4°C.

### *Assessment of AM fungal colonization*

The cleaned roots were cut into 1 cm long pieces and stained with trypan blue, according to the procedure described by Phillips and Hayman (1970). The roots were boiled in 10% KOH for 1 h, acidified with 5 N HCl, and stained overnight with 0.5% trypan blue. The roots were then destained with 70% glycerol. An assessment of mycorrhizal infection was carried out by the slide method; root segments were randomly selected from the stained samples and observed for the presence or absence of functional structures (mycelia,

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arbuscules, and vesicles) of AMF. A minimum of 100 root segments were used for this enumeration, and the colonization by AMF was calculated using the following formula.

$$\text{Per cent colonization (\%)} = \frac{\text{Total number of root segments colonized}}{\text{Total number of root segments studied}} \times 100$$

### *Isolation and identification of AM fungal spores*

AM fungal spores were extracted using wet sieving and the decanting method (Gerdemann and Nicolson 1963). Then 10 g of soil was suspended in tap water, and the mixture was decanted through stacked sieves of sizes ranging from 32 mm to 420 mm. Spores from bottom and middle sieves were collected on a Petri dish and counted under stereo zoom microscope. These were then mounted with polyvinyl alcohol-lactic acid-glycerol (PVLG) for the identification of AM fungal species, which were photographed and identified up to the level specified by Schenck and Perez (1990). The photographs were compared according to the species description provided in INVAM.[1]

## Results and discussion

The study revealed the differences in per cent root colonization among 25 medicinal plant species and that the spore population in rhizospheric soil varied from plant to plant (Table 1). The plants

**Table 1** List of the medicinal plants with per cent AM association and spore population/10 g of dry soil

| Name of the plant | Per cent AM association (%) | Nature of the structure | Spore population/10 g of dry soil |
|---|---|---|---|
| *Acalypha indica* L. | 78.25 | A, V, M | 65 |
| *Achyranthes aspera* L. | 76.67 | A, V, M | 78 |
| *Anisochilus carnosus* Wall. | 90.00 | A, V, M | 63 |
| *Asparagus racemosus* Willd. | 72.50 | A, V, M | 45 |
| *Asystasia gangetica* (L.) T.Anderson | 72.50 | A, V, M | 70 |
| *Blepharis maderaspatensis* (L.) B. Heyne ex Roth | 92.50 | A, V, M | 98 |
| *Calotropis procera* (Ait.) Ait. f. | 63.65 | A, V, M | 69 |
| *Clitoria ternatea* L. | 85.00 | A, V, M | 35 |
| *Desmodium triflorum* (L.) DC. | 90.00 | A, V, M | 110 |
| *Dodonaea viscosa* (L.) Jacq. | 00.00 | – | 00 |
| *Eclipta alba* (L.) Hass | 82.50 | A, V, M | 76 |
| *Grangea maderaspatana* (L.) Poir | 70.00 | A, M | 58 |
| *Indigofera tinctoria* L. | 85.00 | A, M | 89 |
| *Ionidium suffruticosum* (Ging.) | 87.50 | A, V, M | 78 |
| *Ipomaea muricata* (L.) Jacq. | 61.25 | A, V, M | 45 |
| *Leucas aspera* (willd) Link | 67.50 | A, V, M | 99 |
| *Morus alba* L. | 95.00 | A, V, M | 145 |
| *Ocimum sanctum* L. | 80.00 | A, V, M | 125 |
| *Oxalis corniculata* L. | 00.00 | – | 00 |
| *Phyllanthus amarus* L. | 65.00 | A, M | 35 |
| *Santalum album* L. | 85.00 | A, M | 92 |
| *Tinospora cordifolia* Miers | 00.00 | – | 00 |
| *Tribulus terrestris* L. | 65.00 | A, M | 105 |
| *Tridax procumbens* L. | 90.00 | A, V, M | 135 |
| *Tylophora asthmatica* Wight & Arn. | 77.50 | A, V, M | 45 |

A – arbuscules; M – mycelium; V – vesicle

[1] Details available at <http://invam.caf.wvu.edu>

were colonized by a number of AM fungal species. The highest percentage of colonization was observed in *Morus alba* (95%), followed by *Blepharis maderaspatensis* (92.5%), *Anisochilus carnosus* (90%), *Desmodium triflorum* (90%), and *Tridax procumbens* (90%). The lowest colonization was observed in *Ipomaea muricata* (61.25%) (Figure 1).

The maximum number of AM fungal spores was recovered from rhizospheric soil of *M. alba* (145 spores/10 g of dry soil), followed by *T. procumbens* (135 spores), *Ocimum sanctum* (125 spores), and *D. triflorum* (110 spores) (Figure 2). This shows the correlation between the spore population and per cent root colonization. The higher the per cent root colonization,

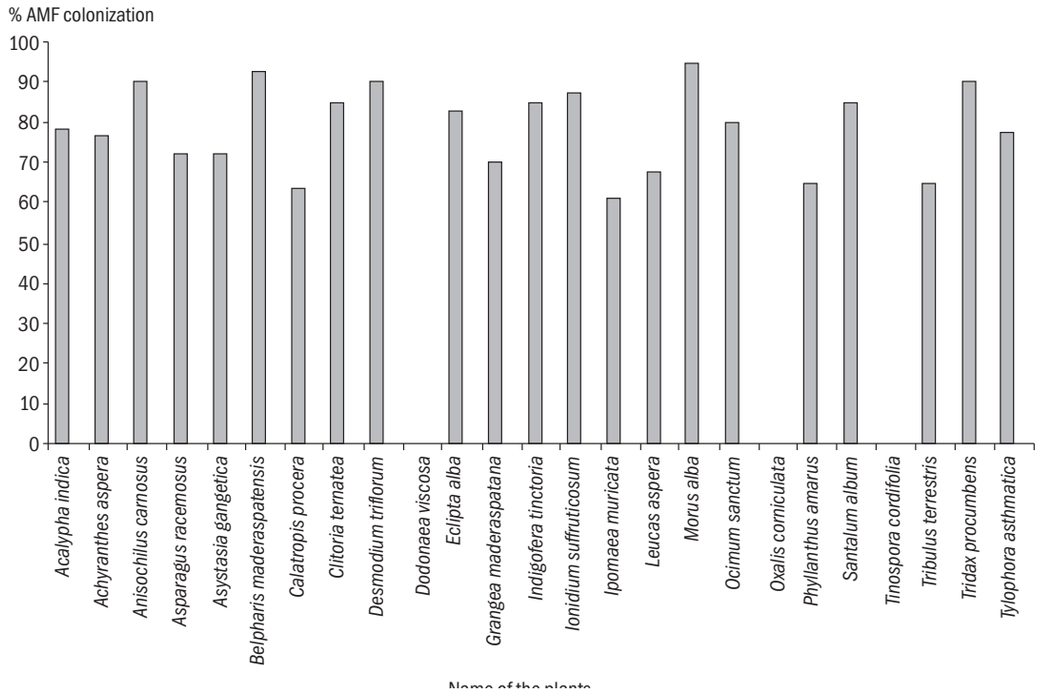


**Figure 1** Percentage of AMF colonization in some medicinal plants

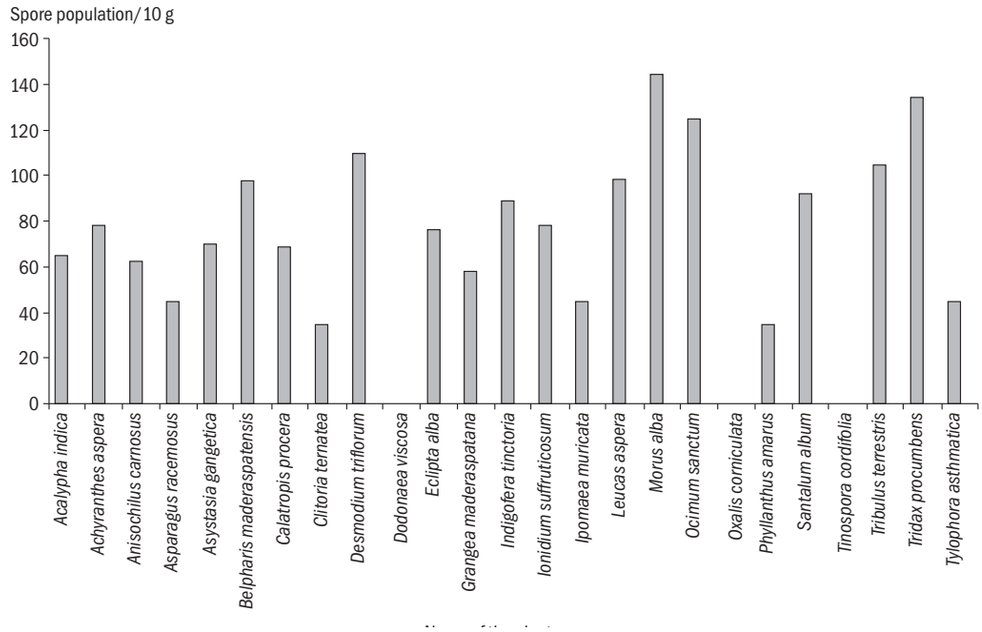


**Figure 2** Spore population/10 g of rhizospheric soil

the more the total number of AM fungal spores. The dominant AM fungal genera found in most of the soil samples were *Glomus*, *Gigaspora*, and *Acaulospora*, which were found in most of the soil samples. Among these three, *Glomus* was found to be more dominant, followed by *Acaulospora* and *Gigaspora*. This is the first report on the status of AMF associated with some medicinal plants found in this region. It is clearly evident from the data that almost all of the medicinal plants selected for the study were invariably found to harbour AM fungal association.

