MYCORRHIZA NETWORK

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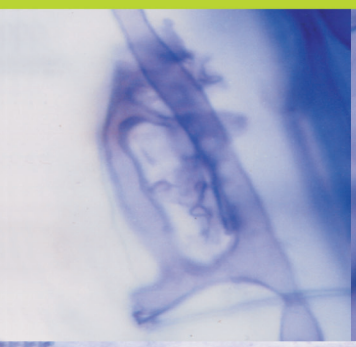

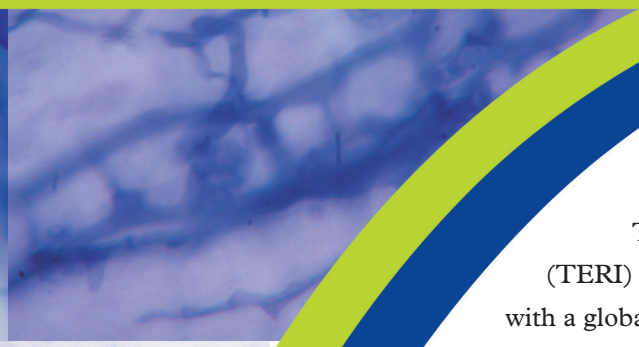

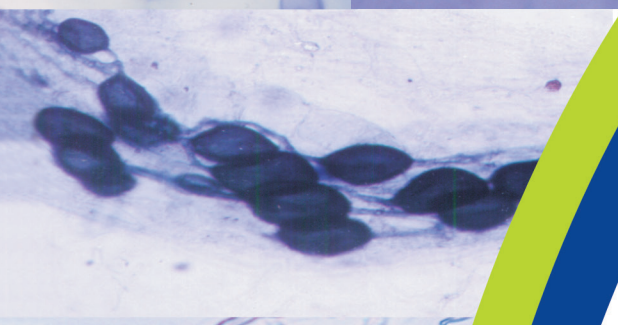

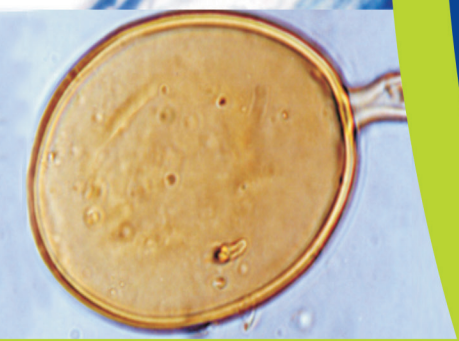

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TERI's Mycorrhiza Network is primarily responsible for establishing the Mycorrhiza Information Centre (MIC), the Centre for Mycorrhiza Culture Collection (CMCC), and publishing *Mycorrhiza News*. The Network helps scientists carry out research in mycorrhiza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

Mycorrhiza News

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

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Research finding papers

Mycorrhizal Colonization of Understorey Plants Growing in Rubber Plantation of Tripura in Northeast India

Krishna Talapatra[a], *Asim Das*[a], *Aparajita Roy Das*[b], *Ajay Krishna Saha*[b], *and Panna Das*[a]*

Introduction

Arbuscular Mycorrhizal (AM) fungi, which form mycorrhizal symbioses with two out of three of all plant species (Trappe 1987), are believed to be obligate biotrophs that are wholly dependent on the plant partner for their carbon supply (Smith and Read 1996). It has also been reported that the maintenance of diverse plant communities may be highly dependent on mycorrhizal diversity (van der Heijden, Klironomos, and Ursic *et al.* 1998).

Dark Septate Endophytes (DSE) are a group of heterogeneous root-associated endophytic fungi, which are characterized by melanized intercellular and intracellular runner hyphae and so-called microsclerotia (Jumpponen and Trappe 1998). Studies indicate that the DSE fungal colonization enhances plant growth, biomass accumulation, nutrient acquisition, or fitness of the inoculated seedlings (Rodriguez, White, and Arnold *et al.* 2009).

Hevea brasiliensis Müll. Arg. is the main tree species exploited in the world for the production of natural rubber (Compagnon and D'Auzac 1986). Rubber is an important commercial crop in Tripura.

In this article, we have focused on AM and DSE fungal colonization in understorey plants of rubber plantation and its associated AM fungal species composition.

Materials and Methods

Location of Study Site and Sample Collection

The plantation was located near Rose Valley Park, Amtali, West Tripura. The roots of the plants were collected from herbs and shrubs during the season from January to April, 2014. Root samples were collected using a trowel by digging at a depth of 0–15 cm. Likewise, soil was collected from rhizosphere of each plant. All the soil collected from different plants was mixed and 500 g were collected in polythene bags. The sample was labelled and was brought to the laboratory for further analysis.

Soil Physico–chemical Properties

From fresh soil sample, soil pH, and electrical conductivity were measured by taking 10 g of soil dissolved in 50 ml of distilled water and stirred for 20 min. The solution was kept for overnight. Measurement of the soil pH and electrical conductivity was done by using a digital pH meter. Soil moisture content was determined by drying 10 g fresh soil sample at 60°C for 24 hr in a hot-air oven. For determination of the soil properties, air-dried soil samples were ground to fine powdered and sieved. The organic carbon was estimated using Walkley-Black method (1934). The soil available nitrogen was estimated (Black 1982). Available phosphorus, potassium, and calcium of soil samples were determined using the method of Jackson (1978).

