MYCORRHIZA NEWS

The Quarterly Newsletter of Mycorrhiza Network

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About TERI

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

TERI's Mycorrhiza Network

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

Mycorrhiza News

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

Contents

Research finding papers

Taxonomic series: other *Glomeromycotan* fungi from Goa

*Sharda W Khade**

1. *Ambispora gerdmannii* (Rose, Daniels,and Trappe) Walker, Vestberg, and Schuessler, in Walker, Vestberg, Demircik, Stockinger, Saito, Sawaki, Nishmura, and Schüßler

Mycological Research **111**: 137–153, 2007 (Plate 1)
= *Glomus gerdmannii Rose,* Daniels, and Trappe, *Mycotaxon* **8**: 297, 1979
= *Acrhaeospora gerdemanii* (Rose, Daniels, and Trappe) Morton and Redecker, *Mycologia* **93**: 186, 2001
= *Appendicispora gerdemannii* (Rose, Daniels, and Trappe) Spain, Oehl and Sieverding, in Spain, Sieverding and Oehl, *Mycotaxon* **97**: 174, 2006

The saccule is sporiferous, white to hyaline, globose to sub-globose, and 200–300 µm in diameter. Chlamydospores are formed singly in soil, globose, 160–240 µm in diameter (Plate 1[a]). The chlamydospore is cream in colour when young, turning orange to light brown. The spore wall structure consists of four layers. The outermost wall (Wall 1) is hyaline when young and turns orange to brown with age. It is friable and consists of wavy margins showing shallow ridges or fissures, and is 5–12 µm thick (Plate 1[b]). Wall 2 is 2–4 µm thick, has a smooth surface and is semi-flexible. Wall 3 is 1–3 µm thick and has smooth surface. It has crystalline properties and produces numerous fractured lines and polygonal shards when the spores are broken (Plate 1[b]). Wall 4 is smooth, hyaline, semi-flexible, 3–7 µm thick, and finely laminated. The contents of the spore are oily.

Distinguishing feature

Outermost spore wall consists of wavy margins and shows shallow ridges or fissures. Wall 3 has crystalline properties and produces numerous fractured lines and polygonal shards when the spores are broken.

Distribution

Recorded from Collem in December with 16.66% frequency of occurrence.

Association

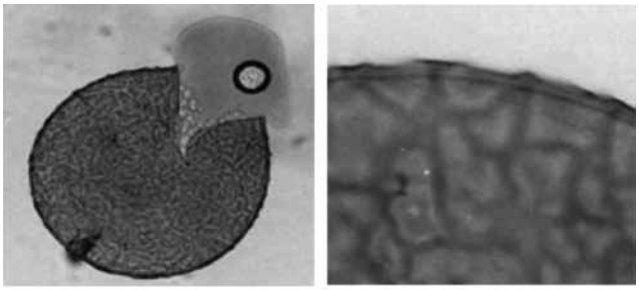
Found in association with *Carica papaya* L. plants from the Western Ghats of Goa, India.

2. *Racocetra gregaria* (Schenck and Nicolson) Oehl, de Souza, and Sieverding

Mycotaxon **106**: 311–360, 2008 (Plate 2)
= *Gigaspora gregaria* Schenck and Nicolson, *Mycologia* **71**: 178–198, 1979
= *Scutellospora gregaria* (Schenck and Nicolson) Walker and Sanders, *Mycotaxon* **27**: 169–182, 1986

Spores are found singly in the soil and are terminally to somewhat eccentrically on a bulbous sporogenous cell (Plate 2[a]). They are reddish brown to dark brown in colour, globose to subglobose, and 250–350 µm in

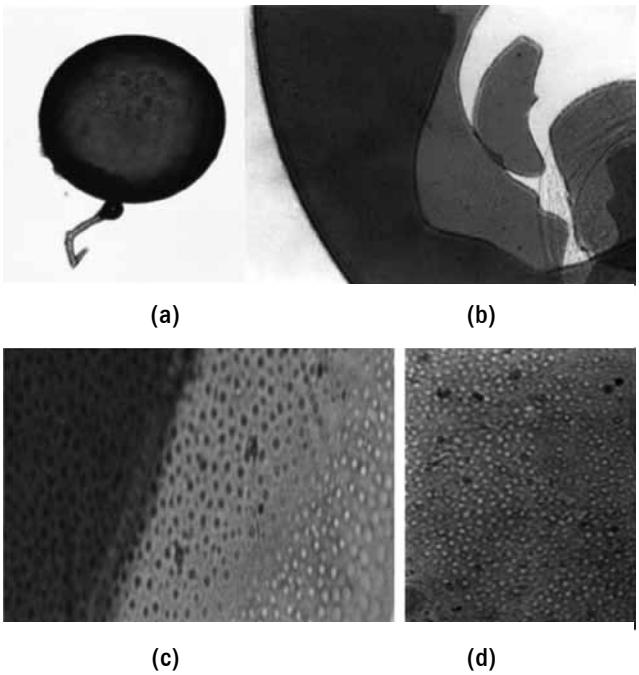
* Dr Khade S W, Department of Botany, Goa University, Taleigao plateau, Goa – 403 206, India
E-mail: shardakhade12@rediffmail.com



(a) (b)

Plate 1(a) A crushed spore of *Ambispora gerdmannii* with globular spore contents (× 200).
Plate 1(b) A portion of spore devoid of its contents, showing ridges or deep fissures in the outermost spore wall, along with fractured lines and polygonal shards of Wall 3 on the spore surface (× 400).

diameter. The spore wall consists of four sub-walls (1–4) divided into two groups (A and B). Wall group A (Plate 2[b]) is composed of three closely appressed walls—an outer unit wall (Wall 1) and two laminated walls (Wall 2 and Wall 3). Wall 1 is brittle, brown, 1–3 µm thick excluding the closely packed warts situated on its outer surface (Plate 2[c] and 2[d]). The warts are pale brown to brown in colour, 2–4 µm high with rounded tips (Plate 2[d]), 4–7 µm diameter at the base, and are crowded together in groups. Wall 2 is brittle, yellow, 3–5 µm thick, Wall 3 brittle, pale yellow to nearly colourless, 5–13 µm thick. Group B of a single membranous hyaline wall (Wall 4) 1–2 µm thick enclosing the spore contents. The sporogenous cell is borne terminally on the septate subtending hyphae (Plate 2[a]). The sporogenous cell is pale brown, 45–78 µm wide, with one or two thick- or thin-walled hyaline projections that are borne terminally. Walls of the sporogenous cell are 2–4 µm thick. The subtending hypha is 4–8 µm in diameter. The germination shield is globose to sub-globose, irregular, pale brown, and 85–100 × 144–156 µm thick.



(a) (b)

(c) (d)

Plate 2(a) A spore of *Racocetra gregaria* with vertically attached bulbous suspensor (× 100).
Plate 2(b) A portion of spore of *Racocetra gregaria* showing group wall A (× 400).
Plate 2(c) Spore wall of *Racocetra gregaria* showing warts (× 1000).
Plate 2(d) Spore wall of *Racocetra gregaria* showing warts (× 1000).

Distinguishing feature

Presence of small warts on the spore surface.

Distribution

Recorded from (1) Valpoi, Old Goa, Collem, and Quepem in December with 66.66% frequency of occurrence; (2) Valpoi in July and October with 37.50% frequency of occurrence; (3) Kodar in January and October with 25% frequency of occurrence; (4) Old Goa in January, April, July, and October with 62.50% frequency of occurrence; and (5) Old Goa in June, July, August, and October with 80% frequency of occurrence.

