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### **About TERI**

A dynamic and flexible organization with a global vision and a local focus, TERI (The Energy and Resources Institute, formerly known as the Tata Energy Research Institute) was established in 1974. TERI's focus is on research in the fields of energy, environment, and sustainable development, 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.

### **Biotechnology and Management of Bioresources Division**

Focusing on ecological, environmental, and food security issues, the activities of the Biotechnology and Management of Bioresources Division include working with a wide variety of living organisms, sophisticated genetic engineering techniques, and, at the grassroots level, with village communities.

### **Mycorrhiza Network**

The Biotechnology and Management of Bioresources Division's Mycorrhiza Network works through its three wings—the MIC (Mycorrhiza Information Centre), the CMCC (Centre for Mycorrhizal Culture Collection), and *Mycorrhiza News*.

The MIC is primarily responsible for establishing databases on Asian mycorrhizologists and on mycorrhizal literature (RIZA), which allows information retrieval and supplies documents on request. The CMCC has the main objectives of procuring strains of both ecto and vesicular arbuscular mycorrhizal fungi from India and abroad; multiplying and maintaining these fungi in pure cultures; screening, isolating, identifying, multiplying, and maintaining native mycorrhizal fungi; developing a database on cultures maintained; and providing starter cultures on request. Cultures are available on an exchange basis or on specific requests at nominal costs for spore extraction or handling.

### **Mycorrhiza News**

*Mycorrhiza News* – a quarterly newsletter publishing articles compiled from RIZA – invites short papers from budding and senior mycorrhizologists, giving methodologies being used in mycorrhizal research, providing information on forthcoming events on mycorrhiza and related subjects, listing important research references published during the quarter, and highlighting the activities of the CMCC.



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## Taxonomy of arbuscular mycorrhizal fungi

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The foundation of taxonomy of Glomalean fungi is stable and exhibits hierarchical patterns of morphological divergence, and for this reason alone, a detailed and thorough analysis is warranted. Because of the simplicity of fungal design and organization, most morphological changes have occurred in the form of asexual spore formation and differentiation of spore subcellular structures. Even though arbuscular fungi are asexual organisms, immediate fixation and strong heritability of whatever neutral or positive genotypic and phenotypic traits evolved over time assure that all progeny generations can be grouped diagnostically as a genealogically conserved morpho-species. The study of morphology provides insights into initial hypotheses of speciation in arbuscular fungi. The number of species in Glomales has long been considered to be low (154), especially relative to that observed in other symbiotic fungal groups such as ectomycorrhizal fungi (20 000) as well as plant groups (250 000). Since speciation in arbuscular fungi involves divergence only in component parts of single somatic cells that become spores, the number of species known today actually seems miraculously large.<sup>1</sup>

### Classification of arbuscular mycorrhizal fungi

AMF (arbuscular mycorrhizal fungi) have been placed in class Zygomycetes and the characteristics

that place them in this class include (1) the presence of chitin in the cell wall (Bonfante-Fasolo, Faccio, Perotto, *et al.* 1990; Weijman and Meuzelaar 1979), (2) presence of nonseptate and coenocytic mycelium, (3) formation of non-motile spores, the chlamydo spores, (4) formation of putative zygosporangia in *Gigaspora decipiens* (Tommerup and Sivasithamparam 1990), and (5) features of nuclei in spores similar to the spores of other Zygomycetous fungi (Maia 1991).

### History of the group

German mycologist, Link (1809), placed the AMF in *Endogone*. Tulasne and Tulasne (1845) described genus *Glomus* comprising two species (*Glomus microcarpum* and *Glomus macrocarpum*). The genus *Sclerocystis* was described by Berkeley and Broome in 1873. Thaxter (1922) revised the family Endogoneae, placing all members of *Glomus* in the genus *Endogone*, while maintaining the genus *Sclerocystis*. Bucholtz (1922) placed Endogoneaceae in the Mucorales, due to the affinities of *Endogone* with the members of the Mortierellaceae. The family Endogoneaceae was placed in its own order, Endogonales, by Moreau (1953), which was later validated by Benjamin (1979).<sup>2</sup>

The first comprehensive Linnaean classification was proposed by Gerdemann and Trappe (1974) who resurrected *Glomus* in the Endogoneaceae and moved

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several species from *Endogone* to *Glomus*. They also described the genera *Acaulospora* and *Gigaspora* in 1974. Ames and Schneider (1979) described the genus *Entrophospora*. Later Berch (1985) emended the genus *Acaulospora*, while Walker and Sanders (1986) transferred member species of *Gigaspora* to another genus *Scutellospora*, based on the presence of subcellular structures associated with germination. Further, the present-day family Glomaceae was formally erected by Pirozynski and Dalpé in 1989.

Another landmark publication was by Morton and Benny (1990) who erected the order Glomales along with two suborders Glomineae and Gigasporineae, and two other families Acaulosporaceae and Gigasporaceae. The new order Glomales is characterized by the unique ability of its members to form arbuscular mycorrhizae in mutualistic association with the root of the host plants. The taxonomy is further divided into suborders based on the (1) presence of vesicles in the root and presence of chlamydospores (thick wall, asexual spore) borne from subtending hyphae for the suborder Glomineae or (2) absence of vesicles in the root and formation of auxiliary cells and zygospores (spores resembling a zygospore but developing asexually from a subtending hypha resulting in a distinct bulbous attachment) in the soil for the suborder Gigasporineae. The classification proposed by Morton and Benny (1990) comprised six genera of AMF (Table 1).

**Table 1** Classification of arbuscular mycorrhizal fungi

Classification of Gerdemann and Trappe (1974), Benjamin (1979), and Warcup (1990)	Classification of Morton and Benny (1990)
Order: Endogonales	<b>1. Order: Endogonales</b>
Family: Endogonaceae	Family: Endogonaceae
Genera: <i>Endogone</i>	Genera: <i>Endogone</i>
<i>Sclerogone</i>	<i>Sclerogone</i>
<i>Glomus</i>	<b>2. Order: Glomales</b>
<b><i>Sclerocystis</i></b>	Suborder: Glomineae
<i>Acaulospora</i>	i) Family: Glomaceae
<i>Entrophospora</i>	Genera: <i>Glomus</i>
<i>Gigaspora</i>	<b><i>Sclerocystis</i></b>
<i>Scutellospora</i>	ii) Family: Acaulosporaceae
	Genera: <i>Acaulospora</i>
	<b><i>Entrophospora</i></b>
	Suborder: Gigasporineae
	Family: Gigasporaceae
	Genera: <i>Gigaspora</i>
	<i>Scutellospora</i>

## Recent advances in taxonomy

The genus *Glomites* was erected by Taylor, Remy, Hass, *et al.* (1995) to describe fossil fungi that closely resemble modern-day *Glomus* species. The genus *Gigaspora* was re-described by Bentivenga and Morton (1995), incorporating developmental patterns of morphological characters.

The separation between *Glomus* and *Sclerocystis* became controversial in the early 1990s. Almeida and Schenck (1990) placed all *Sclerocystis* species in *Glomus* with the exception of *Sclerocystis coremioides*. They were of the opinion that an unbroken continuum of morphological characters existed between sporocarpic *Glomus* species and all the *Sclerocystis* species except one (*S. coremioides*). Almeida and Schenck (1990) considered *S. coremioides* unique and, therefore, separate from the *Glomus* clade, based on the following four morphological traits: (1) spore formation on separate subtending hyphae rather than from branching sporophores, (2) a well-defined septum at the same position near the spore base, (3) arrangement of spores in hemispherical layer, and (4) new sporocarps formed from older sporocarps to often fuse into columns.

