MYCORRHIZA NETWORK

MYCORRHIZA NEWS

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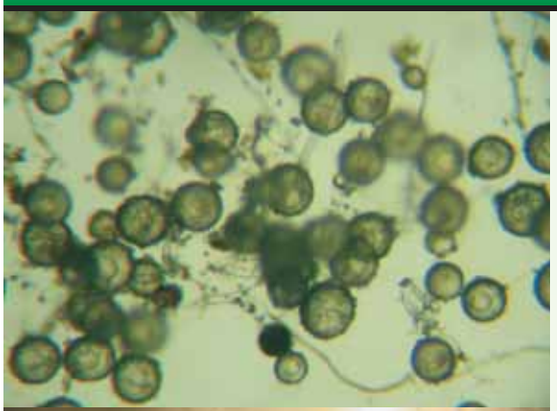

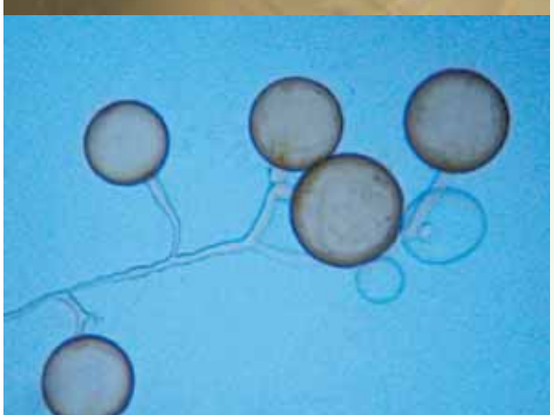

About TERI

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TERI's Mycorrhiza Network

TERI's Mycorrhiza Network is primarily responsible for establishing the MIC (Micorrhiza Information Centre), the CMCC (Centre for Mycorrhiza Culture Collection), and publishing Mycorrhiza News. The Network helps scientists carry out research in mycorrhiza and promotes communication among mycorrhiza scientists.

Mycorrhiza News

The Mycorrhiza News provides a forum for dissemination of scientific information on mycorrhiza research and activities; publishes state-of-the-art papers from eminent scientists; notes on important breakthroughs; brief accounts of new approaches and techniques; publishes papers complied from its RIZA database; provides information on forthcoming events on mycorrhiza and related subjects; lists important research references published during the quarter; and highlights the activities of the CMCC.

Contents

TAXONOMIC FINDINGS [SERIES 2]

Gigaspora from Goa

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1. Gigaspora decipiens Hall and Abbott

Spores were formed singly in soil (Figure 1a) and borne terminally on a bulbous sporogenous cell (Figure 1b). They are creamish white when young and turn yellowish-brown when mature and reddish-brown when old. Further, they are globose to subglobose and 340–425 µm in diameter (Figure 1a and 1b). The spore wall is 28–47 µm thick with 11–15 sub-equal laminations (Figure 1e). It is 20–34 µm thick in young spores (Figure 1c) and up to 47 µm thick in mature spores. It consists of three layers (Figure 1d). The outer permanent rigid layer (Wall 1) is smooth, 2–3 µm thick, adherent to sub-layers of wall 2, and often hard to see in relation to wall 2. Wall 2 consists of sub-layers (or laminae) that increase in number with thickness. This layer is yellow in newly formed spores and turns dark brownish-yellow with age and storage. It is 25–45 µm thick (Figure 1e). Wall 3 is a 'germinal' layer, which is concolorous and adherent to the laminate layer. Numerous 'warts' or 'papillae' form on the inner surface of this layer, and they are especially concentrated in regions where germ tubes form (usually in close proximity to the suspensor cell). Warts are 1.2–5 µm high in germinating spores and 2.5–3 µm wide. Sporogenous cell terminal is present on the subtending hyphae. The sporogenous cell is light brown and 65 µm wide. One or more lateral hyphae are often attached to the cell. The sporogenous cell wall is 5–6.8 µm thick near the spore and 1.2–2.0 µm beyond the sporogenous cell. The subtending hyphae is 8–10 µm in diameter. Germ tubes formed in the vicinity of warty protuberances make up the innermost layer of the spore wall. The germ tube hole that passes through all layers of the spore wall is 6–9 µm wide (Figure 1b). The closure is by a plug concolorous with the laminate layer of the spore wall.

***Distinguishing feature*:** Wall 2 of the spore is 25–45 µm thick with 11–15 sub-equal laminations.

***Distribution*:** Recorded from Kodar in April and January with 25% frequency of occurrence. Recorded from Old Goa in October and December with 12.5% and 20% frequency of occurrence, respectively.

***Association*:** Found in association with *Carica papaya* L. plants from plateaus and coastal areas of Goa, India.

2. Gigaspora margarita Becker and Hall

Spores were formed singly in the soil and borne terminally on a bulbous sporogenous cell. The spores are creamish-white when young and turn yellowish-brown when mature and reddish-brown

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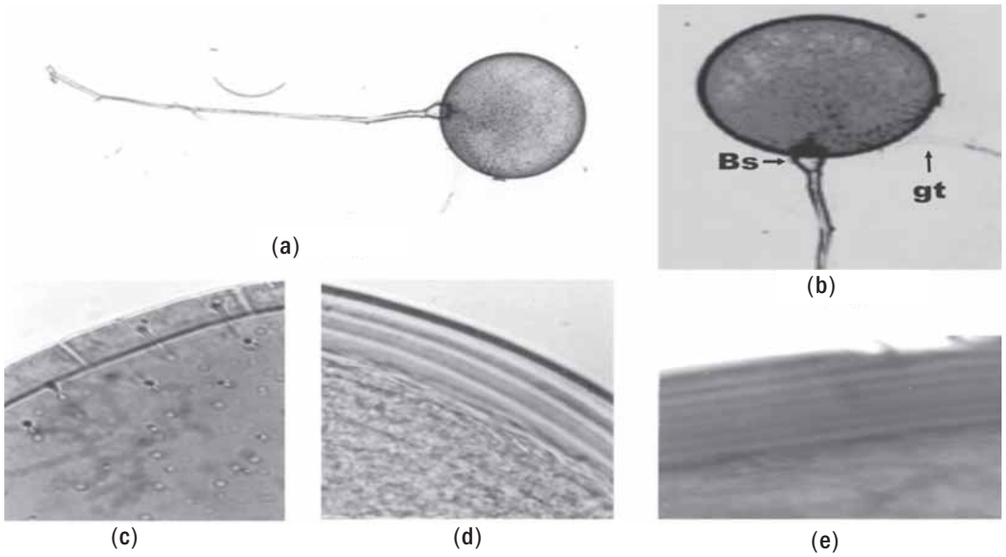

Figure 1a A spore of *Gigaspora decipiens* (× 100).
Figure 1b A spore of *Gigaspora decipiens* borne terminally on bulbous suspensor (Bs), along with the germ tube (gt) originating from the spore (× 200).
Figure 1c A portion of spore with single wall layer in young spore (× 400).
Figure 1d A portion of mature spore with three wall layers (× 400).
Figure 1e A portion of old spore with 11–15 sub-equal laminations of the middle wall of the spore (× 400).

when old. They are globose to subglobose and 300–480 µm in diameter (Figure 2a and 2c). The spore wall is smooth and composed of 4–8 (rarely 10) fused laminations (Figure 2b and 2d). Each lamination is 1.5–4 µm thick. The spore wall is 5–15 µm thick in young spores (Figure 2b) and up to 24 µm thick in mature spores (Figure 2d). Wall 1 is an outer permanent rigid layer, which is smooth, pale brownish-yellow, 2 µm thick, and adherent to the inner laminae. Wall 2 consists of

