

**Vol. 11 No. 2**  
**July 1999**

### **About TERI**

A dynamic and flexible organization with a global vision and a local focus, TERI was established in 1974. While in the initial period the focus was mainly on documentation and information dissemination activities, research activities in the fields of energy, environment, and sustainable development were initiated towards the end of 1982. The genesis of these activities lay in TERI's firm belief that 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.

### **The Bioresources and Biotechnology Division**

Focusing on ecological, environmental, and food security issues, the Division's activities include working with a wide variety of living organisms, sophisticated genetic engineering techniques, and, at the grassroots level, with village communities. The Division functions through four areas: Microbial Biotechnology, Plant Molecular Biology, Plant Tissue Culture, and Forestry/Biodiversity. The Division is actively engaged in mycorrhizal research. The Mycorrhiza Network has specifically been created to help scientists across the globe in carrying out research on mycorrhiza.

### **The Mycorrhiza Network and the Centre for Mycorrhizal Culture Collection**

Established in April 1988 at TERI, New Delhi, the Mycorrhiza Network first set up the MIC (Mycorrhiza Information Centre), the same year, and the CMCC (Centre for Mycorrhizal Culture Collection) – a national germplasm bank of mycorrhizal fungi – in 1993. The general objectives of the Mycorrhiza Network are to strengthen research, encourage participation, promote information exchange, and publish the quarterly newsletter, *Mycorrhiza News*.

The MIC has been primarily responsible for establishing an information network, which facilitates information sharing among the network members and makes the growing literature on mycorrhiza available to researchers. Comprehensive databases on Asian mycorrhizologists and mycorrhizal literature (RIZA) allow information retrieval and supply documents on request.

The main objectives of the CMCC are to procure strains of both ecto and VA mycorrhizal fungi from India and abroad; multiply and maintain these fungi in pure culture; screen, isolate, identify, multiply, and maintain native mycorrhizal fungi; develop a database on cultures maintained, and provide starter cultures on request. Cultures are available on an exchange basis or on specific requests at nominal costs for spore extraction/handling.



## Contents

### Methods for culturing and isolating arbuscular mycorrhizal fungi

*Chris Walker* ... 2

### Research findings

Occurrence and distribution of AMF associated with garlic rhizosphere soil ... 4

VA-mycorrhiza fungi occurring in the mangrove vegetation of Pichavaram forest ... 6

Cellulase activity in mycorrhizal cowpea roots ... 7

CMCC List of cultures ... 9

Fourth National Conference on Mycorrhiza ... 13

### New approaches

A technique for ectomycorrhizal inoculation ... 15

Measurement of pH of AM fungi ... 15

Forthcoming events ... 16

News from members ... 17

Recent references ... 17

## Methods for culturing and isolating arbuscular mycorrhizal fungi

*Chris Walker*

Biological Research and Imaging Laboratory, 2 Penny Hedge, New Milton, Hants, BH25 7TB, UK

<Walker@globalnet.co.uk>

### Introduction

Arbuscular mycorrhizal fungi are known as obligate symbionts. Many attempts have been made, to establish them in axenic culture(s) on a variety of media, but none have so far been a proven success. A few reports claim the growth of AMF (arbuscular mycorrhizal fungi) on defined media, but none of these withstand critical evaluation. Sophisticated dixenic methods using transformed root organ cultures have been successful, but they are difficult and expensive, and are not useful for general studies of mycorrhizae.

It is nevertheless possible (in some instances easy) to obtain cultures of AMF open pot culture (Gilmore 1968). Cultures may be produced by various methods, which are listed below.

### Soil trap cultures

The simplest kind of culture to produce; soil is collected from the area of interest and mixed with a disinfected substrate, which could be soil, sand, or expanded montmorillonite clay. This mixture is then put in a suitable container, which is not contaminated by AMF. Either seeds are sown or seedlings (free of any existing mycorrhizae) planted in the mixture. The plants are then maintained for a period of time suitable for the establishment of mycorrhizae (usually one to six months). The usual result of soil trap cultures is a mixed-species pot culture. Such a culture can then be used as base for further purification.

### Pot substrate cultures

The use of substrate from existing pot cultures is another kind of 'soil trap', though in this case, cultures would (or at least should) already be of only one

species. In this type of culture, a small quantity of substrate from an existing pot culture is either mixed through the disinfected substrate, or added in a depression into which either a mycorrhiza-free plant is placed or seeds are sown. The cultures usually establish quickly, and spores are normally produced within a month or two of the sub-culturing attempt.

### Plant trap cultures

Another method of producing cultures is to lift small plants from the area of interest carefully, thoroughly wash the roots to remove all traces of soil and external mycelium, and then plant them in a suitable sterile substrate. The result is usually a mixed culture, since plants may have more than one symbiont, but sometimes, apparently, single-species cultures are also obtained in this way. This method has the distinct advantage for ecological studies that the species obtained are highly likely to have been in symbiosis with the plant (though it is possible that the occasional propagule from some other symbiosis may remain adherent to the roots after washing).

### Root fragment cultures

In this method, small roots fragments are taken from the species of interest and placed in contact with the roots of mycorrhiza-free hosts in a suitable sterile substrate. This method shares the same advantage as the plant trap culture, but is perhaps, more likely to yield single-species cultures if root fragment is sufficiently small.

### Multi-spore cultures

Spores are extracted from soil or a pot culture substrate. They are sorted under a dissecting microscope into

specimens that are superficially similar. Several (often up to 100) such specimens are then introduced to mycorrhiza-free host plant. There are several methods of introduction. For example, Gerdemann (1955) used a small funnel lined with metal foil. The neck of the funnel was filled with a substrate and the spores were placed at the funnel base on the soil. The funnel was then filled and seeded or planted with a suitable host. After a period of time for the symbiosis to establish, the plants were removed from the funnel, and if mycorrhization was successful, they were transferred to a pot containing disinfested substrate.

Another way is to simply place a pre-germinated mycorrhiza-free plant in a depression, in a pot of sterile substrate; then pipette the spores in sterile water on to the roots, and finally cover with more substrate, and water the pot. This method has one major disadvantage. It is impossible to be certain about the identity of the AMF spore at the dissecting microscope level. Consequently, one can never be sure that the derived culture is of one species. Even if only one species is present, it may be from more than one genet and so may be mixed genotypically. As with plant and soil traps, even when only one type of spore is produced from such a culture, it is possible that other species might be present in a non-sporulating state (fragments of hyphae can adhere to spores and act as propagules; dead spores may appear live under the dissecting microscope and may contain living spores of other species).

### *Single-spore cultures*

These are prepared in exactly the same way as multi-spore cultures, but, as their name implies, are produced from a single spore. The proportion of successes with single-spore cultures may be very low depending on the species of fungus and the condition of the spores. Often, only one or two per cent of attempts will be successful, especially from field collected spores. In this case, contamination may be due to (1) fragment of hyphae, (2) other species sporulating inside dead spores, and (3) production of a culture of a species other than the one thought to have been used for inoculation. With spores from a healthy pot culture, the success rate is usually reasonably high, and may reach up to 80%, with particularly 'aggressive' species. It should be noted that a distinction should be made between cultures from a single spore and those from a single sporocarp, or single cluster of spores. Even with a single spore attempt, a successful culture may result which is not the same species as the spore attempt. Sometimes, a fragment of hyphae can adhere to the spore, and this may act as a propagule rather than the spore itself. Quite often, although a spore appears healthy, it is

actually dead and contains a spore of a different species of glomalean fungus in it, leading again to a successful culture of a different species from that introduced. When sporocarps or spore clusters are used, the danger of such contamination is high. It is therefore, a good idea to establish second generation single-spore cultures from any original successful isolation attempts.

### **Controlling contamination in pot cultures**

Open pot cultures are inevitably prone to contamination. Propagules, which are usually spores or possibly hyphal fragments or roots particles, can be transferred in many ways. For example, people may probe a pot with a finger to assess the soil moisture level and then repeat the action in another pot. Pests such as mites and insects can carry propagules from one pot to another. Watering with a forceful jet or spray can result in particles of substrate containing propagules being splashed from one pot to another. Root contact from plants on a bench or a capillary matting can result in cross-contamination. If it is absolutely necessary to maintain cultures in open pots, contamination must be reduced by proper watering and controlling pests. Control is obviously easier in a growth chamber than in an open green house. Pots should be kept at a distance from each other (at least 10–15 cm) and raised from the bench or ground on a mesh grid (preferably metal) to encourage 'air pruning' of roots and eliminate root contact. Staff should be instructed in the basic biology of fungi so that they understand the need to perform operations to eliminate contamination completely.

In temperate climates, growth chambers or places where sufficient shade can be provided; a system of pot culture production in sealed bags has proved successful (Walker and Vesberg 1994). In this method, small plants in a proprietary plastic bag (Sunbags® from Sigma) incorporated with a micro-filter are used. The pot cultures are produced normally, but are enclosed in the bags. It is sufficient to fasten the bags loosely with plastic clips so that they may be opened easily for inspection, watering, and other maintenance. If desired, the system can be used completely dixenically, though this is not necessary for routine culture maintenance.