AMF displayed higher host specificity in this study. The highest number of mycorrhizal spores in rhizospheric soil and AM fungal infection in the roots of *M. alba*, *A. carnosus, T. procumbens,* and *D. triflorum* indicated that these plant species might be considered good hosts for AMF under the prevailing conditions. This may be attributed to the root exudates of these plants, which might have stimulated the germination of mycorrhizal spores and increased the infection (Azaizeh, Marschner, Römheld, *et al.* 1995).

This study confirms the wide occurrence of AMF in almost all medicinal plants studied. It also extends the range of host plants of AMF, which may play an essential ecological role in highly stressed environments (Chaurasia, Pandey, and Palni 2005). Further, earlier reports indicated that AM fungal association not only enhances the growth of medicinal plants but also improves the productivity of medicinal compounds (Karthikeyan, Joe, and Jaleel 2009). It was observed that the medicinal plant biomass, protein content, and chlorophyll content were higher and that the mycorrhization level was also higher in AM-inoculated plants compared to non-inoculated plants. Hence, there is a need to carry out research for improving the quality and quantity of drugs produced from native medicinal plants, in a relatively shorter period and at a lower expense, by using AMF.

## Acknowledgement

The first author is thankful to the authorities of University of Mysore for sanctioning minor research project and providing financial assistance to carry out the research work.

## References

Allen E B and Allen M F. 1986. **Water relations of xeric grasses in the field: interactions of mycorrhizae and competition**. *New Phytologist* **104**: 559–571

Auge R M. 2001. **Water relations, drought, and vesicular-arbuscular mycorrhizal symbiosis**. *Mycorrhiza* **11**: 3–42

Azaizeh H A, Marschner H, Römheld V, Wittenmayer L. 1995. **Effects of a vesicular-arbuscular mycorrhizal fungus and other soil microorganisms on growth, mineral nutrient acquisition, and root exudation of soil-grown maize plants**. *Mycorrhiza* **5**: 321–327

Chaurasia B, Pandey A, and Palni S L M. 2005. **Distribution, colonization, and diversity of arbuscular mycorrhizal fungi associated with central Himalayan rhododendrons**. *Forest Ecology and Management* **207**: 315–324

Douds D D and Millner P. 1999. **Biodiversity of arbuscular mycorrhizal fungi in agroecosystems.** *Agriculture, Ecosystems, and Environment* **74**: 77–93

Gerdemann J W and Nicolson T H. 1963. **Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting**. *Transactions of the British Mycological Society* **46**: 235–244

Hart M M and Klironomos J N. 2002. **Diversity of arbuscular mycorrhizal fungi and ecosystem functioning**. In *Mycorrhizal Ecology*, edited by M G A van der Heijden and I R Sanders. New York: Springer. pp. 225–242

Jaleel C A, Gopi R, Gomathinayagam M, Panneerselvam R. 2009. **Traditional and non-traditional plant growth regulators alters phytochemical constituents in *Catharanthus roseus***. *Process Biochemistry* **44**: 205–209

Karthikeyan B, Joe M M, and Jaleel C A. 2009. **Response of some medicinal plants to vesicular arbuscular mycorrhizal inoculations**. *Journal of Scientific Research* **1**(1): 381–386

López-Sánchez M E and Honrubia M. 1992. **Seasonal variation of vesicular-arbuscular mycorrhizae in eroded soils from southern Spain**. *Mycorrhiza* **2**: 33–39

Phillips J M and Hayman D S. 1970. **Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection**. *Transactions of the British Mycological Society* **55**: 158–161

Schenck N C and Perez Y. 1990. ***Manual for the Identification of VA Mycorrhizal Fungi***. Gainesville, Florida: Synergistic Publications Inc.

Smith S E and Read D J. 1997. ***Mycorrhizal Symbiosis***, 2nd edn. London, UK: Academic Press

Sundar S K, Palavesam A, and Parthipan B. 2010. **AM fungal diversity in selected medicinal plants of Kanyakumari District, Tamil Nadu, India**. *Indian Journal of Microbiology* **51**(3): 259–265

van der Heijden M G A, Klironomos J M, Ursie M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders I R. 1998. **Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity**. *Nature* **396**: 69–72

# Application of arbuscular mycorrhizal fungal inoculant on growth enhancement of *Solanum melongena* Linn. at different phosphorous level

*A Irfan, M Ayoob, and P K Jite**

## Introduction

Arbuscular mycorrhiza (AM) is a symbiotic association between fungus and roots of plants and is primarily responsible for nutrient transfer (Brundrett 2004). AM fungi (AMF) promote water and nutrient uptake in plants, especially of insoluble soil phosphate (Pi) fraction (Clark and Zeto 2000; Marschner and Dell 1994). Agricultural and horticultural crops have been shown to benefit from vesicular-arbuscular mycorrhizae (VAM) on a worldwide basis as these are most prevalent and universally associated with horticultural and agricultural crops (Mosse 1973). Nelson and Achar (2001) analysed the effect of AMF on the growth performance of cabbage. Ferreira and Janick (1995) analysed the effect of AMF (*Glomus fasciculatum)* along with phosphate level on the growth and artemisinin content of *Artemisia annua*. Similarly, Karthikeyan, Jaleel, Changxing, *et al*. (2008) analysed the effect of AMF and *Glomus mosseae* along with phosphate levels on the growth and alkaloid content of *Catharanthus roseus*.

This study was carried out to investigate the effect of AMF on *Solanum melongena* Linn. with respect to morphological, physiological, and biochemical parameters under three different phosphate levels over non-inoculated plants.

## Materials and methods

Seeds of *S. melongena* L. were obtained from College of Agriculture, Pune, Maharashtra. Cultures of *G. fasciculatum* (Thaxter) Gerd. and Trappe emend. Walker and Koske and *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe were obtained from D J Bhagaraj and maintained on bajra for three months and used as inoculum (containing rhizospheric soil, AM-colonized roots, and 10–15 spores/g of soil) for seedling of *S. melongena* L.

### *Experimental design*

The experimental design consisted of eight treatments having four non-AM-inoculated and four AM-inoculated *S. melongena* L. plants with three phosphate levels ($K_2HPO_4$: 50 mg, 100 mg, and 150 mg in 100 ml of distilled water) per 8 kg of sterilized soil. Pots were arranged in completely randomized block design. Nine replicates of each treatment were grown, and 72 pots were arranged. One-month-old seedlings of *S. melongena* L. were used for experiment. $K_2HPO_4$ was used as phosphate source. After 30 days of AM inoculation, P treatment was given at seven days interval, and it was continued till the last observation was taken. Observations were recorded after 60, 90, and 120 days after AM inoculation. Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS) software and the Duncan Multiple Range Test (DMRT).

### *Morphological parameters*

Shoot length, root length, fresh weight, dry weight, leaf area, and per cent root colonization were studied.

### *Physiological parameters*

Total chlorophyll was determined by Arnon's method (Arnon 1949). The amount of total chlorophyll was expressed in mg/g fresh weight. Total P content was measured as described by Fiske and Subbarao's method (Fiske and Subbarao 1925). The amount of phosphorus was expressed in mg/g dry weight.

### *Determination of carbohydrates and reducing sugars*

Total carbohydrates were determined by Hodge and Hofreiter's (Hodge and Hofreiter 1962) method, and total reducing sugar was determined by Miller's method (Miller 1959). Total carbohydrates and total reducing sugars were expressed in mg/g fresh weight.

## Results and discussion

AM fungal inoculation had a significant effect on all measured plant growth variables as shown in Tables 1 and 2. The present study revealed that plants grown in the presence of AMF have larger biomass than non-AMF plants. Shoot and root length increased significantly in *Gf*+*Gm*+2P as compared to C+2P after 60, 90, and 120 days of AM inoculation (Table 1). *Solanum* plants inoculated with *Gf*+*Gm* (in combination treatment) along with phosphate showed increased shoot and root length as compared to non-inoculated *Solanum* plants. This may be attributed to either the mechanism of mycorrhizal infection and

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**Table 1** Morphological parameters of *Solanum melongena* L. inoculated with *Glomus fasciculatum and Glomus mosseae* under three different phosphate levels