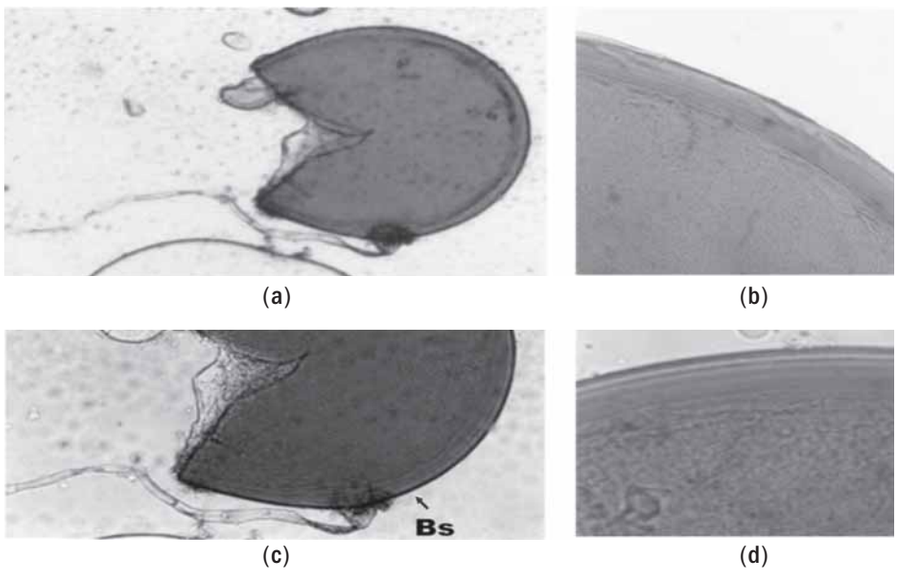

Figure 2a Crushed spore of *Gigaspora margarita* showing oil contents (× 200).
Figure 2b Laminated wall of young spore of *Gigaspora margarita* (× 400).
Figure 2c Crushed spore of *Gigaspora margarita* with hyaline bulbous suspensor and septate subtending hyphae (× 200).
Figure 2d Laminated wall of in mature spore of *Gigaspora margarita* (× 400).

sub-layers (or laminae) that increase in number with thickness, yellow to brownish-yellow, and 13–22 µm thick. Wall 3 is a 'germinal' layer, which is concolorous and adherent with laminate wall 2. Numerous 'warts' or 'papillae' form on the inner surface of wall 3 and they are especially concentrated in regions where germ tubes arise (usually in close proximity to the suspensor cell). The warts are 1. 2–5 µm high in germinating spores and 2.5–3 µm wide. The content of the spores is white and composed of many small oil droplets (Figure 2a), particularly in germination regions. Subtending hyphae generally septate below the sporogenous cell, which is 8–10 µm in diameter. The sporogenous cell is smooth, hyaline to light brown in colour, and 30–65 µm in diameter. Sporogenous cell walls are 2–6 µm thick and are thicker at the point of attachment to the spore. The pore at the point of attachment to the spore base is occluded by a plug concolorous with the laminate layer of the spore wall. The germ tube is formed in the vicinity of warty protuberances on inner surface of the germinal spore wall. The germ tube hole passes through the spore wall, which is 6–8 µm wide.

Distinguishing feature: The spore wall is smooth and composed of 4–8 (rarely 10) fused laminations, which are 15–24 µm thick.

***Distribution*:** Recorded from Collem in December with 66% frequency of occurrence; Valpoi, Kodar, and Collem in December with 50% frequency of occurrence; Valpoi in October and Kodar in April with 12.5% frequency of occurrence; and in April, June, July, August, September and January with 100 % frequency of occurrence from Old Goa**.**

***Association*:** Found in association with *C. papaya* L. plants from Western Ghats, plateaus and coastal area of Goa India.

3. *Gigaspora margarita* Becker and Hall = *Gigaspora ramisporophora* Spain, Sieverding, and Schenck

The spores were formed at the apex of bulbous sporogenous cell. They are brown, (Figure 3a) globose, and 200–450 µm in diameter. The spore wall is brown (Figure 3d) and 9–32 µm thick. The spore wall structure of the three walls is in a single group (Figure 3d). Wall 1 is a unit wall, hyaline to subhyaline, and 1–5 µm thick. Wall 2 is laminate, yellow to yellowish-brown, 4–28 µm, and adherent to wall 3. Wall 3 is 1–3 µm thick. The spore contents are oily (Figure 3a). There are two bulbous sporogenous cells (Figure 3b and 3c). The first is an apical cell, which is globose and 40–60 µm diameter.

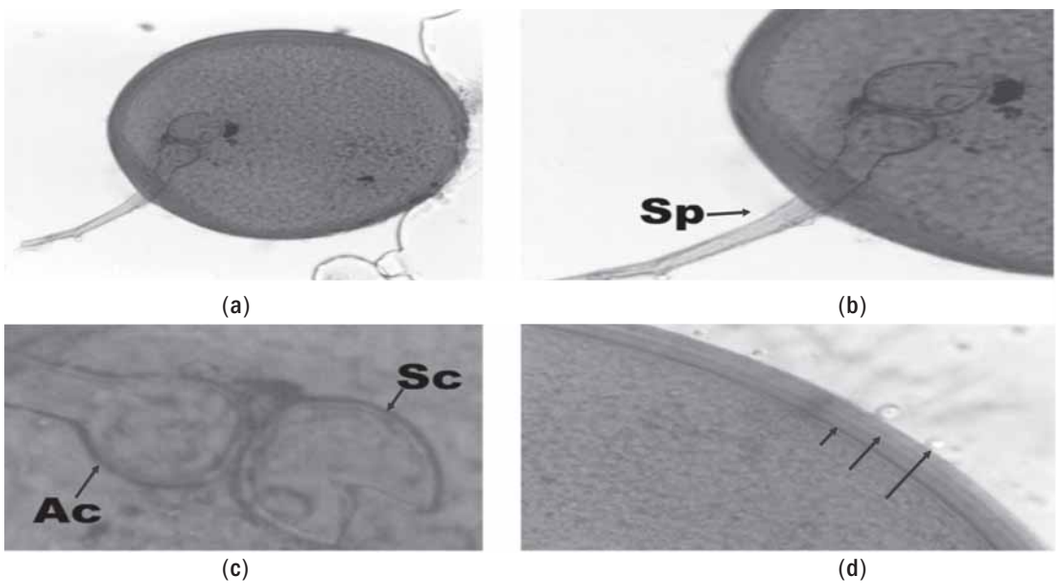

Figure 3a Brown spore of *Gigaspora ramisporophora* with oily spore contents (× 100).
Figure 3b Brown colour spore of *Gigaspora ramisporophora* with curved sporophore (Sp) (× 200).
Figure 3c Double sporogenous cell contiguous with each other (× 1000).
Figure 3d Laminated brown spore wall with three wall (arrows) layers (× 200).

The second is a subapical cell, similar in dimensions to the apical cell, and usually (Figure 3c) contains three walls, which are 6–14 µm in thickness. The sporophore is curved (Figure 3b), 8–15 µm diameter, with a wall that is 1.5–3 µm thick.

Distinguishing feature: Presence of double bulbous suspensor.

***Distribution*:** Recorded in September from Old Goa with 12.5% frequency of occurrence.

***Association*:** Found in association with *C. papaya* L. plants from coastal area of Goa India.

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RESEARCH FINDING PAPERS

Screening of different arbuscular mycorrhizal fungi for raising jamun (*Syzygium cuminii*) rootstocks

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Introduction

Jamun (*Syzygium cuminii* Skeels, Myrtaceae) is an important unexploited, indigenous fruit crop of the country. The ripe fruits are tasty and good sources of minerals, sugars, and proteins. The seed powder has antidiabetic properties and the lotion is used to control ringworms (Dastur 1952). AMF (arbuscular mycorrhizal fungi) are highly evolved, mutualistic associations with plant roots. It is estimated that 95% of plant species characteristically form mycorrhizae. AMF have shown to improve productivity in soils of low fertility, increase plant vigour, and reduce transplant injury. There has been a shift in the horticultural scenario, which necessitates raising the rootstocks organically (from the nursery itself) to ensure higher graft-take, better growth, and, in particular, more stem girth. The present study was carried out to select AMF most suitable for jamun.