In hot or sunny climates, where temperatures in the 'Sunbags®' may become too high for plant or fungal growth, a similar method could be used by replacing plastic bags with bags made of mosquito or curtain netting. A small cane inserted in the pot would allow the bag to be fastened around it with a plant-tie. The netting would help to keep the cultures free of contamination and provide shade for the plants. Drip-feed irrigation would probably work well in such a system.

Finally, the plants must be kept reasonably healthy, adequately watered, and not overfed. This is amongst the best ways to retain cultures and enhance sporulation.

## References

Gerdemann J M. 1955

**Relation of a large soil-borne spore to phycomycetous mycorrhizal infections**

*Mycologia* 47: 619–632

Gilmore A E. 1968

**Phycomycetous mycorrhizal organisms collected by open-pot culture methods**

*Hilgardia* 39: 87–105

Walker C and Vestberg M. 1994

**A simple and inexpensive method for producing and maintaining closed pot cultures of arbuscular mycorrhizal fungi**

*Agricultural Science in Finland* 3: 233–240

---

## Research findings...

*Under this column appear short notes on important breakthroughs/significant achievements in original research of high calibre in the field of mycorrhizae, which have not yet been published.*

---

## Occurrence and distribution of AMF associated with garlic rhizosphere soil

*Kunwar I K, Reddy P J M, Manoharachary C*

Botany Department, Osmania University,  
Hyderabad – 500 007

### Introduction

The AMF (arbuscular mycorrhizal fungi) form obligate symbiotic association with the roots and other underground parts of most of the plants. They are more prevalent in soils deficient in phosphorus. AMF are capable of scavenging phosphorus from the soil into the root system mainly through exploration of the soil by extraradical hyphae beyond the root hairs and the phosphorus depletion zone. Their potential to enhance plant growth and biomass is well known. Thus, AMF are also known as biofertilizers. There are reports on the natural colonization of underground parts of ginger (Taber and Trappe 1982; Mago, Pasricha and Mukerji 1993), turmeric (Iqbal and Nasim 1991; Sampath and Sullia 1992), colacasia (Martin and Birchfield 1972; Bhat and Kaveriappa 1997), *Pueraria tuberosa* (Rodrigues 1996), and garlic (Kunwar and Manoharachary 1998).

Garlic is an important bulb crop. There are no reports on the association of AMF with the garlic rhizosphere soil; hence, an attempt has been made to investigate the qualitative and quantitative composition of AMF associated with garlic.

### Materials and methods

In the present investigation, garlic rhizosphere soil samples were collected from fields and kitchen gardens of eight farmers in Andhra Pradesh and Uttar Pradesh. AMF propagules were collected by wet sieving and decanting method (Gerdemann and

Nicolson 1963) and counted. They were then identified with the help of relevant literature (Schenck and Perez 1987, Morton and Benney 1991, Mehrotra and Bajjal 1994). The physico-chemical characteristics of soil samples such as pH, moisture content, soil texture, and nitrogen (Subbiah and Asija 1956), phosphorus (Jackson 1973), and potassium (Jackson 1958) were estimated.

### Results and discussion

Thirty-five species of AMF fungi were isolated and identified from the rhizosphere soils of garlic (Table 1). It is a clear indication of their diversity. Species belonging to all the six genera of AMF were isolated from garlic soils even though garlic is known to have antifungal and antibacterial properties. The presence of so many species of AMF from ecologically diverse locations is interesting. *Glomus* spp. dominated the rhizosphere soil supporting garlic. Eleven species of the genus *Acaulospora*, one species of *Entrophospora*, four species of *Gigaspora*, 14 species of *Glomus*, two species of *Sclerocystis*, and three species of *Scutellospora* were isolated and identified.

The physico-chemical characteristics of garlic rhizosphere soils are presented in Table 2. The population of AMF in different soils was determined in terms of resting spores and sporocarps in the soil. The AMF endophytes were widespread in all the soils investigated but varied both in number and the type of spores and sporocarps. No relationship was found between pH and spore numbers. Reddy (1993) and Prabhakar (1995) also made similar observations. The authors observed a positive effect of increasing moisture content on spore formation. There are contradictory reports on the effect of moisture content on VAM (vesicular–arbuscular mycorrhiza) fungal propagules. A negative effect of increasing moisture content on spore formation was observed by Reid and Bowen (1979). Durga (1995)

**Table 1** Arbuscular mycorrhizal fungi isolated from rhizosphere soils

Arbuscular mycorrhizal fungi	Rhizosphere soils of garlic from selected areas							
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	S <sub>6</sub>	S <sub>7</sub>	S <sub>8</sub>
<i>Acaulospora bireticulata</i> Rothwell and Trappe	-	-	-	+	-	+	-	-
<i>A. foveata</i> Trappe and Janos	+	+	-	+	-	+	-	-
<i>A. lacunosa</i> morton	+	-	-	+	-	-	+	-
<i>A. laevis</i> Gerdemann and Trappe	+	-	-	-	-	-	-	-
<i>A. morrowae</i> Spain and Schenck	+	-	-	-	-	-	-	-
<i>A. myriocarpa</i> Spain, Sieverding and Schenck	-	+	-	-	-	-	-	-
<i>A. nicolsonii</i> Walker, Reed and Sanders	-	-	+	+	-	-	-	-
<i>A. rehmsii</i> Sieverding and Toro	-	-	+	+	-	-	-	-
<i>A. ruqosa</i> Morton	-	-	-	+	-	-	-	-
<i>A. sporocarpia</i> Berch	-	-	-	-	-	+	-	-
<i>A. tuberculata</i> Janos and Trappe	-	-	-	+	-	-	-	-
<i>Entrophora schenckii</i> Sieverding and Toro	-	-	-	+	-	-	-	-
<i>Gigaspora decipiens</i> Hall and Abbott	-	-	-	-	-	-	+	-
<i>G. gigantea</i> (Nicol. and Gerd) Gerdemann and Trappe	-	-	-	+	-	-	-	-
<i>G. margarita</i> Becker and Hall	-	-	-	+	-	-	-	-
<i>G. rosea</i> Nicolson and Schenck	-	-	-	+	-	-	-	-
<i>Glomus ambisporum</i> Smith and Schenck	+	-	-	-	-	+	-	-
<i>G. citricolum</i> Tang and Zang	+	-	-	+	-	-	-	-
<i>G. constrictum</i> Trappe	-	-	-	+	-	-	-	-
<i>G. delhiense</i> Mukerji, Bhattacharjee, and Tewari	-	-	-	+	-	-	-	-
<i>G. diaphanum</i> Morton and Walker	-	-	-	-	-	+	-	-
<i>G. etunicatum</i> Becker and Gerdemann	-	-	-	-	-	-	+	+
<i>G. fasciculatum</i> (Thaxter sensu Gerd.) Gerdemann and Trappe	-	-	+	+	+	+	-	-
<i>G. geosporum</i> (Nicol. and Gerd.) Walker	-	-	-	+	-	-	-	-
<i>G. hoi</i> Berch and Trappe	-	+	-	-	-	-	+	-
<i>G. monosporum</i> Gerdemann and Trappe	-	-	-	-	-	+	-	-
<i>G. mosseae</i> (Nicol. and Gerd) Gerd. and Trappe	-	-	-	+	-	-	-	-
<i>G. pustulatum</i> Koske, Friese, Walker, and Dalphe	-	+	-	-	-	-	-	+
<i>G. reticulatum</i> Bhattacharjee and Mukerji	-	-	+	-	-	-	+	-
<i>G. tortuosum</i> Schenck and Smith	-	-	-	+	-	-	-	-
<i>Sclerocystis pakistanica</i> Iqbal and Bushra	-	+	-	-	-	+	-	-
<i>S. sinuosa</i> Gerd. and Bakshi	-	+	-	-	-	+	-	-

<i>Scutellospora calospora</i> (Nicol. And Gerd.) Walker and Sanders	-	-	-	+	-	-	-	-
<i>S. erythropha</i> (Koske and Walker) Walker and Sanders	-	-	-	+	-	-	-	-
<i>Scutellospora</i> Sp.	-	-	-	+	-	-	-	-

S<sub>1</sub>-Karimnagar (AP) S<sub>2</sub>-Haldwani (UP) S<sub>3</sub>-Bareilly (UP)  
S<sub>4</sub>-Gorakhpur (UP) S<sub>5</sub>-Banda (UP) S<sub>6</sub>-Deoria (UP)  
S<sub>7</sub>-Khammam (AP) S<sub>8</sub>-Hyderabad (AP)

**Table 2** Physico-chemical characteristics of rhizosphere soils of garlic in relation to number of AMF propagules

Factors	Soil samples							
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	S <sub>6</sub>	S <sub>7</sub>	S <sub>8</sub>
AMF (propagules/100 g soil)	360	82	95	210	190	182	240	110
PH	7.6	7.9	7.8	7.4	7.6	7.9	8.1	7.3
Moisture (%)	11.0	9.0	8.0	10.5	9.5	9.0	12.5	10.0
Sand (%)	60.0	0.0	10.0	55.0	50.0	54.0	50.0	0.0
Salt (%)	30.0	80.0	60.0	35.0	30.0	20.0	37.0	75.0
Clay (%)	10.0	20.0	30.0	10.0	20.0	26.0	13.0	25.0
(in mg/kg)								
Nitrogen	850.0	750.0	850.0	950.0	1100.0	600.0	770.0	1050.0
Phosphorus	4.3	12.4	11.2	5.9	7.6	9.3	5.4	10.5
Potassium	360.0	390.0	440.0	600.0	580.0	490.0	420.0	530.0

S<sub>1</sub> to S<sub>8</sub> are the rhizosphere soils of garlic from S<sub>1</sub>-Karimnagar (AP) S<sub>2</sub>-Haldwani (UP) S<sub>3</sub>-Bareilly (UP) S<sub>4</sub>-Gorakhpur (UP) S<sub>5</sub>-Banda (UP) S<sub>6</sub>-Deoria (UP) S<sub>7</sub>-Khammam (AP) S<sub>8</sub>-Hyderabad (AP)

recorded significant positive correlation between moisture content and VAM fungal spores in *Terminalia* rhizosphere soil indicating positive influence.