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Processing of Roots and Assessment of AM and DSE Fungi

Collected plant root samples were washed thoroughly with tap water and taken in a beaker. Then the washed root samples were cut into small pieces, approximately 1 cm in size. After that, the roots were cleared and stained (Das and Kayang 2008). The root sample were mounted on slide and observed under compound microscope (Olympus C X 21i) for assessment of mycorrhizal structures. The estimation of mycorrhizal colonization was done by the magnified intersection method (McGonigle, Miller, and Evans *et al.* 1990).

Isolation of AM Fungal Spore and Identification

AM fungal spores in the soil sample were isolated by following the modified wet sieving and decanting method (Muthukumar, Senthilkumar, and Rajangam *et al*. 2006). The 25 g of soil sample was suspended in water and passed through a sieve of 35 µm in size. The residues on the sieve were then filtered using the filter paper. Then the spores were picked up with needle in 1–2 drops of polyvinyl alcohol-lactoglycerol under a dissecting microscope (Koske and Tessier 1983) for enumeration. Taxonomic identification of spores up to the species level was done on the basis of sporocarpic size, colour, ornamentation, and wall characteristics of the isolated spores by matching with original descriptions from the websites (www.lrz-muenchen.de/~schuessler/amphylo & www.invam.caf.wvu.edu)

Statistical Methods

Standard errors of means were calculated. Abundance and relative abundance were calculated. The calculation was done in Statistica 9.0.

Results

The soil characteristic of the study site is shown in Table 1. The root samples collected from different plants from the plantation exhibited the presence of AM and DSE fungal colonization (Plate 1). The structural colonization of AM and DSE fungal is presented in Table 2. AM fungal structure such as arbuscules, vesicles, and hyphae were observed in *Lygodium flexuosum*, *Pteris vittata*, *Calamus viminalis,* and *Pavetta indica* and other 10 species possessed fungal structure such as vesicles and hyphae. The maximum AM fungal colonization was observed in *Mucona pruriens*, with lowest colonization observed in *Calamus viminalis.* All the plants possessed hyphae of DSE fungi, of which maximum colonization was recorded in *Phaulopsis dorsiflora* and minimum in *Pavetta indica.*

AM fungal spore density in 25 g of soil was 265.66 ±39.01. A total of nine species were isolated from the rhizosphere of understorey plants from rubber plantation (Table 3). Of these, *Glomus aggregatum* showed highest abundance. The lowest abundance was represented by three species, i.e., *Acaulospora* sp 1, *Ambispora* sp 1, and *Rhizophagus irregulare*. The genera Glomus represented the higher abundance (Figure 1). Here, genus *Glomus* was represented by six species followed by *Acaulospora*, *Ambispora,* and *Rhizophagus* by one species each (Plate 2).

Table 1: Soil properties of rubber plantation

Moisture content (%)	9.18
pH	5.22
Electrical conductivity (mSm^{-1})	091
Organic carbon (%)	0.52
Available nitrogen (kg/ha)	215.48
Available phosphorus (kg/ha)	14.61
Available potassium (kg/ha)	65.51
Available calcium (kg/ha)	52.48

Discussion

Out of 14 species, AM fungal colonization was recorded in 12 species (reported earlier in Table 1). This is the first report of AM fungal colonization in *Calamus viminalis* and *Hemidesmus indicus.* The absence of arbuscules in eight species may be due to its ephemeral nature that is often absent or hard to see in field-collected roots (Brundrett 2004). Moreover, this is the first report of DSE colonization in all the 11 species such as *Lygodium flexuosum*, *Dryopteris filixmas*, *Phaulopsis dorsiflora*, *Hemidesmus indicus*, *Calamus viminalis*, *Ananas comosus*, *Pavetta indica*, *Sida cordifolia*, *Zizyphus mauritiana*, *Gardenia jasminoides,* and *Mucuna pruriens*.

Mycorrhizal networks are common among between mature overstorey trees and juvenile understorey plants (Visser 1995; Jonsson, Dahlberg, and Nilsson *et al.* 1999; Kennedy, Izzo, and Bruns 2003). Mycorrhizal colonization of such understorey plants in rubber plantation site has been studied. Here, the mycorrhizal colonization (%) range variedly which is also similar to the study by Sewnet and Tuju (2013) conducted in understorey coffee plants and within the range studied by Debnath, Sinha, and Saha *et al.* (2014); whereas, Yoshimura *et al.* (2013) observed a lesser percentage of mycorrhizal colonization of understorey plants of *Plantago asiatica*. The colonization rate of understorey plants of desert halophytes in China (He 2002) was also lower than this study.