Material and methods

The experiment was conducted at the nursery of the Department of Pomology, Kittur Rani Channamma College of Horticulture, Arabhavi, Belgaum district, Karnataka during 2004–06, 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, and FYM (2: 1: 2). The culture of nine different AMF were obtained from the Department of Agricultural Microbiology, Kittur Rani Channamma College of Horticulture, Arabhavi. The AMF inoculation was carried out by spreading 5 g of inoculum (consisting of 80 to 88 infective propagules per gram of soil) uniformly at 5 cm depth and putting a thin layer of soil above the inoculum. Seeds were placed and covered with soil (2–3 cm). The polybags of respective treatments were labelled and kept apart from each other in order to avoid AM fungal cross contamination.

Germination count was recorded on a daily basis till 65 DAS (days after sowing). The appearance of plumule was taken as criterion for germination and the GVI (germination vigour index) was computed using the following equation.

$$GVI = \frac{x_1}{d_1} + \frac{x_2}{d_2} + \frac{x_3}{d_3} + \ \ldots \ + \frac{x_n}{d_n} \qquad \ldots(1)$$

where x_1, x_2, x_3, …, x_n are the number of seeds germinated on d_1, d_2, d_3, …, d_n days taken for germination.

Rootstock height (cm), stem diameter (mm), and number of leaves were recorded at monthly

intervals. Rootstock (seedling) vigour index was calculated as given by Bewly and Black (1982).

Rootstock vigour index = Height of seedling (rootstock) × germination percentage

Extramatrical chlamydospores produced by different AMF were determined by adopting method given by Gerdemann and Nicolson (1963). Per cent root colonization was determined using the method provided by Phillips and Hayman (1970) and RMD (relative mycorrhizal dependency) was worked out as follows.

RMD (%) =

$$\frac{\text{Parameter with AMF} - \text{parameter without AMF}}{\text{Parameter with AMF}} \times 100 \quad \ldots(2)$$

Results and discussion

It was observed that jamun responded positively when inoculated with AMF species. Inoculation with *Glomus fasciculatum* and *Glomus intraradices* (89% each) resulted in maximum seed germination. Significantly minimum germination (77.5%) was noticed in seeds inoculated with *Acaulospora laevis* (Table 1). Increased seed germination on AM fungal inoculation was recorded in charoli (Kareddy 2003), citrus (Venkat 2004), mango (Bassanagowda 2005), papaya (Duragannavar, Patil, Patil, *et al*. 2004), and Aonla (Swamy, Patil, and Athani 2005). There were highly significant differences among different treatments for germination vigour index.

Increase in plant height, stem diameter, and number of leaves (Table 1) were recorded in 90 and 180 DAS rootstocks inoculated with *G. fasciculatum* (199.50 mm and 319.40 mm of stem height, 5.38 mm and 8.96 mm of stem diameter, and 19.75 number of leaves and 27.67 number of leaves, respectively). In all the growth phases, uninoculated control registered least growth of rootstocks (Silva and Sigueira 1991; Bassanagowda 2005), citrus (Vinayak and Bagyaraj 1990; Venkat, Swamy, Patil, *et al*. 2004), and sapota (Sreeramulu, Gowda, and Bagyaraj 1998). This increased growth of vegetative parameters is the consequence of increased root proliferation via increased nutrient and water uptake due to mycorrhizal inoculation. The rootstock vigour index was highest in rootstocks inoculated with *G. fasciculatum* (2842.45) and least in uninoculated control (1739.27).

Table 1 Effect of different AMF on germination and rootstock parameters in jamun

	Germination		*Rootstock height* (mm)		*Stem diameter* (mm)		*Number of leaves*		
Treatment	%	*Vigour index*	90 DAS	180 DAS	90 DAS	180 DAS	90 DAS	180 DAS	*Rootstock vigour index*
Glomus bagyaraji	87.00 (68.92)	2.245	155.40	236.40	4.82	7.96	13.65	22.90	2056.40
Glomus leptotichum	84.50 (66.85)	1.968	163.00	224.40	4.69	7.83	13.45	21.13	1895.80
Acaulospora laevis	77.50 (61.72)	1.873	185.40	236.50	4.73	7.78	14.80	21.53	1834.02
Sclerocystis dussii	87.00 (68.92)	1.776	193.30	298.40	5.31	8.71	17.85	26.63	2595.87
Glomus mosseae	83.00 (65.73)	1.966	188.90	271.60	4.79	7.85	19.20	25.23	2256.62
Gigaspora margarita	85.50 (67.69)	1.787	182.50	253.60	4.98	8.06	19.65	24.81	2166.49
Glomus monosporum	84.50 (67.23)	1.907	190.50	282.40	5.08	8.00	19.35	26.27	2384.55
Glomus intraradices	89.00 (70.68)	2.290	191.50	297.90	5.22	8.68	19.20	26.30	2651.20
Glomus fasciculatum	89.00 (70.68)	2.164	199.50	319.40	5.38	8.96	19.75	27.67	2842.45
Control	80.00 (63.48)	2.315	151.00	217.60	4.41	6.45	13.53	20.17	1739.27
S.Em±	1.258	0.032	0.528	0.830	0.124	0.145	0.687	0.645	766.72
CD (5%)	3.630	0.091	1.525	2.397	0.356	0.419	1.983	1.863	221.43
CD (1%)	4.892	0.123	2.054	3.228	0.480	0.563	2.670	2.509	298.102
CV (%)	2.97	3.17	5.84	6.29	5.02	3.084	8.03	5.32	6.890

AMF – arbuscular mycorrhizal fungi; DAS – days after sowing

The data in Table 2 shows the influence of AMF on chlamydospore development and root colonization, which was found to be significant. Spore population at 180 DAS in the rhizosphere inoculated with *G. intraradices* (889.75), *G. fasciculatum* (880.25), *G. monosporum* (855.75), and *Sclerocystis dussii* (849.25) was significantly higher than other AMF and that of uninoculated rootstock (99.5). More root colonization was seen in plants having *G. fasciculatum* (94%), *S. dussii* (91%), *G. intraradices* (90.75%), and *G. monosporum* (90%) at 180 DAS.

Higher RMD in terms of germination (10.11%), height (31.88 %), stem diameter (28.08 %), and number of leaves (27.1%) were registered in rootstocks inoculated with *G. fasciculatum*. The least RMD was noted in *Acaulospora laevis* (-3.23% and 17.12%, respectively).

Jamun seeds are recalcitrant and lose viability faster than other seeds due to their small size and thin seed coat. Being a seasonal fruit, its duration is also very short. Thus, increasing germination within stipulated time is of utmost importance. Enhancing the growth of rootstock to attain graftable size as early as possible is an emerging concern. From the present experiment, it is observed that the preferred species by jamun are *G. fasciculatum*, *S. dussii*, *G. intraradices*, and *G. monosporum*.