Association

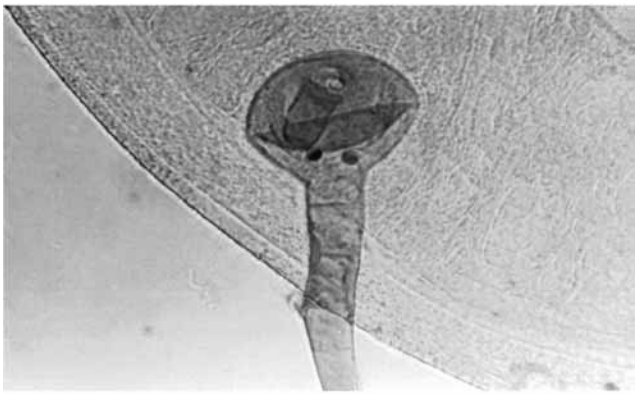
Found in association with *C. papaya* plants from Western Ghats, plateaus and coastal areas of Goa, India.

3. Racocetra verrucosa (Koske and Walker) Oehl, de Souza, and Sieverding

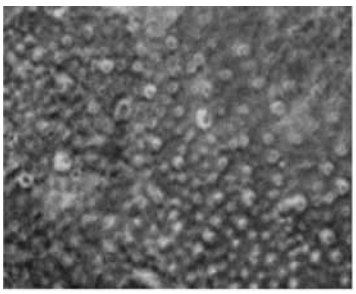
Mycotaxon **106**: 311–360, 2008 (Plate 3)
=*Gigaspora verrucosa* Koske and C Walker, *Mycologia* **77**: 702–720, 1985
= *Scutellospora verrucosa* (Koske and Walker) Walker and Sanders, *Mycotaxon* **27**: 169–182, 1986

The spores are formed singly in soil, and terminally or somewhat laterally on a bulbous sporogenous cell. They are pale straw yellow to dark yellow in colour, globose to sub-globose, and 220–360 µm in diameter. The spore wall of the three walls (1–3) is divided into two groups (A and B) (Plate 3[a]). Group A has 2 walls (Wall 1 and Wall 2). Wall 1 is hyaline to pale yellow in colour, brittle, and ornamented. Wall 2 is

(a)



(b)



(c)

(d)

Plate 3(a) A portion of spore of *Racocetra verrucosa* with two wall groups (× 100).
Plate 3(b) Sporogenous cell of *Racocetra verrucosa* (× 200).
Plate 3(c) Warts on the spore surface of *Racocetra verrucosa* (× 1000).
Plate 3(d) Germination shield in *Racocetra verrucosa* (× 400).

3 µm thick including the crowded, low, rounded warts (Plate 3[c]), mostly 0.5–1.5 × 0.5–1.5 µm at the base, 0.5–1.5 µm high, spaced 1–3 µm apart on the surface, and tightly adherent to the inner wall (Wall 2). Wall 2 is pale yellow to orangish brown in colour, laminated, and 4–10 µm thick. Group B consists of a single membranous hyaline wall (Wall 3), which is 0.5–1 µm thick. The sporogenous cell is borne terminally on a coenocytic to sparsely septate subtending hyphae. The sporogenous cell is yellowish brown and often darker than the spore (Plate 3[b]), 50–65 µm diameter with one or two thick-walled peg-like hyphal projections that are 20–45 × 6–10 µm thick. The subtending hypha is 4–6 µm in diameter. The germination shield is dark brown, often darker than the spore, oval in shape, 69 × 114 µm thick. The germ tube emerges from the germination shield (Plate 3[d]).

Distinguishing feature

Wart-like structures on outer spore surface.

Distribution

Recorded from (1) Kodar in January with 12.5% frequency of occurrence; (2) Old Goa in April with 62.50% frequency of occurrence; (3) Old Goa in June, August, and September with 75% frequency of occurrence.

Association

Found in association with *C. papaya* plants from plateau region and coastal areas of Goa, India.

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Mycorrhizal cheating: mutualistic facultative myco-heterotrophy in Chameleon plant (*Houttuynia cordata* Thunb.)

*Vipin Parkash** and *Priya Dhungana*

Introduction

Houttuynia cordata Thunb. (Chameleon plant) is an aromatic ethnomedicinal plant of lizard's tail family (Saururaceae). It is a species native to Japan, South-East Asia, and the Himalayas (Watson and Dallwitz 1992). It is known as *Jamyrdoh* in *Khasi* dialect and is traditionally used by the *Khasi* tribe of Nongkhyllem Reserve Forest Nongpoh, Meghalaya, India, in treating cholera, dysentery, stomach infection, and flatulence. The whole plant is also consumed in the local domestic culinary preparation. It is a rhizome-bearing aromatic medicinal herb with creeping root stock, broad leaves, ovate–cordate, naked flowers in dense spikes that are subtended by four white and petalloid bracts (Bora 2001), and is restricted to specialized moist habitats. The root, young shoots, leaves, and sometimes the whole plant are traditionally used to cure various diseases throughout South-East Asia. It is a well-known and traditionally used medicinal material in China and Japan and is listed in the Chinese Pharmacopoeia.[1] In Indo–China, the entire plant is considered for cooling and emmenagogue. The leaves are recommended for measles, dysentery, and gonorrhea and are used in the treatment of eye troubles, skin diseases, hemorrhoids, and in certain diseases that strike only women. Some recent findings on its medicinal properties are anti-inflammatory (Lu, Liang, Yi, *et al.* 2006), anti-allergic (Kim, Kim, Kim, *et al.* 2007), virucidal (Chiang, Chang, Chen, *et al.* 2003), anti-oxidative (Ng, Yen, Liao, *et al.* 2007), and anti-cancer (Chang, Chiang, Chen, *et al.* 2001; Kim, Ryu, No, *et al.* 2001).

Recently, during the period of SARS outbreak, *H. cordata* was one of the ingredients in the SARS prevention formulae recognized by the Ministry of Public Health and the State Administrative Bureau of Traditional Chinese Medicine [TCM] (2003). The plant exhibited significant inhibitory effects on SARS–CoV 3C-like protease (3CLpro) and RNA-dependent RNA polymerase (RdRp), which was non-toxic to laboratory animals following oral administration at 16 g/kg (Lau, Lee, Koon, *et al.* 2008). The steam distillate of fresh *H. cordata* could inhibit herpes simplex virus type-1 (HSV-1), influenza virus, and human immunodeficiency virus type-1 (HIV-1) without showing cytotoxicity (Hayashi, Kamiya, and Hayashi 1995). Traditionally, the aerial part of the plant is used for medicinal purposes while the root portion was recently considered usable in the Chinese Pharmacopoeia.[2]

The assessment of availability, ecological feature, and habitat preference of the medicinal herb *H. cordata* Thunb. in the Brahmaputra Valley of Assam, India, was carried out by Bhattacharya and Sarma (2010), but little or almost no work was carried out on the rhizospheric mutualistic facultative myco-heterotrophy. Thus, the present investigation attempted to provide mutualistic facultative myco-heterotrophy details of *H. cordata* through assessment of natural occurrence of endomycorrhizal status by qualitative and quantitative analyses.

Materials and methods

Soil sample collection

Rhizospheric soil samples of the roots/rhizomes were collected from road-side streams and moist and damp benches from the Nongkhyllem Reserve Forest Nongpoh, Meghalaya, during August–September 2010 by digging out a small amount of soil close to plant roots up to a depth of 15–30 cm. These samples were kept in sterilized polythene bags in the laboratory at 5–10°C for further processing and for mycorrhizal quantification and root colonization.