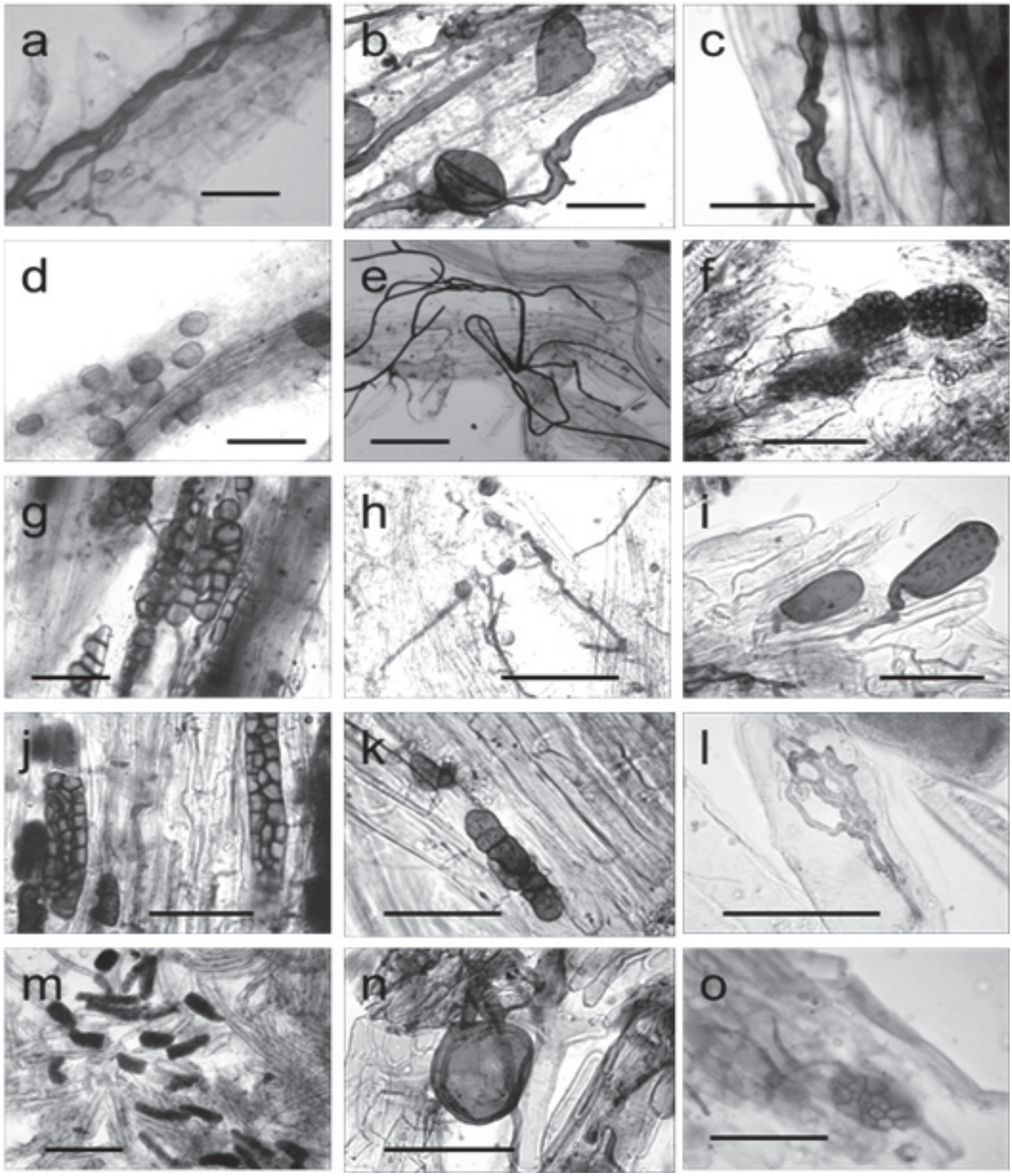

Plate 1: Mycorrhizal colonization in understorey plants of rubber plantation. (a) Root segment of *Ananas comosus* showing AM fungal hyphae (200 µm), (b) Presence of vesicles attached with hyphae in *Calamus viminalis* (200 µm), (c) Root portion of *Christella dentata* showing hyphae of AM fungi (100 µm), (d) Root segment of *Dryopteris fillxmas* showing vesicles (200 µm), (e) Extraradical hyphae in *Gardenia jasminoides* (300 µm), (f) Microsclerotia in the root segment of *Hemidesmus indicus* (100 µm), (g) Root segment of *Lygodium flexuosum* showing microsclerotia (100 µm), (h) Hyphae with vesicles in *Mucona pruriens* (300 µm), (i) Root portion of *Pavetta indica* showing vesicles (100 µm), (j) Root segment of *Pavetta indica* showing microsclerotia of DSE (100 µm), (k) Presence of DSE in *Phaulopsis dorsiflora* (100 µm), (l) Root segment of *Pteris vittata* showing intracellular hyphae (50 µm), (m) Root portion of *Sida cordifolia* showing microsclerotia of DSE (200 µm), (n) Root segment of *Tamarindus indica* showing vesicle (50 µm), and (o) Microsclerotia of DSE in the root segment of *Zizyphus mauritiana* (100 µm)

Table 2: Mycorrhizal colonization (%) in the roots of 15 plants from rubber plantation

Plant name	Family	AM Fungi				DSE Fungi	
		RLA%	RLV%	RLH%	Previous Report	DSH%	Previous Report
Lygodium flexuosum (L.) Sw.	Lygodiaceae	28.75 ±2.60	17.33 ±2.05	65.43 ±2.91	Khade and Rodrigues 2002	22.18 ±3.27	–
Dryopteris filixmas (L.) *Schott*	Dryopteridaceae	0.00 ±0.00	38.43 ±2.48	74.10 ±2.53	Harley and Harley 1987	12.53 ±1.93	–
Pteris vittata L.	Pteridaceae	3.00 ±1.49	36.56 ±1.46	76.06 ±2.38	Khade and Rodrigues 2002	23.94 ±2.38	Zhang, Li, and Li *et al*. 2011
Christella dentata (Forssk.) Brownsey & Jeremy	Thelypteridaceae	0.00 ±0.00	24.01 ±3.07	60.74 ±4.20	Khade and Rodrigues 2002	24.36 ±2.21	Ghanta, Dutta, and Mukhopadhyay 2012
Phaulopsis dorsiflora (Retz.) Sant.	Acanthaceae	0.00 ±0.00	13.65 ±1.81	53.82 ±1.72	Debnath, Saha, and Das 2014	24.42 ±2.02	–
Tamarindus indica L.	Fabaceae	0.00 ±0.00	36.19 ±2.32	68.69 ±3.06	Bâ, Plenchette, and Danthu *et al*. 2000	22.52 ±1.95	Zhang, Li, and Li *et al*. 2011
Hemidesmus indicus (L) R.Br.	Periplocaceae	0.00 ±0.00	24.18 ±2.32	61.06 ±2.62	–	11.16 ±1.88	–
Calamus viminalis Willd.	Arecaceae	0.84 ±0.46	24.14 ±1.92	48.93 ±3.29	–	20.01 ±2.55	–
Ananas comosus (L.) Merr.	Bromeliaceae	0.00 ±0.00	22.33 ±4.70	57.04 ±5.57	Aziz, Yuen, and Habte1990	9.25 ±2.00	–
Pavetta indica L.	Rubiceae	11.67 ±3.98	37.76 ±4.07	80.16 ±3.22	Muthukumar and Udaiyan 2000	8.17 ±2.23	–
Sida cordifolia L.	Malvaceae	0.00 ±0.00	34.28 ±2.79	73.63 ±3.76	Muthukumar and Udaiyan 2000	11.31 ± 2.21	–
Zizyphus mauritiana Lam.	Rhamnaceae	0.00 ±0.00	25.60 ±2.84	59.96 ±2.86	Bâ, Plenchette, and Danthu *et al*. 2000	17.41 ±2.14	–
Gardenia jasminoides J. Ellis	Rubiaceae	0.00 ±0.00	24.06 ±3.07	50.07±4.57	Wang and Jiang 2015	17.33 ±3.02	–
Mucuna pruriens (L) Dc	Fabaceae	0.00 ±0.00	38.89 ±3.06	90.88 ±2.82	Jemo, Nolte, and Nwaga 2007	9.13 ±2.82	–