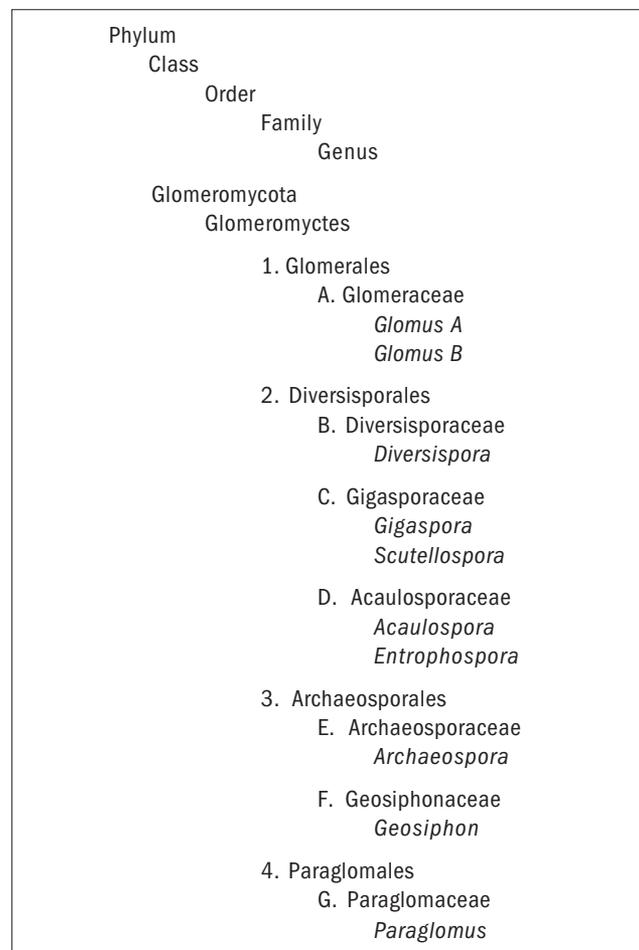
Wu (1993) resisted this change on the basis of comparative studies of spore ontogeny and sporocarps morphology of the *Sclerocystis* species, which was carried out to show that the above-mentioned traits were shared to varying degrees by other *Sclerocystis* species. Wu (1993) hypothesized a model of a smooth evolutionary transition between relatively unorganized *Glomus*-like sporocarps of *S. rubiformis* and intermediate forms like *S. clavispora*, *S. liquidambaris* and *S. sinuosa* to *S. coremioides*. He concluded that *S. coremioides* was not unique. This series of transformations led Wu (1993) to reject the changes of Almeida and Schenck (1990) and revert to Gerdemann and Trappe's (1974) classification scheme.

Molecular studies carried out by Simon, Bousquet, Lèvesque, *et al.* (1993), Gehrig, Schüßler, and Kluge (1996), and Redecker, Morton, and Bruns (2000a) suggested that *Glomus* is a polyphyletic conglomerate of distantly related lineages, and some morphological characters previously used to define the genus may not be sufficiently informative. Later, Redecker, Morton, and Bruns (2000b) carried out phylogenetic analysis of 18S ribosomal unit of *G. sinuosum* (= *S. sinuosa*) and *S. coremioides*, which revealed that both species are the close relatives and fall within the *Glomus* clade. Their studies reported that formation of complex sporocarps is an advanced character of some *Glomus* species, but the sporocarpic trait is not sufficiently unique to group these species into a separate genus *Sclerocystis*.

Further, two new families were erected by Morton and Redecker (2001), based on some atypical

morphological characters like striking immunological and fatty acid distance, and 18S rDNA sequence divergence. Two dimorphic sister species with similar ontogenetic sequences, *Acaulospora gerdemannii* and *Glomus gerdemannii* (*sensu lato*), together with *Acaulospora trappei* (*sensu lato*) were transferred to Archaeosporaceae. Two ancestral species previously classified in *Glomus*, *G. occultum*, and *G. brasilianum* (*sensu lato*) were grouped in a sister family, Paraglomaceae.

More recently, Schüßler, Schwarzott, and Walker (2001) transferred AMF from the polyphyletic phylum Zygomycotina to newly erected monophyletic phylum Glomeromycota (Figure 1). They analysed AMF and the endocytobiotic fungus *Geosiphon pyriformis* phylogenetically by their SSU (small subunit) rRNA gene sequences. They reported that Glomeromycota probably diverged from the same common ancestor as the Ascomycota and Basidiomycota. They also erected new orders like *Archaeosporales* (Archaeosporales, Geosiphonaceae), *Paraglomerales* (Paraglomeraceae), *Diversisporales* (Acaulosporaceae, Diversisporaceae fam. ined., Gigasporaceae), and Glomeraceae



**Figure 1** Classification of arbuscular mycorrhizal fungi by Schüßler, Schwarzott, and Walker (2001)

(*Glomus* – Group A, *Glomus* – Group B), with their respective families given in parenthesis.

### Characteristics used for identification of arbuscular mycorrhizal fungi

The validity and importance of morphological characteristics in establishing taxonomic species are of considerable importance to construct a workable system of identification. Undoubtedly, certain characteristics will be of greater importance than other in describing taxonomic species and estimating the extent to which two species are similar or dissimilar. Characteristics used for identification of AMF can be broadly classified into macro- and micro-characteristics (Table 2).

#### Macro-characteristics

The macro-characteristics can be used to characterize the visual difference in the gross descriptive morphology of a species.

#### Sporocarp

Spores of AMF are produced singly and/or in sporocarps in soil or roots. Sporocarps have spores organized into loose or compact structure formed in soil, root, empty seed coats, insect carapaces or rhizomes. Peridium may be present around sporocarps

**Table 2** Macro and micro taxonomic characteristics used for identification of arbuscular mycorrhizal fungi

Macro-characteristics	
Sporocarp morphology	Size Shape Peridium
Spore	Colour Shape Size
Subtending hypha	Shape Width Pore occlusion
Auxiliary cells	Size
Mycorrhizal anatomy	Hyphal characters Intraradical spores
Micro-characteristics	
Spore wall structure	Colour Dimension Number Type Ornamentation
Spore germination	Direct Indirect

in the form of loosely or compact interwoven hyphae, a patchy covering over the sporocarps or as hyphal network covering single or small clusters of spores. The presence or absence of peridium accounts for much of the variation observed in size of sporocarps.

### Spore

Characteristics such as spore colour, shape, and size may vary considerably depending on the developmental stage and environmental conditions. Spore colour varies in hyaline through white to yellow, red, brown, and black, with all intermediate shades. Spore size varies considerably within the same species and, hence, both immature and mature spores are taken into account while describing the species. Shape of the spores is mainly governed by the genotype of the fungus and the substrate in which the spore is formed. Intraradical spores are mainly globose, sub-globose to ellipsoidal, while extraradical spores may be globose, sub globose, ellipsoidal, oblong ovate to highly irregular shaped.

### Subtending hyphae

At generic level of classification, the shape of the subtending hyphae or the sporophore assumes great importance. The subtending hyphae may be simple to recurved or sometimes swollen in *Glomus* species. The sporophore in *Gigaspora* and *Scutellospora* is bulbous. Sometimes, the spores are sessile (*Acaulospora* and *Entrophospora*) or may bear small pedicel (*Acaulospora*) or a swollen sporophore (*Entrophospora*). The width of the hyphae varies considerably within different genera and species of AMF.

The mechanism of pore occlusion at the point of attachment of the subtending hypha to the spore has some taxonomic importance. Walker (1992) suggested three distinct lines with regard to the occlusion of the spore content in *Glomus*: (1) spore possessing a complete endospore formed by more or less flexible inner wall group, (2) spores sealed by the in growing and thickening of the wall layer of the subtending hyphae, and (3) occlusion by a septum usually somewhat distal to the spore base.

### Auxiliary cells

The size and shape of the auxillary cells have been found to be of little importance in differentiating species *Gigaspora* or *Scutellospora*. In *Gigaspora*, the auxillary cells are echinulate with spines that are forked dichotomously (Bentivenga and Morton 1995), whereas in *Scutellospora*, the projections on the surface of the auxiliary cells are highly variable in shape and size (Morton 1995).

### Mycorrhizal anatomy

Colonization of the root with AMF initiates a series of developmental process culminating in a

morphologically and functionally unique symbiosis. It is possible to differentiate among certain AMF using visual differences in morphology of fungal hyphae and vesicles within roots (Abbott and Gazey 1994). It has been suggested that certain hyphal characteristics such as long infection units with 'H' connections between parallel strands of hyphae in *Glomus* (Abbott and Robson 1979), pale staining of intraradical hyphae by trypan blue in *Acaulospora* (Bentivenga and Morton 1995), constriction near branch points in hyphae of *Acaulospora* and *Entrophospora*, and irregularly coiled swollen hyphae with lateral projections or knobs in *Gigaspora* or *Scutellospora* may be utilized as diagnostic features to identify genera in mycorrhizal roots (Morton and Bentivenga 1994). Intraradical spores in Glomaceae usually are globose, subglobose to elliptical, whereas those in *Acaulospora* are pleomorphic, knobby, and stain lightly in trypan blue.

### Micro-characteristics

The micro-characteristics will demonstrate a degree of intra-specific difference in developmental morphology (Mehrotra 1997).

### Spore wall structure

Spore wall characteristics have been universally accepted as more stable and reliable criteria than other spore features (Mehrotra 1997). A spore wall is defined as the first individual structure to be formed, originating from the wall of sprogenous hypha and differentiating into phenotypically distinctive layers (Morton 1995). Seven wall layer types, that is, evanescent, laminated, membranous unit (Walker 1983), expanding (Berch and Koske 1986), coriaceous (Walker 1986), amorphous (Morton 1986), and germinal (Spain, Sieverding, and Schenck 1989) have been described so far. They are distinguished mainly on the basis of their morphological features and their reaction to certain chemicals such as lactophenol and Melzer's reagent.

The number, width, and position of wall layers differ among species and they have been increasingly relied upon for identification purposes. Differentiation of subcellular morphological characteristics in spores of *Gigaspora* (Bentivenga and Morton 1995) and *Scutellospora* species (Morton 1995) is used for identification. Ornamentation on the spore wall layer may sometime prove to be an important taxonomic criterion in identification of species, especially when other morphological characteristics are overlapping.