It is a well known fact that the nutritionally deficient soils, phosphorus soils in particular, harbour more AMF. The authors' findings also support this. This is corroborated by the findings of Reddy (1993), Janaki Rani and Manoharachary (1994), Prabhakar (1995), and Manoharachary et al. (1996).

No AMF have been reported so far from garlic rhizosphere soils. The present study provides an interesting and original account of AMF species associated with garlic rhizosphere soils, and it is an indication of the mycorrhizal dependency of the plant.

## Acknowledgements

The authors are grateful to the University Grants Commission for financial financial assistance under the SAP programme.

## References

- Bhat R P and Kaveriappa K M. 1997  
**Occurrence of vesicular-arbuscular mycorrhizal fungi in the tubers of *Colocasia esculenta* (L.) Schott**  
*Mycorrhiza News* 9(2): 12-13
- Durga V V K. 1995  
**Studies on VAM fungi associated with *Tectona grandis* Linn F. and *Terminalia arjuna* (Roxb.)**

## Wight & Arn.

Ph D Thesis, Osmania University, Hyderabad. pp. 273

Gerdemann J W and Nicolson T H. 1963

### Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting

*Transactions of the British Mycological Society* 46: 235–246

Iqbal S M and Nasim G. 1991

### Are underground non-root portions of tropical plants vesicular-arbuscular mycorrhizal?

*Transactions of the Mycological Society of Japan* 32: 467–476

Jackson M L. 1958

*Soil chemical analysis* New Jersey: Prentice-Hall International, Inc.

Jackson M L. 1973

*Soil chemical analysis*, New Delhi: Prentice Hall, India.

Janaki Rani S and Manoharachary C. 1994

### Occurrence and distribution of VAM fungi associated with Safflower

*Indian Phytopathology* 47(3): 263–265

Kunwar I K and Manoharachary C. 1998

### Colonization by arbuscular mycorrhizal and mycophyllous fungi in roots and scale leaves of garlic

*Journal of Indian Botanical Society* 77

Mago P, Pasricha P, and Mukerji K G. 1993

### Survey of vesicular-arbuscular mycorrhizal fungi in scale leaves of modified stems

*Phytomorphology* 43: 81–85

Manoharachary C, Vani P, Satya Prasad K, Prakash P. 1996

### Vesicular-arbuscular mycorrhizal symbiosis with special reference to castor production

In *Persp. Biol. Sci.* pp. 197–216

Martin M J and Birchfield W. 1972

### Endogone S P in roots of sweet potato and soyabean in Louisiana

*Plant Disease Reporter* 56(1): 58

Mehrotra V S and Bajjal U. 1994

### Advances in the taxonomy of vesicular-arbuscular mycorrhizal fungi, pp. 227–286

In *Biotechnology in India*, edited by Dwivedi B K and Pandey G Allahabad: Bioved Research Society.

Morton J B and Benny G L. 1990

### Revised classification of arbuscular mycorrhizal fungi (zygomycetes): a new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae

*Mycotaxon* XXXVII: 471–491

Prabhakar B. 1995

### Taxonomy and ecology of vesicular-arbuscular mycorrhizal fungi associated with *Casuarina equisetifolia* forest plantations

Ph D Thesis, Osmania University, Hyderabad. 102 pp.

Reddy P J M. 1993

### Studies on vesicular-arbuscular mycorrhizal fungi associated with *Terminalia alata* Heync

Ph D Thesis, Osmania University, Hyderabad. 91 pp.

Reid C P P and Bowen G D. 1979

### Effects of soil moisture on VA mycorrhiza formation and root development in *Medicago*

In *The soil-root interface*, edited by Harley J L and Russel R S London: Academic Press. pp. 211

Rodrigues B F. 1996.

### Occurrence of vesicular-arbuscular mycorrhizal fungi in the tubers of *Pueraria tuberosa* (Willd.) Dc.

*Mycorrhiza News* 8(2): 9

Sampath P and Sullia S B. 1992

### The occurrence of VAM fungi in the scale leaves of turmeric

*Mycorrhiza News* 4(1): 5

Schenck N C and Perez Y. 1987

*Manual for identification of VA mycorrhizal fungi* Florida: Fifield Hall University. 241 pp.

Subbiah B V and Asija G L. 1956.

### A rapid procedure for determination of available nitrogen in soils

*Current Science* 25: 259–260

Taber R A and Trappe J M. 1982.

### Vesicular-arbuscular mycorrhiza in rhizomes, scale-like leaves, roots and xylem of ginger

*Mycologia* 74(1): 158–161

---

## VA-mycorrhizal fungi occurring in the mangrove vegetation of Pichavaram forest

Lingan V K, Tholkappian P, Sundaram M D

Department of Agricultural Microbiology,  
Faculty of Agriculture, Annamalai University,  
Annamalainagar – 608 002, India

### Introduction

Mangrove ecosystems are very peculiar formations that develop best along coastal, estuarine, and deltaic areas, where brackish water results from the mixing of sea water with land run-off. The tropical or sub-tropical

parts such as saline wet lands are inhabited by mangroves, a climax formation of hydrohalophytes belonging to several families. The occurrence of VAM (vesicular-arbuscular mycorrhizal) fungi in the soils of the Chennai sea coast was reported by Mohankumar et al. in 1988. This study presents the survey undertaken for VAM fungi in different mangrove tree species of Pichavaram mangrove forests.

### Materials and method

The Pichavaram mangrove forest is situated on the Coromandal coast (Bay of Bengal Sea Board), India (latitude 11°N, longitude 79°46' E). It lies about 225 km south of the Chennai coast and 40 km south of the Cuddalore coast between the Vellar and

Coleroon estuarine complex system. These mangroves, covering an area of about 14 km<sup>2</sup> (199 hectares), are highly productive ecosystems, and support luxuriant vegetation.

Roots and rhizosphere soil samples were collected from mangrove tree species at Pichavaram mangrove forest which include *Rhizophora apiculata*, *Rhizophora mucronata*, *Aegiceras curriculature*, *Bruguiera cylindrica*, *Exoecaria agallocha*, *Avicennia officinalis*, *Rhizophora*, and *Avicennia marina*. The soil characteristics are presented in Table 1.

**Table 1** Soil characteristics of Pichavaram mangrove forests

Properties	Saline soil collected from mangrove forest
Texture	Sandy loam
pH	8.5 – 9.0
Electrical conductivity (ds/m)	5.2
Available nitrogen (kg/ha)	82
Available phosphorus (kg/ha)	34
Available potassium (kg/ha)	178
Bulk density	1.60

VAM spores in the soil were enumerated by wet sieving and decanting technique (Gerdemann and Nicolson 1963), and the root colonization of VAM fungi was estimated by trypan blue technique (Phillips and Hayman 1970).

## Results and discussion

Among the eight mangrove tree species tested, five were found to have higher root colonization of VAM fungi and the remaining three showed very low VAM colonization (Table 2). Quantitative variations of VAM spores were also observed. *Glomus fasciculatum*, *G. mosseae* and *G. aggregatum* and two unidentified species of *Gigaspora* were noted. Among the mangroves, *Bruguiera cylindrica* was moderately infected with VAM fungi showing 55% root colonization and 221 spores per 100 gm of rhizosphere soil. Rozema et al. (1986) also investi-

**Table 2** VAM fungi status of some mangrove tree species

Mangrove tree species	Spores/100 g soil	Per cent root colonization				VAM species
		base	middle	lateral	total	
<i>Rhizophora apiculata</i>	6	2	—	—	2	Gf
<i>Rhizophora mucronata</i>	4	1	1	—	2	Ga
<i>Rhizophora</i> sp.	13	4	2	2	8	Gf, Ga
<i>Bruguiera cylindrica</i>	221	33	13	9	55	Gf, Ga
<i>Aegiceras curriculature</i>	3	1	1	—	2	Ga
<i>Exoecaria agallocha</i>	147	29	8	6	43	Gf, Gm
<i>Avicennia officinalis</i>	65	16	5	2	23	Gf, Ga
<i>Avicennia marina</i>	105	21	6	5	32	Gf, Gm

Gf—*Glomus fasciculatum* Ga—*Glomus aggregatum* Gm—*Glomus mosseae*

gated the VAM status of salt marshes at Bargaon Opsoom Kwade Hoek.