| Phosphate level | Treatment | Leaf area ($cm^2$) | Shoot length (cm) | Root length (cm) | Fresh weight (g) | Dry weight (g) |
|---|---|---|---|---|---|---|
| C | 60 | 30.96±1.95e | 14.00±2.00e | 7.00±1.00e | 6.57±1.41d | 0.54±0.05d |
| | 90 | 37.29±5.10d | 20.33±2.08f | 8.67±0.58e | 8.74±0.89f | 1.30±0.05d |
| | 120 | 40.96±2.62d | 24.00±2.65f | 10.33±2.08e | 10.04±0.83c | 1.51±0.07e |
| C+1P | 60 | 32.20±3.13de | 16.67±3.79de | 8.00±1.00e | 7.82±1.74cd | 0.67±0.12d |
| | 90 | 41.53±4.24d | 23.67±3.06ef | 10.00±1.00e | 10.81±1.18ef | 1.43±0.07cd |
| | 120 | 43.86±3.78d | 27.00±1.00ef | 12.33±1.53de | 13.68±2.01b | 1.60±0.06de |
| C+2P | 60 | 37.58±4.62cde | 17.33±3.06de | 9.00±1.00de | 8.53±1.43cd | 0.90±0.14c |
| | 90 | 43.24±2.53cd | 24.33±4.16ef | 11.67±0.58de | 13.19±0.57de | 1.50±0.34cd |
| | 120 | 47.36±4.21cd | 29.00±2.00ed | 13.67±1.53cd | 14.77±0.49b | 1.66±0.10de |
| C+3P | 60 | 40.69±2.67bcd | 20.33±1.15d | 11.33±1.53cd | 10.91±1.16c | 1.06±0.27b |
| | 90 | 45.36±5.08cd | 26.67±2.52de | 14.00±1.00cd | 15.11±0.59d | 1.54±0.11cd |
| | 120 | 47.36±4.21cd | 32.00±1.00d | 16.00±2.00bc | 16.08±1.90b | 1.74±0.12de |
| *Gf+Gm* | 60 | 45.74±2.20abc | 25.00±3.61c | 12.67±1.53bc | 15.44±2.03b | 1.25±0.16b |
| | 90 | 50.41±7.82bc | 31.00±1.00cd | 15.67±1.53bc | 18.73±1.01c | 1.68±0.06bc |
| | 120 | 54.07±6.76bc | 36.33±2.08c | 18.00±1.00ab | 22.86±0.99a | 1.84±0.11cd |
| *Gf+Gm*+1P | 60 | 47.55±8.10ab | 26.67±2.08bc | 15.00±1.00ab | 16.07±2.07b | 1.27±0.05b |
| | 90 | 54.55±4.50ab | 34.00±4.36bc | 18.67±2.52ab | 21.40±2.09bc | 1.88±0.10b |
| | 120 | 60.22±7.68a | 38.00±3.00bc | 20.33±1.53a | 23.58±3.33a | 1.91±0.12bc |
| *Gf+Gm*+2P | 60 | 51.50±7.12a | 30.33±1.53ab | 16.33±2.08a | 18.28±1.53ab | 1.32±0.07ab |
| | 90 | 58.50±3.18ab | 37.67±4.04ab | 19.67±2.08a | 23.96±4.07ab | 1.92±0.09ab |
| | 120 | 60.83±5.69ab | 42.33±1.53a | 21.33±3.06a | 25.40±2.66a | 2.33±0.31a |
| *Gf+Gm*+3P | 60 | 53.99±4.03a | 32.00±2.00a | 17.00±2.65 | 20.16±2.08a | 1.48±0.11a |
| | 90 | 61.32±3.42a | 40.67±2.52a | 20.67±3.06a | 25.97±1.60a | 2.17±0.09a |
| | 120 | 63.32±1.44a | 42.67±1.53ab | 19.00±1.00ab | 25.37±1.56a | 2.14±0.041ab |

C – control; C+1P – control with first phosphate level; C+2P – control with second phosphate level; C+3P – control with third phosphate level; *Gf+Gm* – *G. fasciculatum* + *G. mosseae; Gf+Gm*+1P – *G. fasciculatum* + *G. mosseae* with first phosphate level; *Gf+Gm*+2P – *G. fasciculatum* + *G. mosseae* with second phosphate level; *Gf+Gm*+3P – *G. fasciculatum* + *G. mosseae* with third phosphate level

development in the host tissue (Sanders, Tinker, Black, *et al*. 1977) or the improvement in phosphate uptake as a result of AM infection (Jeffries 1987). Similar observations were made by Chiramel, Bagyaraj, and Patil (2006), who reported that plants inoculated with *Glomus leptotichum*, *Glomus etunicatum,* and *G. mosseae* showed higher plant height as compared to non-inoculated plants. Present findings are also in agreement with Elahi, Mridha, and Aminuzzaman (2010), who investigated the effects of inoculation with VAM fungi on growth and total biomass of *S. melongena*. At high phosphate level (*Gf+Gm*+3P), *Solanum* plants showed decreased shoot and root length as compared to *Gf+Gm*+2P after 120 days of AM inoculation. Fresh and dry weights of AM-inoculated plants increased in all phosphate levels studied. After 120 days of AM inoculation, fresh and dry weights decreased in *Gf+Gm*+3P level but was still higher in AM-inoculated *Solanum* as compared to non-AM-inoculated plants because at high phosphate level, plant growth gets suppressed due to which there is reduced accumulation of minerals in mycorrhizal plants (Rubio, Borie, Schalchli, *et al*. 2002) (Table 1).

Per cent mycorrhizal colonization increased with an increase in phosphate level in early stages of colonization (Table 2). In this study, there was significant increase in the per cent root colonization during 60 and 90 days of *G. fasciculatum and G. mosseae* inoculation. It decreased after 120 days of AM inoculation in *Gf+Gm*+3P level because of high P condition. It is reported that high soil P level reduces both intraradical as well as extraradical AM development (Abbott and

**Table 2** Biochemical parameters of *Solanum melongena* L. inoculated with *Glomus fasciculatum and Glomus mosseae* under three different phosphate levels

| Phosphate level | Treatment | Per cent root colonization | Total chlorophyll (mg/g) | Phosphate content (mg/g) | Total carbohydrates (mg/g) | Total reducing sugar (mg/g) |
|---|---|---|---|---|---|---|
| C | 60 | 0.00 | 0.436±0.023d | 0.36±0.07d | 9.36±0.326d | 6.17±0.88d |
| | 90 | 0.00 | 1.165±0.062f | 0.70±0.096 | 11.42±1.290f | 8.77±1.45g |
| | 120 | 0.00 | 1.396±0.131b | 0.76±0.04e | 12.013±2.757f | 9.67±2.06f |
| C+1P | 60 | 0.00 | 0.919±0.185c | 0.58±0.13cd | 15.02±3.08cd | 7.56±1.34d |
| | 90 | 0.00 | 1.352±0.057ef | 1.24±0.08b | 17.41±0.49e | 11.54±1.39fg |
| | 120 | 0.00 | 1.518±0.199b | 1.49±0.24d | 18.83±0.83e | 12.69±1.99ef |
| C+2P | 60 | 0.00 | 1.066±0.145c | 0.65±0.10cd | 16.24±1.32c | 9.02±1.46cd |
| | 90 | 0.00 | 1.502±0.070de | 1.34±0.13b | 20.04±1.74d | 13.67±1.66ef |
| | 120 | 0.00 | 1.675±0.107b | 1.80±0.12d | 21.62±0.92d | 15.55±1.28de |
| C+3P | 60 | 0.00 | 1.279±0.253bc | 0.67±0.09cd | 16.56±1.082c | 10.74±1.93c |
| | 90 | 0.00 | 1.579±0.045d | 1.58±0.10a | 22.68±0.83c | 15.49±1.21de |
| | 120 | 0.00 | 1.726±0.159b | 1.89±0.19c | 23.35±1.53cd | 18.27±1.78cd |
| *Gf+Gm* | 60 | 27.00±4.58c | 1.493±0.199b | 0.76±0.19cd | 19.35±2.73bc | 14.62±1.78b |
| | 90 | 47.33±6.80b | 1.985±0.152c | 1.05±0.14a | 24.56±1.08c | 17.38±1.07cd |
| | 120 | 52.66±3.05b | 2.263±0.358a | 1.42±0.07bc | 25.02±0.68c | 20.42±1.20bc |
| *Gf+Gm*+1P | 60 | 34.00±2.00b | 1.539±0.101b | 0.97±0.19b | 22.92±2.88ab | 14.88±1.33b |
| | 90 | 54.66±2.30a | 2.224±0.100b | 1.52±0.18a | 28.79±1.08b | 20.11±2.42bc |
| | 120 | 69.66±7.09b | 2.559±0.204a | 1.97±0.16abc | 31.69±0.83b | 23.24±1.31ab |
| *Gf+Gm*+2P | 60 | 38.66±3.51a | 1.858±0.115a | 1.15±0.19a | 23.50±2.79a | 16.12±1.61ab |
| | 90 | 68.66±3.51a | 2.374±0.208b | 1.61±0.07a | 34.11±0.83a | 22.80±1.62ab |
| | 120 | 79.66±1.15a | 2.659±0.325a | 2.09±0.05ab | 34.14±0.87a | 25.01±2.90a |
| *Gf+Gm*+3P | 60 | 39.00±2.64a | 1.996±0.200a | 1.17±0.17a | 25.22±3.31a | 17.79±1.80a |
| | 90 | 69.00±3.60a | 2.752±0.125a | 2.01±0.08a | 34.83±1.08a | 24.59±2.97a |
| | 120 | 64.33±3.05a | 2.414±0.182a | 2.16±0.10a | 33.51±2.06a | 22.11±2.45ab |

C – control; C+1P –control with first phosphate level; C+2P –control with second phosphate level; C+3P –control with third phosphate level; *Gf+Gm* – *G. fasciculatum* + *G. mosseae; Gf+Gm*+1P – *G. fasciculatum* + *G. mosseae* with first phosphate level; *Gf+Gm*+2P – *G. fasciculatum* + *G. mosseae* with second phosphate level; *Gf+Gm*+3P – *G. fasciculatum* + *G. mosseae* with third phosphate level.

*Note* Data were analysed by Duncan's Multiple New Range Test. Small alphabet indicate significant differences at p = 0.05 level.