Collection of spores

Collection of vesicular–arbuscular mycorrhiza (VAM) spores was done by wet sieving and decanting (Gerdemann and Nicolson 1963) technique. Approximately 100 g of soil were suspended in ≥1 litre of water. Heavier particles were allowed to

* Corresponding author: Rain Forest Research Institute (ICFRE), Autonomous council of Ministry of Environment and Forests, Government of India, Deovan, Jorhat – 785 001, Assam, India.
Email: bhardwajvpnpark@rediffmail.com

[1] The Pharmacopoeia Committee of China 2005

[2] The Pharmacopoeia Committee of China 2005

settle for a few seconds to overnight and the liquid was decanted through sieves of different sizes in the order 150 µm, 120 µm, 90 µm, 63 µm, 45 µm, and 20 µm, removing large particles of organic matter but coarse enough to allow the desired spores to pass through. The sieving retained on different sieves was collected on Petri dishes. The trapped spores were then transferred to Whatman filter paper number 1 by repeated washings with water. The spores were picked by hydrodermic needles under stereo-binocular microscope.

Arbuscular mycorrhizal quantification

For quantitative estimation of VAM spores, the Gaur and Adholeya (1994) modified method was used. The filter paper was divided into many small sections by marking with a ballpoint pen. The total number of spores was counted by adding the number of spores present in each sector under stereo-binocular microscope.

Identification of VAM fungi

For the identification of AM spores, the criteria used were conventional morphological character (that is, colour, size, shape, wall structure, surface, ornamentation of spores), nature and size of subtending hyphae, bulbous suspensor, the number and arrangement of the spores in the sporocarp. These VAM spores were identified by using the keys of Schenck and Perez (1990); Morton and Benny (1990); and Mukerji (1996).

Clearing and staining of root segments

Rhizospheric root samples were stained according to the Phillips and Hayman (1970) method. The roots were first cleaned with 10% (w/v) KOH at 98°C for 1 hour. The roots were then bleached with 5% H_2O_2 (v/v) solution for 10 minutes. All samples were acidified with 1% (v/v) HCl for 5 minutes and stained with 0.05% (w/v) Trypan Blue in acidic glycerol by heating them at 98°C for 15 minutes. The stained roots were stored in acidic glycerol for further study.

Analyses of the root samples

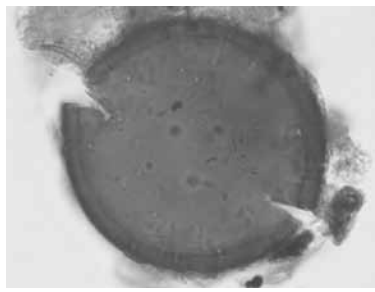
Ten stained root pieces, approximately 1 cm long, were mounted on a slide in glycerol and were examined with a light trinocular microscope (Labovision, BIOXL). A total of three replicates were made. Typical structures that indicated the presence of mycorrhizae or other root-associated fungi were documented and micro-photographed with a light trinocular microscope attached digital camera (Canon make A3100IS) and Image-Zoom Browser Ex analysis software for Windows 2008. The mycorrhizal type present in each sample was designated according to Harley and Smith (1983) classification. The criteria used in this study for the determination of arbuscular mycorrhiza (AM) was the presence of vesicles, arbuscules, and mycelium in at least one individual root segment and the occurrence of typical AM structures in the rest of the samples (intracellular or intercellular hyphae, vesicles, coils, and arbuscules), which were classified as Arum or Paris-type (Smith and Smith 1997). The intensity of length colonized by arbuscular mycorrhizal fungi (AMF) was estimated according to the following formula.

$$\text{Intensity of length colonized} = \frac{\text{Total length of mycorrhizal infection}}{\text{Total length of root segments}} \times 100$$

Results and discussion

The rhizospheric sample of this plant was collected during August–September 2010 to study its mutualistic facultative myco-heterotrophic nature. The population of *H. cordata* was observed to occur only in shady and well-moistened places under a canopy of trees or on the forest floor (Plate 1[a]). The soil properties are important as the plant studied was from moist and marshy habitats. Therefore, the physical properties of the soil were analysed. The AM fungal diversity, spore count, and per cent root colonization in *H. cordata* Thunb. are shown in Table 1. The temperature of soil was 20.5°C ± 0.0

(a)



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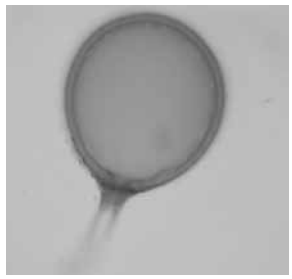

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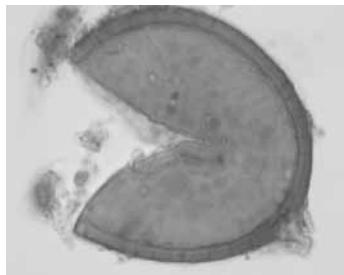

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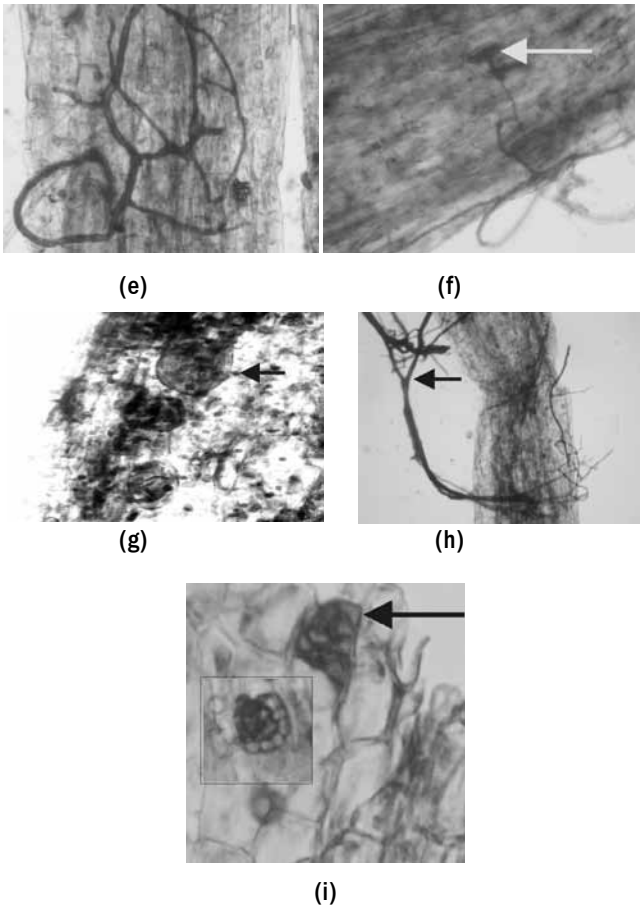

Plate 1(a) *Houttuynia cordata* habitat (inset: flower).
Plate 1(b) *Glomus* sp. (× 400).
Plate 1(c) *Glomus pallidum* arrow showing flat septum (× 400).
Plate 1(d) *Glomus clarum* (× 400).
Plate 1(e) Extraradicle mycelia on the root surface (× 100).
Plate 1(f) Mycelium entering the root hair and forming arbuscule inside the cortex (× 100).
Plate 1(g) Round to elliptical vesicles (× 100).
Plate 1(h) Arrow shows a typical peloton in a cortex cell (× 400; inset: typical hyphal loop/coils, × 400).
Plate 1(i) External mycelium around the root (× 100).

and the pH was slightly acidic (6.89 ± 0.0). The quantitative analysis of *H. cordata* was examined for endomycorhizal association. The spore quantification of the rhizospheric sample was 40 spores/25 g of soil and the diversity of endomycorrhiza includes three genera (that is, *Glomus, Acaulospora*, and *Gigaspora*). The *Glomus* species (that is, *G. albidum, G. clarum, G. geosporum, G. pallidum, G. macrocarpum*) were more dominant than the *Acaulospora* species (that is, *A. laevis*) and the *Gigaspora* species (Plate 1[b]–1[d]).