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Table 2 Effect of different AMF on chlamydospore (number/50 g soil), per cent root colonization, and relative mycorrhizal dependency

			Relative mycorrhizal dependency (%)			
Treatments	*Chlamydospores*	*Root colonization* (%)	*Seed Germination*	*Rootstock height*	*Stem diameter*	*Number of leaves*
Glomus bagyaraji	830.50	86.50	8.05	7.99	18.99	11.90
Glomus leptotichum	801.00	84.75	5.33	3.05	17.62	4.53
Acaulospora laevis	810.25	84.50	3.23	8.01	17.12	6.30
Sclerocystis dussii	849.25	91.00	8.05	27.09	25.93	24.04
Glomus mosseae	800.00	81.50	3.61	19.92	17.81	20.04
Gigaspora margarita	815.00	78.25	6.43	14.20	20.02	18.68
Glomus monosporum	855.75	90.00	5.33	22.96	25.05	23.22
Glomus intraradices	889.75	90.75	10.11	26.97	25.69	23.36
Glomus fasciculatum	880.25	94.00	10.11	31.88	28.08	27.10
Control	99.50	36.50	–	–	–	–
S.Em±	9.464	0.941	–	–	–	–
CD (5%)	27.332	3.205	–	–	–	–
CD (1%)	36.796	4.372	–	–	–	–
CV (%)	2.48	2.30	–	–	–	–

AMF - arbuscular mycorrhizal fungi

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Diversity pattern of arbuscular mycorrhizal fungi in some contaminated sites of Kerala

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Introduction

The rapid growth of various industries in recent years is creating more wastelands as effluents containing toxic elements and heavy metals are disposed of on the land. While some of them serve as nutrients for plants, most of them are phytotoxic, not allowing the plants to establish on such wastelands (Hetrick, Wilson, and Figge 1994). The use of plant-based systems (phytoremediation) for the treatment of contaminated soil has been increasing in recent years (Cunningham, Shann, Croweley, *et al*. 1997).

AMF (arbuscular mycorrhizal fungi) are being harnessed for afforestation and rehabilitation of wasteland (Giri, Kapoor, Agrawal *et al*. 2004). Mycorrhizal symbiosis offsets toxicity by acting as a biofilter for contaminants (Leyval, Turnau, and Haselwandter 1997). Common anthropogenic contaminants have been shown to decrease AM colonization and influence community composition (Egerton-Warburton and Allen 2000). However, in some areas contaminated with heavy metals, no effect on mycorrhizal colonization was observed (Shetty, Hetrick, Figge, *et al*. 1994), resulting in the survival of tolerant strains of AMF (Weissenhorn, Leyval, and Berthelin 1993). The present study reports the diversity of AM species in five contaminated sites in Kerala.

Materials and methods

Soil samples were collected from five contaminated sites (Table 1) in the districts of Alappuzha and Kottayam. Ten replicate samples were drawn from each site from a depth of 10 cm, kept in polythene bags, labelled, and stored at 4 °C till analysis. The physico-chemical characteristics of the soils were analysed as per standard procedure (Byju 2001). AM fungal species were recovered from the soil samples by wet-sieving and decanting method (Gerdemann and Nicolson 1963). The finest sieve used was 45 µm. The spores were collected on a grid-patterned (4×4) filter paper, washed three times with distilled water to spread evenly over the entire grid, and counted using a dissecting microscope at 30× magnification. For identifying spore characters, spores were mounted on glass slides in PVLG (polyvinyl alcohol–lactoglycerol) and PVLG + Melzer's reagent (1:1, v/v). The slides were examined at 100× magnification under a binocular research microscope and then identified to species level using current taxonomic criteria (Schenck and Pérez 1990).

Results

Wide variations were found in the physico-chemical characteristics of the soils under study (Table 1). The soils of sites 1 and 4 showed acidic tendency, while in other sites, they were alkaline. The soil moisture was slightly high in site 2. Irrespective of sites, the soils in general had high levels of OC (organic carbon). Higher values for N (nitrogen) and P (phosphorous) were recorded in sites 2 and 3, respectively, whereas, site 5 showed a high value for K (potassium).

Table 1 Physico-chemical characteristics of contaminated soils (mean ± SD, n = 10)

Site	*Location*	*Source of contamination*	*Temperature* (˚C)	*pH*	*Moisture* (%)	*Organic carbon* (%)	*Nitrogen* (kg/ha)	*Phosphorous* (kg/ha)	*Potassium* (kg/ha)
S1	Kuttanad	Agricultural	27.31±1.38	4.42±0.51	12.29±9.53	1.19±9.53	431.9±04.40	14.46±8.37	24.91±20.12
S2	Nattakam	Cement dust	29.12±1.05	7.71±0.65	9.86 ±3.76	1.27±0.44	520±91.32	63.28±56.28	86.48±56.88
S3	Kakkazham	Seafood effluent	27.50±1.00	7.69±0.51	8.31± 2.63	1.28± 0.36	425.6±86.35	315.90±111.33	109.40±92.45
S4	Kattachira	Coir retting effluent	27.80±0.40	5.55±1.28	21.56±5.02	1.03±0.16	378±83.06	149.90±118.62	76±55.11
S5	Alappuzha	Municipal solid waste	28.10±0.53	7.72±0.47	9.75±1.64	1.43±0.56	450.8±70.50	309.30±134.95	129.20±122.19

Table 2 Spore load and AM species diversity in contaminated soils

Site	*Source of contamination*	*Spore load/ 50 ml soil*	*AM species*
S1	Agricultural run-off	7.50 ± 3.00	*Entrophospora* sp. *Glomus* sp., *Sclerocystis* sp.
S2	Cement dust	3.50 ± 2.33	*Acaulospora thromi, Glomus deserticola, Glomus fasciculatum, Glomus* sp.
S3	Sea food effluent	3.50 ± 2.33	*Glomus* sp.
S4	Coir retting effluent	6.70 ± 2.36	*Glomus australe, Glomus fragilistratum, Glomus* sp., *Scutellispora perisca*
S5	Municipal solid waste	10.83 ± 4.49	*Acaulospora delicata, Glomus microaggregatum, Glomus* sp., *Scutellispora perisca*

AM – arbuscular mycorrhizal

Spore population of AMF in soils varied with sites. Maximum spore load was observed in soil contaminated with municipal solid waste while lowest spore load was recorded in the soil contaminated with cement dust. The spore load/50 ml soil of other sites was on par. From the five contaminated soils, 11 species of AMF were isolated and identified (Table 2). Maximum number of AM species were encountered in sites 2, 4, and 5. The predominant fungal species in all the contaminated sites was *Glomus* species.

The study reveals that AMF can survive and sustain in various contaminated soils, though their distribution and composition vary. The study warrants the need for identifying and propagating AM species/strains, which can endure soil contamination so that they can be effectively utilized in the reclamation of degraded land.

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Arbuscular mycorrhizal association in popular banana (*Musa* sp.) variety from the state of Goa

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Introduction

Banana (*Musa* sp.), a large herbaceous perennial plant, is so popular that backyards of practically every house in the rural areas of Goa contain banana plants. Family Musaceae, to which banana belongs, contains two genera—*Musa* and *Ensete*. *Ensete* is used for extracting fibre and as a vegetable. *Musa* is best divided into four sections. Of these, *Emusa* contains majority of edible banana derived from *Musa accuminata* (AAA) and *Musa balbisiana* (BBB). At present, in Goa, banana plantation occupies an area of 1875 hectares, with corresponding production of 10 650 tonnes, which is sufficient to meet 40% of the banana requirement of the state. The balance 60% is met by import from neighbouring states.

Symbiotic mycorrhizal association between fungi and roots of higher plants is a common feature and is gaining importance due to the fact that it enhances plant productivity. The ecological and economical value of arbuscular mycorrhizae can be directly inferred from the fact that about four-fifths of all land plants, including the agronomically important crops, form this type of mycorrhiza (Azcón-Aguilar and Barea 1997). AM (arbuscular mycorrhizal) symbiosis influences several aspects of plant physiology, such as mineral nutrition, plant development, and plant protection (Gianinazzi, Trouvelot, and Gianinazzi-Pearson 1990). The primary aim of AM symbiosis is to increase the supply of mineral nutrients, particularly those, the ionic forms of which have poor mobility rate or those that are present in low concentration in soil solution. This mainly includes phosphate, ammonium, zinc, and copper (Barea 1991).