### Spore germination

The germination of spores in Glomales takes place by two methods: (1) direct germination takes place when the inner wall layers protrude through outer wall layer as a germ tube initially and later elongating into a

typical hypha (*Glomus* and *Gigaspora*) and (2) indirect germination takes place by the development of germination shield prior to emergence of germ tube (*Acaulospora*, *Entrophospora*, and *Scutellospora*). Germination shields in Acaulosporaceae form below a semi-rigid unit wall layer and above the beaded membranous wall layer, while in *Scutellospora*, they are formed between membranous layer and coriaceous wall layers (Franke and Morton 1994; Spain 1992).

## Genera of arbuscular mycorrhizal fungi

Species of AMF are placed under seven genera—*Acaulospora*, *Entrophospora*, *Archaeospora*, *Glomus*, *Paraglomus*, *Gigaspora*, and *Scutellospora*. Four set of characteristics are complementary in identifying a fungus/fungi to genus level<sup>3</sup>: (1) mycorrhizal structures when the roots are available for study, (2) mode of formation of spores extracted from a soil sample (culture or field), (3) properties of spore and subcellular structures, and (4) mode of spore germination. The species belonging to common genera of AMF like *Acaulospora*, *Gigaspora*, *Glomus*, and *Scutellospora* are described taxonomically in the forthcoming series.

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## Research finding papers

# Incidence of *Glomus Claroideum* Schenck and Smith Emend. Walker and Vestberg in *Sorghum Bicolor* L. from metal contaminated soils adjoining Kanpur Tanneries, Uttar Pradesh

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### Introduction

Phytoremediation of soil and water contaminated by organic and inorganic waste has been the focus of recent biotechnological research (Hayes, Chaudhry, Buckney, *et al.* 2003). The accumulation of metals, at high concentrations, in soil can be due to industrial activities (release of effluent into the soil) or anthropogenic activities (application of sewage sludge into the soil). This latter practice has been widely used for nutrient recycling and is accepted for waste disposal in agricultural soil. However, the addition of sludge considerably increases the amount of heavy metals in soil, causing changes in soil properties, which could be toxic for micro-organisms. In this context, there is an increasing concern about the possible side effects on microbial populations, especially after long-term sludge applications to acidic soil. (Del Val, Barea, and Azcón-Aguilar 1999).

Soil micro-organisms are known to play a key role in the mobilization and immobilization of metal cations, thereby changing their availability to plants (Birch and Bachofen 1990). AMF (arbuscular mycorrhizal fungi) are soil micro-organisms that establish mutual symbioses with the majority of higher plants, thus providing a direct physical link between soil and plant roots (Barea and Jeffries 1995). Only a few studies have been carried out involving interactions between AMF and metals as a source of soil disturbance. Most of the results already obtained are derived from laboratory and pot experiments, with metal salts used as the source of heavy metals, which are not very representative of natural field conditions where metals usually accumulate in a less-available chemical form (Del Val, Barea, and Azcón-Aguilar 1999). Therefore, the present study was undertaken to investigate the AM (arbuscular mycorrhizal) status of *Sorghum bicolor* L. growing on metal contaminated sites adjoining Kanpur tanneries in Uttar Pradesh.

### Materials and methods

IAJMAU, a well-known famous site for tannery effluent discharge in Kanpur, Uttar Pradesh, was selected for the study. This place is highly contaminated with very high levels of chromium and continuous loading of tannery effluent and sludge since the British time. It is situated on the shores of Ganges, Lucknow highway, between Kanpur and Lucknow. The effluent is discharged after a brief processing at the Indo-German plant with alum. *S. bicolor* L. was cultivated in monoculture and in large areas.

Soil samples, along with the roots (feeder roots), were randomly collected from the rhizosphere region of several plants growing on metal contaminated soil from the study site, during August 2004. These samples were packed in polyethylene bags, labelled, and brought to the laboratory. The root samples were freshly analysed whereas the soil samples were stored at 4 °C until processed. The roots were cleaned and stained in 0.05% trypan in lactoglycerol (Phillips and Hayman 1970) and the degree of colonization was estimated by slide method (Giovannetti and Mosse 1980). Spores of AMF were isolated by wet sieving and decanting method (Gerdemann and Nicolson 1963) and quantification of spore density was carried out (Gaur and Adholeya 1994). Identification of AMF was carried out based on spore morphology and wall characteristics ascertained using a compound microscope. AMF were identified to species level using bibliographies provided by Schenck and Perez (1990) and Walker and Vestberg (1998). Taxonomic identification of spores was matched with the descriptions provided by the International Collection of Vesicular Arbuscular Mycorrhizal Fungi.<sup>1</sup>

Rhizosphere soil samples per plant species were taken for analysis. The pH of the soil was measured in 1:2 soil water suspension using a pH meter. Electrical

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

conductivity was measured at room temperature in 1:5 soil suspension using a conductivity meter. Standard soil analysis techniques such as Walkley and Black's (1934) rapid titration method, micro-Kjeldahl method (Jackson 1971) and Oleson, Cole, Watanabe, *et al.* (1954) were employed for determination of organic carbon, total nitrogen, and available phosphorus respectively. Available potassium was estimated by ammonium acetate method (Hanway and Heidel 1952), using a flame photometer. Metals such as aluminium, arsenic, cadmium, chromium, copper, iron, manganese, nickel, lead, and zinc were quantified using an atomic absorption spectrophotometer.

## Results and discussion

As far as the edaphic factors are concerned, the pH (7.45), electrical conductivity (0.64 mmhos/cm), and organic carbon content (0.85%) were optimum, whereas the available phosphorus (15.5 mg/kg) and total nitrogen (0.018%) levels were low. However, available potassium content (180 mg/kg) of the soil

was very high. Further, the rhizosphere soil of the sample plants recorded very high levels of iron (19596 mg/kg), zinc (618.45 mg/kg), chromium (359.89 mg/kg), arsenic (40.25 mg/kg), and nickel (618.45 mg/kg) while aluminium (12339.84 mg/kg), manganese 9169.80 mg/kg, copper (36.5 mg/kg), lead (19.13 mg/kg), and cadmium (1.54 mg/kg) were within permissible limits.

Studies on AM association revealed the presence of extramatrical spores, hyphae, vesicles, and intraradical spores (Plate 1). Different stages of root colonization encountered in the present study may be attributed to the time of sampling (monsoons), indicating that conditions were favourable for their formation and that mycorrhizal strategies of plants may be correlated with environmental conditions (Khade and Rodrigues 2003).

The present study recorded 50% mean total root colonization in *S. bicolor* L. which supports the findings of Weissenhorn and Leyval (1995) who reported root colonization of AMF upto 40% in spite of high

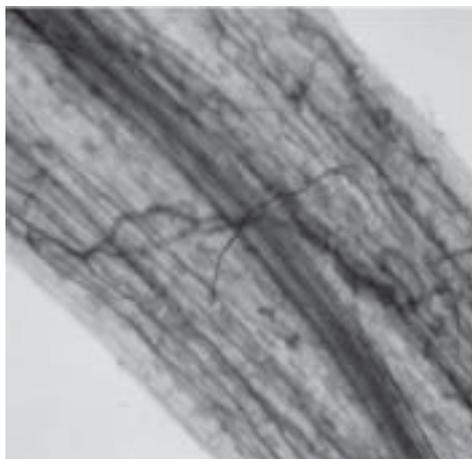


Plate 1(a) Hyphal colonization of AM fungi ( $\times 200$ ).



Plate 1(c) A single spore of *Glomus claroideum* ( $\times 400$ ).

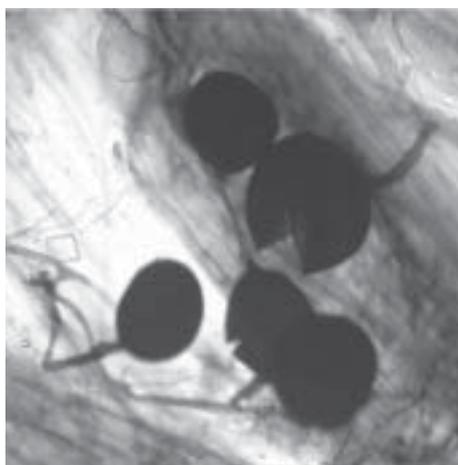


Plate 1 (b) Intraradical spores of AM fungi ( $\times 400$ ).