After qualitative analysis, it was found that all the root segments were colonized by symbiotic endomycorrhizae. The per cent external mycelium colonization was 95% whereas that of internal mycelium colonization was 100%. Most of the internal colonization was of Paris type and typical peloton-like arbusculate infection (mostly reticulate) (70%) was present in the cortical cells, while the vesiculate type of infection (20%) was mostly of elliptical shape. The intensity of mycorrhizal colonization in roots, whether internal or external, was more prevalent (100%) and traverse profusely in the cortical region (Table 1; Plates 1[e]–1[i]).

Many scientists and researchers worked on the natural association of mycorrhizal fungi on different types of vegetations (Trimurtulu and Johri 1998; Muthukumar and Udaiyan 2001; Brundrett, Ashwath, and Jasper 1996; Brundrett 1991; Bagyaraj 1992). A survey of the AMF status of plants growing in Western Ghats regions of southern India was undertaken by Muthukumar and Udaiyan (2000). They examined the root and soil samples of plants growing in four vegetation types—forest, grassland, scrub, and cultivated lands. VAM association was recorded in several families that were assumed to be non-mycorrhizal in nature, for example, Amaranthaceae, Capparidaceae, Commelinaceae, Cyperaceae, and Portulacaceae. They also found VAM fungal species highest richness in scrub and lowest in agriculture and plantation soil.

Occurrence of AMF in rhizosphere soils of some medicinal plants was also studied by Babu

Table 1 Endomycorrhizal diversity, spore count, and per cent root colonization in *H. cordata* Thunb.

Plant name	Intensity of length colonization[a]	Per cent of root colonization[a]				Spore count (per 25 g of soil)[a]	Physical analysis[a]		Diversity of spore[a]
		External	Internal	Arbusculate	Vesiculate		pH	Temp. (°C)	
Houttuynia cordata Thunb.	100 ± 0.0	90 ± 0.0	100 ± 0.0	70 ± 0.0	20 ± 0.0	40.5 ± 8.55	6.89 ± 0.0	20.5 ± 0	Ga, Gc, Gp, Gg, Gma, Gm, Al, Gis

± – standard deviation; Ga – G. albidum Walker and Rhodes; Gc – G. clarum Nicol. and Schenck; Gg – G. geosporum (Nicol. and Gerd.) Walker; Gma – G. macrocarpum Tulasne and Tulasne; Gm – G. mossae (Nicol. and Gerd.) Gerd. and Trappe; Gp – G. pallidum Hall; Al – Acaulaspora laevis Gerdemann and Trappe; Gis – Gigaspora sp Gerd. and Trappe
[a] Average of five replications

and Manoharachary (2003). Distribution of AMF in the rhizosphere soils of betel vine was studied by Dwivedi, Yadav, Vyas, *et al.* (2003). VAM association of medicinal plants from the Maruthamalai hills of Western Ghats was examined by Muthukumar and Udaiyan (2001). The results of the study indicated that soil phosphorus influences mycorrhizal root colonization.

Similarly, it was found in this study that the roots of the Chameleon plant were heavily infected by endomycorrhizal fungi. Typical peloton-like arbusculate (reticulate) and vesiculate structures were seen in the root cortical region. As reported by Leake (1994), the facultative (or partial) myco-heterotrophy exists when a plant is capable of photosynthesis, but parasitizes fungi as a supplementary food supply. This plant is also capable of photosynthesis, but parasitizes and forms symbiosis with endomycorrhizae for supplementary food supply as it is evident from its endomycorrhizal colonization and typical peloton-like arbusculate (reticulate) and vesiculate structures present in the root cortical region. Peloton structures are mostly seen in Orchids root infections. The roots of orchids join with mycorrhizas, which play a critical role in the orchid development. Mycorrhizal fungal associations with orchids are of particular interest because of their essential role in seed germination and protocorm development and their similarity in many respects to biotrophic fungal infections. The majority of orchids are photosynthetic at maturity. However, more than 100 species of orchids are completely dependent on their fungal partners throughout their lifetime (Dearnaley 2007). These mycorrhizal fungi are characterized by forming fungal coil formation known as Pelotons, which are to be digested by the host cells resulting in a degenerated hyphal mass surrounded by host plasma membrane (Senthilkumar, Krishnamurthy, John Britto, *et al.* 2000).

From the present study, it is envisaged that the Chameleon plant has a great ethnomedicinal importance as it is widely used for different ailments by tribal people. Besides traditional and commercial importance, it has tremendous ecological significance because of its mutualistic facultative myco-heterotrophic nature and soil-binding abilities in its rhizosphere due to endomycorrhizal fungi. The pattern of typical mycorrhizal colonization shows the mutualistic facultative myco-heterotrophic nature of this herb, known as "myco-heterotroph" or "mycorrhizal cheater".

Acknowledgement

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Diversity of arbuscular mycorrhizal fungi associated with the rhizosphere of tea [*Camellia sinensis* (L.) O. Kuntze] plantation in upper Assam, India

D S Toman and D K Jha#*

Introduction

Arbuscular mycorrhizae (AM) are generally aseptate fungi classified under Glomeromycota. Arbuscular mycorrhizal fungi (AMF) have a loose external mycelium, extending the hyphae into the soil beyond the depletion zone, from which penetration of the host root cells takes place (Baruah and Borthakur 1997; Jacobsen 2004). The well-known effect of soil microorganisms includes mineralization of organic matter and release of nutrient elements for plant use, production of growth substances, nitrogen fixation, ammonification, nitrification, and improvement in nutrient uptake. Interrelation of these fungi with their host plants may be important in determining the welfare of the hosts. Growth of many plant species maybe enhanced in poor soils by adding spores of the AMF to the soil, which in turn mobilizes and transports nutrients (Ravikumar, Ananthakrishnan, Appasamy, *et al.* 1997; Kelly, Morton, and Cumming 2005).

Tea, a most popular non-alcoholic beverage, is made from the processed young leaves of *Camellia sinensis*. India is the foremost producer, consumer, and exporter of tea. Though little research work has been carried out on the subject (Singh, Pandey, Chaurasia, *et al.* 2008; Pandey and Palni 1996, 2004), the role played by AM fungi in tea soil is not clear and needs extensive study. The general outcome of this kind of symbiosis in other crops (Singh and Jha 2005) has tempted workers to believe in similar probabilities in tea. There is enough scope to exploit AMF to its maximum advantage in the improvement of growth, yield, and quality of tea cultivars, especially in the extensive tea-growing belt of Assam (that is, the Brahmaputra Valley). Therefore, keeping the above-mentioned significance of AMF association in mind, an attempt has been made to study the occurrence and distribution of AMF in the roots of *C. sinensis* (L.) O. Kuntze, in the agroclimatic zone referred above.

Materials and methods

Study sites

The present study was carried out in the Upper Brahmaputra Valley Zone (Figure 1), basically dominated by commercial tea cultivation and comprising five administrative districts of Assam (Tinsukia, Dibrugarh, Sivasagar, Jorhat, and Golaghat). Three tea gardens from each district were selected randomly for the present investigation.