AM colonization in several banana varieties has been studied in India by Girija and Nair (1988). The AM populations associated with banana have been studied by Arias, Balanco, Vargas, *et* al. (1998) in the Caribbean region of Costa Rica, and the ones associated with seasonality have been studied by Khade and Rodrigues (2004) in the state of Goa. In the present study, the AM associations and distribution of AMF (arbuscular mycorrhizal fungi) in popular banana cultivar, for example, Saldatti (AAB) from North Goa, have been investigated.

Materials and methods

Sample collection

Commonly occurring Saldatti variety from agricultural farms of Mapusa, Old Goa, and Valpoi was surveyed for AM association. Selection of the sites was carried out on the basis of the type of soil. All the plants selected for the study at these sites were medium to tall fruit-bearing plants. Five plants per variety were selected for study from each site. At each site, roots and rhizospheric soil samples of Saldatti variety were collected from the field at a depth of 10–20 cm, placed in plastic bags, labelled, and transported to the laboratory. All the samples were collected in May 1998 when the air temperature varied between 34.6 ˚C and 27.9 ˚C and humidity ranged from 66% to 77%.

Root samples were freshly processed, whereas, the soil samples were stored at 4 ˚C until further analysis. The roots were cleared 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). AMF were identified to species level using bibliographies provided by Schenck and Perez (1990), Almeida and Schenck (1990), and Walker and Vestberg (1998). Standard deviation was calculated for mean root colonization and mean spore density of AMF.

Rhizosphere soil samples per plant species were used for analysis. Soil pH was measured in 1:2 soil water suspension by using a pH meter. Electrical conductivity was measured at room temperature in 1:5 soil suspension by using a conductivity meter. Standard soil analysis techniques, for example, Walkley and Black's (1934) rapid titration method, micro-Kjeldahl method (Jackson 1971), and a method by Oleson, Cole, Watanabe, *et al.* (1954) were employed for

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determination of organic carbon, total nitrogen, and available phosphorous, respectively. Available potassium was estimated by ammonium acetate method (Hanway and Heidel 1952) by using flame photometer. Metals like aluminium, arsenic, cadmium, chromium, copper, iron, manganese, nickel, lead, and zinc were quantified by using atomic absorption spectrophotometer.

Diversity indices

Species richness per site is the mean number of AM fungal species associated with each site (Beena, Raviraja, Arun, *et al.* 2000).

Results

Data on rhizospheric soil analysis of three study sites is presented in Table 1. The soil was acidic to near neutral and electrical conductivity was found to be satisfactory. The total nitrogen and available phosphorous levels were limiting, while the available potassium levels were low to medium. Data on micronutrient analysis of soils samples at the study sites is presented in Table 2. All the four micronutrients, that is, Zn, Cu, Fe, and Mn, were found in high levels in the rhizosphere soil of Saldatti variety. Data on mycorrhizal association in Saldatti variety is presented in Table 3. Root colonization was characterized by the presence of hyphae, arbuscules, and vesicles.

Root colonization ranged from 37% (Mapusa) to 58% (Old Goa), while spore density ranged from 91.04 (Mapusa) to 403.65 (Old Goa) spores 100 g/rhizosphere soil.

Data on the distribution of AMF associated with Saldatti variety from three study sites is presented in Table 4. A total of 11 species of AMF belonging to two genera, that is, *Acaulospora* and *Glomus*, were recorded. *Glomus claroideum* Schenck and Smith emend. Walker and Vestberg was the predominant species followed by *Glomus* sp. in

Table 1 Comparative account of edaphic factors and macronutrients in the rhizosphere soil of Saldatti (*Musa* sp.) variety

Variety	*Locality*	*Type of soil*	*pH**	*EC**(mhos/cm)
Saldatti	Mapusa	Lateritic soil	6.3 ± 0.21	0.04 ± 0.00
Saldatti	Valpoi	Clayey loam	6.6 ± 0.25	0.98 ± 0.01
Saldatti	Old Goa	Alluvial soil	5.7 ± 0.07	0.86 ± 0.03

*Values indicate n = 5 ± 1 SD

Table 3 Comparative account of spore density and root colonization in Saldatti *(Musa* sp.) variety

Variety	*Locality*	*Spore density** (100g/rhizosphere soil)	*Root colonization** (%)
Saldatti	Mapusa	91.04 ± 11.71	37 ± 2.89
Saldatti	Valpoi	381.96 ± 30.45	55 ± 4.56
Saldatti	Old Goa	403.65 ± 21.55	58 ± 5.77

*Values indicate n = 5 ± 1 SD

Table 4 Distribution of AMF in Saldatti variety from the study sites

AMF	*Mapusa*	*Valpoi*	*Old Goa*
Acaulospora nicolsonii Walker, Reed and Sanders	–	+	–
Glomus claroideum Schenck and Smith emend. Walker and Vestberg	+	+	+
Glomus geosporum (Nicol. and Gerd.) Walker	–	–	+
Glomus globiferum Koske and Walker	–	–	+
Glomus heterosporum Smith and Schenck	–	–	+
Glomus monosporum Gerdemann and Trappe	–	–	+
Glomus clavisporum (Trappe) Almeida and Schenck	–	+	–
Species richness	**1**	**3**	**5**

AMF – arbuscular mycorrhizal fungi

Table 2 Comparative account of micronutrients in the rhizosphere soil of banana (*Musa* sp. of Saldatti) variety

		*Micronutrients** (µg/gm)			
Variety	*Locality*	*Zn*	*Cu*	*Fe*	*Mn*
Saldatti	Mapusa	1.72 ± 0.68	2.84 ± 0.18	13.84 ± 1.28	22.65 ± 3.36
Saldatti	Valpoi	1.67 ± 0.12	3.75 ± 0.18	18.55 ± 1.66	23.84 ± 0.83
Saldatti	Old Goa	2.95 ± 0.32	3.84 ± 0.28	12.63 ± 1.62	19.51 ± 2.62

Zn – zinc; Cu – copper; Fe – iron; Mn – mangenese
*Values indicate n = 5 ± 1 SD

rhizospheric soils of Saldatti variety. The species richness of AMF in the present study ranged from one species (Mapusa) to nine species (Old Goa).

Discussion

In the present study, all the samples of Saldatti variety selected at the study sites recorded AM colonization. Similarly, Girija and Nair (1988) reported the natural incidence of AM colonization in commonly cultivated *Musa sapientum* L. from Kerala. The root colonization levels reported by them varied from 22.7% to 60.9%. The spore density in the present study ranged from moderate (Mapusa) to high levels (Valpoi and Old Goa). Similarly, high levels of AM populations were reported by Khade and Rodrigues (2004) in Savarbondi variety from Valpoi during summer. Our study recorded the presence of single species of *Acualospora* and 10 species of *Glomus* associated with Saldatti variety. Similarly, Khade and Rodrigues (2004) recorded three species of *Acualospora* and four species of *Glomus* associated with Savarbondi variety from Valpoi during summer. Our study supports the findings of Arias, Balanco, Vargas, *et al.* (1998), who reported that *Glomus* was dominant (nine species) followed by *Acaulospora* (four species) in three banana plantations in Caribbean region of Costa Rica.

In the present study, the soil types varied at the study sites. Lateritic soils of Mapusa recorded low levels of root colonization, spore density, and distribution of AMF, while clayey loam and alluvial soils of Valpoi and Old Goa recorded high levels of root colonization, spore density, and distribution of AMF. In general, the AM association and distribution in identical host cultivar under identical environmental conditions may be influenced by soil types.