Robson 1984; Liu, Hamel, Hamilton, *et al*. 2000). Total chlorophyll content consequently increased in AM-inoculated plants than in non-AM-inoculated control plants at first and second phosphate levels (50–100 mg/kg of soil) after 60, 90, and 120 days of inoculation. But at high phosphate level (150 mg/kg of soil), total chlorophyll content decreased in AM-inoculated plants (*Gf+Gm* +3P) after 120 days of inoculation, which means that mycorrhization or P fertilization influenced the concentration of photosynthetic pigments (Table 2). These results are in agreement with those obtained by some earlier works (Giri, Kapoor, and Mukerji 2003; Kapoor and Bhatnagar 2007). Similarly, Elahi, Mridha, and Aminuzzaman (2010) investigated the effects of inoculation with VAM fungi on chlorophyll content of *S. melongena* and observed highest chlorophyll content in AM-treated *Solanum* plants than in non-treated *Solanum* plants. This investigation revealed that P content increased significantly in AM *Solanum* plants as compared to non-AM plants. P content increased after 60, 90, and 120 days of AM inoculation in all phosphate levels (Table 2). The increased P uptake may be a result of increased phosphorylase activity on the surface of mycorrhizal roots. It may also be due to the increased absorbing area contributed by fungal hyphae (Pearson and Tinker 1975). The increment was observed to be greater in AM-inoculated plants than in non-AM-inoculated control plants. After 120 days of

AM inoculation, it was found to be more in *Gf*+*Gm*+3P as compared to C+3P treatment (Table 2). Our results are in agreement with the observations of Labour, Jolicoeur, and St-Arnaud (2003) and Dhanda, Sethi, and Behl (2004), who reported that plants with AMF had higher phosphorus content in shoots than in non-AMF plants.

## Conclusion

This study revealed that when *Solanum* plants were inoculated with AMF, they showed significantly good growth as compared to control plants, both morphologically and biochemically. This suggests that AMF can act as bio-fertilizer and can certainly prove beneficial for increasing biomass and improving production of agricultural crops. Hence, proper selection of mycorrhizal strain could play an important role in optimizing the growth of *S. melongena*.

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## References

Abbott L K and Robson A D. 1984. **The effect of root density, inoculum placement, and infectivity of inoculum on the development of vesicular–arbuscular mycorrhiza.** *New Phytologist* **97**: 285–299

Arnon D I. 1949. **Copper enzymes in isolated chloroplasts. Polyphenoloxidase in beta vulgaris**. *Plant Physiology* **24**: 1–15

Brundrett M C. 2004. **Diversity and classification of mycorrhizal associations**. *Biological Reviews of the Cambridge Philosophical Society (London)* **79**: 473–495

Chiramel T, Bagyaraj D J, and Patil C S P. 2006. **Response of *Andrographis paniculata* to different arbuscular mycorrhizal fungi**. *Journal of Agricultural Technology* **2**(2): 221–228

Clark R B and Zeto S K. 1996. **Iron acquisition by mycorrhizal maize infected with a VAM fungus *Glomus fasiculatum***. *Plant Physiology* **101**: 865–871

Dhanda S S, Sethi G S, and Behl R K. 2004. **Indices of drought tolerance in wheat genotypes at early stages of plant growth**. *Journal of Agronomy and Crop Science* **190**(1): 6–12

Elahi F E, Mridha M A U, Aminuzzaman. 2010. **Influence of AMF inoculation on growth, nutrient uptake, arsenic toxicity and chlorophyll content of eggplant grown in arsenic amended soil**. *Advances in natural and applied sciences* **4**(2): 184–192

Ferreira F F S and Janick J. 1995. **Floral morphology of *Artemisia annua* with special reference to trichomes**. *International Journal of Plant Sciences* **156**: 807–815

Fiske C H and Subbarao Y. 1925. **The colorimetric determination of phosphorus**. *Journal of Biological Chemistry* **66**: 375–400

Giri B, Kapoor R, and Mukerji K G. 2003. **Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of *Acacia auriculiformis***. *Biology and Fertility of Soils* **38**: 170–175

Hodge J E and Hofreiter B T. 1962. **Determination of reducing sugars and carbohydrates**. In *Methods in Carbohydrate Chemistry*, edited by R L Whistler, M L Wolrom, J N BeMiller, and F Shafizadeh. New York: Academic Press

Jeffries D. 1987. **Use of mycorrhizae in agriculture**. *Critical Reviews in Biotechnology* **15**: 319–358

Kapoor R and Bhatnagar A K. 2007. **Attenuation of cadmium toxicity in mycorrhizal celery (*Apium graveolens* L.)**. *World Journal of Microbiology and Biotechnology* **23**: 1083–1089

Karthikeyan B, Jaleel C A, Changxing Z, Joe M M, Srimannarayan J, Deiveekasundaram M. 2008. **The effect of AM fungi and phosphorus level on the biomass yield and ajmalicine production in *Catharanthus roseus***. *EurAsian Journal of BioSciences* **2**: 26–33

Labour K, Jolicoeur M, and St-Arnaud M. 2003. **Arbuscular mycorrhizal responsiveness of in vitro tomato root lines is not related to growth and nutrient uptake rates**. *Canadian Journal of Botany* **81**(7): 645–656

Liu A, Hamel C, Hamilton R I, Ma B L, Smith D L. 2000. **Acquisition of Cu, Zn, Mn, and Fe by mycorrhizal maize (*Zea mays L.*) grown in soil at different P and micronutrient levels**. *Mycorrhiza* **9**: 331–336

Marschner H and Dell. 1994. **Nutrient uptake in mycorrhizal symbiosis**. *Plant and Soil* **159**: 89–102

Miller G L. 1959. **Use of dinitrosalicylic acid reagent for determination of reducing sugar**. *Analytical Chemistry* **31**: 426–428

Mosse B. 1973. **Advances in the study of vesicular-arbuscular mycorrhiza**. *Annual Review of Phytopathology* **11**: 171–196

Nelson R and P N Achar. 2001. **Stimulation of growth and nutrient uptake by VAM fungi in *Brassica oleracea* var. capitata**. *Biologia Plantarum* **44**: 277–281

Pearson V and P B Tinker. 1975. **Measurement of phosphorus fluxes in the external hyphae of endomycorrhizae**. In *Endomycorrhizas*, edited by F E Sanders, B Mosseae, and P B Tinker, pp. 277–287. New York: Academic Press

Rubio R, Borie F, Schalchli C, Castillo C, Azcon R. 2002. **Plant growth responses in natural acidic soil as affected by arbuscular mycorrhizal inoculation and phosphorus sources**. *Journal of Plant Nutrition* **25**: 1389–1405

Sanders F E, Tinker P B, Black R L B, Palmerley S M. 1977. **The development of endomycorrhizal root systems: I. Spread of infection and growth-promoting effects with four species of vesicular-arbuscular endophytes**. *New Phytologist* **78**: 257–268

# Effect of *Glomus intraradices* AM fungi on the shoot dry matter, seed dry yield, and nitrogen and phosphorus uptake of soybean (*Glycine max* [L.] Merrill) inoculated with cultivar-specific *Bradyrhizobium japonicum*

*K Prasad**

## Introduction

Soybean (*Glycine max* [L.] Merrill) is one of the most widely grown legumes, which is a source of protein, oil, and minerals for humans and livestock. In India, most of the land used for food production has non-productive alluvial soil characterized by low pH and nutrient availability, particularly of N and P. In fact, leguminous plants are most sensitive to P deficiency, and their capability to absorb P nutrient is very low. This is due to their magnoloid root system (Mosse 1981). The combination of soybean and phosphate (super phosphate and rock phosphate) fertilizer have been used widely to improve soybean production. There is a possibility of reducing the use of P fertilizers and using eco-friendly and cheaper bio-fertilizers such as arbuscular mycorrhizal fungi (AMF) and nitrogen-fixer *Bradyrhizobium japonicum* for better growth and yield. Association of soybean with AMF increases the uptake of nutrients, particularly P (Gavito, Schweiger, and Jakobsen 2003; Xavier and Germida 2003), zinc (Chen, Li, Tao, *et al*. 2003; Xie, Staehelin, Vierheilig, *et al*. 1995), and nitrogen as well as increasing crop production (Raverkar and Tilak 2002). The association of bradyrhizobial strains with the roots of soybean plants also improves soil health and nitrogen fixation, thus increasing further crop production (Jaarsveld, Smith, and Kruger 2002; Bethlenfalvay, Schreiner, and Mihara 1997; Troeh and Loynachan 2009; Nelson, Niedziela, Pitchay, *et al*. 2010). Synergistic effects of AMF and *B. japanicum* have a high potential to improve the nutrient supply of soybean, including phosphorus and soil quality (Tilak, Rathi, and Saxena 1995).

AMF, when associated with higher plants, play an important role in improving the intake of macronutrients and micronutrients, which are of low mobility in soil solution. That AMF promote plant growth and nutrient uptake is well established in *Glomus* species, in which growth and uptake increased 20 fold and 8 fold, respectively, compared to control (Lukiwati, Hardjosoewignjo, Fakuara, *et al.* 1994). Uptake of nutrient (Waidyanatha, Yogoratnam, and Ariyaratne 1979) and mineral content (Lukiwati and Hardjosoewignjo 1998) of mycorrhizal plants was higher compared to non-mycorrhizal plants. Symbiotic associations between AMF and leguminous plants were more responsive to rock phosphate than to other types of P fertilizers (Dodd, Arias, Koomen, *et al.* 1990). In each plant species, plant growth and root geometry differ, as well as their response to treatment (Kerridge and Ratcliff 1982). Strains and species of AMF have been shown to differ with regard to the extent to which they increase nutrient uptake and plant growth. Variations in mycorrhizal efficiency are determined by differences in the number of active arbuscules as a proportion of the total fungal biomass within the root system (Smith and Smith 1995).