## References

- Gerdemann J W and Nicolson T H. 1963  
**Spores of mycorrhizal *Endogone* spp. extracted from soil by wet sieving and decanting**  
*Transactions of the British Mycological Society* **46**: 235–244
- Mohankumar V, Ragupathy S, Nirmala C B, Mahadevan A. 1988.  
**Distribution of VAM in sandy beach soil of Chennai coast**  
*Current Science* **57**(7): 367–368
- Phillips J M and Hayman D S. 1970  
**Improved procedure for clearing roots and obtaining parasite and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection**  
*Transactions of the British Mycological Society* **55**: 158–161
- Rozeina J, van Digglen W A, van Eshrock M, Brockman R, Punte H. 1986  
**Occurrence and ecological significance of vesicular–arbuscular mycorrhiza in the salt marsh environment**  
*Acta Botanica Neerlandica* **35**(4): 457–467

## Cellulase activity in mycorrhizal cowpea roots

Thiagarajan T R

University College of Belize, Belize, Central America

### Introduction

Production of endoglucanase and exoglucanase enzymes has been reported (Garcia-Garrido et al.

1992) in the roots of lettuce and onion during the penetration of the host by the VAM (vesicular–arbuscular mycorrhizal) fungus *Glomus mosseae*. The intracellular establishment of VAM symbiosis between fungus and plant roots requires penetration of the host cell by the fungus. This experiment was carried out to examine the possibility of production of cell wall hydrolyzing enzymes (cellulases) during the process of intracellular establishment.

## Methodology

Five germinated spores of *Glomus pallidum* were placed on germinating cowpea seedling roots and later carefully transplanted to growth pouches with sterile vermiculite and nutrient solution. Plants were grown in a growth chamber (16 hours light at 30 °C, 8 hours dark at 20 °C) up to 45 days. Non-mycorrhizal plants were also maintained in the same conditions. The plants were harvested in one-week intervals and their roots washed in sterile distilled water. About 2 g of the fresh root material was ground with 10 ml of 0.1M acetate buffer (pH 5.0) and centrifuged at 10 000 rpm for 10 minutes. The supernatant was again subjected to 80% ammonium sulphate precipitation. The precipitate was then re-dissolved in 5 ml of the same buffer and desalted by dialysis. Cellulase activity was measured according to Wood et al. (1989). The reaction mixture was incubated at 37 °C for seven days, then 1 ml of the supernatant was assayed for total sugar and protein content according to standard methods. The enzyme activity is expressed in micrograms of reducing sugar produced per millilitre supernatant per milligram protein.

## Results

Typical VAM colonization was noted in inoculated cowpea roots. The percentage colonization increased with the age of the plants (Figure 1). Cellulase activity

was evident in all the harvests, in mycorrhizal as well as in non-mycorrhizal roots (Figure 2). In mycorrhizal roots, the enzyme activity gradually increased and attained maximum on the 21st day and then declined. A significant increase in enzyme activity was noticed in mycorrhizal roots from 14 days after inoculation. The possibility of increase in cellulase activity in the mycorrhizal cowpea roots could be due to the fungal partner, which may secrete extracellular cellulase to penetrate the host cell wall.

**Figure 2** Cellulase activity in mycorrhizal and non-mycorrhizal plants

## References

- Garcia-Garrido J M, Garcia-Romera I, and Ocampo J A. 1992  
**Cellulase productions by the vesicular-arbuscular mycorrhizal fungus *Glomus mossea* (Nicol. & Gerd.) Gerd. and Trappe.**  
*New Phytologist* 121(2): 221–226
- Wood T M, McCreae S I, and Mahalingeshwara K. 1989  
**The mechanism of fungal cellulase action. Synergism between components of *Penicillium pinophilum* cellulase in solubilizing hydrogen bond-ordered cellulose**  
*Biochem Journal* 260: 37–43

**Figure 1** Percentage VAM colonization for different time intervals



## Centre for Mycorrhizal Culture Collection (CMCC)

### List of cultures available from CMCC

S No.	Germplasm bank code	Name of the fungus	Host
1.	EM-1001	<i>Alpova diplophloeus</i>	<i>Pseudotsuga menziesii</i> /Alnus rubra
2.	EM-1145	<i>Amanita murina</i>	<i>Eucalyptus bridgerina</i>
3.	EM-1060	<i>Amanita muscaria</i>	—
4.	EM-1069	<i>Amanita muscaria</i>	—
5.	EM-1084	<i>Amanita muscaria</i>	<i>Pinus patula</i>
6.	EM-1146	<i>Amanita muscaria</i>	<i>Sous resineux</i>
7.	EM-1100	<i>Amanita muscaria</i> var. <i>famosa</i>	<i>Populus tremuloides</i>
8.	EM-1148	<i>Boletus cavipes</i>	—
9.	EM-1277	<i>Boletus cavipes</i>	—
10.	EM-1072	<i>Boletus edulis</i>	—
11.	EM-1035	<i>Cenococcum geophyllum</i>	<i>Pseudotsuga menziesii</i>
12.	EM-1036	<i>Cenococcum geophyllum</i>	<i>Tsuga mertensiana</i>
13.	EM-1088	<i>Cenococcum geophyllum</i>	—
14.	EM-1150	<i>Cenococcum geophyllum</i>	Picea sp.
15.	EM-1149	<i>Cenococcum geophyllum</i>	—
16.	EM-1151	<i>Cenococcum geophyllum</i>	Picea sp.
17.	EM-1252	<i>Cenococcum geophyllum</i>	Douglas fir
18.	EM-1253	<i>Chalciporus piperatus</i>	—
19.	EM-1153	<i>Ectendomycorrhizae</i>	<i>Pinus sylvestris</i>
20.	EM-1154	<i>Ectendomycorrhizae</i>	—
21.	EM-1156	<i>Elaphomyces granulatus</i>	Chene
22.	EM-1157	<i>Elaphomyces granulatus</i>	Chene
23.	EM-1108	<i>Gautieria otthii</i>	<i>Pinus banksiana</i>
24.	AM-1005	<i>Gigaspora margarita</i>	—
25.	AM-1001	<i>Glomus etunicatum</i>	—
26.	AM-1119	<i>Glomus geosporum</i>	—
27.	AM-1004	<i>Glomus intraradices</i>	—
28.	AM-1006	<i>Glomus mosseae</i>	—
29.	EM-1159	<i>Hebeloma calyptosporum</i>	—
30.	EM-1160	<i>Hebeloma circinans</i>	<i>Picea</i>
31.	EM-1168	<i>Hebeloma crustuliniforme</i>	Chene
32.	EM-1165	<i>Hebeloma crustuliniforme</i>	—
33.	EM-1163	<i>Hebeloma crustuliniforme</i>	—
34.	EM-1008	<i>Hebeloma crustuliniforme</i>	<i>Pinus monticola</i> /Tsuga heterophylla

S No.	Germplasm bank code	Name of the fungus	Host
35.	EM-1015	<i>Hebeloma crustuliniforme</i>	<i>Pseudotsuga menziesii</i>
36.	EM-1169	<i>Hebeloma crustuliniforme</i>	Picea sp.
37.	EM-1164	<i>Hebeloma crustuliniforme</i>	—
38.	EM-1161	<i>Hebeloma crustuliniforme</i>	Picea
39.	EM-1172	<i>Hebeloma cylindrosporum</i>	Picea
40.	EM-1173	<i>Hebeloma cylindrosporum</i>	—
41.	EM-1175	<i>Hebeloma edurum</i>	Hetre
42.	EM-1176	<i>Hebeloma ingratum</i>	<i>Tremble fevillus</i>
43.	EM-1177	<i>Hebeloma mesophaeum</i>	—
44.	EM-1182	<i>Hebeloma sinapizans</i>	Hetre
45.	EM-1183	<i>Hebeloma truncatum</i>	—
46.	EM-1184	<i>Hebeloma vaccinum</i>	—
47.	EM-1091	<i>Laccaria amethystina</i>	<i>Pseudotsuga menziesii</i>
48.	EM-1103	<i>Laccaria bicolor</i>	<i>Picea glauca</i>
49.	EM-1102	<i>Laccaria bicolor</i>	<i>Picea muricina</i>
50.	EM-1121	<i>Laccaria farinacea</i>	<i>Tsuga mertensiana</i>
51.	EM-1083	<i>Laccaria fraterna</i>	<i>Eucalyptus globulus</i>
52.	EM-1009	<i>Laccaria laccata</i>	Alpine
53.	EM-1033	<i>Laccaria laccata</i>	<i>Pseudotsuga menziesii</i>
54.	EM-1076	<i>Laccaria laccata</i>	<i>Pinus patula</i>
55.	EM-1016	<i>Laccaria laccata</i>	Subalpine fir
56.	EM-1067	<i>Laccaria laccata</i>	—
57.	EM-1079	<i>Laccaria laccata</i>	<i>Pinus patula</i>
58.	EM-1090	<i>Laccaria laccata</i>	—
59.	EM-1031	<i>Laccaria laccata</i>	<i>Pseudotsuga menziesii</i>
60.	EM-1032	<i>Laccaria laccata</i>	Hemlock
61.	EM-1058	<i>Laccaria laccata</i>	—
62.	EM-1065	<i>Laccaria laccata</i>	—
63.	EM-1066	<i>Laccaria laccata</i>	—
64.	EM-1085	<i>Laccaria laccata</i>	<i>Pinus patula</i>
65.	EM-1042	<i>Laccaria laccata</i>	<i>Pseudotsuga menziesii</i>
66.	EM-1104	<i>Laccaria laccata</i>	Mixed hardwood forest
67.	EM-1105	<i>Laccaria laccata</i>	<i>Pinus resinosa</i>
68.	EM-1191	<i>Laccaria laccata</i>	<i>Eucalyptus</i> sp.
69.	EM-1192	<i>Laccaria laccata</i>	<i>Picea sitchensis</i>
70.	EM-1086	<i>Laccaria proxima</i>	—
71.	EM-1196	<i>Laccaria tortilis</i>	Bouleau
72.	EM-1263	<i>Lactarius quietus</i>	Chene
73.	EM-1052	<i>Lactarius rufus</i>	<i>Larix laricina</i> , <i>Picea mariana</i>
74.	EM-1124	<i>Melanogaster tuberiformis</i>	—
75.	EM-1073	<i>Paxillus involutus</i>	—