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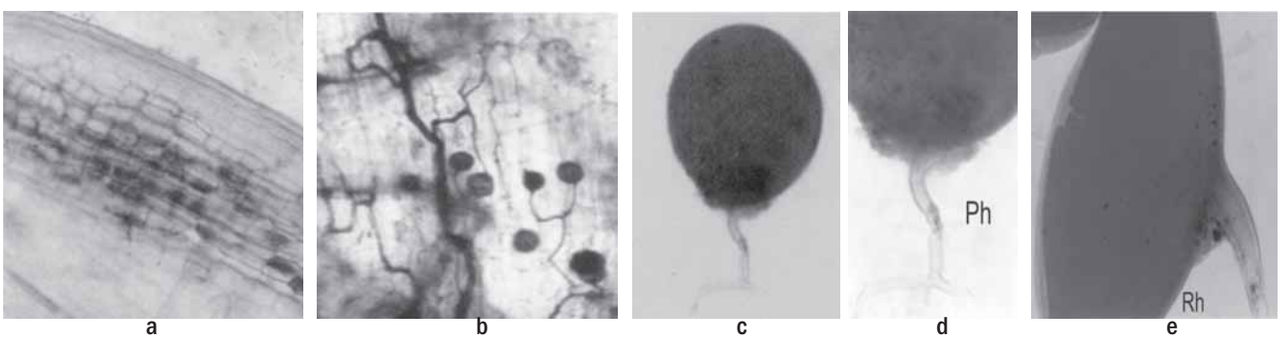

Figure 1(a) Arbuscular colonization of arbuscular mycorrhizal fungi (× 100).
Figure 1(b) Vesicular colonization of arbuscular mycorrhizal fungi (× 100).
Figure 1(c) A spore of *Glomus globiferum* Koske and Walker (× 100).
Figure 1(d) A portion of spore of *Glomus globiferum* attached to parent hyphae (Ph) Koske and Walker (× 400).
Figure 1(e) Crushed spore of *Glomus monosporum* with recurved hyphae (Rh) Gerdemann and Trappe (× 400).

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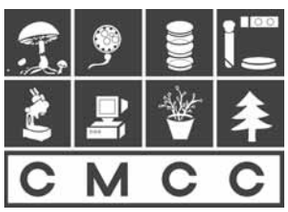

CENTRE FOR MYCORRHIZAL CULTURE COLLECTION

Influence of Arbuscular mycorrhizal fungi on plant biomass of *Euphorbia prostrata*

Veeranna A Channashettar, Harminder Singh Uppal, and *Alok Adholeya**

Introduction

Symbiotic associations between AMF (arbuscular mycorrhizal fungi) and plant roots are widespread in the natural environment and can provide a range of benefits to the host plant. These include improved yield, nutrition, enhanced resistance to soil-borne pests and disease, improved resistance to drought, tolerance of heavy metals, and better soil structure (Gosling, Hodge, Goodlass, *et al*. 2006). Many agricultural crops are mycorrhizal and there is widespread, although equivocal, evidence that crop plants benefit from the AM (arbuscular mycorrhizal) association in the same way.

Organic farming has developed from a wide number of disparate movements across the world, into a more uniform group of farming systems. Though the

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production methods vary considerably, general principles include the exclusion of most synthetic biocides and fertilizers, management of soils through addition of organic materials, and use of crop rotation (IFOAM 1998). Within this paradigm, AMF are usually considered to play an important role, and it is assumed that they can compensate for the reduced use of fertilizers (Galvez, Douds, Drinkwater, *et al*. 2001).

AMF are known to increase the yield and mineral nutrition of associated plants. Therefore, efforts must be made to optimize the beneficial effects of AMF in sustainable agriculture (Sieverding 1991). A greenhouse experiment was conducted to study the influence of the mycorrhiza and vermicompost on biomass of *Euphorbia prostrata* plant.

Materials and methods

The greenhouse experiment was conducted at TERI, Gual Pahari, Gurgaon, using complete randomized design with five replications. The experiment was carried in plastic trays (38 cm × 27 cm) filled with sterilized soil to investigate the influence of AMF on biomass of *E. prostrata*. There were eight treatments with different levels of vermicompost and AMF (Table 1).

Vermicompost application

The area of each tray was 0.103 m^2. The vermicompost was mixed thoroughly in the soil, along with the treatments. A full dose (100%) was considered as 1 tonne/acre.

Application of AMF inoculum and sowing of Euphorbia prostrata *seed*

The mycorrhiza inoculum was applied at a depth of 1.5–2 cm in rows made in the tray. The seeds were sown in the same rows.

Table 1 Description of treatments used

Symbol used	*Details of treatment*
T1	Absolute control (without vermicompost and mycorrhiza)
T2	Control (only vermicompost)
T3	AMF (GI) + vermicompost (100%)
T4	AMF (GI) + AMF vermicompost (50%)
T5	AMF consortium + vermicompost (100%)
T6	AMF consortium + vermicompost (50%)
T7	Only AMF consortium (without vermicompost)
T8	Only AMF (GI) (without vermicompost)

GI – *Glomus intraradices;* AMF – arbuscular mycorrhizal fungi
[AMF consortium – mixed AMF isolates]

Harvesting of plants

The plants were harvested after 60 days, and the plant shoot and root biomass were recorded.

Statistical analysis

The treatment effects were determined with the help of a one-way ANOVA (analysis of variance) using a completely randomized design and Costat software. Significant differences between treatments were confirmed by the DMRT (Duncan's multiple range test).

Results and discussion

The plant shoot and root biomass was significantly increased with the inoculation of AMF (Figures 1 and 2). The highest shoot biomass was observed with the AMF consortium and vermicompost (100%) treatment. There was 34.6% increase in plant biomass as compared to the 100% vermicompost treatment. Plants with a treatment of *Glomus intraradices* + vermicompost (100%) showed 20.1% increase in shoot biomass. Root biomass was also significantly higher with the treatment of AMF and vermicompost (100%), as compared to the control.

The AMF treatment significantly increased root and shoot biomass, as compared to the absolute control. The results indicate that both mycorrhizae increased the fresh weight of the plant. However, the mixed consortium of AMF increased the weight of the plant the most. It is seen that inoculation of soils with multiple AMF promotes plant growth better

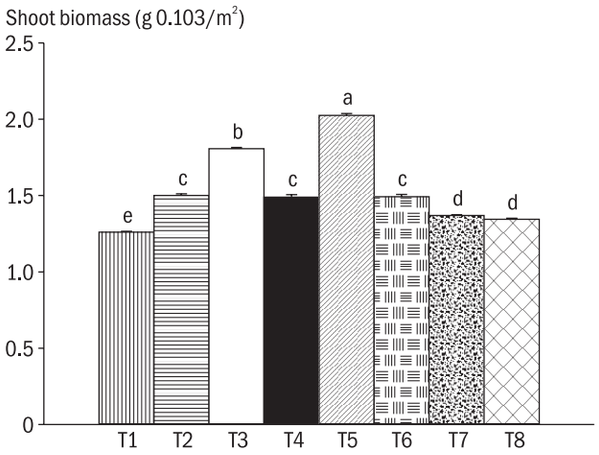

Figure 1 Shoot biomass of *Euphorbia prostrata* plant as influenced by arbuscular mycorrhizal fungi
[The values are the mean of five replicates. Bars with different alphabets indicate significant difference using the Duncan's multiple range test at $p < 0.01$].