Table 3: Distribution of spores extracted from the rubber plantation

Fungal species	Abundance	Relative abundance
Acaulospora sp 1	1	1.41
Ambispora sp 1	1	1.41
Glomus aggregatum	24	33.80
Glomus macrocarpum	6	8.45
Glomus sp 1	16	22.54
Glomus sp 2	11	15.49
Glomus sp 3	4	5.63
Glomus sp 4	7	9.86
Rhizophagus irregulare	1	1.41
	71	**100.00**

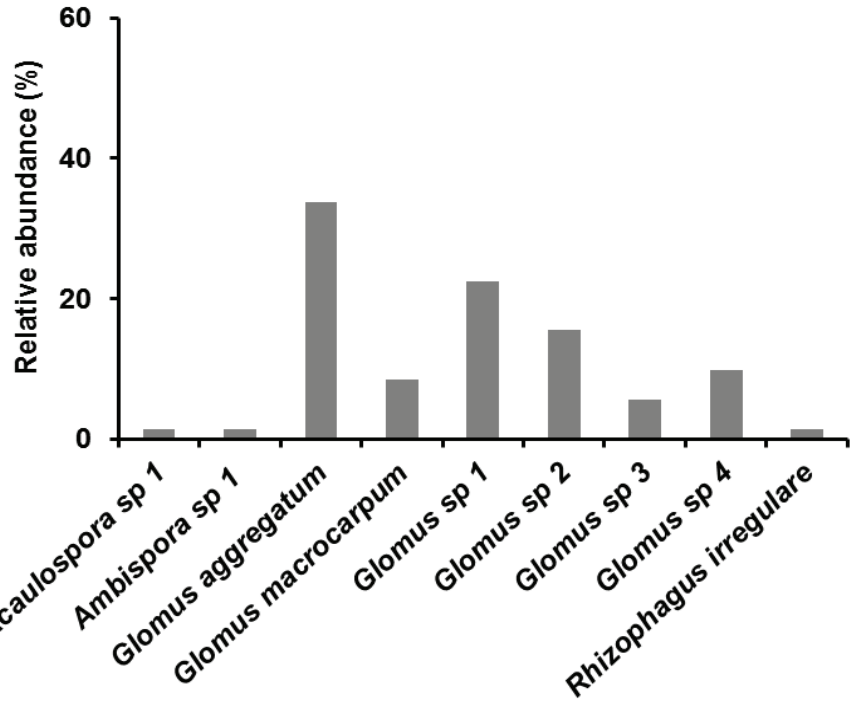

Figure 1: Relative abundance of AM fungal species associated with understorey plants of rubber plantation