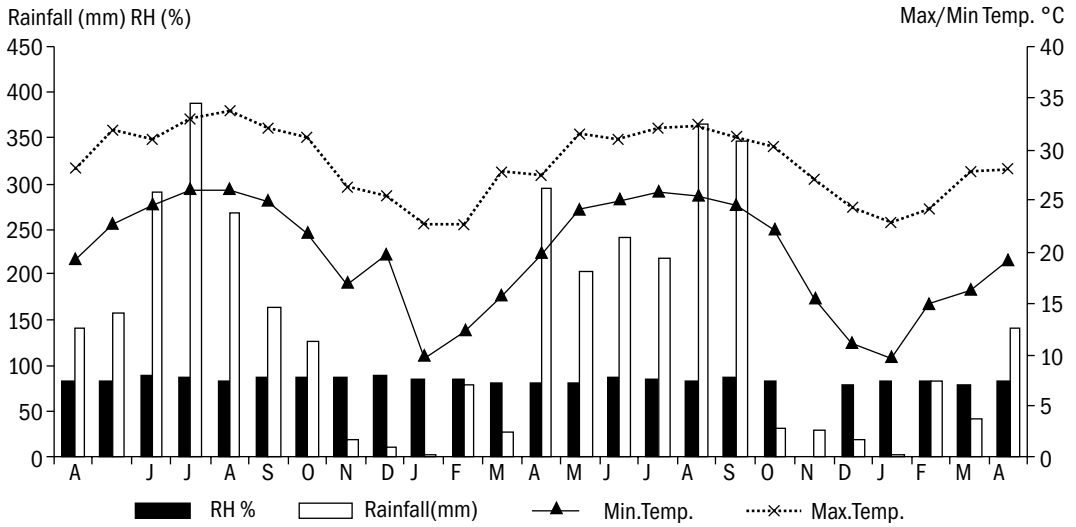

Figure 1 Average meteorological data of the Upper Brahmaputra Valley Region
Courtesy: Assam Agricultural University, Jorhat, Assam

* Department of Botany, DCB Girls' College, Jorhat – 785 001, Assam, India (*E-mail* tomanrds@yahoo.co.in)
\# Department of Botany, Gauhati University, Assam – 786 004, India

Methods

Collection of root and soil samples

To demonstrate true seasonal patterns of infection and spore distribution, the study was continued for three years—October 2006 to September 2009. From each tea garden, three samples of tea root and soil were collected at random. Each sample had five replicates. Fine roots of mature trees were excavated after tracing larger roots from the stem collar of tea bush. These replicates were later bulked together and kept for further study. Soil samples (~200 g) were collected from 0–10 cm depth in the adjoining regions of the roots as most spores were reported to be concentrated in this zone (Ingleby, Diagne, Deans, *et al.* 1997). They were packed in polythene bags, along with the roots, and kept in a refrigerator in the laboratory until they were analysed.

Physico-chemical analysis of soil

The soil pH was measured with a digital pH meter. Soil organic carbon was determined by wet digestion method (Walkley and Black 1934), available nitrogen was estimated by alkaline potassium permanganate method (Subbiah and Asija 1956), available phosphorus was determined after Brary and Kurtz (1945), and potassium was estimated following Schollenberger and Simon (1945).

Mycorrhizal infection

Trypan blue method (Phillips and Hayman 1970) was used for the determination of the intensity of root infection. This method was also suitable for the determination of the rate of colonization and comparison of structures. The colonization was calculated using the following formula.

$$\text{Per cent colonization} = \frac{\text{Total number of root segments colonized}}{\text{Total number of root segments examined}} \times 100$$

Spore extraction and identification

Spore extraction from the soil was carried out using wet sieving and decanting technique (Gerdemann and Nicolson 1963). Sieves of sizes ranging from 200 µm to 46 µm were used to separate the spores from the soil samples. The sieved spores were placed in water and observed with a stereo-zoom microscope under reflected light. The spore density was expressed as the total number of spores occurring in 50 g of soil. The spores were then observed for distinguishing morphological characters such as size, shape, and wall characteristics under a compound microscope (model: Leica DME). The spores were identified according to the manual of identification of vesicular–arbuscular mycorrhizal (VAM) fungi by Schenck and Perez (1990). The INVAM worksheet for spore characterization was used for diagnosing the spores. Additional species not included in the manual were identified as per the description given in the INVAM website.

Results and discussion

Pioneer research work on VAM in tea was conducted by Tunstall (1926, 1930), who reported that the young tea roots were associated with vesicular–arbuscular endotrophic mycorrhiza. The mycelia were found extended in the cell of the primary cortex, except in the root tips. Similarly, *Glomus, Acaulospora, Gigaspora* were identified in the tea soil.[1]

The present study showed significant variation in root colonization percentage and spore accumulation as well, in dormant and active growth seasons (April–September). However, comparatively higher root colonization was recorded during dormant period (October–March).

Colonization of AMF in the present study was found to be significantly influenced by seasonal changes and more or less by the physico-chemical characteristics of soil (Table 1). During the *Banji* stage or dormant period, both spore density and root colonization were recorded higher than the active growth period (April–September) in all the studied areas (Figures 2 and 3). While Golaghat district showed highest spore density, Sivasagar district had the lowest. Interestingly, considerably higher root colonization could be seen in the Sivasagar district compared to Golaghat.

Overall 20 AMF morphotypes were detected from these study areas. The genus *Glomus* was found to be dominating in the upper Brahmaputra Valley, along with its 15 morphotypes. *Acaulospora* is the second moderately distributed genus, along with its five morphotypes. Impact of land use pattern may

Table 1 Physico-chemical analysis of soil

District	pH	Organic carbon (%)	Average P (ppm)	Average K (ppm)	Average N (%)
Tinsukia	5.6	1.69	12.14	60	0.189
Dibrugarh	5.6	1.72	14.34	55	0.193
Sivasagar	5.4	1.12	8.09	51	0.145
Jorhat	5.5	1.09	6.09	22	0.129
Golaghat	5.6	1.82	6.05	60	0.198

[1] Annual Report Tea Research Association, Tocklai Experimental Station, 1981, 1982; 44, 57

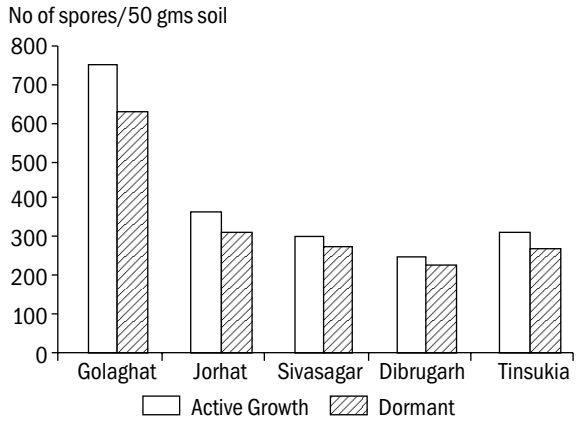

Figure 2 Number of arbuscular mycorrhizal fungi spores per 50 g of soil

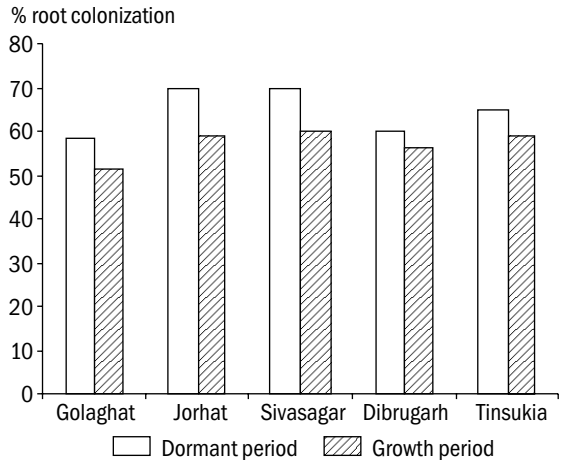

Figure 3 Per cent root colonization in different districts

have certain effects on the diversity and tolerance of AMF population (Oehl, Sieverding, Ineichen, *et al.* 2003). Similarly, regular application of higher doses of nitrogen and phosphorus fertilizer also decreases the number of large-spored species (Egerton-Warburton, Graham, Allen, *et al.* 2001) such as *Gigaspora*. The dominance of *Glomus* supports the earlier explanation regarding the phenomenon of selection pressure, where various agricultural practices might have induced tolerance in selective genus. However, the present investigation observed a contrasting finding to Singh, Pandey, Chaurasia, *et al.* (2008), where 51 AMF morphotypes were detected in tea soil.