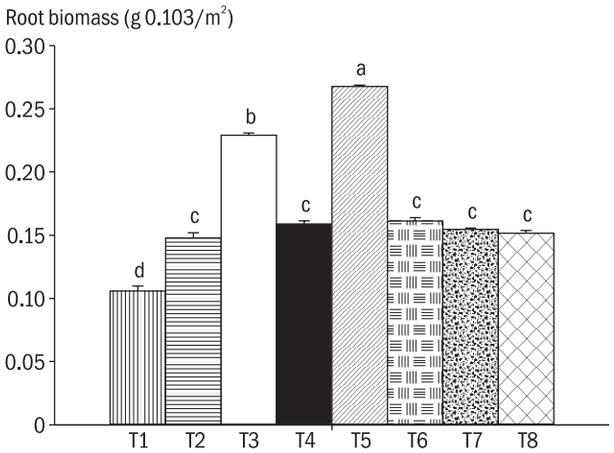

Figure 2 Root biomass of *Euphorbia prostrata* plant as influenced by arbuscular mycorrhizal fungi
[The values are the mean of five replicates. Bars with different alphabets indicate significant difference using the Duncan's multiple range test at $p < 0.01$].

than single isolates in earlier studies (Daft and Hogarth 1983; Koomen, Grace, and Hayman 1987).

Earlier results showed that direct inoculation of either the host plant or soil with AMF is capable of increasing the phosphorous uptake. In some cases, direct inoculation increases the yield and reduces disease in AM dependant crops. Though the experiments have been conducted in the glasshouse and, therefore, have questionable relevance to the field situation (Xavier and Germida 1997), there are examples of field experiments where inoculation has successfully increased nutrient uptake and/or yield or reduced disease severity of different plants (Al-Karaki, McMichael, and Zak 2004; Douds, Nagahashi, Pfeffer, *et al*. 2005; Mohammad, Mitra, and Khan 2004). Water retention, nutrient supply, and minor elements in vermicompost-amended soil increases better plant development (Atiyeh, Lee, Edwards, *et al*. 2002). Additionally, it is possible that AMF with large microbial population in vermicompost might have increased plant growth with synergetic effect. Recent investigations have brought to light instances where biological activities are markedly enhanced in two or three-membered associations of organisms. Synergistic effects of AMF and *Bradyrhizobium japonicum* have a high potential to improve the nutrient supply of soybean, including phosphorous and soil quality (Tilak, Saxena, and Sadasivam 1995). It has been reported that microbial biomass and dehydrogenase activities increased in vermicompost-amended soil compared to inorganic-fertilized soil, and the size of the microbial biomass was positively correlated with yields of pepper plants (Arancon, Lee, Edwards, *et al*. 2005). It is also reported that humic acids in the vermicompost had positive effect on the growth of maize plants and peppers in laboratory and greenhouse experiments (Atiyeh, Lee, Edwards, *et al*. 2002).

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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.

- *Agronomy Journal*
- *Annals of Forest Science*
- *Applied Soil Ecology*
- *Biotechnology Letters*
- *Botany-Botanique*
- *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*
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- *Geochimica ET Cosmochimica Acta*
- *International Journal of Phytoremediation*
- *Journal of Arid Environments*
- *Journal of Biological Education*
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- *Journal of Experimental Botany*
- *Journal of Plant Growth Regulation*
- *Journal of Plant Nutrition*
- *Mycorrhiza*
- *Mycoscience*
- *National Academy Science Letters-IndiaPlant Cell*
- *Plant Physiology*
- *Sylwan*

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)
Aroca R, Vernieri P, Ruiz-Lozano J M*. 2008	**Mycorrhizal and non-mycorrhizal *Lactuca sativa* plants exhibit contrasting responses to exogenous ABA during drought stress and recovery** *Journal of Experimental Botany* **59**(8): 2029–2041 [*Ruiz-Lozano J M, Estac Expt Zaidin CSIC, Department of Microbiol Suelo and Sistemas Simbiot, Prof Albareda 1, E–18008 Granada, Spain]
Balogh-Brunstad Z*, Keller C K, Dickinson J T, Stevens F, Li C Y, Bormann B T. 2008.	**Biotite weathering and nutrient uptake by ectomycorrhizal fungus, *Suillus tomentosus*, in liquid-culture experiments** *Geochimica ET Cosmochimica Acta* **72**(11): 2601–2618 [*Balogh-Brunstad, Z, Washington State University, School of Earth and Environmental Science, POB 642812, Pullman, WA 99164]

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Cappellazzo G, Lanfranco L*, Fitz M, Wipf D, Bonfante P. 2008	**Characterization of an amino acid permease from the endomycorrhizal fungus *Glomus mosseae*** *Plant Physiology* **147**(1): 429–437 [*Lanfranco L, University Turin, Dipartimento Biol Vegetale, I–10125 Turin, Italy]
Chen B D, Roos P, Zhu Y G, Jakobsen I*. 2008	**Arbuscular mycorrhizas contribute to phyto stabilization of uranium in uranium mining tailings** *Journal of Environmental Radioactivity* **99**(5): 801–810 [*Jakobsen I, Tech University of Denmark, Riso Natl Lab, Biosyst Department, DK – 4000 Roskilde, Denmark]
de Boulois H D, Joner E J, Leyval C, Jakobsen I, Chen B D, Roos P, Thiry Y, Rufyikiri G, Delvaux B, Declerck S*. 2008.	**Impact of arbuscular mycorrhizal fungi on uranium accumulation by plants** *Journal of Environmental Radioactivity* **99**(5): 775–784 [*Declerck S, University of Catholique Louvain, Unite Microbiol, Croix Stud 3, B – 1348 Louvain, Belgium]
Garg N* and Manchanda G. 2008	**Effect of arbuscular mycorrhizal inoculation on salt-induced nodule senescence in *Cajanus cajan* (pigeonpea)** *Journal of Plant Growth Regulation* **27**(2): 115–124 [*Garg N, Department of Botany, Punjab University, Chandigarh –160014, India]
Gavito M E* and Olsson P A. 2008	**Foraging strategies of the external mycelium of the arbuscular mycorrhizal fungi *Glomus intraradices* and *Scutellospora calospora*** *Applied Soil Ecology* **39**(3): 282–290 [*Gavito M E, Univ Nacl Autonoma Mexico, Ctr Invest Ecosistemas, Apartado Postal 27–3, Morelia 58090, Michoacan, Mexico]
Lilleskov E *, Wargo P M, Vogt K A, Vogt D J. 2008	**Mycorrhizal fungal community relationship to root nitrogen concentration over a regional atmospheric nitrogen deposition gradient in the north-eastern USA** *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **38**(5): 1260–1266 [*Lilleskov E A, US Forest Serv, USDA, No Res Stn, Houghton, MI 49931]
Manoharachary C*. 2008	**Mycorrhiza in agriculture and forestry** *National Academy Science Letters–India* **30**(11–12): 341–353 [Manoharachary C, Department of Botany, Osmania University, Hyderabad – 500007, Andhra Pradesh, India]
Massicotte H B*, Melville L H, Tackaberry L E, Peterson R L. 2008	**A comparative study of mycorrhizas in several genera of Pyroleae (*Ericaceae*) from western Canada** *Botany-Botanique* **86**(6): 610–622 [*Massicotte H B, Univ No British Columbia, Ecosyst Sci and Management Program, Prince George, BC V2N 4Z9, Canada]
Mathimaran N, Falquet L, Ineichen K, Picard C, Redecker D, Boller T, Wiemken A*. 2008	**Microsatellites for disentangling underground networks: Strain-specific identification of *Glomus intraradices*, an arbuscular mycorrhizal fungus** *Fungal Genetics and Biology* **45**(6): 812–817 [*Wiemken A, University Basel, Institute of Botany, Hebelstr 1, CH – 4056 Basel, Switzerland]
Minerdi D*, Moretti M, Gilardi G, Barberio C, Gullino M L, Garibaldi A. 2008	**Bacterial ectosymbionts and virulence silencing in a *Fusarium oxysporum* strain** *Environmental Microbiology* **10**(7): 1725–1741 [Minerdi D, University of Turin, Ctr Competence Innovat Agroenvironm Field Agroinn, Via Leonardo Da Vinci 44, I–10095 Grugliasco, Italy]