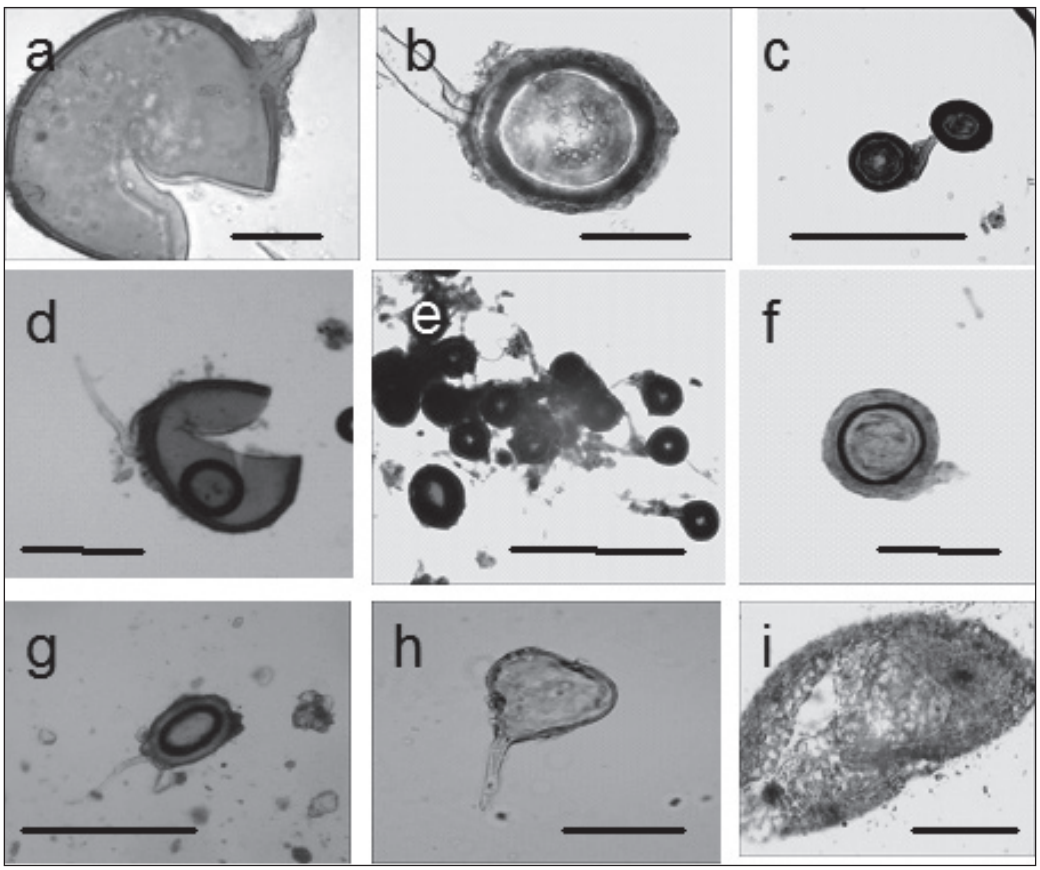

Plate 2: Spores of AM fungi isolated from the rhizospheric soil of understoreys plants of rubber plantation (a) *Glomus* sp 1 (scale bar = 50 µm), (b) *Glomus* sp 2 (scale bar = 50 µm), (c) *G. macrocarpum* (scale bar = 100 µm), (d) *Glomus* sp 3(scale bar = 50 µm), (e) *Glomus aggregatum* (scale bar = 200 µm), (f) *Ambispora* sp 1 (scale bar = 100 µm), (g) *Glomus* sp 4 (scale bar = 100 µm), (h) *Rhizophagus irregulare* (scale bar = 100 µm), and (i) *Acaulospora* sp 1 (scale bar = 50 µm)

In the present study, four genera with nine AM fungal species were identified to be similar with the study by Sewnet and Tuju (2013). A total of approximately 266 spores were found in this investigation. In contrast, Debnath, Sinha, and Saha *et al.* (2014) extracted 195 spores per 25 g soil with three genera and eight AM fungal species from rubber plantation of Takhmachara, Tripura. However, the observation of spore populations alone may not provide adequate information about AM fungal community structure, because of the differences in growth and sporulation among AM fungal species (Land and Schönbeck 1991).

Acknowledgements

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Seasonal Dynamics of Arbuscular Mycorrhizal Fungi Associated with Four Medicinal Plants

Sapana Sharma[a]★, *Sandeep Sharma*[b], *and Ashok Aggarwal*[c]

Introduction

Human society today demands production of high quality products in the most sustainable way that causes least damage to the environment. Typically, fertilizers and pesticides are being used at high levels in the intensive production of plants but these are extremely costly and harmful for mankind as well as to beneficial microbes, especially Vesicular Arbuscular Mycorrhizal (VAM) fungi. However, to implement such a plan, we must develop plant systems that can efficiently scavenge and utilize soil nutrients present at low levels with the help of different biofertilizers. One such category of biofertilizers is 'Arbuscular Mycorrhizal Fungi'. These biotools act as an extension of root system as well as help in the uptake of water and mineral nutrients; hence, they hold importance in commercial agriculture and forestry (Rajan *et al.* 2000; Singh and Jha 2005). AM fungi have been observed to be associated with medicinal and aromatic plants and are used as biofertilizers at commercial level (Adholeya 2004). Diversity and functioning of AMF communities have traditionally been based on root colonization estimates and AM spore count. Apart from this, spore population dynamics is also regulated by various biotic and abiotic factors which include host species, cropping pattern, crop rotations, crop management practices, use of fertilizers, nutrient limitation, stress, etc. High colonization occurred during the peak growing season and low colonization during early and late growing season of a plant (Lekberg *et al.* 2008; Mandyam and Jumpponen 2008). Soil moisture (Ruotsalainen *et al.* 2002), temperature (Lugo and Cabello 2003), growth rate, and turnover of plant roots are among the most-frequently proposed drivers of AM seasonality. AMF community may vary with host plant even within the same ecosystem with different cropping patterns (Lekberg and Koide 2005). These differences are significant not only in terms of species composition but also in their seasonal dynamics (Muthukumar and Udaiyan 2002). A study of seasonal changes in AM fungal community may help in providing fundamental insights into the basic ecology of these glomalean fungi. Keeping in view the above information, the present study was undertaken to assess the seasonal change in the AMF associated with four selected medicinal plants namely *Stevia rebaudiana, Sapindus mukorossi, Centella asiatica,* and *Albizzia lebbek* to predict the most suitable conditions for the growth and germination of AMF.

Materials and Methods

Collection of Samples

Fine roots and soil from the rhizosphere of medicinal plants under study were collected from 15–30 cm deep soil at the middle of each month. Three different plants of each type were randomly selected for study. A composite sample was prepared for each plant. Fifty gram of the rhizospheric soil along with fine roots was taken for analysis.

Estimation of Mycorrhizal Spore Population

AM spores were isolated from the soil samples by using 'Wet Seiving and Decanting Technique' (Gerdemann and Nicolson 1963).