S No.	Germplasm bank code	Name of the fungus	Host
76.	EM-1208	<i>Paxillus involutus</i>	—
77.	EM-1047	<i>Phialocephala fortinii</i>	Pinus sp.
78.	EM-1219	<i>Piloderma nigra</i>	—
79.	EM-1010	<i>Pisolithus tinctorius</i>	<i>Pinus taeda</i>
80.	EM-1004	<i>Pisolithus tinctorius</i>	<i>Pinus virginiana</i>
81.	EM-1034	<i>Pisolithus tinctorius</i>	<i>Pinus taeda</i>
82.	EM-1081	<i>Pisolithus tinctorius</i>	<i>Eucalyptus tereticornis</i>
83.	EM-1005	<i>Pisolithus tinctorius</i>	Sawtooth oak
84.	EM -1002	<i>Pisolithus tinctorius</i>	<i>Tsuga canadensis</i>
85.	EM-1006	<i>Pisolithus tinctorius</i>	Pine oak
86.	EM-1080	<i>Pisolithus tinctorius</i>	<i>Eucalyptus tereticornis</i>
87.	EM-1070	<i>Pisolithus tinctorius</i>	<i>Eucalyptus tereticornis</i>
88.	EM-1057	<i>Pisolithus tinctorius</i>	—
89.	EM-1059	<i>Pisolithus tinctorius</i>	—
90.	EM-1064	<i>Pisolithus tinctorius</i>	—
91.	EM-1013	<i>Rhizopogon arenicola</i>	Sitka spruce/lodge pole pine
92.	EM-1028	<i>Rhizopogon clavitisporus</i>	<i>Pinus ponderosa</i>
93.	EM-1044	<i>Rhizopogon colossus</i>	<i>Pinus ponderosa/Pseudotsuga menziesii</i>
94.	EM-1029	<i>Rhizopogon cusickensis</i>	<i>Arbutus menziesii/Pinus ponderosa</i>
95.	EM-1038	<i>Rhizopogon ellenae</i>	<i>Arbutus menziesii, Pinus ponderosa</i>
96.	EM-1046	<i>Rhizopogon ellenae</i>	<i>Pseudotsuga menziesii</i>
97.	EM-1019	<i>Rhizopogon fuscorubens</i>	<i>Pinus contorta</i>
98.	EM-1125	<i>Rhizopogon fuscorubens</i>	<i>Pinus radiata</i>
99.	EM-1027	<i>Rhizopogon hawkeriae</i>	<i>Pseudotsuga menziesii, Abies, Pinus ponderosa</i>
100.	EM-1113	<i>Rhizopogon hawkeriae</i>	Douglas-fir
101.	EM-1040	<i>Rhizopogon mutabilis</i>	<i>Pinus ponderosa</i>
102.	EM-1017	<i>Rhizopogon occidentalis</i>	<i>Pinus ponderosa</i>
103.	EM-1118	<i>Rhizopogon occidentalis</i>	<i>Pinus ponderosa</i>
104.	EM-1026	<i>Rhizopogon ochraceorubens</i>	<i>Pinus contorta</i>
105.	EM-1012	<i>Rhizopogon ochraceorubens</i>	<i>Pinus contorta/Abies lasiocarpa</i>
106.	EM-1114	<i>Rhizopogon ochraceorubens</i>	<i>Pinus contorta</i>
107.	EM-1011	<i>Rhizopogon parksii</i>	<i>Pseudotsuga menziesii/Pinus lambertiana/Abies concolor</i>
108.	EM-1020	<i>Rhizopogon parksii</i>	<i>Picea sitchensis/Tsuga heterophylla</i>
109.	EM-1053	<i>Rhizopogon parksii</i>	<i>Pseudotsuga menziesii/Abies amabilis/Tsuga heterophylla</i>
110.	EM-1126	<i>Rhizopogon reaii</i>	<i>Pinus caribaea var. hondurensis</i>
111.	EM-1049	<i>Rhizopogon rubescens</i>	<i>Pinus contorta, Abies engelmannii, Picea lasiocarpa</i>
112.	EM-1050	<i>Rhizopogon rubescens</i>	<i>Abies lasiocarpa/Pinus contorta</i>
113.	EM-1061	<i>Rhizopogon rubescens</i>	—

S No.	Germplasm bank code	Name of the fungus	Host
114.	EM-1109	<i>Rhizopogon rubescens</i>	<i>Pinus banksiana</i>
115.	EM-1043	<i>Rhizopogon smithii</i>	<i>Pinus contorta</i>
116.	EM-1111	<i>Rhizopogon smithii</i>	<i>Pinus contorta</i>
117.	EM-1127	<i>Rhizopogon smithii</i>	Shasta fir
118.	EM-1022	<i>Rhizopogon subareolatus</i>	<i>Pseudotsuga menziesii</i>
119.	EM-1048	<i>Rhizopogon subcaerulescens</i>	<i>Abies lasiocarpa</i> , <i>Picea engelmannii</i> , <i>Pinus contorta</i>
120.	EM-1018	<i>Rhizopogon subcaerulescens</i>	<i>Abies grandis</i> / <i>Pseudotsuga menziesii</i>
121.	EM-1024	<i>Rhizopogon subcaerulescens</i>	<i>Pseudotsuga menziesii</i>
122.	EM-1007	<i>Rhizopogon subcaerulescens</i>	<i>Abies grandis</i> var. <i>subpannosus</i>
123.	EM-1117	<i>Rhizopogon vinicolor</i>	Mixed conifer forest
124.	EM-1039	<i>Rhizopogon vulgaris</i>	<i>Pinus ponderosa</i>
125.	EM-1023	<i>Rhizopogon vulgaris</i>	<i>Pinus contorta</i> / <i>Abies amabilis</i>
126.	EM-1063	<i>Rhizopogon vulgaris</i>	—
127.	EM-1115	<i>Rhizopogon vulgaris</i>	<i>Pinus jeffreyi</i> / <i>Abies concolor</i>
128.	EM-1232	<i>Rhizopogon vulgaris</i>	—
129.	EM-1082	<i>Scleroderma citrinum</i>	<i>Pinus patula</i>
130.	EM-1107	<i>Scleroderma citrinum</i>	Mixed hardwood forest
131.	EM-1030	<i>Suillus americanus</i>	<i>Pinus strobus</i>
132.	EM-1074	<i>Suillus bovinus</i>	—
133.	EM-1120	<i>Suillus brevipes</i>	<i>Pinus contorta</i>
134.	EM-1123	<i>Suillus brevipes</i>	<i>Pinus contorta</i>
135.	EM-1119	<i>Suillus granulatus</i>	<i>Pinus ponderosa</i>
136.	EM-1110	<i>Suillus lakei</i>	Douglas-fir, Hemlock
137.	EM-1071	<i>Suillus luteolus</i>	—
138.	EM-1136	<i>Suillus luteus</i>	<i>Pinus</i> sp.
139.	EM-1116	<i>Suillus punctatipes</i>	<i>Tsuga mertensiana</i> / <i>Abies lasiocarpa</i>
140.	EM-1051	<i>Suillus tomentosus</i>	<i>Pinus ponderosa</i>
141.	EM-1021	<i>Suillus tomentosus</i>	Alder, Spruce, Lodgepole pine mix.
142.	EM-1122	<i>Suillus tomentosus</i>	<i>Pinus monticola</i> / <i>Tsuga heterophylla</i>
143.	EM-1075	<i>Suillus variegatus</i>	—
144.	EM-1003	<i>Thelephora americanus</i>	<i>Pseudotsuga menziesii</i>
145.	EM-1077	<i>Thelephora terrestris</i>	<i>Pinus patula</i>
146.	EM-1062	<i>Thelephora terrestris</i>	—
147.	EM-1041	<i>Tuber melanosporum</i>	—
148.	AM-1001	<i>Glomus etunicatum</i>	—
149.	AM-1006	<i>Glomus mosseae</i>	—
150.	AM-1004	<i>Glomus intraradices</i>	—
151.	AM-1005	<i>Gigaspora margarita</i>	—
152.	AM-1119	<i>Glomus geosporum</i>	—

## Fourth National Conference on Mycorrhiza

The Fourth National Conference on Mycorrhiza was held at the Barkatulla University from 5 to 7 March 1999. It was organized under the auspices of the Mycorrhiza Network, TERI, at the Institute of Microbiology and Biotechnology, Barkatulla University, Bhopal. Over 75 delegates from India and one each from Sri Lanka, Italy, and Zambia participated in the conference.