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Murillo-Williams A* and Pedersen P. 2008	**Arbuscular mycorrhizal colonization response to three seed-applied fungicides** *Agronomy Journal* **100**(3): 795–800 [*Murillo-Williams A, University of Costa Rica, CIGRAS, San Jose, Costa Rica]
Al Agely A* and Sylvia D M. 2008	**Compatible host/mycorrhizal fungus combinations for micropropagated sea oats: II. Field evaluation** *Mycorrhiza* **18**(5): 257–261 [*Al Agely A, University of Florida, Department of Soil and Water, 2196 McCarty Hall A, POB 110290, Gainesville, FL 32611]
Alarcon A, Davies F T*, Autenrieth R L, Zuberer D A. 2008	**Arbuscular mycorrhiza and petroleum-degrading microorganisms enhance phytoremediation of petroleum-contaminated soil** *International Journal of Phytoremediation* **10**(4): 251–263 [*Davies F T, Texas A and M University, Department of Horticultural Science, Fac Mol and Environment Plant Science, College Stn, TX 77843]
Aleksandrowicz-Trzcinska M. 2008.	**Mycorrhiza of forest tree seedlings in clear-cut regenerations** *Sylwan* **151**(11): 3–9 [Aleksandrowicz-Trzcinska M, Wydzial Lesny SGGW, Katedra Ochrony Lasu and Ekol, Ul Nowoursynowska 159, PL – 02776 Warsaw, Poland]
Azul A M*, Ramos V, Pato A, Azenha M, Freitas H. 2008	**Mycorrhizal types in the Mediterranean Basin: safety teaching and training** *Journal of Biological Education* **42**(3): 130–137 [*Azul A M, University of Coimbra, Department of Botany, Ctr Fundamental Ecol, P – 3000 Coimbra, Portugal]
Bernatchez F, Jeannotte R, Begg C B M, Hamel C, Whalen J K*. 2008	**Soil fertility and arbuscular mycorrhizal fungi related to trees growing on smallholder farms in Senegal** *Journal of Arid Environments* **72**(7):1247–1256. [*Whalen J K, McGill University, Department of Natural Resource Science, Macdonald Campus, Ste Anne De Bellevue, PQ H9X 3V9, Canada]
Deng Z Q and Suzuki A*. 2008	**Effects of pH, NH_4-N concentration, temperature, and storage period on basidiospore germination in an ectomycorrhizal ammonia fungus *Hebeloma vinosophyllum*** *Mycoscience* **49**(3): 178–184 [*Suzuki A, Chiba University, Fac Educ, Inage Ku, 1–33 Yayoi Cho, Chiba – 2638522, Japan]
Ducic T, Parlade J, and Polle A*. 2008	**The influence of the ectomycorrhizal fungus *Rhizopogon subareolatus* on growth and nutrient element localization in two varieties of Douglas fir (*Pseudotsuga menziesii* var. menziesii and var. glauca) in response to manganese stress** *Mycorrhiza* **18**(5): 227–239 [*Polle, A, Univ Gottingen, Inst. Forstbot, Busgenweg 2, D – 37077 Gottingen, Germany]
Fan Y Q, Luan Y S*, An L J, Yu K. 2008	**Arbuscular mycorrhizae formed by *Penicillium pinophilum* improve the growth, nutrient uptake and photosynthesis of strawberry with two inoculum-types** *Biotechnology Letters* **30**(8): 1489–1494 [*Luan Y S, Dalian University of Technology, School of Environmental and Biological Science and Technology, Dalian, China]

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Genre A, Chabaud M, Faccio A, Barker D G, Bonfante P*. 2008	**Prepenetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago truncatula* and *Daucus carota*** *Plant Cell* **20**(5): 1407–1420 [*Bonfante P, University of Turin, Ist Protezione Piante Consiglio Nazl Ric, Department of Plant Biology, I–10125 Turin, Italy]
Hao L F, Zhang J L*, Chen F J, Christie P, Li X L. 2008	**Response of two maize inbred lines with contrasting phosphorous efficiency and root morphology to mycorrhizal colonization at different soil phosphorous supply levels** *Journal of Plant Nutrition* **31**(6): 1059–1073 [*Zhang J L, China Agricultural University, College of Resources and Environmental Science, Department of Plant Nutrition, Beijing 100094, China]
Hilszczanska D*, Malecka M, and Sierota Z. 2008	**Changes in nitrogen level and mycorrhizal structure of Scots pine seedlings inoculated with *Thelephora terrestris*** *Annals of Forest Science* **65**(4): 409 [*Hilszczanska D, Forest Research Institute, Department of Forest Pathology, Braci Lesnej 3, PL–05090 Sekocin Stary, Raszyn, Poland]
Jokipii S*, Haggman H, Brader G, Kallio P T, Niemi K. 2008	**Endogenous PttHb1 and PttTrHb, and heterologous Vitreoscilla vhb haemoglobin gene expression in hybrid aspen roots with ectomycorrhizal interaction**. *Journal of Experimental Botany* **59**(9): 2449–2459 [*Jokipii S, University of Oulu, Department of Biology, POB 3000, Oulu 90014, Finland]
Landry C P*, Hamel C, and Vanasse A, 2008.	**Influence of arbuscular mycorrhizae on soil P dynamics, corn P nutrition and growth in a ridge-tilled commercial field** *Canadian Journal of Soil Science* **88**(3): 283–294 [*Landry C P, IRDA Inc, 2700 Rue Einstein, Quebec City, PQ G1P 3W8, Canada]

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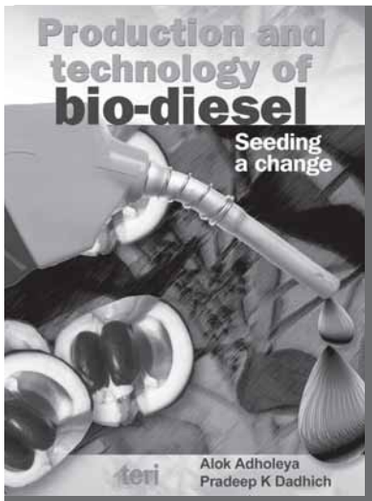

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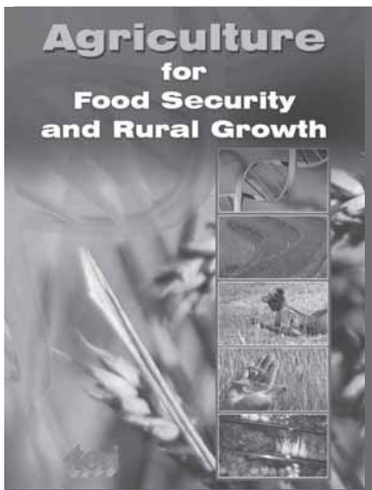

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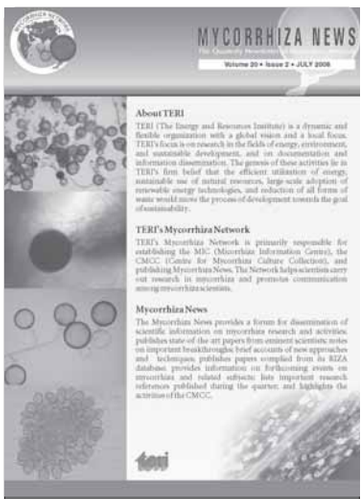

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