Estimation of AM Root Colonization

Percent mycorrhizal root colonization was analysed by 'Rapid Clearing and Staining Method' (Phillips and Hayman 1970).

The pH variation was also recorded for each sample by mixing soil and distilled water (1:2) using digital pH meter.

Temperature of the soil was also recorded by soil thermometer in the morning.

Results

In case of *Stevia rebaudiana*, it is clear from Table 1 that AM spore count and root colonization vary with monthly sampling in a year. Mycorrhizal spore population reached to maximum in January (132.66 ±2.51) and minimum in July (51 ±1.00). Decline in spore number was observed from April to August and increase in mycorrhizal spore number started from September (114.66 ±5.50) to January (132.66 ±2.51). AMF colonization was highest in September (100 ±0.00) and then declined steadily; lowest colonization was observed in November

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Table 1: Seasonal dynamics of endomycorrhizal fungi associated with *Stevia rebaudiana*

Sl. No.	Month	AM Spore No./50 g of soil	Mycorrhizal Root Colonization (%)	Temperature	pH
1	January	132.66 ±2.51	51.10 ±3.85	15.1	7.1
2	February	89.66 ±3.05	60 ±0.00	16.0	7.2
3	March	110 ±11.53	64.40 ±3.81	21.3	7.1
4	April	73 ±9.84	73.33 ±0.00	13.5	7.4
5	May	79.66 ±6.11	95.53 ±3.86	16.2	7.4
6	June	68.66 ±3.51	84.42 ±10.18	25.5	8.0
7	July	51 ±1.00	80 ±0.00	30.0	7.2
8	August	94 ±1.73	93.33 ±0.00	27.0	6.9
9	September	114.66 ±5.50	100 ±0.00	29.0	6.9
10	October	97 ±5.29	68.88 ±3.85	38.0	7.2
11	November	95 ±2.00	48.83 ±3.86	19.0	6.9
12	December	123 ±2.00	64.40 ±3.81	14.9	7.0

(48.83 ±3.86). Root colonization increased from May to September and then it declined. This showed that root colonization was maximum in late summer and lowest in winter season. Spore density was found maximum at 14.9°C and minimum at 30°C. AM spore population and root colonization were found to be maximum in neutral (pH 7.0) and nearly neutral soil (pH 6.9), respectively.

In case of *Centella asiatica*, it is evident from Table 2 that spore density was maximum in May (83.33 ±4.16) and decreased steadily and again increased in September (80.33 ±13.61). It gradually declined and minimum spore count was recorded in April (30 ±9.16). It means that maximum increase in spore population was registered in summer and lowest in late winter. Percent arbuscular mycorrhizal root colonization was found to be highest in the month of September (77.77 ±10.18) and then declined up to April with lowest value (22.21 ±7.69). Then it again increased in May (73.33 ±6.67). In *C. asiatica*, almost same seasonal pattern was observed with regard to spore number and root colonization. Spore population was highest at 15.2°C and at slightly alkaline pH (7.1) and minimum at nearly neutral pH (6.8) and 13.4°C. Root colonization was highest at pH (7.4) and 28.3°C. With regard to third selected medicinal plant,

Table 2: Seasonal dynamics of endomycorrhizal fungi associated with *Centella asiatica*

Sl. No.	Month	AM Spore No./50 g of soil	Mycorrhizal Root Colonization (%)	Temperature	pH
1	January	53 ±2.00	31.10 ±3.85	14.4	7.3
2	February	59 ±3.60	28.88 ±10.18	16.3	7.4
3	March	50.66 ±3.78	53.33 ±0.00	20.3	6.9
4	April	30 ±9.16	22.21 ±7.69	13.4	6.8
5	May	83.33 ±4.16	73.33 ±6.67	15.2	7.1
6	June	66.66 ±7.63	57.77 ±3.85	26.6	7.2
7	July	40.53 ±8.96	60 ±0.00	29.8	6.7
8	August	36.33 ±5.03	71.10 ±3.85	26.5	6.3
9	September	80.33 ±13.61	77.77 ±10.18	28.3	7.4
10	October	50 ±2.64	57.77 ±3.85	24.7	7.6
11	November	45 ±1.00	68.88 ±3.85	18.7	6.8
12	December	48 ±1.00	68.88 ±10.18	13.8	6.6

Sapindus mukorossi, spore count and root colonization were also observed to be varying with months of the year, soil temperature, and soil pH. It is envisaged from Table 3 that AM spore count was found to be maximum in August (116.33 ±8.38) and September (115 ±19.13) and minimum in July (24.66 ±6.35). Highest spore density was observed in peak growing season and lowest in early and late growing season. Percent colonization was maximum in March (100 ±0.00); then it declined up to July (44.44 ±3.85); again increased in August (77.77 ±3.85) and then declined gradually. AM spore count was maximum nearly neutral soil (pH 6.8) at 29.6°C and minimum in soil having pH 6.9 and 30.2°C. But root colonization was highest in alkaline soil having pH 7.4 at 25.2°C and minimum in nearly neutral soil (pH 6.9) at 30.2°C.