### Inaugural session

The inaugural session was presided by Prof. M S Sodha, Vice Chancellor of Barkatulla University. Dr Anil Prakash, Director of the Conference, accorded a warm welcome to the delegates. Dr Alok Adholeya welcomed the delegates on behalf of

TERI, New Delhi. Prof. M S Sodha, in his presidential address, emphasized the need for a multi-disciplinary coordinated strategy for future research on mycorrhiza. He specifically stressed on increasing the use of mathematics in research modelling and various aspects of application technology for commercial production of mycorrhiza biofertilizers. The chief guest, Prof. R K S Chauhan, Vice Chancellor, Vikram University, Ujjain, highlighted the chronological developments in mycorrhizal research in India.

The keynote address was delivered by Prof. D J Bagyaraj, Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK Campus, Bangalore. He highlighted the interaction of different categories of soil microflora and microfauna with VAM (vesicular arbuscular mycorrhizal) fungi.

### Proceedings of the conference

The deliberations in the conference were held under five sessions.

#### **Session 1: Association and distribution of mycorrhizal fungi under varying edapho climatic conditions**

Chair Prof. R G Kapooria • Rapporteur Dr Ragini Gothwal

Oral presentations 3 • Poster presentations 16

Topics covered: Research on orchid mycorrhiza; influence of tannery, paper board, and sewage water on VAM fungi; and ecological studies on VAM fungi in coastal dune environs.

#### **Session 2: Plant growth responses and mycorrhizal dependency**

Chair Dr C Manoharachary • Rapporteur Dr P K Pandey

Oral presentations 9 • Poster presentations 16

Topics covered: Mycorrhizal dependency of *Acacia* spp. (in coal mine soils) and *Sesbania grandiflora* (in saline soils); mycorrhiza responsiveness of rice varieties in nutrient deficient soils; VAM studies on coffee, neem, and tissue culture plantlets of banana and Alocasia; and failure of *Pisolithus tinctorius* to form mycorrhiza with eucalyptus in alkaline soil.

#### **Session 3: Physiology and biochemistry**

Chair Prof. D J Bagyaraj • Rapporteur Mrs Vandana Tandon

Oral presentations 5 • Poster presentations 3

Topics covered: Effects of VAM inoculation on (i) biomass yield and sucrose content of sugarcane; (ii) growth, nutrient uptake, and biochemical constituents of *Pueraria phaseoloides*; (iii) phosphorus kinetics in sweet potato; and (iv) enhancement of colonization in upland rice. A significant presentation was made on the appearance of wounds as a regular feature near or behind the growing tips of extraradical hyphae of *Gigaspora margarita* cultured on RiT-DNA transformed carrot roots.

#### **Session 4: Interactions between mycorrhiza and other rhizosphere flora and fauna**

Chair Mr Sujjan Singh • Rapporteur Ms Reema

Oral presentations 5 • Poster presentations 14

Topics covered: Interaction of VAM and phosphorus-solubilizing microbes and Bradyrhizobium on uptake and concentration of nitrogen in soybean; VAM and phosphorus solubilizers on growth and nutrition of teak, and VAM and rhizobium with varying phosphorus levels on response of mung bean. A presentation on the development of a protocol to freeze dry the vegetative mycelia of ectomycorrhizal fungi was a significant breakthrough in long-term preservation technology for mycorrhizal fungi. Out of the 15 mycorrhizal fungi subjected to lyophilization, 14 responded positively. *Pisolithus tinctorius* was found sensitive to freezing.

#### **Session 5: Maintenance of mycorrhizal cultures and application of mycorrhizal technology**

Chair Prof. B N Johri • Oral presentations 6 • Poster presentations 5

Topics covered: Application of *Tricholoma imbricatum* to banana; application of VAM fungi to tuber crops; and vegetative mycelial inoculum production of *Laccaria laccata* by submerged cultivation.

The posters highlighted on various aspects of mycorrhiza research.

## Recommendations

The panel discussion was chaired by Prof. S Chaudhuri, Bidhan Krishi Viswavidyalaya, Kalyani, West Bengal. He emphasized the need to set future agenda for mycorrhiza research and bring mycorrhizal science to the status of applicable technology in the near future. Mr Sujan Singh, Dr Satyanarayana and Dr Marcello Intini represented ectomycorrhizal research; and Dr Alok Adholeya, Dr B N Johri, Dr Bagyaraj, and Dr Manoharachari represented VAM research. The recommendations are summarized below.

### Ectomycorrhiza

- Selection of available and efficient species/strains of ectomycorrhizal fungi for different soil ecologies.
- Scaling up of fermentation technology for the production of mycelial inoculum of promising ectomycorrhizal fungi.
- Research on augmenting the production of sporocarps of ectomycorrhizal fungi under field conditions and evolving techniques for the utilization of spore inoculum in forest nurseries.

### Vesicular arbuscular mycorrhiza

- Continued efforts to develop mass production technology for VAM fungi.
- Attempts to exploit AMF in nutrient-deficient areas.
- AM dependency of plant species should be intensified in horticultural, medicinal, and forestry plants particularly where low productivity is obtained due to low input system of production and management.
- Initiate intensive research programmes for the integration of mycorrhizal research with other micro-organism-based biofertilizers, biocontrol agents, etc. for holistic plant nutrition.
- Involve expert group in the development of the taxonomy of VAM employing molecular study tools. All described cultures should be maintained live and deposited with the Centre for Mycorrhizal Culture Collection at TERI.
- Determine maximum phosphorus level at which the benefits from VAM inoculation can accrue to plants for each plant species which is dependent on mycorrhiza under different edaphoclimatic conditions.

## Guest lectures

Four lectures were delivered by the guest speakers. Mr Sujan Singh highlighted the salient points in ectomycorrhizal research that was conducted at the Forest Research Institute, Dehra Dun. Special mention was made of the inoculation of exotic hard pine

nurseries in dome areas of Orissa, Madhya Pradesh, and Andhra Pradesh. He also highlighted the successful use of mycorrhizal inoculation coupled with the application of hormones in reducing the nursery period from 4.5 years to 3.5 years in *Abies pindrow* nurseries in Himachal Pradesh. He appraised the delegates about the various activities of the Mycorrhiza Network, Asia.

Prof. C Manoharachary, in his lecture, highlighted the role of AMF in boosting plant growth. In India, 118 species of AMF belonging to the genera *Acaulospora*, *Glomus*, *Gigaspora*, *Entrophospora*, *Sclerocystis*, and *Scutellospora* have been recorded. For correct delimitation of species, the use of molecular tools like polymerase chain reaction, restriction fragment length polymorphism, genetical markers, biochemical markers, and immunological techniques were emphasized. According to him, weeds like *Parthenium* have 70%–80% AM infection, crop plants 40%–60%, and forest trees below 40%. Several AM species are now known to enhance plant growth and nutrient uptake, especially phosphorus; biomass and yield increases in crops, forest trees, and aromatic, medicinal, and horticultural plants; and thus have a great potential as biofertilizers. For their mass production, the life-cycle of AMF needs to be studied. Root organ culture has given some information but further studies are needed for the commercial production of AM Fungi.

Dr Alok Adholeya, in his lecture, presented the details of RiT-DNA technique. In this technique, the carrot roots are first transformed by rhizogene bacteria, the transformed roots are then freed from bacteria, grown in pure culture, and inoculated with contamination-free AM spores. The successive stages of mycorrhizal establishment and AM spore production was discussed. Pure culture of enormous number of AM spores can be produced in petriplates using this technique. He also presented his work on successful vegetation of fly ash dumps by forest species like *Eucalyptus*, *Populus*, neem, *Dalbergia sissoo*, and many floriculture plants with the help of VAM inoculation.

Dr R G Kapooria, in his lecture, gave a general account of climate, soil, and vegetation types of Zambia. Ectomycorrhizae in Zambia been recorded on trees belonging to Caesalpinaceae, Papilionaceae, Dipterocarpaceae, Euphorbeaceae, and Proteaceae. Several species belonging to *Baikiaea*, *Colophospermum*, *Erythrophleum*, *Guibourtia*, and *Pterocarpus* supported endomycorrhizae representing species of *Glomus*, *Acaulospora*, and *Gigaspora*. *G. mosseae* occurs predominantly while *Acaulospora denticulata*, *A. mellea*, *Glomus etunicatum*, and *Gigaspora margarita* occurred in insignificant numbers.

## Valedictory session

The session was chaired by Prof. P S Bisen, Director, Institute of Microbiology and Biotechnology, Barkatullah University, Bhopal. Dr Anil Prakash thanked all the delegates for making the deliberations a great success. Mr Sujan Singh thanked the delegates on behalf of the Mycorrhiza Network, TERI, New

Delhi. He also thanked Dr R K Pachauri, Director, TERI, for his guidance; Prof. M S Sodha, Vice Chancellor and Prof. Besin for organizing the conference at Barkatullah University; and Dr Anil Prakash and Dr V S Mehrotra for successfully organizing the conference. The vote of thanks was proposed by Dr V S Mehrotra, Secretary of the conference.