Various edaphic factors also seem to play a significant role in the distribution of soil microflora in tea. The commercial tea plantations, where application of chemical fertilizer is a regular occurrence, also influence the AMF population. The present study indicates that higher NPK fertilizer concentrations decrease AMF population in Sivasagar and Dibrugarh districts. A similar report was presented by Barthakur, Dutta, Sarmah, *et al.* (2005). Lesser spore density in Sivasagar district may suggest a stress situation (Sastry and Johri 1999) due to oil exploration and drilling activities. Higher temperature and rainfall (during the active growth period) seems to be ideal situation when soil microflora establish themselves; density and root colonization further increase in the subsequent *Banji* period. The studied areas had pH ranges of 5.0–5.6, which was quite favourable for the growth of AMF (Singh, Pandey, Chaurasia, *et al.* 2008).

Conclusion

Following are the important experimental findings of the present investigation.

- The genus *Glomus* is a common species that occurs widely in the soils of tea plantations in the upper Brahmaputra Valley.
- The AMF population considerably increased during the dormant period.
- The over-application of NPK fertilizers has a negative impact on the AMF population.
- Oil exploration activities may also have certain effect on soil microflora.

North-east India is regarded as one of the richest biodiversity hotspots in the world. However, little attention has been given to the microflora of this region, which contributes silently in various ways to the ecosystem management process. These associations have great economic and agricultural significance, leading to ecological success for both fungus and plants to exploit habitats in which neither may grow successfully. Thus, the successful establishment and vigorous growth of many major groups of plants, including crop species, is favoured by mycorrhiza formation (Cooke 1977). Therefore, a comprehensive and detailed study of the microflora is required to identify, assess, and utilize the same for future generation. A well-organized web-enabled database, including physical and genetical characterizations, backed by an active user-friendly retrieval system may be useful to evaluate nature's unique bioresources for mankind.

Acknowledgement

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Website

http://invam.caf.wvu.edu/Myc_Info/ Taxonomy/species.htm

Growth and secondary metabolite in mycorrhiza-treated Ashwagandha plants

*Milan M Chandarana and Y T Jasrai**

Introduction

Mycorrhiza is a symbiotic association between plant root and fungi. This symbiosis is characterized by the bidirectional movement of nutrients as carbon flows to the fungus and inorganic nutrients move to the plants. Arbuscular mycorrhiza (AM) plays a key role in increasing nutrient uptake (Baqual, Das, and Katiyar 2005), drought tolerance (Devachandra, Patil, Patil, *et al.* 2008), and well-developed root system (Bhattacharya, Paul, Saha, *et al.* 2002). Moreover, it was reported that there is a positive correlation between fungal infection and accumulation of secondary metabolites in roots (Maier, Peipp, Schmidt, *et al.* 1995; Abu-Zayed, Khan, and Khoo 1999).

Ashwagandha (*Withania somnifera* L) is an important medicinal plant of India belonging to family Solanaceae. It is also known as Indian ginseng and its roots are the source of certain drugs. The annual production of these drug is 14 000–15 000 tonnes both from wild as well as cultivated sources. The present market rate of Ashwagandha roots varies ₹70–80 per kg. Withanolides A, Withaferin A, and 12-deoxywithastramonolide are the active constituents obtained from the roots and leaves of this plant. Withaferin A shows antioxidant activity (Bhattacharya, Satyan, and Ghosal 1997) and anti-proliferative activity on human tumor cell lines (Jayaprakasham, Zhang, Seeram, *et al.* 2003). Kuboyama, Tohda, and Komatsu (2005) reported that Withanolide A induces neurotic regeneration and synaptic reconstitution.

The study was initiated with an aim to study the effect of applied mycorrhiza on plant growth and accumulation of secondary metabolites in roots of Ashwagandha (*Withania somnifera* L).

Materials and methods

Ashwagandha (*W. somnifera* var. WS-134) saplings were brought from the Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand. The saplings were transplanted in pots containing garden soil and farmyard manure (1:1 v/v). Mycorrhizal consortium (K C P Sugar Industries Corporation Ltd. Vuyyuru based on TERI - DBT) was applied as per the manufacturer's instructions to one set of plants. Saplings in the same conditions but without mycorrhizal treatment served as the control. Both sets were kept in the botanical garden of the Department of Botany, Gujarat University, Ahmedabad.

Quantification of growth

After 120 days of plantation, both the control and AM-treated plants were uprooted carefully without damaging the root system and growth parameters like shoot length; number of branches; root length; fresh weight and dry weight of root, shoot, and leaves; and chlorophyll content were recorded. For chlorophyll, 100 mg of freshly harvested leaf tissue (without mid-vein) was crushed in 80% acetone and quantified (Arnon 1949).

HPLC analysis

HPLC analysis was carried out, along with three standards, namely, Withanolides A, Withaferin A, and 12-deoxywithastramonolide at the Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand.

Quantification of root colonization

Freshly harvested roots were carefully washed with running tap water, and AM fungal infection in roots was determined (Sylvia 1994). The percentage colonization by AMF was determined (Nicholson 1960).

$$\text{Per cent root colonization} = \frac{\text{Number of infected segments}}{\text{Total number of segments observed}} \times 100$$

Results and discussion

The results revealed a significant increase in the growth of AM-inoculated plants over control plants after 120 days (Table 1). Numbers of leaves and branches were 23.24% and 58.27% more in AM-inoculated plants over control plants, respectively. However, there was no significant difference in the shoot length and shoot fresh/dry weight of both sets of plants. The chlorophyll content was 27.22% more in

* Department of Botany, School of Sciences, Gujarat University, Ahmedabad – 380 009, India

Table 1 Growth differences in AM-treated and non-treated plants after harvesting

	Treatment	
Growth parameter	**Control**	**AM**
Shoot length (cm)	11.975 ± 1.253	10.896 ± 1.386
Root length (cm)	11.109 ± 0.448	11.568 ± 0.529
Number of leaves	29.65 ±6.059	36.54 ± 7.231
Number of branches	0.937 ± 0.731	1.483 ± 0.907
Dry weight (g)		
Root	0.186 ± 0.036	0.21 ± 0.069
Shoot	0.292 ±0.059	0.263 ±0.074
Leaves	0.203 ±0.056	0.361 ± 0.041
Fresh weight (g)		
Root	1.124 ±0.161	1.526 ± 0.135
Shoot	1.426 ± 0.112	1.331 ± 0.159
Leaves	1.461 ± 0.109	1.931 ± 0.144
Chlorophyll content (mg/g)	11.109 ± 0.015	14.133 ± 0.186

AM-inoculated plants than in non-inoculated plants. The fresh weights of root and leaves were 35.77% and 32.17% more in AM-inoculated plants than in non-inoculated plants. The dry weights of root and leaves were 12% and 77.83% more in AM-inoculated plants than in non-inoculated plants. The root length was 4.13% more in AM-inoculated plants than in non-inoculated plants.