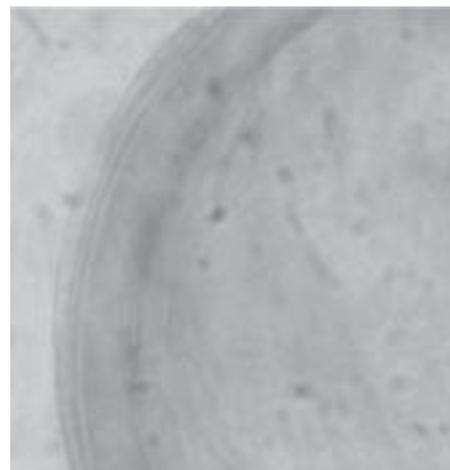


Plate 1(d) A portion of spore wall of *Glomus claroideum* showing wall layers ( $\times 1000$ ).

cadmium (1220 mg/kg) and lead (895 mg/kg) concentrations. The mean spore density of AMF recorded in the present study was low (24 spores/100 g soil). Similarly, Leyval, Singh, and Joner (1995) reported low spore density of (3–46 spores/100 g soil) in Norwegian soils collected from heavy-metal polluted areas.

In the present study, single species of AMF such as *Glomus claroideum* Schenck and Smith emend. was recorded from the rhizosphere soil of *S. bicolor* L. growing on metal contaminated site. Similarly, Weissenhorn, Leyval, and Berthelin (1995) isolated only *Glomus mosseae* from the heavy-metal polluted soils. Another unique species of AMF, such as *Scutellospora dipurpurascens*, has been reported from the rhizosphere of *Agrostis capillaries* growing in contaminated surroundings of zinc refinery in the Netherlands (Griffioen 1994).

## Conclusion

Record of a single AM fungal species, that is, *G. claroideum* shows that very few species of AMF are tolerant to heavy metal soil pollution. *G. claroideum* appears to be a highly tolerant AM species to metals in polluted soil. Also, a selection of heavy metal tolerant AM species by growing trap plants in polluted soils appears to be a better alternative as to select AM species under artificial conditions. Further, work would be needed to utilize *G. claroideum* for bioremediation of polluted soils in symbiosis with roots of different crops plants.

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## Mass multiplication of AMF using soilless substrates

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### Introduction

The word mycorrhiza, first used by a German researcher Frank in 1885, originates from the Greek word mycos, meaning fungus and rhiza, meaning root. AM (arbuscular mycorrhizae) are symbiotic associations, formed between soil fungi and plant, roots that play an essential role in plant growth, plant protection, and soil quality. Because AM fungi have obligate symbiotic status, they need to have an association with plant roots for their own growth and proliferation (Dalpé and Monreal 2004). It is well known that AMF (arbuscular mycorrhizal fungi) improve the growth of plants by increasing the absorptive surface through the extra-radical hyphae compared with root hairs, and thus help in the absorption of relatively immobile ions in soil (Bagyaraj 1992). Being obligate in nature, these fungi have limitation in bulk propagation and as such, their propagation relies on maintenance of the soil-based substrate (Ridgway, Kandula, and Stewart 2006). Pot cultivation remains the preferred propagation technique because it provides a convenient and relatively economic method to produce mycorrhizal inoculum on a large scale (Sahay, Sudha, Varma, *et al.* 1998). However, this carrier is quite bulky and transportation is a problem.

A soilless medium has also been tried. An ideal soilless mixture should hold sufficient water for plant growth and simultaneously permit good aeration. Beads

have a porous texture with numerous air spaces into which the mycorrhizal propagules fit well. Mixing of air-dried inoculum with inert carriers such as sand, vermiculite, and soil-rite has also been documented (Millner and Kitt 1992). Bark calcined clay and perlite provide good aeration. Peat and vermiculite hold more water than these materials but allow air to penetrate better than sand. Dry perlite and vermiculite are very light while sand has high bulk density (Sharma, Singh, and Akhauri 2000). According to Ridgway, Kandula, and Stewart (2006), the density of the media had the greatest effect on spore formation. The size of particles in a soilless substrate plays an important role in optimizing the production of AM inocula (Gaur and Adholeya 2000). Plenchette, Furlan, and Fortin (1982) recommended the use of calcined montmorillonite clay in pot cultures. Other materials that may be used in artificial mixes include bark, peat, perlite, pumice, and vermiculite. In the present study, several partially artificial substrates in various combinations were compared with a sand:soil mixture (1:1 by volume; Bagyaraj and Manjunath 1980) for their suitability as growth medium for mass multiplication of the AM fungi.

Horticultural, ornamental, and fruit-tree crops are usually grown in containers on soilless media prepared with organic substrates and inorganic conditioners which lack AMF propagules. Most of these crops are dependent on AM symbiosis when they are transplanted from the nursery to the field.

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Inoculation with mycorrhizae in the containers is feasible and requires little inoculum. An effort was therefore made to optimize the substrate for the development of AM inocula.

## Materials and methods

### Potting medium

Vermiculite, perlite, FYM (farmyard manure), and soil were used in different proportions as the growing medium for plants.

1. VSF (Vermiculite: Soil: FYM [2:1:1])
2. VS (Vermiculite: Soil [3:1])
3. VFP (Vermiculite: FYM: Perlite [2:1:1])
4. VPS (Vermiculite: Perlite: Soil [1:1:2])
5. VPFS (Vermiculite: Perlite: FYM: Soil [1:1:1:1])

### Host plant and growth conditions

Seeds of *Sorghum bicolor* L. were surface sterilized with 0.5% NaOCl (Sodium hypo chloride) before sowing in autoclaved substrate mixture. The seeds were germinated on towel paper and then sown in the substrate filled in a half kg pot.

### Mycorrhizal inoculum preparation

Mycorrhizal inoculum of *Glomus intraradices*, *Glomus etunicatum*, and *Glomus mosseae* was multiplied on maize for three cycles each of 60 days in soil:sand (1:1) mixture. The soil from an apple orchard was collected, sieved, and filled in 2 kg capacity pots. Maize (*Zea mays* L.), *Vigna mungo*, and marigold were used as trap plants. The trap plants were also grown for three cycles of 60 days each.

### Inoculation

Inoculation was done by making hole in pots with a 1 ml pipette tip and inoculum was provided at 1 g of *G. intraradices*, *G. etunicatum*, *G. mosseae*, and apple trap consisting of soil, extraradical spores, hyphae, and infected root pieces in each pot. The germinated seeds were kept on the top of the inoculum and covered with the same substrate. The pots were kept in glasshouse conditions and plants were watered as and when required. All pots were given a half-strength of Hoagland solution (Hoagland and Arnon 1938), weekly.

### I-set

In the first set, three different substrates—perlite, vermiculite, and FYM—were tested. The substrates were autoclaved (121 °C) for 3 h on alternate days, kept for a week, and then transferred into half kg pots. Five different types of combinations were used with five replications. The experiment was carried

**Table 1** Substrate levels used as carrier in the experiment

Serial number	Substrate	Inoculum
1	VSF (2:1:1)	<i>Glomus intraradices</i>
2	VS (3:1)	<i>Glomus intraradices</i>
3	VFP (2:1:1)	<i>Glomus intraradices</i>
4	VPS (1:1:2)	<i>Glomus intraradices</i>
5	VPFS (1:1:1:1)	<i>Glomus intraradices</i>

VSF - Vermiculite: Soil: FYM; VS - Vermiculite: Soil; VFP - Vermiculite: FYM: Perlite; VPS - Vermiculite: Perlite: Soil; VPFS - Vermiculite: Perlite: FYM: Soil

out for two cycles of 60 days each (Table 1).

### II-set

In the second set, *G. intraradices*, *G. mosseae*, *G. etunicatum*, and apple trap inoculum were used as a mycorrhizal inoculum. The best results in terms of spore production and infection was obtained from VPFS and therefore, this substrate was used for evaluating the inoculum production by different mycorrhizal species having five replications. Sorghum seeds were sown as described above. The experiment was carried out for two cycles of 60 days each.

### Observations

Four plants from each treatment were harvested after eight weeks. At each harvest, shoots were cut just above the crown and roots were washed with tap water. To calculate percentage mycorrhizal colonization (Biermann and Lindermann 1981), the roots were stained with trypan blue according to the procedure described by Phillips and Hayman (1970). Isolation of spores was done using wet sieving and decanting method (Gerdemann and Nicolson 1963) and spores were counted under a stereozoom microscope (Nikon SMZ 1500). The data was analysed using a two-factor complete randomized design.

### Results

#### I-set

The spore count/g of substrate was significantly higher in VPFS than in the other treatments (Table 2). The highest spore count after eight weeks was found in VPFS, followed by VPS. The spore count/g of soil after eight weeks was significantly lower in VSF, in the first cycle.

It is evident from Table 1 that the AM fungus colonized 48.97% of the roots when plants were grown in VPFS for the first cycle, whereas it was significantly lower (31.98%) in plants grown in

VSF. The same trend was found in the second cycle as higher with VPFS and lower with VSF.

Mycorrhizal plants grown in VPFS produced the highest spore count/g of soil and VSF produced a lower spore count/g of soil, in both the cycles.