Two symbiotic associations between legumes and microorganisms are extremely important for the production of legumes in alluvial soil because effective nodulation can be a problem in low-pH soil. Dual inoculation of AMF and *Bradyrhizobium* is a promising technique to overcome this problem. Very few studies on symbiosis among plants, AMF, and *Rhizobium* have been reported although symbiosis between legume and *Rhizobium* has been studied extensively. In legumes, AMF not only stimulate plant growth but also modulate nitrogen fixation. This appears to be because of better phosphorus nutrition in legumes as a consequence of mycorrhizal association. In fact, it has been suggested that symbiosis in legumes should now be considered a tripartite symbiosis among legumes, *Rhizobium*, and mycorrhizal entophyte. Associated plant species in *Rhizobium* and *Bradyrhizobium* and *Frankia*, are normally mycorrhizal. Mycorrhizal and nodules symbioses typically act synergistically, both on infection rate and on mineral nutrition and plant growth. Where nitrogen and phosphorus are limiting, AMF may improve P uptake. The higher P concentration in plants benefits the bacterial symbiont and the functioning of its nitrogenase, leading to increased nitrogen fixation, which in turn promotes root and mycorrhizal development (Asimi, Gianinazzi-Pearson, and Gianinazzi 1980; Robson and Abbott 1987; Lee and Pankhurst 1992). This study was carried out under greenhouse condition. The influence

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of *Glomus intraradices* and *B. japonicum* inoculation on the shoot dry matter, yield production, N and P uptake, AMF colonization in root, and AMF spores density in the alluvial soil of soybean-growing areas.

## Materials and methods

### *AM fungal inoculum preparation*

Individual AMF spores showing hyphal connection were isolated using the modified wet sieving and decanting method of Daniels and Skipper (1982) and Gerdemann and Nicolson (1963) from air-dried rhizospheric soil samples collected from soybean-cultivated fields in Jabalpur, Madhya Pradesh. Characterization of individual AMF spores was carried out after being subjected to morphogenetic and micrometric analysis based on their colour, diameter, shape, wall layers, surface content, hyphal colour, hyphal width, and hyphal attachment with the wall. On this basis, *Glomus* was categorized as the dominant genus and identified at species level (*G. intraradices*) with the help of relevant literature (Morton and Benny 1990; Schenck and Perez 1990; Wu, Hao, Lin, *et al*. 2002) and was cultured for AMF inoculum preparation and utilization.

Isolated AMF spores were surface sterilized for 20 min with 2% chloramines T and streptomycin sulfate (200 μg/ml) and then rinsed in several changes of sterile water. The pure inoculum was produced by single spore cultivation. Single AMF spore cultures were purified and maintained using the standard funnel technique (Menge and Timmer 1982) with surface-sterilized seeds of *Zea mays* cv. Shakti as the host plant. Then they were maintained on surface-sterilized seeds of *Paspalum notatum* and the sterilized substrate (soil and sand in the ratio of 1:1 V/V) was used. The substrate containing spores and root pieces served as a stock culture of AMF inoculum.

### *Bradyrhizobium inoculant preparation and seed treatment*

*B. japonicum* isolates of soybean (JS335) are being maintained from a collection of 10 cultivars specific. These isolates were selected on the basis of non-significant difference with regard to their symbiotic efficiency, which was determined earlier based on results of various pot and mist house experiments (data not shown here). *Bradyrhizobial* isolates were maintained in 20E medium (Werner, Wilcockson, and Zimmermann 1975). A mixture of phosphorus-free sterilized charcoal (pH 6.8) and sand (3:1) was used as carrier for pure *B. japonicum* inoculant production. Sterilized carrier was inoculated with exponentially growing bradyrhizobial cultures. Carrier inoculant having around 1010 bacterial cells per gram was applied to surface-sterilized soybean seeds with 0.05% KOH (Call and Davis 1982) before sowing by using 10% sugar (jaggery) solution (Subba Rao 1988) as a sticker material for proper seed pelleting. Seeds without bacterial treatment served as controls.

### *Earthenware pot preparation and inoculation*

Air-dried and sieved-autoclaved alluvial soil collected from a non-legume cultivated field was filled in earthen pots (8 kg per pot). In each pot, 10 g of mycorrhizal inoculum (containing 20 spores per gram) of *G. intraradices* AMF was placed at a depth of 2 cm below the seed-sowing level and covered with sieved-autoclaved soil. Thus each pot received 200 AMF spores. There were four treatment combinations for soybean cultivar (control, that is, no AMF or CSBJ, single treatment of AMF, single treatment of CSBJ, and dual treatment of AMF and CSBJ). There were six replicates of each treatment. Plants were grown in the pots for 90 days in a greenhouse (having temperature in the range of 27–35°C and relative humidity in the range of 70%–80%) under natural illumination and were watered as needed. The plants were harvested after the yield matured, and data pertaining to shoot dry matter, seed yield, shoot nitrogen and phosphorus content, intensity of AMF colonization in roots, and infestation in soil were recorded.

### *Estimation of AMF colonization*

Freshly collected roots were washed in water, cleaned with 10% KOH, acidified with 1 N HCl, and stained in 0.05% trypan blue (Phillips and Hayman 1970). Mycorrhizae infection in the roots was expressed as per cent of segments containing fungal structures like mycelia, vesicles, and arbuscles (100 root segments per sample were evaluated for mycorrhizal infection).

$$\text{Per cent of mycorrhizal association (\%)} = \frac{\text{Number of mycorrhizal segments}}{\text{Total number of segments screened}} \times 100$$

### *Chemical analysis of soil and estimation of shoot nitrogen and phosphorus*

The local alluvial soil filled in the pots was analysed before experimentation for pH (soil suspension, 1:4, W/V), EC (soil suspension, 1:4, W/V), organic carbon, available nitrogen (Jackson 1973), and phosphorus (Olsen, Cole, Wantabe, *et al*. 1954). Shoot N and P were determined by the Kjeldahl method and the ammonium molybdate vanadate method, respectively, as described by Jackson (1973).

### *Statistics*

The data were statistically examined by analysis of variance (ANOVA). A least significant difference (LSD) test was carried out according to Misra and Misra (1983) to detect differences between treatment means when the ANOVA indicated significant treatment effects. In all procedures, probabilities less than 0.05 were considered to indicate statistical significance. Significant increase over control has been accordingly marked in Tables 1 and 2. The data on shoot N and P were also statistically analysed to obtain the correlation matrices for various parameters such as shoot dry matter, seed weight, and AMF colonization using the STATISTI XL 1.7 program.

## Results

### *Soil characteristics*

Selected properties of the soil used are as follows: light brown in colour; alluvial; sandy loam; pH: 8.1; EC: 0.56 $dsm^{-1}$; OC: 0.17%; available nitrogen: 62.91 mg $kg^{-1}$; and Olsen P: 16.00 mg $kg^{-1}$.

Shoot dry matter, dry matter yield, and nitrogen and phosphorus uptake of soybean plants were significantly influenced by inoculation treatment (Table 1). Shoot dry matter, seed yield, and nitrogen and phosphorus uptake of soybean plants with *G. intraradices* inoculation and dual inoculation (*G. intraradices* + *B. japonicum*) were significantly higher compared to non-inoculated soybean plants. Shoot dry matter, dry matter yield, and nitrogen and phosphorus uptake of soybean plants inoculated with *G. intraradices* were increased by 2.39, 2.94, 2.87, and 5.24 fold, respectively, compared to non-mycorrhizal plants. Shoot dry matter, dry matter yield, and nitrogen and phosphorus uptake of dually inoculated (*G. intraradices* + *B. japonicum*) plants were increased by 2.76, 3.22, 9.1, and 6.53 fold, respectively, compared to non-inoculated plants (Table 1). *B. japonicum* can significantly increase nitrogen uptake of soybean plants compared to non-inoculated plants. However, shoot dry matter, seed yield, and P uptake of soybean plants inoculated with *B. japonicum* were not significantly different compared to non-inoculated plants. Dual inoculation (*G. intraradices* + *B. japonicum*) significantly increased the shoot dry matter, yield production, and nitrogen and phosphorus concentration compared to plants inoculated with *G. intraradices* and *B. japonicum* separately.

AMF root colonization and spore density in soil were significantly influenced by inoculation treatment. AMF root colonization and spore density were not found in the treatments of control (soil without inoculation) and *B. japonicum* inoculation (Table 2). Mycorrhizal root colonization and spore population of *G. intraradices* were significantly higher with dual inoculation (*G. intraradices* + *B. japonicum*) compared to single *G. intraradices* inoculation.