In case of *Albizzia lebbek* (Table 4), mycorrhizal spore abundance was observed to be maximum in October (116 ±5.00) then it declined gradually and minimum number of spores was found in June (23.66 ±3.21) while root colonization was maximum in August (86.66 ±0.00) and lowest in November–December (31.10 ±3.85). It was observed that root colonization declined from August to December and then again increased. It was highest in summer and

Table 3: Seasonal dynamics of endomycorrhizal fungi associated with *Sapindus mukorossii*

Sl. No.	Month	AM Spore No./50 g of soil	Mycorrhizal Root Colonization (%)	Temperature	pH
1	January	60 ±3.60	55.55 ±10.18	15.2	7.3
2	February	85.66 ±6.80	57.77 ±7.69	18.6	7.4
3	March	84.66 ±9.07	100 ±0.00	25.2	7.4
4	April	91 ±8.71	51.10 ±3.85	27.9	7.2
5	May	75 ±11.53	51.10 ±16.77	29.3	7.3
6	June	89.33 ±7.63	46.66 ±6.66	32.4	7.4
7	July	24.66 ±6.35	44.44 ±3.84	30.2	6.9
8	August	116.33 ±8.38	77.77 ±3.85	29.6	6.8
9	September	115.66 ±19.13	62.22 ±3.84	28.3	7.0
10	October	59.33 ±10.01	68.88 ±7.69	25.8	7.1
11	November	104.66 ±10.21	71.06 ±3.86	19.4	6.7
12	December	57.33 ±5.13	60 ±0.00	19.7	7.0

Table 4: Seasonal dynamics of endomycorrhizal fungi associated with *Albizzia lebbek*

Sl. No.	Month	AM Spore No./50 g of soil	Mycorrhizal Root Colonization (%)	Temperature	pH
1	January	53 ±5.00	53.33 ±6.66	18.5	7.4
2	February	59.33 ±4.04	44.44 ±3.84	21.1	7.6
3	March	38.66 ±4.04	71.10 ±3.85	27.9	7.3
4	April	24 ±5.56	84.44 ±3.84	30.3	7.5
5	May	24.33 ±3.05	80 ±0.00	31.8	7.4
6	June	23.66 ±3.21	66.66 ±0.00	30.6	6.9
7	July	33.66 ±3.78	82.21 ±7.69	30.0	9.3
8	August	90.33 ±3.05	86.66 ±0.00	29.6	8.1
9	September	105.66 ±4.61	59.99 ±6.65	27.5	8.0
10	October	116 ±5.00	40 ±0.00	25.0	8.1
11	November	75.33 ±5.50	31.10 ±3.85	20.0	7.3
12	December	52.66 ±2.08	31.10 ±3.85	18.0	7.1

lowest in winter. With regard to pH, it was observed that highly alkaline pH (8.16) favoured the spore number and lowest spore count was observed in soil having pH 6.9. AM root colonization was also found to be highest at alkaline pH (8.10) and lowest at pH 7.3. When effect of temperature was studied, it was found that spore population was highest at 25.0°C and lowest at 30.6°C while root infection was maximum at 29.6°C and lowest at 18.0°C.

Discussion

It is clear from the above results that different months of the year, varied soil temperatures and different soil pH values affected the AM spore population, diversity and AM colonization at variable rate. A wide range of differences was observed in each plant. This suggests that AM spore diversity and distribution are seasonal and a wide range of spore density and root colonization might be due to a wide range of hosts. Sporulation rate of AM fungi has also been found to be host dependent and due to this the plants have shown different results. Mycorrhizal colonization is influenced by moisture content of the soil (Ghosh *et al.* 2007). Results of present investigation with regard to *C. asiatica* are also in accordance with this. Ghosh *et al.* (2007) also studied the seasonal dynamics of AMF in *C. asiatica* and found that the colonization percentage was not affected by moisture content and changed soil conditions had little effect on colonization in *C. asiatica*. But in present investigation, changed soil conditions such as soil temperature and soil pH have affected AMF population and colonization significantly. Different morphological structures such as arbuscules and vesicles were also influenced by different environmental factors and season of the year. Lugo and Cabello (2003) found maximum number of vesicles in summer. Same were the observations in our study. It can be said from the above results that soil texture, optimum moisture, pH, and temperature all might have created favourable conditions for maximum sporulation of AM fungi. Influence of pH on AM spore population may be due to alteration in the concentration of many nutrients and ions in the soil solution. The response of AM fungi to soil pH may depend on the species and strains constituting the indigenous AM flora (Robson and Abbott 1989). Soil pH varied significantly between species of plants and with various sampling seasons. This might be the reason of difference in distribution and diversity of AM fungi in all selected medicinal plants and these variations could be attributed to the host mediated changes in the rhizosphere. Millar (2000) indicated that a significant seasonal trend for AM colonization exists regardless of water gradient. Seasonal variations in mycorrhizal colonization levels are also linked to plant phenological events including new root growth, active plant growth, flowering, fruiting, and seedling establishment (Carvalho *et al.* 2001).

Santos-Gonzalez *et al.* (2007) found the highest AMF richness in May in case of *Prunella vulgaris* and *Antennaria dioica*. Similar results were seen in case of *C. asiatica* in present investigation. Lugo and Cabello (2002) observed maximum sporulation in dry season and lowest in wet season. In the present study, similar results were found in case of *C. asiatica* only but other plants did not follow this pattern. Studies of Apple *et al.* (2005) have indicated that colonization was highest in the spring season in case of *Ambrosia dumosa* and found further that AM colonization was also influenced by rainfall. Staddon *et al.* (2002) has recorded that mycorrhizal fungi respond to increase and decrease in soil temperature. Similar are the results in the present study with regard to all the four plants investigated. Fitter *et al.* (2004) also studied the effect of temperature on AMF community and found that AM fungi are temperature sensitive. Different studies have shown that plants forming associations with AMF had improved plant growth in acid soils and a positive AM fungal effect was found in acid soils. But in the present investigation, maximum AM fungal population was found in neutral soil in case of *S. rebaudiana*, in slightly alkaline soil (pH 7.1) in *C. asiatica*, in nearly neutral soil (pH 6.8) in *S. mukorossi* and in highly alkaline soil (pH 8.16) in case of *A. lebbek*. This represents a wide range of pH influencing endomycorrhizal population. Mohammad *et al.* (2003) reported that biotic factors are relatively less important than abiotic factors for establishing AM population pattern and found that the duration of moisture availability determines the level of AMF colonization. This might be the reason for higher root colonization in rainy season in *S. rebaudiana*, *C. asiatica,* and *A. lebbek* in the present study. Improvements in soil fertility especially increase in pH, availability of nutrients, and soil moisture provided conducible environment for mycorrhizal formation. It is also clear from literature that when soil conditions are congenial for spore germination, mycorrhizal colonization increases and spore number decreases (He *et al.* 2002). The percentage of vesicles showed the highest number in summer followed by autumn, winter, and spring. The percentage of arbuscules and coils was higher in the period between summer and winter whereas percent entry points were highest between summer and winter which corresponds to higher root colonization (Lugo and Cabello 2003).