---

## New approaches...

*Under this column appear brief accounts of new techniques, modifications of available techniques, and new applications of other known techniques, etc. in mycorrhiza research that have been published in reputed journals during the last two or three years.*

---

### A technique for ectomycorrhizal inoculation

Based on the principle that root exudation can stimulate the germination and growth of mycorrhizal fungi; Hua Xiaomei, Guolong Liu, Xiaolin Zhang, and Laiyou Zhengi proposed a method for ectomycorrhizal inoculation and its effect on field performance (*Proceedings of the First International Conference on Mycorrhizae*, 4–9 August, 1996 Berkeley, California). The method consists of growing pine seedlings by cutting their apexes and inoculating them with ectomycorrhizal fungus. This method has been compared with the traditional method or other mycorrhizal inoculation techniques to raise pine seedlings. The results show that this technique can promote the development and growth of root system and ectomycorrhizae formation, increase the biomass of seedlings, improve quality and yield of plantable seedlings, and enhance the survival rate and growth of plantlings significantly. The benefit analysis confirms that this method has significant economic and social benefits with a countable value of 44662.5 yuan/hm<sup>2</sup> in nursery alone.

### Measurement of pH of AM fungi

Intracellular pH was determined for (1) the germ tubes of *Gigaspora margarita* and (2) extraradical hyphae and germ tubes of *Glomus intraradices* using BCECF-AM (molecular probes) dye under radiometric measurements at 450 and 490 nm excitation by Mario J, Germette S, Gaudette M, Perrier M, and Beccard G. This method was developed to perform real time analysis of cytosolic pH of AM (arbuscular mycorrhizal) fungal cells submitted to various experimental conditions. The use of nigericin led to an *in vitro* calibration curve. Minimum loading time, concentration of the dye, and signal to noise optimization were determined so that valid pH measurement could be made for a steady-state period on viable cells. A characteristic pH profile pattern was observed along the hyphae of AM fungi. For *G. margarita*, the pH of the apex (0–6 µm) was typically 6.8 rapidly raising up to 7.1 behind this region (10 µm), and then slowly decreasing the next 300 µm to reach a constant value of 6.7. A similar pattern was observed for *G. intraradices* hyphae. The pH profile of *G. margarita* germ tubes was higher when cultured in the presence of *Daucus carota* hairy root (non-mycorrhizal). For extraradical hyphae of *G. intraradices*, the presence of root exudates also raised the pH of hyphal cytosol. Preliminary results indicate that the method of cytosolic pH measurement could be useful for studying signal perception and/or H<sup>+</sup>/co-transport of nutrients by AM hyphae.

---

**Source** Proceedings of the First International Conference on Mycorrhiza, 4–9 August 1996, Berkeley, California. <<http://plantbio.berkeley.edu/~burns/icom.htm>>

#### Erratum

See, Volume 11, Number 1, P.15, column 2, line 7: 'Expercentra' should read 'Experientia'.

## Forthcoming events

Conferences, congresses, seminars, symposiums,  
workshops

---

- Curitiba, Brazil  
1-7 September 1999  
**Seminar on Sustainability of Plantations**  
Dr Carlos Ferreira, National Center of Forest Research, EMBRAPA, XX,  
Curitiba, Brazil  
*Fax* +55 41 766 1276 • *E-mail* bellote@cnpf.embrapa.br
- Gottingen, Germany  
9-23 September 1999  
**27th International Forestry Students Symposium: Forest History – the Link to our Future**  
IFSS 99 Organizing Team, IFSA Secretariat, Buesgenweg 2, 37077 Gottingen,  
Germany  
*Fax* +49 551 379 6992 • *E-mail* ifss@ifsa.net
- Hanoi, Vietnam  
18-22 October 1999  
**Impact of Logging on Biodiversity**  
Titiek Setyawati, Research Fellow, CIFOR, PO Box 6596, JKPWB Jakarta 10065,  
Indonesia  
*Fax* +55 41 766 1276 • *E-mail* t.setyawati@cgnet.com
- Santo Domingo,  
Dominican Republic  
18-23 October 1999  
**II Latin American Symposium on Advances in the Production of Forest Seeds**  
Rodolfo Salazar, CATIE, Turriable, Costa Rica  
*Fax* +506 556 556 7766 • *E-mail* rsalazar@catie.ac.cr
- New Delhi, India  
14-18 February 2000  
**International Conference on Managing Natural Resources for Sustainable Agricultural Production in the 21st century**  
Dr A K Singh, Secretary General, International Conference on Managing Natural Resources, Indian Society of Soil Science, Indian Agricultural Research Institute, New Delhi – 110 012, India  
*Fax* +91 11 578 8682, 575 5529 • *Tel.* +91 11 578 6790, 573 1494  
*E-mail* icmnr@iari.ernet.in
- Cartagena-Almería, Spain  
7-11 March 2000  
**International Symposium on Protected Cultivation in Mild Winter Climates: Current Trends for Sustainable Technologies**  
Universidad de Murcia, Departamento de Ingeniería Aplicada, Paseo Alfonso XIII, 52, 30203 Cartagena, Spain  
*Fax* +34 968 325435 • *Tel.* +34 968 325446  
*E-mail* juanfern@plc.um.es
- Tasmania, Australia  
19-24 March 2000  
**The Future of Eucalyptus for Wood Production**  
Secretariat, Conference Design Pty Ltd, PO Box 342, Sandy Bay, Tasmania, Australia, 7006  
*Tel.* +3 6224 3773 • *E-mail* mail@cdesign.com.au
- Los Baños, The Philippines  
31 March-3 April 2000  
**International Rice Research Conference**  
Shaobing Peng, Chair, Organizing Committee, International Rice Research Conference 2000, International Rice Research Institute, PO Box 3127, 1271 Makati City, The Philippines  
*Fax* +63 2 891 1292 • *E-mail* s.peng@cgiar.org
- Ontario, Canada  
13-15 April 2000  
**Conference 2000 Taking Agriculture into the next Millennium**  
AIC '99 Conference Coordinator, Margaret Weeks, PO Box 2000, Charlottetown, PEI, Ottawa, Ontario, Canada, C1A 7N8  
*Tel.* +902 368 6677 • *E-mail* aic99@gov.pe.ca



## News from members

Dr Seema Bhadauria, Senior Lecturer, Microbiology, Department of Botany, Raja Balwant Singh College, Agra, chaired a session in the National Conference on *Sustainable Ecosystem and Environment* held at Bhooma Niketan, in Haridwar from 2 to 3 November 1998.

Dr Archana Singh was awarded the 'Young Scientist Medal' (gold medal) at this conference for presenting a paper titled **Conservation of alkali-resistant trees and their utilization in watershed development**, edited by Dr Seema Bhadauria and Archana Singh.

## Recent references . . .

*Latest additions to the Network's database on mycorrhiza are published here for information of the members. The Mycorrhiza Network will be pleased to supply any of the available documents to bonafide researchers at a nominal charge.*

Name of the journal (volume no, issue no, and year)	Name of the article (page numbers)	Authors and correspondence address
<i>Agronomy Journal</i> 91(1), 1999	Plant determinants of mycorrhizal dependency in soybean. pp.135–141	Khalil S, Loynachan T E*, and Tabatabai M A. Iowa State University, Department of Agronomy, Ames, IA 50011 USA.
<i>American Journal of Botany</i> 86(4), 1999	Mycorrhizal status of the genus <i>Carex</i> (Cyperaceae). pp. 547–553	Miller R M*, Smith C I, Jastrow J D, Bever J D. Argonne National Laboratory, Division of Environmental Research, Argonne, IL 60439 USA. <rmiller@anl.gov>
<i>Applied and Environmental Microbiology</i> 65(5), 1999	Structure and dynamics of experimentally introduced and naturally occurring <i>Laccaria</i> sp. discrete genotypes in a Douglas fir plantation. pp. 2006–2014	Selosse M A*, Martin F, Bouchard D, LeTacon F. INRA, Centre Recherche Nancy, F-54280 Champenoux, France.
<i>Biology and Fertility of Soils</i> 29(1), 1999	Influence of long-term conservation tillage on soil and rhizosphere microorganisms. pp. 81–86	Hoflich G*, Tauschke M, Kuhn G, Werner K, Frielinghaus M, Hohn W. ZALF EV, Inst Mikrobielle Okol & Bodenbiol, Eberswalder Str 84, D-15374 Muncheberg, Germany.
	Influence of arbuscular mycorrhizae on growth, leaf yield, and phosphorus uptake in mulberry ( <i>Morus alba</i> L.) under rainfed, lateritic soil conditions. p. 98–103	Setua G C*, Kar R, Ghosh J K, Das K K, Sen S K. Central Sericultural Research and Training Institute, Mulberry Agronomy and Soil Science Section, Berhampore – 742 101, India
<i>Bioresource Technology</i> 69(3), 1999	The influence of vesicular–arbuscular mycorrhizal fungi alone or in combination with <i>Meloidogyne incognita</i> on <i>Hyoscyamus niger</i> L. pp. 275–278	Pandey R*, Gupta M L, Singh H B, Kumar S. Council of Scientific and Industrial Research, Central Institute of Medicinal & Aromatic Plants, PO Box CIMAP, Lucknow – 226015, India