**Table1** Impact of AM fungi and CSBJ on shoot dry matter,seed dry matter, N and P uptake of soybean under greenhouse conditions

| Treatments | SDM | DMSY | N uptake | P uptake |
|---|---|---|---|---|
| Control | 2.16d±0.47 | 1.00c±0.08 | 18.29c±0.12 | 1.94c±0.05 |
| *Glomus intraradices* | 5.12b±0.14 | 2.94b±0.15 | 52.56b±0.08 | 10.16b±0.12 |
| CSBJ | 4.13c±0.32 | 1.29c±0.11 | 62.26b±0.21 | 3.12c±0.11 |
| *Glomus intraradices*+CSBJ | 5.96a.±0.16 | 3.22a±0.04 | 166.48a±0.14 | 12.66a±0.14 |

SDM – shoot dry matter (g plant-1); DMSY – dry matter seed yield (g plant-1); control – soil without inoculation; *Glomus intraradices* – AM fungus; CSBJ – cultivar specific *Bradyrhizobium japonicum*

*Notes* Values are means of six replicates ± SE; Values in a column followed by the same letter are significantly different with DMRT 5%

**Table 2** AMF root colonization and spore density with *Glomus intraradices* and CSBJ inoculated soybean

| Treatments | AMF root colonization (%) | AMF spore density (spore 100g-1 dry soil) |
|---|---|---|
| Control | 0.00c±0.00 | 0.00c±0.00 |
| *Glomus intraradices* | 44.40b±0.23 | 61.25b±0.26 |
| CSBJ | 0.00c±0.00 | 0.00c±0.00 |
| *Glomus intraradices*+CSBJ | 57.45a±0.25 | 88.26a±0.11 |

Control – soil without inoculation; *Glomus intraradices* – AM fungus; CSBJ – cultivar specific *Bradyrhizobium japonicum*

*Notes* Values are means of six replicates ± SE; Values in a column followed by the same letter are significantly different with DMRT 5%

## *Discussion*

Shoot dry matter, seed yield, and N and P uptake of soybean plants with dual inoculation (*G. intraradices* + *B. japonicum*) were significantly higher compared to control (soil without inoculation), *G. intraradices*, and *B. japonicum* inoculation. Meghvansi, Prasad, Harwani, *et al*. (2008) reported that shoot dry matter, dry yield, and N and P uptake of soybean were significantly higher with dual inoculation (AMF + CSBJ) compared to AMF and CSBJ separately. According to Asimi, Gianinazzi-Pearson, and Gianinazzi (1980), the mycorrhizal and nodule symbiosis typically act synergistically, both on infection rate and on mineral nutrition and plant growth. Where nitrogen and phosphorus are limiting, AMF may improve P uptake. The higher P concentration in plants benefits the bacterial symbiont and the functioning of its nitrogenase, leading to increased nitrogen fixation, which in turn promotes root and mycorrhizal development. Furlan and Bernier-Cardou (1989) and Marschner and Dell (1994) reported that mycorrhizal fungi increased the efficiency of mineral uptake, especially of P, in P-deficient soils and other nutrients that result in enhanced plant growth and yield production. Enhancement of nutrient uptake in mycorrhizal legumes is most likely due to the external fungal hyphae acting as an extension of the rooting system. External hyphae length prolong up to 7–10 m per gram of soil (Allen, Weinbaun, Morri, *et al*. 1992). Accordingly, mycorrhiza-inoculated legumes absorb nutrients from soil and translocate them to the host root more efficiently (more extensive and better distributed) than non-mycorrhizal legumes (Linderman 1992). Better growth in mycorrhiza-inoculated plants than in non-mycorrhizal plants (non-inoculated control) may be determined by differences in the number of active arbuscules as a proportion of the total fungal biomass within the root system. The arbuscules may indeed play a major role in P transfer (Smith and Smith 1995; Turk, Assaf, Hameed, *et al.* 2006). Lukiwati, Hardjosoewignjo, Fakuara, *et al.* (1995) reported that *Glomus* sp. could increase dry matter production, crude protein and P uptake of *Flemingia macrophylla*, *Stylosanthes guianensis*, and *Pueraria phaseoloides*. *Glomus* sp. was found to be most effective for a range of crops and pastures, at low pH and at a wide range of N, P, and K levels (Howeler, Sieverding, and Saif 1987; Pirdashti, Motaghian, and Bahmanyar 2010.).

AMF root colonization percentage and spore population of G. *intraradices* with dual inoculation (*G. intraradices* + *B. japonicum*) were higher compared to *G. intraradices* inoculation alone. The symbiotic association increases mycorrhizal root colonization, spore population, as well as nodulation. This dual inoculation can increase external hyphae, which is important for the development of spore and increases root cell permeability.

## Conclusion

Dual inoculation (*G. intraradices* + *B. japonicum*) increased the yield of soybean compared to *G. intraradices* or *B. japonicum* with and without inoculation. AMF and cultivar-specific *Bradyrhizobium* could be selected and utilized for improved production of soybean in a sustainable manner.

## References


Allen M F, Weinbaun B, Morri S J, Allen E B. 1992. **Techniques for following the hyphae of VA mycorrhiza fungi**, pp. 24–25. In *Proceedings of International Symposium on Management of Mycorrhizas in Agriculture, Horticulture, and Forest*. Perth, Western Australia: Kluwer Academic

Asimi S, Gianinazzi-Pearson V, and Gianinazzi S. 1980. **Influence of increasing soil phosphorus levels on interactions between vesicular-arbuscular mycorrhizae and Rhizobium in soybeans.** *Canadian Journal of Botany* **58**: 2200–2205

Bethlenfalvay G J, Schreiner R P, and Mihara K L. 1997. **Mycorrhizal fungi effects on nutrient composition and yield of soybean seeds**. *Journal of Plant Nutrition* **20** (4–5): 581–591

Call C A and Davis F T. 1982. **Effect of vesicular arbuscular mycorrhizae on survival and growth of perennial grasses in lignite overburden in Texas**. A*griculture Ecosystem and Environment* **24**: 395–405

Chen B D, Li X L, Tao H Q, Christie P, Wong M H. 2003. **The role of arbuscular mycorrhiza in zinc uptake by red clover rowing in a calcareous soil spiked with various quantities of zinc**. *Chemosphere* **50**: 839–846

Daniels B A and Skipper H D. 1982. **Methods for the recovery and quantitative estimation of propogules from soil**. In *Methods and Principles of Mycorrhizal Research*, edited by N C Schenck, pp. 29–35. St. Paul, Minnesota, USA: APS Press

Dodd J C, Arias I, Koomen I, Hayman D S. 1990. **The management of populations of vesicular-arbuscular mycorrhizal fungi in acid-infertile soil of a savanna ecosystem. I. The effect of pre-cropping and inoculation with VAM fungi on plant growth and nutrition in the field**. *Plant and Soil* **122**: 229–240

Furlan V and Bernier-Cardou M. 1989. **Effects of N, P, and K on formation of vesicular-arbuscular mycorrhizae, growth and mineral content of onion**. *Plant and Soil* **113**: 167–174

Gavito M E, Schweiger P, and Jakobsen I. 2003. **P uptake by arbuscular mycorrhizal hyphae: effect of soil temperature and atmospheric $CO_2$ enrichment**. *Global Change Biology* **9**: 106–116

Gerdemann J W and Nicolson T H. 1963. **Spores of a mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting**. *Transactions of the British Mycological Society* **46**: 235–244

Howeler R H, Sieverding E, and Saif S. 1987. **Practical aspects of mycorrhizal technology in some tropical crops and pastures**. *Plant and Soil* **100**: 249–283

Jaarsveld C M V, Smith M A, and Kruger G H. 2002. **Interaction amongst soybean (*Glycine max* [L.] Merrill) genotype, soil type and inoculant strain with regard to $N_2$ fixation**. *Journal of Agronomy and Crop Science* **188**: 206–211

Jackson M L. 1973. ***Soil Chemical Analysis***. New Delhi: Prentice Hall of India Ltd.

Kerridge P C and Ratcliff D. 1982. **Comparative growth of four tropical pasture legumes and guinea grass with different phosphorus sources**. *Tropical Grasslands* **16**(1): 33–40

Lee K E and Pankhurst C E. 1992. **Soil organisms and sustainable productivity**. *Australian Journal of Soil Research* **30**: 855–892

Linderman R G. 1992. **Vesicular-arbuscular mycorrhizae and soil microbial interaction**. *Mycorrhizae in Sustainable Agriculture* **54**: 45–70 [ASA special publication]

Lukiwati D R and Hardjosoewignjo S. 1998. **Mineral content improvement of some tropical legumes with Glomus sp. fungi inoculation and rock phosphate fertilization**. Presentation made at *International Workshop on Mycorrhiza* [Guangzhou, People's Republic of China, 6 September–31 August 1998]

Lukiwati D R, Hardjosoewignjo S, Fakuara Y, Anas I. 1994. **Dry matter yield of forage legumes by VAM and rock phosphate fertilizer in the latosolic soil**. Presentation made at the *Workshop on Plantation Forestry and the Application of New Biotechnology* [Perlis, Malaysia, 28 November–1 December 1994]

Lukiwati D R, Hardjosoewignjo S, Fakuara Y, Anas I. 1995. **Effect of VAM and rock phosphate on productivity of forage legumes**. In *Proceedings of the Second Symposium on Biology and Biotechnology of Mycorrhizae and Third Asian Conference on Mycorrhiza (ACOM III)*, edited by Supriyanto and J T Kartana, Yogyakarta, Indonesia

Marschner H and Dell B. 1994. **Nutrient uptake in mycorrhizal symbiosis**, pp. 89–102. In *Proceedings of an International Symposium on Management of Mycorrhizas in Agriculture, Horticulture, and Forest*, edited by A D Robson, L K Abbott, and N Malajczuk. Perth: Kluwer Academic Publishers

Meghvansi M K, Prasad K, Harwani D, Mahna S K. 2008. **Response of soybean cultivars toward inoculation with three arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* in the alluvial soil**. *European Journal of Soil Biology* **44**: 316–323

Menge J and Timmer L W. 1982. **Procedures for inoculation of plants with vesicular-arbuscular mycorrhiza in the laboratory greenhouse**. In *Methods and Principles of Mycorrhizae Research*, edited by N C Schenck, pp. 59–68. St. Paul, Minnesota, USA: APS Press

Misra B N and Misra M K. 1983. ***Introductory Practical Biostatistics***. Kolkata, India: Darbari Prakashan

Morton J B and Benny G L. 1990. **Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, glomales, two new suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an anemendation of Glomaceae**. *Mycotaxon* **37**: 471–491