### //-set

The percent root colonization and spore count by mycorrhizal inoculum varied with inoculum *G. intraradices*, *G. mosseae*, *G. etunicatum*, and apple trap. The highest root colonization after eight weeks was found with *G. intraradices*. Apple trap was the second best inoculum with reference to spore count and percent root infection. The highest spore count was found in case of *G. intraradices* followed by apple trap, *G. etunicatum*, and *G. mosseae* (Table 2).

## Discussion

Enhanced production in the substrates may be related to better soil aeration, drainage, oxygen supply, and root growth. Soil aeration is a key factor

in driving infection, colonization, and metabolic activity of the AMF (Saif 1981). Perlite-soil mix (1:1) was found to be the best substrate on the basis of root colonization and spore production and of the infective propagules of the pot ball (Sreenivasa and Bagyaraj 1988). According to Hawkins and George (1997), the root colonization percentages for *Triticum aestivum* (73%), *S. bicolor* (36%), and *Linum usitatissimum* (65%) were within the range of colonization rates obtained with soil substrate culture in perlite. Guo, Zhang, Christie, *et al.* (2006) reported an increase in shoot dry weight, shoot length, sheath diameter, root N (nitrogen) and P (phosphorus) content, shoot N and P concentrations and content in *Allium cepa* L., grown in Perlite as affected by the AM fungi *Glomus versiforme* and *G. intraradices* BEG 141 and by ammonium:nitrate ratios of 3:1, 1:1, and 1:3 in 4 mM solutions. Yun-Jeong and Eckhard (2005) reported that roots of lettuce (*Lactuca sativa* var. capitata) plants were highly colonized by the AMF, *G. mosseae* (BEG 107) after four weeks in the NFT

**Table 2** Effect of different soilless substrates on production of spores of *G. intraradices* and per cent root infection

Treatment	Number of spores/g soil			AMF infection (%)		
	First cycle	Second cycle	Mean	First cycle	Second cycle	Mean
VSF (2:1:1)	3.04 <sup>a</sup>	6.20 <sup>bc</sup>	4.62 <sup>a</sup>	31.98 <sup>a</sup>	49.88 <sup>e</sup>	40.93 <sup>a</sup>
VS (3:1)	4.16 <sup>ab</sup>	7.36 <sup>c</sup>	5.76 <sup>b</sup>	37.83 <sup>b</sup>	52.10 <sup>f</sup>	44.97 <sup>b</sup>
VFP (2:1:1)	5.32 <sup>b</sup>	8.43 <sup>c</sup>	6.88 <sup>c</sup>	41.49 <sup>c</sup>	58.71 <sup>g</sup>	50.10 <sup>c</sup>
VPS (1:1:2)	6.08 <sup>bc</sup>	9.85 <sup>d</sup>	7.97 <sup>d</sup>	46.93 <sup>d</sup>	63.39 <sup>h</sup>	55.16 <sup>d</sup>
VPFS (1:1:1:1)	6.20 <sup>bc</sup>	11.42 <sup>e</sup>	8.81 <sup>e</sup>	48.97 <sup>e</sup>	66.59 <sup>i</sup>	57.78 <sup>e</sup>
Mean	4.96 <sup>a</sup>	8.65 <sup>b</sup>		41.44 <sup>a</sup>	58.13 <sup>b</sup>	

VSF - Vermiculite: Soil: FYM; VS - Vermiculite: Soil; VFP - Vermiculite: FYM: Perlite; VPS - Vermiculite: Perlite: Soil; VPFS - Vermiculite: Perlite: FYM: Soil

Note: CD (5%)

Number of spores Cycles - 0.52, Treatments - 0.83, Interaction - 1.17

AMF infection Cycles - 0.53, Treatments - 0.83, Interaction - 1.17

**Table 3** Effect of different arbuscular mycorrhizal fungi on mycorrhizal spores and per cent root infection using Vermiculite + Perlite + FYM + soil as substrate

Treatment	Number of spores/g soil			AMF infection (%)		
	First cycle	Second cycle	Mean	First cycle	Second cycle	Mean
<i>Glomus mosseae</i>	4.22 <sup>a</sup>	9.68 <sup>d</sup>	6.95 <sup>a</sup>	37.11 <sup>a</sup>	56.63 <sup>d</sup>	46.87 <sup>a</sup>
<i>Glomus etunicatum</i>	6.32 <sup>b</sup>	10.49 <sup>d</sup>	8.41 <sup>b</sup>	43.88 <sup>b</sup>	59.92 <sup>e</sup>	51.90 <sup>b</sup>
Apple trap	7.66 <sup>c</sup>	12.40 <sup>e</sup>	10.03 <sup>c</sup>	54.57 <sup>c</sup>	64.10 <sup>f</sup>	59.33 <sup>c</sup>
<i>Glomus intraradices</i>	8.14 <sup>c</sup>	14.46 <sup>f</sup>	11.30 <sup>d</sup>	64.55 <sup>f</sup>	77.36 <sup>g</sup>	70.96 <sup>d</sup>
Mean	6.59 <sup>a</sup>	11.76 <sup>b</sup>		50.03 <sup>a</sup>	64.50 <sup>b</sup>	

Note:

CD (5%)

Number of spores Cycles - 0.55, Treatments - 0.78, Interaction - 1.10

AMF infection Cycles - 0.88, Treatments - 1.25, Interaction - 1.76

(nutrient film technique) culture system, following an initial phase of five weeks in inoculated Perlite.

In the present study, percent root colonization and spore count were highest in VPFS particles, which could be because of their porous property, plant biomass, more water absorbing capacity, and different particle size. Greater spore formation in media of this particle size may be attributed to optimal aeration, drainage, and oxygen supply (Saif 1981). An ideal substrate for AM mass production is expected to be low in organic matter and nutrients; that is, inadequate mineral nutrient composition may affect fungal development (Dehne and Backhaus 1986). Water levels allowing for the greatest plant growth would, therefore, result in the highest amount of photosynthate available for fungal growth and development.

Application of Hoagland solution without P enhanced both spore production and percent infection. Douds and Schenck (1990) also demonstrated that low levels of P help maintain high levels of infectious AMF populations. Mass production of *G. intraradices* was highest in both I and II cycle and lowest in case of *G. mosseae*. One might conclude that increased sporulation and percent infection in VPFS mixture are due to increased root growth. According to Menge, Johnson, and Platt (1978) and Mosse (1977), the different substrates differ in their susceptibility to mycorrhizal infection and spore production. A well-aerated substrate has been recommended, such as coarse texture sandy soil (Gaur and Adholeya 2000), mixed with vermiculite or perlite or Turface (Dehne and Backhaus 1986).

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## Effect of mycorrhizal inoculation on mulberry saplings of Goshoerami variety under temperate climatic conditions

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### Introduction

Sericulture in the state of Jammu and Kashmir is age old and the bivoltine silk produced in the valley of Kashmir is famous for its lustre and quality. Although a good number of silkworm rearers are involved in the venture, the non availability of mulberry leaf in abundance under field/farmers conditions continues to be the biggest impediment for the effective development of sericulture. With this in mind, the technology of raising mulberry plants under polyhouse conditions was generated (Baqual, Sheikh, Qayoom, *et al.* 2004) wherein 60%–65% rooting of mulberry variety (*Goshoerami*), which is otherwise a hard rooter under open conditions but the most promising and popular variety in valley, was achieved (Munshi, Baqual, Malik, *et al.* 2003). Through this technology mulberry plants with a height of 5.5–6 ft and fit for distribution among the beneficiaries are produced in two years time, as against the traditional way of producing plants of the same height in more than five years (through grafts). Further, the polyhouse

technology is not only effective but economically far cheaper than the existing system of multiplication.

On the other hand, the use of biofertilizers, accompanied with the reduction in the use of chemical fertilizers in mulberry cultivation is highly fruitful. VAM (vesicular arbuscular mycorrhizal) fungi have been reported to colonize mulberry plants (Padma and Sullia 1991). The use of mycorrhizae, *Glomus fasciculatum* and *Glomus mosseae*, phosphate mobilizing fungi was found to be highly effective which not only brings improvement in the quality of foliage (Baqual, Das and Katiyar 2005) but results in enhanced yield as well. Data of 4.5 months old mycorrhizal saplings of S1 mulberry variety revealed an overall better performance in some important qualitative and quantitative characters over that of non-mycorrhizal saplings (Sethua, Sudhakar, Kar, *et al.* 1999). Das, Katiyar, Hanumanthat, *et al.* (1995) have observed an increase in growth, development, and survival of mulberry saplings inoculated with *G. mosseae*. In order to explore the possibility of bringing further reduction in the production cost of plants through use of

mycorrhizal inoculum under temperate climatic conditions, the present study was initiated.

## Material and methods

In the present study, the cuttings (13–15 cm in length and 1–1.5 cm in diameter with 4–5 active buds) were prepared from mulberry plants of the *Goshoerami* variety pruned before 7–8 months. The freshly prepared cuttings were dipped in 0.1% diathane  $M_{45}$  solution for half an hour to ensure their surface disinfection. The cuttings were gently planted in perforated polythene bags containing well-powdered rooting medium comprising of decomposed FYM, sand, and soil in the ratio of 1:7:2. The plantation of cuttings was carried in the month of February 2004 and the polybags along with the cuttings were placed in polyhouse. The growing saplings were retained in polyhouse till the end of May 2004.