\* The corresponding author

Name of the journal (volume no, issue no, and year)	Name of the article (page numbers)	Authors and correspondence address
<i>Canadian Journal of Botany - Revue Canadienne de Botanique</i> 76(11), 1998	Arbuscular mycorrhizal fungi associated with <i>Festuca</i> species in the Canadian high arctic. pp. 1930–1938	Dalpe Y* and Aiken S G. Agr. & Agri Food Canada, Research Branch, Eastern Cereal and Oilseed Research Center, Ottawa, ON K1A 0C6, Canada. <dalpey@em.agr.ca>
	Arbuscular mycorrhizae promote establishment of prairie species in a tallgrass prairie restoration. pp. 1947–1954	Smith MR*, Charvat I, and Jacobson R.L. University of Minnesota, Department of Plant Biology, 1445 Gortner Ave, St Paul, MN 55108 USA.
<i>European Journal of Forest Pathology</i> 29(2), 1999	Effect of three species of bacteria on damping-off, root rot development, and ectomycorrhizal colonization of lodgepole pine and white spruce seedlings. pp. 123–134	Pedersen E A*, Reddy M S, and Chakravarty P. Agrium Inc, Biol, 402-15 Innovat Blvd, Saskatoon, SK S7N 2X8, Canada.
<i>Forest Ecology and Management</i> 119(1-3), 1999	Fine-root biomass and necromass in limed and fertilized Norway spruce ( <i>Picea abies</i> (L.) Karst) stands. pp. 99–10	Helmisaari H S* and Hallbacken L. Finnish Forest Research Institute, Vantaa Research Centre, POB 18, FIN-01301 Vantaa, Finland.
	Growth stimulation of <i>Acacia mangium</i> Willd by <i>Pisolithus</i> sp. in some Senegalese soils. pp. 209–215	Duponnois R* and Ba A M. ORSTOM, Lab BioPedol, BP 1386, Dakar, Senegal. <duponnois@belair.orstom.sn>
<i>Journal of Ecology</i> 87(2), 1999	Competitive interactions between <i>Nardus stricta</i> L. and <i>Calluna vulgaris</i> (L.) Hull: the effect of fertilizer and defoliation on above- and below-ground performance. pp. 330–340	Hartley S E* and Amos L. Institute of Terrestrial Ecology, Banchory Research Station, Banchory AB31 4BY, Kincardine, Scotland.
<i>Journal of Plant Nutrition</i> 22(4-5), 1999	Effect of arbuscular mycorrhizal colonization of four species of <i>Glomus</i> on physiological responses of maize. pp. 783–797	Boucher A*, Dalpe Y, and Charest C. University Ottawa, Department of Biology, PO Box 450, STN A, Ottawa, ON K1N 6N5, Canada.
<i>Journal of Plant Nutrition and Soil Science - Zeitschrift Fur Pflanzenernahrung und Bodenkunde</i> 162(1), 1999	Sand culture of mycorrhizal plants. pp. 107–112	Jentschke G*, Brandes B, Heinzemann J, Marschner P, Godbold D L. University of Wales, School of Agriculture and Forest Science, Bangor LL57 2UW, Gwynedd, Wales.
<i>Molecular Ecology</i> 8(4), 1999	Molecular diversity of arbuscular mycorrhizal fungi colonising <i>Hyacinthoides non-scripta</i> (Bluebell) in a seminatural woodland. pp. 659–666	Helgason T*, Fitter AH, and Young JPW. University of York, Department of Biology, PO Box 373, York YO10 5YW, N Yorkshire, England.
<i>Mycorrhiza</i> 8(6), 1999	The influence of fire frequency an arbuscular mycorrhizal colonization in the shrub <i>Dillwynia retorta</i> (Wendland) Druce (Fabaceae). pp. 289–296	Torpy F R*, Morrison D A, Bloomfield B J. University of Technology Sydney, Department of Environmental Science, Westbourne St, Gore Hill, NSW 2065, Australia.
	Plant growth and ectomycorrhiza formation by transplants on deglaciated land near Exit Glacier, Alaska. pp. 297–304	Helm D J*, Allen E B, and Trappe J M. Univ Alaska Fairbanks, Agr & Forestry Expt Stn, 533 E Fireweed Ave, Palmer, AK 99645 USA.

Name of the journal (volume no, issue no, and year)	Name of the article (page numbers)	Authors and correspondence address
	Glomalean mycorrhizal fungi from tropical Australia I. Comparison of the effectiveness and specificity of different isolation procedure. pp. 305–314	Brundrett M C*, Abbot L K, and Jasper D A. CSIRO Forestry & Forest Prod, Centre for Mediterranean Agricultural Research, Private Bag PO, Wembly, WA 6014, Australia. <m.brundrett@cmar.csiro.au>
	Glomalean mycorrhizal fungi from tropical Australia II. The effect of nutrient levels and host species on the isolation of fungi. pp. 315–321	Brundrett M C*, Jasper D A, and Ashwath N. CSIRO Forestry & Forest Prod, Centre for Mediterranean Agricultural Research, Private Bag PO, Wembly, WA 6014, Australia. <m.brundrett@cmar.csiro.au>
	Influence of laser light on mycelial growth of <i>Hebeloma mesophaeum</i> and ectomycorrhizal development on Scots pine. pp. 323–327	Hilszczanska D*, Oszako T, and Sierota Z. Forest Research Institute Warsaw, Department of Forest and Phytopathology, Ul Bitwy Warszawskiej 1920 R 3, PL-00973 Warsaw, Poland.
	Effects of <i>Pseudomonas fluorescens</i> DF57 on growth and P uptake of two arbuscular mycorrhizal fungi in symbiosis with cucumber. pp.329–334.	Ravnskov S* and Jakobsen I. Riso National Laboratory, Plant Biology & Biochem Department, PBK-300, DK-4000 Roskilde, Denmark.
	Colonization potential of in vitro-produced arbuscular mycorrhizal fungus spores compared with a root-segment inoculum from open pot culture. pp. 335–338	Vimard B*, StArnaud M, Furlan V, Fortin J A. University of Montreal, Institut Recherche Biologie Vegetale, 4101 East, Sherbrooke Street, Montreal, PQ H1X 2B2, Canada.
<i>Oikos</i> 85(1), 1999	Small mammals, ectomycorrhizae, and conifer succession in beaver meadows. pp. 83–94	Terwilliger J* and Pastor J. Vermilion Community Coll, Ely, MN 55731, USA.
<i>Phytochemistry</i> 50(6), 1999	Sterol distribution in arbuscular mycorrhizal fungi. pp.1027–1031	Grandmougin Ferjani A, Dalpe Y, Hartmann M A, Laruelle F, Sancholle M* University Littoral Cote dOpale, Laboratory of Mycology, 17 Ave Bleriot, BP 699, F-62228 Colais, France.
<i>Plant and Soil</i> 207(1), 1998	Influence of arbuscular mycorrhizal fungi and simulated acid rain on the growth and coexistence of the grasses <i>Calamagrostis villosa</i> and <i>Deschampsia flexuosa</i> . pp. 45–57	Malcova R*, Vosatka M, and Albrechtova J. University, Faculty of Science, Department of Plant Physiol, CR-12844 Prague, Czech Republic.
<i>Plant and Soil</i> 206(2), 1998	Mycorrhizas and C and N transformations in the rhizospheres of <i>Pinus sylvestris</i> , <i>Picea abies</i> , and <i>Betula pendula</i> seedlings. pp.191–204	Priha O*, Lehto T, and Smolander A . Finnish Forest Research Institute, PO Box 18, FIN-01301 Vantaa, Finland.
<i>Plant and Soil</i> 206(1), 1998	Citrus root responses to localized drying soil: A new approach to studying mycorrhizal effects on the roots of mature trees. pp.1–10	Espeleta J F*, Eissenstat D M, and Graham J H. University of Georgia, Department of Botany, Athens, GA 30602 USA.

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

## RIZA Database

A bibliographic database on floppy disk/CD Rom has been developed by the Mycorrhiza Information Centre in TERI. It provides information on various aspects of mycorrhiza research covered in books, proceedings of conference, reports, and journals from 1984 onwards. It is so designed that information on a broad subject or its particular aspect can be retrieved easily.

### **Subscription rates**

Individual: Rs 2000/- • Institutional: Rs 5000/- • Annual update: Rs 500/-

## Mycorrhiza News

A quarterly newsletter of the Mycorrhiza Network, it covers state-of-the-art articles on mycorrhiza research, research papers on important breakthroughs/significant achievements in original research, brief accounts of new techniques, news from members, forthcoming events, and recent references on mycorrhiza. An update on Centre for Mycorrhizal Culture Collection is also provided.

### **Subscription rates**

India: Rs 100 (Individual/institutional) • Other countries: US \$40 (airmail)

Developing countries: US \$20 (Individual)

US \$30 (Institutional)

Please remit subscriptions by a bank draft/cheque (local cheques only) favouring TERI and payable at New Delhi.

### ***For more information, please contact***

The Editor, *Mycorrhiza News*

### ***For subscriptions, please contact***

Information Dissemination Services

Tata Energy Research Institute

Darbari Seth Block, Habitat Place, Lodhi Road

New Delhi - 110 003, India

Tel. +91 11 462 2246/460 1550 • Fax +91 11 463 2609/462 1770

E-mail [outreach@teri.res.in](mailto:outreach@teri.res.in) • Web site [www.teriin.org](http://www.teriin.org)



**Subscribe to RIZA database  
and avail one year FREE  
subscription to Mycorrhiza News**

**Editor:** Alok Adholeya

**Assistant Editors:** Anitha Saravanan, Subrata Deb