HPLC analysis

Quantification of active compounds with three standards—Withaferin A, 12-deoxywithastramonolide, and Withanoloid A—clearly indicated that Withanoloid A and 12-deoxywithastramonolide were significantly high in mycorrhizal plants (Figure 1). Withanoloid A was 28.29% and 12-deoxywithastramonolide was 53.77% high in mycorrhizal-treated plants than in control plants. It was also noted that Withaferin A was 15.18% low in mycorrhizal plants than in control plants.

The high percentage of root colonization (72%) in AM-inoculated plants must have resulted in better growth of Ashwagandha plants and production of secondary metabolites like Withaferin A and 12-deoxywithastramonolide. Maier, Peipp, Schmidt, *et al*. (1995) observed that the terpenoid glycoside accumulation occurred in cereals that were inoculated with AM. Moreover, the level of this compound was directly correlated with the degree of root colonization. Srimathi and Kumutha (2009) also observed that AM enhanced the yield of the secondary metabolite forskohlin alkaloid in *Coleus forskohlii*.

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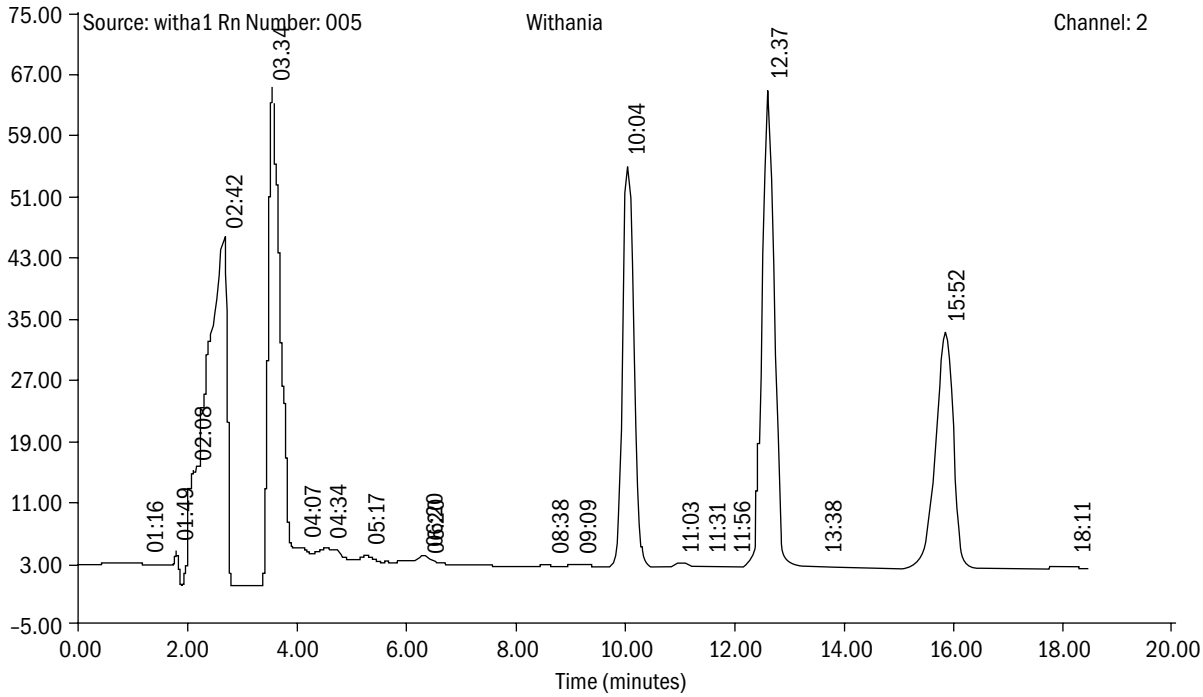

Figure 1(a) HPLC profile of treated and control plants of *Withania:* standard mixture

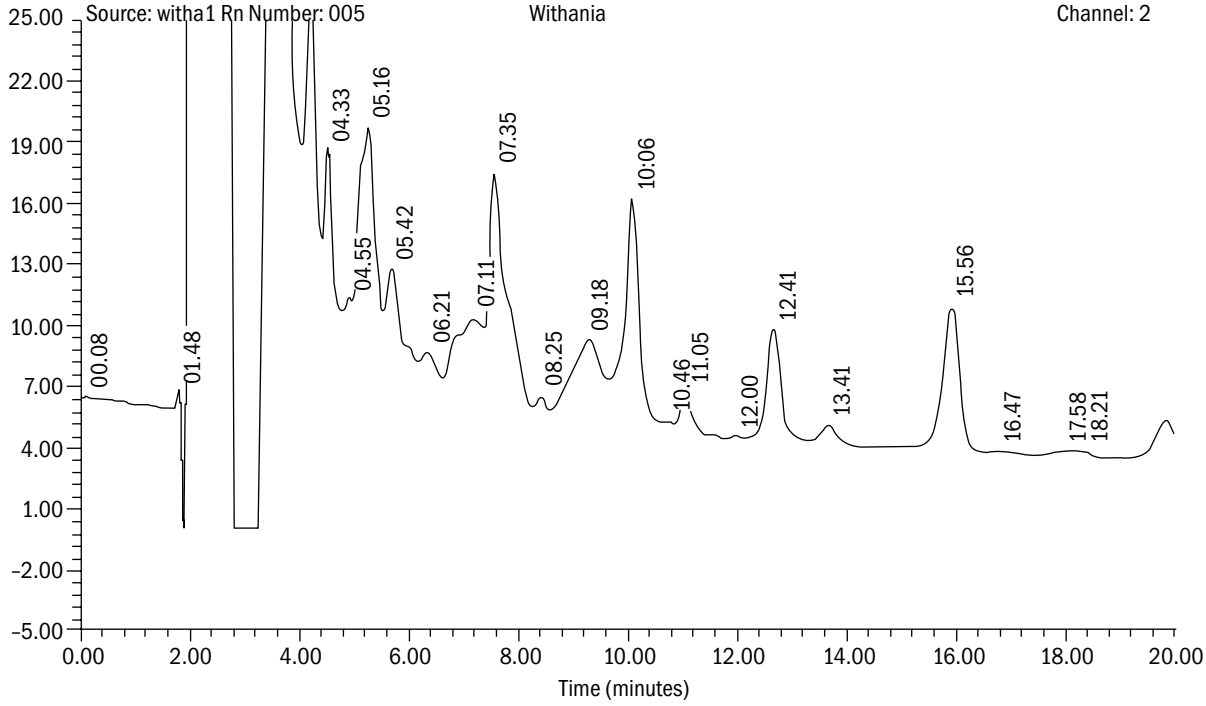

Figure 1(b) HPLC profile of treated and control plants of *Withania: Withania* control