Mosse B. 1981. **Vesicular–arbuscular mycorrhiza research for tropical agriculture**. [Hawaii Institute of Tropical Agriculture and Human Resources, University of Hawaii, Research Bulletin 174]

Nelson P V, Niedziela C E Jr, Pitchay D S, Mingis N C. 2010. **Effectiveness, ammonium impact, and potassium adequacy of soybean-base liquid fertilizer on bedding plants**. *Journal of Plant Nutrition* **33**(5): 724–735

Olsen S R, Cole C V, Wantabe F S, Dean L A. 1954. ***Estimation of available phosphorus in soils by extraction with sodium bicarbonate***. USDA Circular 939: 1–19. Government Printing Office Washington, DC

Phillips J M and Hayman D S. 1970. **Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection**. *Transactions of the British Mycological Society* **55**: 158–161

Pirdashti H, Motaghian A, and Bahmanyar M A. 2010. **Effects of organic amendments application on grain yield, leaf chlorophyll content, and some morphological characteristics in soybean cultivars**. *Journal of Plant Nutrition* **33**(4): 485–495

Raverkar K P and Tilak K V B R. 2002. **Improved mycorrhizal colonization, nodulation status, biomass production, and nutrient uptake in soybean due to inoculation with *Bradyrhizobium japanicum***. *Legume Research* **25**: 32–36

Robson A D and Abbott L K. 1987. **Managing symbiotic association between plants and microorganisms**. In *Temperate Pastures*, edited by J L Wheeler, C J Pearson, and G E Robards, pp. 191–203. Australia: Commonwealth Scientific and Industrial Research Organization

Schenck N C and Perez Y. 1990. ***A Manual for Identification of Vesicular-Arbuscular Mycorrhizal Fungi***, INVAM, 3rd edn. Gainesville, Florida: University of Florida

Smith F A and Smith S E. 1995. **Nutrition transfer in vesicular-arbuscular mycorrhizas: a new model based on the distribution of ATP uses on fungal and plant membranes**. *Biotropia* **8**: 1–10

Subba Rao N S. 1988. **Rhizobium inoculant**. In *Biofertilizers in Agriculture and Forestry*, 2nd edn, pp. 16–76. New Delhi, India: Oxford and IBH Publishing Co. Pvt. Ltd.

Tilak K V B R, Rathi S K, and Saxena A K. 1995. **Field evaluation of different arbuscular mycorrhizae of soybean**. In *Mycorrhizae: biofertilizers for the future*, edited by A Adholeya and S Singh, pp. 339–344. New Delhi: The Energy and Resources Institute (TERI)

Troeh Z I and Loynachan T E. 2009. **Diversity of arbuscular mycorrhizal fungal species in soils of cultivated soybean fields**. *Agronomy Journal* **101**: 1453–1462

Turk M A, Assaf T A, Hameed K M, Tawaha A M. 2006. **Significance of mycorrhiza**. *World Journal of Agricultural Sciences* **2**(1): 16–20

Waidyanatha U P De S, Yogoratnam N, and Ariyaratne W A. 1979. **Effect of mycorrhizal infection on growth and uptake of phosphorus from two rock phosphates**. *New Phytologist* **82**: 147–152

Werner D, Wilcockson J, and Zimmermann E. 1975. **Adsorption and selection of rhizobia with ion-exchange papers**. *Archives of Microbiology* **105**: 27–32

Wu T, Hao W, Lin X, Shi Y. 2002. **Screening of arbuscular mycorrhizal fungi for the revegetation of eroded red soils in subtropical China**. *Plant and Soil* **239**: 225–235

Xavier L J C and Germida J J. 2003. **Selective interactions between arbuscular mycorrhizal fungi and *Rhizobium***. *Biology of Fertile Soils* **37**: 261–267

Xie Z P, Staehelin C, Vierheilig H, Wiemken A, Jabbouri S, Broughton W J, Vogeli-Lange R, Boller T. 1995. **Rhizobial nodulation factors stimulate mycorrhizal colonization of nodulating and non-nodulating soybeans**. *Plant Physiology* **108**(4): 1519–1525

## Bibliography

Giovannetti M and Mosse B. 1980. **An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots**. *New Phytologist* **84**: 489–500


# Centre for Mycorrhizal Culture Collection

## Sample collection: few guidelines to be followed

*Chaitali Bhattacharya and Alok Adholeya**

The Centre for Mycorrhizal Culture Collection (CMCC) has been at the forefront in maintaining a Germplasm Collection Bank of Mycorrhizae since several years. This facility has been designed to collect and maintain live cultures from different parts of the globe towards being characterized at morphological, molecular, biochemical, and functional levels. To achieve this, soil samples are acquired from various sources of diverse habitats and propagated as trap culture using bait plants from which discrete spores are isolated and finally developed into pure cultures with utmost care and by observing necessary precautions.

The bank has enriched its sample collection with the help of various researchers. Their invaluable contributions have been acknowledged by announcing their names in our newsletter. Towards this objective, CMCC also offers to reimburse the cost of shipment if needed. Meticulous documentation along with sample collection is a critical feature for providing appropriate Accession Codes for deposit and maintenance in the bank. It is also a very essential prerequisite for preserving a well-structured Germplasm Bank. In order to facilitate our readers and members, the process that is to be followed for the collection and information recording of the samples is illustrated below.

The two most essential steps involved in the collection include the following.

### 1. Sample collection

While doing sampling, it should be kept in mind that the sample is a representative of the area to be studied. Since the goal is to capture the mycorrhiza diversity,the sampling should be done in a careful manner. The rhizospheric soil should be collected from a depth of 15 cm to 30 cm and should most importantly contain roots of the plants from

| Information | Details to be filled |
|---|---|
| Sample number | |
| Name of the researcher | |
| Name of the laboratory | |
| Address:<br>State:<br>City:<br>Phone number:<br>Email address: | |
| Name and contact address of the sample collector | |
| Date of sample collected | |
| Date of sample packed | |
| Field details from where the sample was collected | |
| Sampling depth | |
| Name of the crop/host | |
| Description of habitat (for example, arid, tropical, temperate, pasture, forest, cropland, and so on) | |
| In case of field: type of fertilization maintained | |
| Vegetation surrounding the plant being sampled | |
| Nearest landmark of the field: town/village/ state/country | |
| Geographic location (latitude/longitude/ altitude) | |
| Type of sample: composite sample (indicate number) or individual sample | |
| Soil properties (for example, colour, texture, pH, organic matter content, nutrient levels such as N, P, K, Ca, and so on) | |
| Reason for deposit | |
| Any other information | |

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where the sampling is done. A root ball would be ideal for this purpose. Each field type needs to be sampled separately. However, if in the same field, the appearance of soil varies, unequal elevation is observed, use of different fertilizers or manuring methods is practised or differential crop growth patterns are encountered, the field should be divided accordingly and each such area is to be sampled separately.

In case the field is uniform, sampling should be done from 8 to 10 places and then a composite sample should be prepared after thorough mixing. The soil should be poured on a piece of clean cloth or paper and it should be mixed thoroughly followed by discarding and retaining only around 0.50 kg to 1 kg mixed soil sample by quartering. Quartering may be done by mixing the sample well, dividing it into four equal parts, then rejecting two opposite quarters, mixing the remaining two portions, re-dividing them into four parts and rejecting two opposite quarters and so on. Removal of any rock or stone particles should be undertaken before packing the samples. The final amount of sample should then be air dried to a point where there is no free moisture. This would prevent the growth of unwanted fungus while being transported to the bank. These air dried samples are then preferably filled in cloth or aerated bags and sealed.

## 2. Labelling and documentation of the samples

Detailed information of the samples that are sent to the bank is of paramount importance for providing an accession code to the depositor, as it has always been observed that data of the collected fungi, their environments, particularly associated plants and soil, if not recorded at the time of collection, are often impossible to retrieve at a later stage. A soil sample information sheet (Table 1) has been provided at the end of the article. This should be suitably filled and sent to the bank along with each sample and corresponding labelling should be done at the right edge of the bag on both the sides with water resistant permanent marker pen. The success of the culture sent to the bank for its maintenance depends a lot on the approach with which the sampling has been performed by the depositor. Underestimating and not appropriately following the guidelines would lead to a contaminant filled culture which is not recommended and approved to be maintained in the bank.

# Recent references

The latest additions to the network's database on mycorrhiza are published here for the members' information. The list consists of papers from the following journals.