The nursery land was prepared by digging it 2 ft deep and the soil was pulverized and levelled. The saplings were thereafter transplanted to the nursery with a spacing of 3 x 1.5 ft by 1 June 2004. During the course of transplantation, the polybags were gently torn out and the saplings planted in predug pits without disturbing the root system. Before plantation of the saplings, 50 g of soil-based VAM inoculum was poured into each pit. The nursery was immediately irrigated for the establishment of saplings. The plantation of cuttings was done following randomized block design with four treatments and six replications. The treatments included application of half a dose of recommended level of phosphorus ( $T_1$ ), 3/4th dose of phosphorus ( $T_2$ ), 1/4th dose of phosphorus ( $T_3$ ), and recommended dose ( $T_4$ ). The recommended dose being 50:25:25 kg NPK/ha/yr (Nitrogen Phosphorus Potassium/hectare/year). The data on various commercial parameters like plant height, stem diameter, leaf weight, branch number, and internodal distance was recorded. However, observations on plant diameter, height, and internodal distance reflected here have been recorded during June 2006; like two years after plantation, where as the rest of the observations

have been recorded during June 2007 after maintaining the plants in the shape of a bush.

## Results and discussion

The data presented in Table 1 indicates superiority of mycorrhizal treatments in almost all parameters over the non-mycorrhizal ones. The height/plant, 141.95 cm, recorded in treatment ( $T_2$ ) receiving 3/4th of phosphorus and inoculation was statistically at par with the uninoculated treatment, 149.76 cm ( $T_4$  - control), receiving full and recommended dose of fertilizer (50:25:25 kg of NPK/ha/yr). A similar trend was recorded with respect to leaf weight/plant and plant diameter (stem girth) with  $T_4$  recording the highest values – 449.45 g/plant and 5.59 cm respectively. However, these values were at par with  $T_2$  recording 421.5 g/plant and 5.12 cm girth. The enhanced productivity of leaf in mycorrhizal treatments might have been due to better nutrient uptake (Mosseae 1973).

Although the treatments  $T_1$ ,  $T_2$ , and  $T_3$  received inoculation, yet the graded curtailment in the application of phosphatic fertilizer indicates its impact on various commercial parameters of mulberry plants. The average branch length/plant, 361.38 cm, was recorded in the control receiving full and recommended dose of NPK, but this was statistically at par with  $T_2$  recording branch length of 325.07 cm.

The internodal distance and the branch number/plant were non-significant (Table 1).

Similar observations were made with respect to parameters like plant diameter (recorded after one year of plantation) and average branch length/plant. However, branch number and internodal distance were non-significant. Leaf weight/plant was also significantly higher in  $T_4$  but at par with  $T_2$ . It was observed that the plant height had direct bearing on leaf yield.

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**Table 1** Effect of mycorrhizal inoculation on the growth of mulberry plants raised through saplings

Treatments	Plant height (cm)	Diameter of main plant (cm)	Branch number/plant	Branch length (cm)	Internodal distance (cm)	Leaf weight/plant (g)
$T_1$	131.6	4.40	2.66	314.42	5.20	392.43
$T_2$	141.95	5.12	2.91	325.07	5.70	421.5
$T_3$	129.09	4.08	32.38	292.29	5.37	369.28
$T_4$	149.76	5.59	3.35	361.38	5.82	449.45
CD @ 5%	9.420	0.699	NS	45.918	NS	31.477

NS - non-significant

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## Response of jamun (*Syzygium cuminii* Skeels) to different arbuscular mycorrhizal fungal species for germination

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### Abstract

An experiment was conducted at the Department of Pomology, Kittur Rani Channamma College of Horticulture, Arabhavi to find out the effect of nine AM (arbuscular mycorrhizal) fungi for germination in jamun. Significantly least number of days for initiation of germination was recorded in seeds inoculated with *Glomus bagyaraji* (14 days), while significantly maximum number of days for completion of germination was observed in uninoculated seeds (56.5 days). Inoculation with *Glomus fasciculatum* and *Glomus intraradices* (89% each) recorded significantly highest germination percentage. Uninoculated seeds had shown significantly highest germination index (2.315) when compared to other treatments.

### Introduction

Jamun (*Syzygium cuminii* Skeels) is one of the under exploited indigenous fruit crops of India which has gained exceptional importance in recent past for its hardy nature, uncomparable medicinal and nutritional properties. The seed powder has anti diabetic properties and also contains curative properties for ringworm (Dastur 1952). Jamun seeds are recalcitrant and lose viability fast due to their

small size and thin seed coat. The fruits are highly season specific in availability and duration is also short. Hence, increasing of per cent germination within a stipulated time period is of utmost importance.

### Material and methods

An investigation was carried out at the nursery of Department of Pomology, Kittur Rani Channamma College of Horticulture, Arabhavi, with four replications in completely randomized design. Non-descriptive uniform size jamun seeds were sown in polybags (8 × 12 cm) containing potting mixture of soil: sand: FYM (farm yard manure) in 2: 1: 2 proportion. Cultures of nine different AM fungi as mentioned in Tables 1 and 2 were obtained from the Department of Agricultural Microbiology, Kittur Rani Channamma College of Horticulture, Arabhavi. AM fungal inoculation was done by spreading five g of inoculum (consisting of 80-88 infective propagules) uniformly at five cm depth and putting a thin layer of soil above the inoculum. Seeds were placed and covered with soil (two-three cm). The polybags of respective treatments were labelled and kept apart from each other to avoid AM fungal cross contamination.

Germination count was recorded daily till 70 DAS (days after sowing). Appearance of plumule was taken as criterion for germination. Days taken for initiation, 50% and completion of germination, and changes in colour of leaves were recorded. GVI (germination vigour index) was computed using the formula,

$$GVI = \frac{x_1}{d_1} + \frac{x_2}{d_2} + \frac{x_3}{d_3} + \dots + \frac{x_n}{d_n}$$

where  $x_1, x_2, x_3, \dots, x_n$  were the number of seeds germinated on  $d_1, d_2, d_3, \dots, d_n$  days taken for germination, respectively.

Seedling vigour and seedling vigour index at 180 DAS were calculated as per the following formulae.

Seedling vigour = per cent germination  $\times$  dry weight of seedling

Seedling vigour index (Bewly and Black 1982) = per cent germination  $\times$  height of seedling

RMD (relative mycorrhizal dependency) was computed as follows.

RMD (%) =

$$\frac{\text{Parameter with AM fungi} - \text{parameter without AM fungi}}{\text{Parameter with AM fungi}} \times 100$$

## Results and discussion

AM fungi significantly influenced the response of jamun for germination. Significantly least number of days for initiation of germination were recorded in seeds inoculated with *G. bagyaraji* (14 days). Among the different AM fungi inoculated to seeds, *Acaulospora laevis* and *Glomus mosseae* took significantly minimum number of days (45.25 days each) for completion of germination, which was statistically on par with *G. intraradices* (46.50 days)

and *Glomus leptotichum* (49.25 days). Significantly maximum days for completion of germination was registered in uninoculated control (56.50 days) (Table 1). Inoculation with *G. intraradices* recorded significantly minimum days (49 days) for completion of colour change when compared to the rest of the treatments.

The influence of AM fungi on germination was found to be significant (Table 2). Inoculation with *G. fasciculatum* and *G. intraradices* recorded significantly maximum germination percentage (89% each), followed by *G. bagyaraji* (87%) and *Sclerocystis dussii* (87%) which were statistically on par with each other. Significantly minimum germination percentage (77.5%) was noticed in seeds inoculated with *A. laevis* which was statistically on par with uninoculated control (80%).

Uninoculated control had shown highest germination index of 2.315 (Table 2) and significantly lowest value was recorded in inoculation with *S. dussii* (1.776) which was statistically on par with *Gigaspora margarita* (1.787).

The rootstock vigour and rootstock vigour index (Table 2) were significantly highest in *G. fasciculatum* (1209.78 and 2842.45, respectively), which was statistically on par with *G. intraradices* (1148.63 and 2651.20, respectively). Significantly least values for both the parameters were recorded in uninoculated control (535.70 and 1739.27, respectively).

Highest RMD for germination was noticed in *G. fasciculatum* and *G. intraradices* (10.11% each) and least was noted in *A. laevis* (-3.23%).