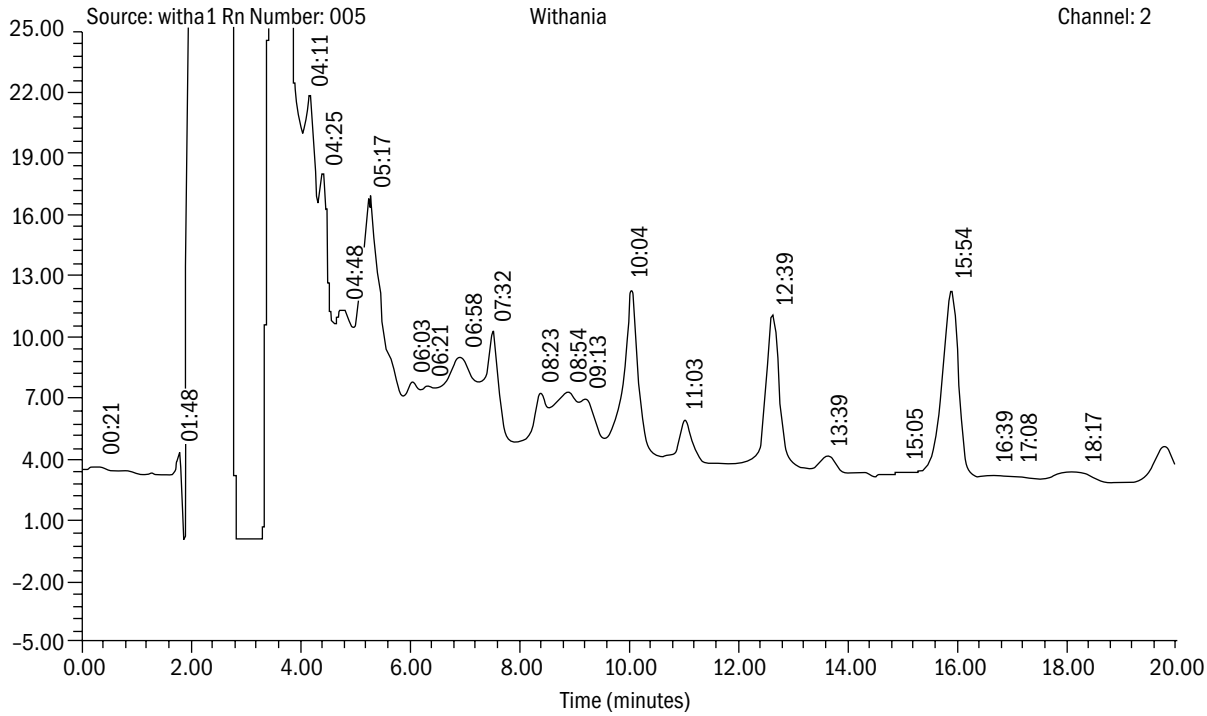

Figure 1(c) HPLC profile of treated and control plants of *Withania: Withania* treated

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Centre for Mycorrhizal Culture Collection

Collection at TERI

*Chaitali Bhattacharya and Alok Adholeya**

Introduction to the germplasm bank

The Centre for Mycorrhizal Culture Collection (CMCC) was established with the objective of conserving the diversity that exists within the ever-increasing imperative fungus—mycorrihiza. The founding of this centre dates back to 1993 with funding from the Department of Biotechnology (DBT), Government of India. Since then, the depository has been enthusiastically involved in collecting and maintaining endomycorrhizal and ectomycorrhizal isolates. The collections are effected from different agroclimatic zones, agricultural fields, and environmentally vulnerable and degraded land (including industrial wastelands) of India. Apart from these cultures, the CMCC possesses collections obtained from various parts of the world. The vision is to create a culture collection bank that would have cultures from all around the world in order to preserve valuable germplasm that could be made available to researchers and the industry as starter cultures for exploring the usage of this fungus in hitherto unrevealed sectors.

The primary dictum of the germplasm bank is to form an invaluable reservoir of genetic diversity of endomycorrhizal and ectomycorrhiza fungi. In order to do so, samples (that is, soil samples) are acquired from various sources of diverse habitats, propagated as trap culture using bait plants, isolated, and finally developed into pure cultures with utmost care. These isolates are then characterized at the morphological, biochemical, molecular, and functional level. These actively growing pot cultures are given unique accession codes. They are regularly maintained under in situ conditions and in vitro cultures of the same are endeavoured to engender.

At present, the CMCC has more than 400 accessions as live cultures collected from northern, central, and western parts of India as well as from Central Europe. A major mandate of the CMCC is to receive submissions from interested researchers and organizations for maintaining their cultures and providing them accession codes. Receipt of such cultures from diverse habitats and extreme regions is envisaged to generate scope for the researchers and to achieve diversity under one roof towards exploring this brawny fungus for benefit in the field of sustainable agriculture and mankind.

Cultures maintained at CMCC

The CMCC collection list includes different isolates of both ectomycorrhiza and endomycorrhiza.

Ectomycorrhiza: The CMCC has a rich collection of more than 250 ectomycorrhiza fungus, which include different species of *Laccaria*, *Hebeloma, Cenococcum, Ectendomycorrhizae, Amanita, Alpova, Elaphomyces, Gautieria, Melanogaster, Paxillus, Pisolithus, Scleroderma, Suillus, Tricholoma,* and many more genera.

Endomycorrhiza: Many isolates of *Glomus, Gigaspora*, and *Scutellospora* are maintained under both in situ and in vitro conditions. Many more isolates are

Objectives of the CMCC

- Exploring the diversity that exists in the world of mycorrhiza.
- Maintaining and documenting mycorrhiza collected from different regions as trap cultures.
- Screening, isolating, identifying, and maintaining native mycorrhiza fungus as pure culture.
- Characterizing the germplasm to determine its molecular, biochemical, and functional properties.
- Accepting submission of cultures and providing them with accession codes.
- Training programmes through workshops related to mycorrhiza culture isolation and maintenance as pure culture.

* Research Associate, Biotechnology and Bioresources, Centre for Mycorrhizal Research, The Energy and Resources Institute, New Delhi – 110 003

in the process of being identified and developed. The details of these isolates are maintained in the database and will be published at frequent intervals and will be made available for research purpose.

Training programme conducted

In order to spread knowledge and expertise on mycorrhiza, different workshops are organized at regular intervals. These workshops are structured by experts who have been exposed to various national and international trainings. A workshop includes different aspects such as sampling methods, spore isolation, identification, maintenance of trap culture, and production of inoculum, of mycorrhiza. The Centre also provides different assays that are used in assessing the vigour of an inoculum like colonization assessment, spore viability, and infectivity potential. Under special conditions, these workshops can be organized as per the requirement of an interested organization according to its necessity (tailor-made workshops). These workshops aim not only to generate interest among budding researchers and interested institutes but also to teach young entrepreneurs and farmers the importance of mycorrhiza in sustainable agriculture.

Recent references

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

- *Applied Clay Science*
- *Applied Soil Ecology*
- *Aquatic Botany*
- *Biological Control*
- *CATENA*
- *Comptes Rendus Biologies*
- *European Journal of Soil Biology*
- *Forest Ecology and Management*
- *Fungal Biology*
- *International Biodeterioration and Biodegradation*
- *Journal of Arid Environments*
- *Journal of Environmental Sciences*
- *Journal of Plant Physiology*
- *Journal of Proteomics*
- *Physiological and Molecular Plant Pathology*
- *Scientia Horticulturae*
- *Soil Biology and Biochemistry*
- *Trends in Plant Science*

Name of the author(s) and year of publication	Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author is marked with an asterisk)
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MYCORRHIZA NETWORK WEBSITE LAUNCHED!

We are pleased to announce the launch of the Mycorrhiza Network website. The objective of the website is to act as a forum that will strengthen research, encourage cooperation, promote exchange of information and exchange of germplasm, and facilitate transfer of technology. This will help promote mycorrhiza research and communication among mycorrhizologists and industries involved in the numerous research fields related to mycorrhiza.

In addition to other information resources, the website also encompasses the Network's three major components: (1) Mycorrhiza Information Centre; (2) Centre for Mycorrhiza Culture Collection; and (3) Mycorrhiza News.

We welcome all mycorrhizologists, scientists, educators, agriculturists, and students to register with the Mycorrhiza Network by filling and submitting the free online registration form available on the website <www.mycorrhizae.org.in>.

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Tel. +11 (0) 24682100, Cellular 9811628889
Fax +44 (0) 24682145 E-mail aloka@teri.res.in
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