- *Acta Ecologica Sinica*
- *Agriculture, Ecosystems and Environment*
- *Applied Clay Science*
- *Applied Soil Ecology*
- *Aquatic Botany*
- *Biological Control*
- *Comptes Rendus Biologies*
- *Ectomycorrhizas and Climate Change*
- *Environmental and Experimental Botany*
- *Environmental Pollution*
- *European Journal of Soil Biology*
- *Field Crops Research*
- *Forest Ecology and Management*
- *Fungal Biology*
- *Fungal Ecology*
- *International Biodeterioration and Biodegradation*
- *Journal of Arid Environments*
- *Journal of Plant Physiology*
- *Pedobiologia*
- *Physiological and Molecular Plant Pathology*
- *Scientia Horticulturae*
- *Trends in Plant Science*

| Name of the author(s) and year of publication | Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author, marked with an asterisk) |
|---|---|
| Adrien Gallou*, Hernán Patricio Lucero Mosquera, Sylvie Cranenbrouck, Juan Pablo Suárez, Stéphane Declerck. 2011 | **Mycorrhiza induced resistance in potato plantlets challenged by *Phytophthora infestans*.** *Physiological and Molecular Plant Pathology* **76**(1): 20–26<br><br>[*Université catholique de Louvain (UCL), Earth and Life Institute, Mycology, Place croix du Sud 3, 1348 Louvain-la-Neuve, Belgium |
| Amanda F Currie, Philip J Murray, and Alan C Gange. 2011 | **Is a specialist root-feeding insect affected by arbuscular mycorrhizal fungi?** *Applied Soil Ecology* **47**(2): 77–83<br><br>[School of Biological Sciences, Royal Holloway University of London, Egham Hill, Egham, Surrey TW20 0EX, UK] |
| Amy L Clark and Samuel B St Clair. 2011 | **Mycorrhizas and secondary succession in aspen–conifer forests: light limitation differentially affects a dominant early and late successional species.** *Forest Ecology and Management* **262**(2): 203–207<br><br>[Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT, United States] |
| Angela Cicatelli, Guido Lingua, Valeria Todeschini, Stefania Biondi, Patrizia Torrigiani, Stefano Castiglione. 2011 | **Arbuscular mycorrhizal fungi modulate the leaf transcriptome of a *Populus alba* L. clone grown on a zinc and copper-contaminated soil.** *Environmental and Experimental Botany* **75**(In progress): 25–35<br><br>[Dipartimento di Chimica, Università di Salerno, Stecca 8, Via Ponte don Melillo, 84 084 Fisciano (SA), Italy] |
| Arocena J M*, Velde B, and Robertson S J. 2011 | **Weathering of biotite in the presence of arbuscular mycorrhizae in selected agricultural crops.** *Applied Clay Science*, In Press, Corrected Proof, Available online 21 July 2011<br><br>[*University of Northern British Columbia, Prince George, BC, Canada V2N4Z9] |
| *Baran Mardukhi, Farhad Rejali, Gudarz Daei, Mohammad Reza Ardakani, Mohammad Javad Malakouti, Mohammad Miransari. 2011. | **Arbuscular mycorrhizas enhance nutrient uptake in different wheat genotypes at high salinity levels under field and greenhouse conditions** *Comptes Rendus Biologies* **334**(7): 564–571<br><br>[*Department of Soil Science, College of Agriculture, Tarbiat Modares University, Tehran, Iran] |

| Name of the author(s) and year of publication | Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author is marked with an asterisk) |
|---|---|
| Barea J M, Palenzuela J, Cornejo P*, Sánchez-Castro I, Navarro-Fernández C, Lopéz-García A, Estrada B, Azcón R, Ferrol N, Azcón-Aguilar C. 2011 | **Ecological and functional roles of mycorrhizas in semi-arid ecosystems of Southeast Spain.** *Journal of Arid Environments* **75** (12): 1292–1301<br><br>[*Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, CSIC, Profesor Albareda 1, 18 008 Granada, Spain] |
| Boldt K, Pörs Y, Haupt B, Bitterlich M, Kühn C, Grimm B, Franken P. 2011 | **Photochemical processes, carbon assimilation and RNA accumulation of sucrose transporter genes in tomato arbuscular mycorrhiza.** *Journal of Plant Physiology* **168**(11): 1256–1263<br><br>[Leibniz-Institute of Vegetable and Ornamental Crops, Theodor-Echtermeyer-Weg 1, D-14 979 Grossbeeren, Germany] |
| Brian J Pickles, Keith N Egger, Hugues B Massicotte, D Scott Green. 2011 | **Ectomycorrhizas and climate change.** *Fungal Ecology*, In Press, Corrected Proof, Available online 12 October 2011<br><br>[Ecosystem Science and Management, University of Northern British Columbia, 3333 University Way, Prince George, BC V2N 4Z9, Canada] |
| Christos N Hassiotis and Evanthia I Dina. 2011 | **The effects of laurel (*Laurus nobilis* L.) on development of two mycorrhizal fungi Original Research Article.** *International Biodeterioration and Biodegradation* **65**(4): 628–634<br><br>[Technical University of Larissa, Department Natural Environment and Forestry, 43 100 Karditsa, Greece] |
| Elżbieta Orłowska*, Wojciech Przybyłowicz, Dariusz Orlowski, Katarzyna Turnau, Jolanta Mesjasz-Przybyłowicz. 2011 | **The effect of mycorrhiza on the growth and elemental composition of Ni-hyperaccumulating plant *Berkheya coddii* Roessler.** *Environmental Pollution* **159**(12): 3730–3738<br><br>[*Materials Research Department, iThemba LABS, PO Box 722, Somerset West 7129, South Africa] |
| Flavia Ercolin and Didier Reinhardt. 2011 | **Successful joint ventures of plants: arbuscular mycorrhiza and beyond** *Trends in Plant Science* **16**(7): 356–362<br><br>[*Department of Biology, University of Fribourg, CH-1700 Fribourg, Switzerland] |
| Fracchia S, Krapovickas L, Aranda-Rickert A, Valentinuzzi V S. 2011 | **Dispersal of arbuscular mycorrhizal fungi and dark septate endophytes by Ctenomys cf. knighti (Rodentia) in the northern Monte Desert of Argentina.** *Journal of Arid Environments* **75**(11): 1016–1023<br><br>[Centro Regional de Investigaciones Científicas y Transferencia Tecnológica, CRILAR- CONICET Entre Ríos y Mendoza, 5301 Anillaco, La Rioja, Argentina] |
| Fritz Oehl*, David Schneider, Ewald Sieverding, Conradin A Burga. 2011 | **Succession of arbuscular mycorrhizal communities in the foreland of the retreating Morteratsch glacier in the Central Alps.** *Pedobiologia* **54**(5–6): 321–331<br><br>[*Agroscope Reckenholz-Tänikon Research Station ART, Ecological Farming Systems, Reckenholzstrasse 191, CH-8046 Zürich, Switzerland] |
| Fuqiang Song*, Ge Song, Airong Dong, Xiangshi Kong. 2011 | **Regulatory mechanisms of host plant defense responses to arbuscular mycorrhiza.** *Acta Ecologica Sinica* **31**(6): 322–327<br><br>[*Key Laboratory of Microbiology, School of Life Science, Heilongjiang University, Harbin 150 080, PR China] |

| Name of the author(s) and year of publication | Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author is marked with an asterisk) |
|---|---|
| Hai-Han Zhang, Ming Tang, Hui Chen, Cui-Ling Zheng, Zhen-Chuan Niu. 2010 | **Effect of inoculation with AM fungi on lead uptake, translocation and stress alleviation of *Zea mays* L. seedlings planting in soil with increasing lead concentrations.** *European Journal of Soil Biology* **46**(5): 306–311<br><br>[*College of Life Sciences, Northwest A & F University, Yangling, Shaanxi 712 100, China] |
| Ibrahim Ortas*, Nebahat Sari, Çağdaş Akpinar, Halit Yetisir. 2011 | **Screening mycorrhiza species for plant growth, P and Zn uptake in pepper seedling grown under greenhouse conditions.** *Scientia Horticulturae* **128**(2): 92–98<br><br>[*Department of Soil Science, Faculty of Agriculture, University of Çukurova, Adana 01330, Turkey] |
| Karim Benabdellah, Younes Abbas, Mohamed Abourouh, Ricardo Aroca, Rosario Azcón. 2011 | **Influence of two bacterial isolates from degraded and non-degraded soils and arbuscular mycorrhizae fungi isolated from semi-arid zone on the growth of *Trifolium repens* under drought conditions: Mechanisms related to bacterial effectiveness.** *European Journal of Soil Biology* **47**(5): 303–309<br><br>[*Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín (CSIC), Profesor Albareda n° 1, 18 008 Granada, Spain] |
| Khade S W. 2011 | **Specific variations of arbuscular mycorrhizal (AM) fungi in potential timber yielding tree species from the Western Ghat region of Goa, India.** *Journal of Sustainable Forestry* **30**(6): 459–479<br><br>[Department of Botany, Goa University, Taleigao Plateau, Goa 403206, India] |
| Ma del Carmen A González-Chávez*, María del Pilar Ortega-Larrocea, Rogelio Carrillo-González, Melina López-Meyer, Beatriz Xoconostle-Cázares, Susana K Gomez, Maria J Harrison, Alejandro Miguel Figueroa-López, Ignacio E Maldonado-Mendoza. 2011 | **Arsenate induces the expression of fungal genes involved in As transport in arbuscular mycorrhiza.** *Fungal Biology*, In Press, Corrected Proof, Available online 1 September 2011<br><br>[*Programa de Edafología, Instituto de Recursos Naturales, Colegio de Postgraduados en Ciencias Agrícolas, Campus Montecillo, Carretera México-Texcoco Km 36.5, Texcoco, México 56 230, Mexico] |
| Mohandas Sukhada*, R Manjula, and R D Rawal. 2011 | **Evaluation of arbuscular mycorrhiza and other biocontrol agents against *Phytophthora parasitica* var. nicotianae infecting papaya (*Carica papaya* cv. Surya) and enumeration of pathogen population using immunotechniques.** *Biological Control* **58**(1): 22–29<br><br>[*Division of Biotechnology, Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bangalore 560 089, India] |
| Monika A Gorzelak*, Sarah Hambleton, and Hugues B Massicotte. 2011 | **Community structure of ericoid mycorrhizas and root-associated fungi of *Vaccinium membranaceum* across an elevation gradient in the Canadian Rocky Mountains.** *Fungal Ecology,* In Press, Corrected Proof, Available online 13 October 2011<br><br>[*Biology Department, University of British Columbia, Okanagan, 3333 University Way, Kelowna, BC, Canada V1V 1V7] |

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