Increased enhancement of germination by AM fungal inoculation had also been recorded in citrus (Venkat 2004); mango (Santosh 2004 and Bassanagowda 2005); papaya (DurgannavarPatil, Patil,

**Table 1** Effect of different AMF on days taken for germination and colour change of leaves

Treatment	Days taken for germination			Days taken for leaf colour change		
	Initiation	50%	Completion	Initiation	50%	Completion
<i>Glomus bagyaraji</i>	14.00	18.25	54.25	24.00	31.75	60.00
<i>Glomus leptotichum</i>	15.50	20.25	49.25	24.75	31.50	55.50
<i>Acaulospora laevis</i>	15.25	19.75	45.25	24.50	31.75	52.25
<i>Sclerocystis dussii</i>	16.00	20.50	52.50	24.25	31.75	57.25
<i>Glomus mosseae</i>	15.25	20.75	45.25	24.25	32.75	54.00
<i>Gigaspora margarita</i>	16.00	24.00	52.25	24.00	37.00	62.75
<i>Glomus monosporum</i>	15.75	22.25	51.75	24.25	33.75	57.75
<i>Glomus intraradices</i>	15.00	19.75	46.50	24.00	32.40	49.00
<i>Glomus fasciculatum</i>	15.00	19.00	50.25	24.00	33.25	55.25
Control	15.00	21.00	56.50	24.25	35.25	62.75
S.Em $\pm$	0.264	0.496	1.618	0.199	0.679	0.479
CD (5%)	0.710	1.431	4.673	NS	1.975	1.382
CD (1%)	0.956	1.927	6.292	NS	2.638	1.862
CV (%)	3.22	4.83	6.42	1.64	4.10	1.69

NS - non-significant

**Table 2** Effect of different AMF on per cent germination and germination index in jamun

Treatment	Germination (%)	Germination index	Seedling vigour	Seedling vigour index	Relative mycorrhizal dependency (%)
Glomus bagyaraji	87.00 (68.92)	2.245	955.22	2056.40	8.05
Glomus leptotichum	84.50 (66.85)	1.968	905.13	1895.80	5.33
Acaulospora laevis	77.50 (61.72)	1.873	885.95	1834.02	3.23
Sclerocystis dussii	87.00 (68.92)	1.776	1108.10	2595.87	8.05
Glomus mosseae	83.00 (65.73)	1.966	960.74	2256.62	3.61
Gigaspora margarita	85.50 (67.69)	1.787	1035.40	2166.49	6.43
Glomus monosporum	84.50 (67.23)	1.907	1068.03	2384.55	5.33
Glomus intraradices	89.00 (70.68)	2.290	1148.63	2651.20	10.11
Glomus fasciculatum	89.00 (70.68)	2.164	1209.78	2842.45	10.11
Control	80.00 (63.48)	2.315	535.70	1739.27	-
S.Em±	1.258	0.032	39.343	766.72	
CD(5%)	3.630	0.091	113.621	221.43	
CD(1%)	4.892	0.123	152.964	298.102	
CV(%)	2.97	3.17	8.02	6.890	

Figures in parenthesis pertains to the angular transformation of the data; NS - non-significant

*et al.* 2004); *aeonla* (Swamy, Patil, and Athani 2005); and *charoli* (Kareddy 2003). During the early phase of germination, the germinating seeds release several compounds including amino acids, organic acids, inorganic ion sugars, phenolics, and proteins (Simon 1984). This solute leakage influences detection of the seed by soil microflora and fauna (beneficial and pathogenic) during germination. These solutes might help keep AM fungal propagules to germinate early. Certain solutes synthesized in the appropriate hosts are reported necessary to initiate mycorrhizal association (Xie, Starhelin, and Vierheilig 1995). The differences observed in the efficacy of germination by different AM fungal species could probably be attributed to leached solutes from the appropriate/preferred host. Thus, leachats/exudates might play a prominent role in early propagation of endomycorrhiza contributing to improved efficiency and early seed germination (Cruz, Ishii, Matsumotto, *et al.* 2003). AM fungi are known to secrete plant growth regulators like gibberellins (Allen, Moore, and Christensen 1980), auxins and cytokinins (Edriss, Davis, and Burger 1989) in turn help the seeds germinate early.

The present study revealed that jamun is responsive to inoculation of AM fungi as evident from enhanced germination and vigour. *G. fasciculatum* and *G. intraradices* were recorded to be more preferred AM species by jamun.

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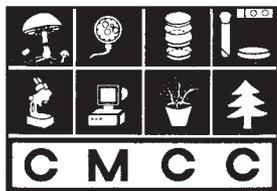
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## Centre for Mycorrhizal Culture Collection

### Impact assessment of Mycorrhiza application on *Oryza sativa* L.

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Centre for Mycorrhizal Research, TERI, Darbari Seth Block, IHC Complex, Lodhi Road, New Delhi-110 003, India

#### Introduction

Chemical fertilizer consumption increased tremendously to maximize grain yield per unit area by cultivation of hybrid crops after green revolution. Estimates of overall efficiency of applied fertilizers have been about 50% or lower for N (nitrogen), <25% for P (phosphorus), about 40% for K (potassium), and <2% for micronutrients. These lower efficiencies are due to significant losses of nutrients by leaching, run-off, gaseous emission, and fixation by soil (Singh and Singh 2003).

The AMF (arbuscular mycorrhizal fungi) are an important component of the soil microbial biomass. These micro-organisms provide nutrition to a plant by sequestering nutrients from the soil and translocating them to the plant, and in return gets carbon from them. This makes the utilization of nutrients highly efficient and reduces the dependence on external chemical inputs. Therefore, mycorrhizal technology offers a biological means of assuring plant production at a low cost without chemical fertilizers.

Soil microorganisms, including AMF, are affected by the presence of high metal concentration in the soil (Giller, Witter, and Macgrath 1998). But the organisms, in turn, influence the availability of metals in soil either directly, through alterations of pH, biosorption or uptake; or indirectly in through their effect on plant growth, root exudation, and resulting rhizospheric chemistry.

A unique trait of AMF is that they form a direct link between soil and root, providing a path for movement of elements in the rhizosphere that help in the absorption of relatively immobile ions in soil such as phosphate, copper, and zinc. In addition, AMF inoculated plants showed greater tolerance to toxic metals, root pathogens, drought, high soil temperature, salinity, soil reaction, and transplant shock than non-inoculated plants (Mosse, Stribley, and Le Tacon 1981;

Bagyaraj 1990; Bagyaraj and Varma 1995). Studies conducted on agro forestry trees and important crops have shown that AMF increase biomass production (Howeler, Sieverding, and Saif 1987; Bagyaraj 1992).

Under field conditions, different plant species live together and hyphae of AMF interconnect the root system of adjacent plants changing the level of AMF colonization. AMF hyphae can mediate nutrient transfer between plants (Bethlenfalvay, Schreiner, Mihara, *et al.* 1996; Ocampo 1986). The plants benefit particularly through enhanced phosphorus, water, and mineral nutrient uptake (Smith and Read 1981), which often result in better growth. The AMF can protect plants against the toxic effects of excessive concentrations of heavy metals (Heggo, Angle, and Chaney 1990; Marschner 1995). As symbionts of plant root, AMF are critical components of soil microbial communities, which influence above-ground productivity and plant community development (Sanders, Clapp, and Wiemken 1996; van der Heijden *et al.* 1998), improve water relations (Allen and Allen 1986; Neumann and George 2004), increase nutrient uptake and stress tolerance (Lapointe and Molard 1997), and assist in stable aggregate formation and enhance C and P dynamics in the rhizosphere (Coleman and Crossley 1996; Sanders, Clapp, and Wiemken 1996; Jeffries, Gianianazzi, and Perotto 2003).

The present investigation was undertaken to find out the impact of different AMF species on plant population, biomass, and yield of *Oryza sativa* L. cv Pant Dhan 4.

#### Materials and methods

Field experiment was laid out in a randomized block design consisting main four blocks as replicates and each block further divided into four plots (5m x 5m each) having different AMF treatments (T1: control; T2: LL2 consortia; T3: ROC1; and T4: Commercial

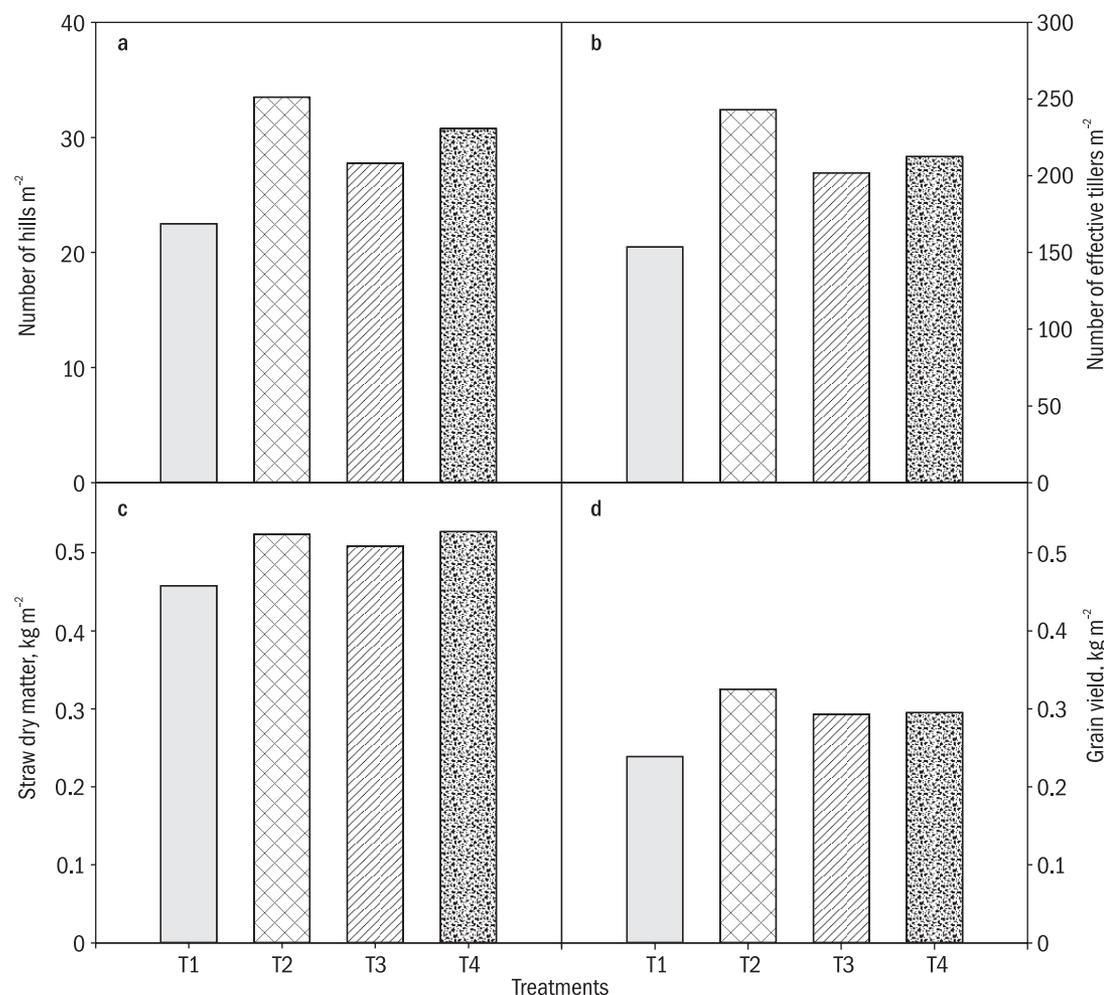
AMF). Rice seed was encapsulated with AMF (@20 infective propagules/seed) for raising the nursery and at the time of transplantation roots were inoculated by dipping in the AMF solution (@20 infective propagules/plant). Farmyard manure was applied @ 3 t/ha at the time of field preparation. Field was maintained by following recommended agronomic practices except chemical fertilizer and pesticide application. The data for agronomic parameters was collected randomly from each plot at the time of harvesting. The number of effective tillers and hills were taken at three places (3 x 1 m<sup>2</sup>) diagonally from each plot. Plant biomass and grain yield were taken from 9 m<sup>2</sup> leaving, 1 m from each side.

## Results and discussion

The AMF inoculated plants showed an increase in number of hills, effective tillers, straw dry matter, and yield compared to uninoculated plants (Figure 1). An increased growth rate of banana with inoculum of mycorrhiza has been reported by many workers (Alonso, Gonzales, Exposito, *et al.* 1995;

Parvathareddy, Nagesh, Rao, *et al.* 1997). Among the treatments, LL2 consortia, ROC1, and commercial AMF inoculated plots showed 49%, 23%, and 37% increase in number of hills, respectively compare to the uninoculated control plots (Figure 1a). A similar trend was observed for a number of effective tillers (Figure 1b). Further, growth of mycorrhizal plants is usually high when the potential for active photosynthesis is high. The high rate of photosynthesis by mycorrhizal plants may be evoked by a number of changes, such as an increase in plant hormones (Miller 1971), stomatal opening, enhanced ion transport, and regulation of chlorophyll level (Johnson 1984).

Maximum straw dry matter was recorded in commercial AMF (Figure 1c). Grain yield increase of 36%, 23%, and 24% was observed in plots inoculated with LL2 consortia, ROC1, and commercial AMF respectively (Figure 1d). Increased uptake of N, P, K, Cu (copper), Mn (manganese), Fe (iron), and Zn (zinc) (Bagyaraj and Manjunath 1980) and increased tolerance to biotic stresses (Abdulla 2002)



**Figure 1** Effect of AMF on (a) number of hills m<sup>-2</sup> (b) number of effective tillers m<sup>-2</sup> (c) straw dry matter, kg m<sup>-2</sup> and (d) grain yield, kg m<sup>-2</sup> in rice

might have contributed to the production of more leaves and greater leaf area and thereby, higher photosynthetic capacity during reproductive phase, and translocation of carbohydrates from other plant parts to reproductive parts, which may have resulted in increased yield and yield attributes.

The present investigation, therefore, clearly indicates that the use of AMF increases the growth and yield of *Oryza sativa* L., which in turn could reduce greenhouse gases emission by reducing the consumption of chemical fertilizers and sequester carbon in soils and biomass.

## Acknowledgments

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## Recent references

The latest additions to the network's database on mycorrhiza are published here for the members' information.

The Mycorrhiza Network will be pleased to supply any of the available documents to bonafide researchers at a nominal charge. This list consists of papers from the following journals.

- *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*
- *Acta Physiologiae Plantarum*
- *American Journal of Botany*
- *Biological Agriculture and Horticulture*
- *Botanical Studies*
- *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*
- *Cereal Research Communications*
- *Chemosphere*
- *Crop Protection*
- *Ecological Monographs*
- *European Journal of Plant Pathology*
- *Fems Microbiology Letters*
- *Forest Ecology and Management*
- *Forest Science*
- *Fungal Genetics and Biology*
- *Hortscience*
- *In Vitro Cellular and Developmental Biology-Plant*
- *Journal of Applied Microbiology*
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- *Journal of Experimental Botany*
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- *Journal of The Faculty of Agriculture Kyushu University*
- *Land Degradation and Development*
- *Mycorrhiza*
- *New Phytologist*
- *Oecologia*
- *Pesquisa Agropecuaria Brasileira*
- *Plant Biology*
- *Planta*
- *Revista Brasileira De Ciencia Do Solo*
- *Soil Biology and Biochemistry*
- *Soil Science and Plant Nutrition*

Copies of papers published by mycorrhizologists during this quarter may please be sent to the Network for inclusion in the next issue.

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)
Alejo-Iturvide F, Marquez-Lucio M A, Morales-Ramirez I, Vazquez-Garciduenas M S, Olalde-Portugal V*. 2008	<b>Mycorrhizal protection of chilli plants challenged by <i>Phytophthora capsici</i></b> <i>European Journal of Plant Pathology</i> <b>120</b> (1): 13–20 [Ctr Invest and Estudios Avanzados, Dept. Biotecnol and Bioquim, Campus Guanajuato, Km 9-6 Libramiento N Carretera I, Guanajuato 36500, Mexico]
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### Conferences, congresses, seminars, symposia, and workshops

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20–23 May 2008

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Eastern Sun Events, PO Box 12612, Centrahil, 6006, Port Elizabeth

*Tel.* +27 (0)41 374 5654 • *E-mail* info@easternsun.co.za  
*Fax* +27 (0)41 373 2042 • *Website* www.easternsun.co.za

Kraków, Poland  
2–6 July 2008

**Plant-Microbial Interactions 2008 (PMI–2008)**

Katarzyna Turnau,  
Conference venue: Strict City Centre

*E-mail* pmi2008@eko.uj.edu.pl  
*Website* http://www.eko.uj.edu.pl/mycorrhiza/pmi/

Honolulu, Hawaii, USA  
12–18 July 2008

**ICOM 2008: International Congress on Membranes and Membrane Processes**

The North American Membrane Society  
ICOM 2008 Secretariat, University of Texas at Austin, Center for Energy and Environmental Resources, 10100 Burnet Road, Building 133-R7100  
Austin, TX 78758

*Website* http://www.membranes.org

Ottawa, Canada  
14–17 June 2008

**The 50th annual meeting of the Canadian Society of Plant Physiologists**  
University of Ottawa

*Website* http://simulium.bio.uottawa.ca/cspp/CSPP2008/index.html

Torino, Italy  
24–29 August 2008

**9th International Congress of Plant Pathology**

*Website* http://www.icpp2008.org

Germany, Bonn  
29 September–3 October 2008

**The 6th International Conference on Mushroom Biology and Mushroom Products**

Tourismus and Congress GmbH, Region Bonn/Rhein-Sieg/Ahrweiler,  
Adenauerallee 131, D-53113 Bonn, Germany

*Tel* +49(0)228-9104149 • *E-Mail* GAMU2008@bonn-region.de  
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Kusadasi, Turkey  
29 October–1 November 2008

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