It has been recorded that maximum spore density occur in summer and lowest in winter with intermediate values in autumn and spring.

A. lebbek also showed the maximum spore density in summer in the present investigation. Spore density and AM root colonization were poorly related to each other but related with prior months and this suggested that high colonization in one season precedes high sporulation in next season (Escudero and Mendoza 2005). Almost similar were the results in case of all four selected plants in present investigation. Posada *et al.* (2008) also studied the seasonal dynamics of AM fungi with reference to physical, chemical, and environmental factors in *Brachiaria decumbens* and found that AM fungi are influenced by all these factors. Dwivedi (2008) studied seasonal variation in mycorrhizal fungi on winter wheat cultivar C-306 in four different sites and found that neutral pH is best for maximum occurrence and development of AM fungi. In present investigation, same were the observations in case of *S. rebaudiana*, *C. asiatica*, and *S. mukorossi*. Heinemeyer and Fitter (2004) investigated the effect of temperature on arbuscular mycorrhizal fungi in *Plantago lanceolata* and *Holcus lanatus* and found that percentage of colonized roots positively correlated with temperature in *P. lanceolata* and no significant effect of temperature was seen in *H. lanatus*. In the present study, no correlation was observed between temperature and AM fungal richness.

Studies regarding the seasonal dynamics of AM fungi are useful as they help to predict the season and edaphic conditions, which are conducible for the maximum growth of AM fungi and for inoculation with mycorrhizal fungi in that particular season which results in maximum propagation of medicinal plants. Such studies will also be useful in the conservation strategies of medicinal plants which are under threat.

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CENTRE FOR MYCORRHIZAL CULTURE COLLECTION

Morphotaxonomy of *Diversispora spurca* (accession–CMCC/AM–1806)

Kiran Sunar[a], *Maunata Ghorui*[b], *and Alok Adholeya*[⋆#]

Glomeromycotan fungi are very diverse and produce relatively large spores with characteristically layered wall containing having several hundreds of nuclei (Be'card and Pfeffer 1993). The spores may be formed in clusters or aggregates or singly in sporocarps (Gerdemann and Trappe 1974). Like most zygomycota, glomeromycotan fungi lack regular septation in their hypha. The phylum glomeromycota comprises about more than 200 described morphospecies that have been categorized by features of the spore wall, spore morphology, type of subtending hyphae, and spore wall layers. The way the spore is formed on the hypha ('mode of spore formation') has been important to designate genera and families, and the layered structure of the spore walls is used to distinguish species (Morton 1988). Walker (1983) established the concept of 'murographs' to describe and compare the different types of structures of the spore walls. Morphotaxonomic studies therefore have become one of the most preferred modes of characterizing these diverse groups of microorganisms. In continuation to our series of articles which have been dedicated to describe valuable Arbuscular Mycorrhizal Fungi (AMF), in this current article we would like to take up the morphotaxonomic analysis of a unique AMF available in Centre for Mycorrhizal Culture Collection bank with the accession number CMCC/AM–1806.

Monosporal Establishment

This culture (CMCC/AM–1806) has been isolated and raised from the soils of Kasarma site in Dungarpur District of Rajasthan. The climate of Dungarpur is quite dry with hot summers but is milder than most of the other Rajasthan cities. The trap cultures were raised for the proliferation of indigenous mycorrhiza in pot conditions. After an adequate growth cycle of the host plant, spores propagated in trap culture were checked by wet-sieving and decanting method (Gerdemann and Nicholson 1963) and categorized on the basis of their morphology. In the next step, all the healthy spores were categorized according to their size, structure, and colour. Similar types of spores were grouped or isolated for obtaining pure single species culture of Arbuscular Mycorrhizal Fungi (AMF). Voucher specimen of all the potential monosporal were prepared and morphotaxonomic analysis of the spore and its wall layers, hyphal attachment, etc., were carried out under compound microscope (10×, 40×, and 100×) after mounting in Polyvinly Lacto glycerol and Polyvinly Lacto glycerol: Melzer's reagents (1:1). Selected healthy single AMF spores were used to raise monospecific cultures which were inoculated to pre-germinated seed of a suitable host. After a successful growth period of 3–6 months, the host roots were evaluated for colonization and sporulation. Cultures showing colonized roots and spores were considered successful cultures for raising monosporals and also considered to be pure when the spores isolated from them were morphologically similar to the voucher specimen prepared from the mother cultures that were used during the initiation of the monosporals (Figure 1).

A detailed characteristic morphotaxonomic description of this accession has been presented as adopted by various workers for identification.

Spore Morphology and Shape

Spores isolated from cultures were borne singly in soil. These were devoid of sporocarp. The colour of spores

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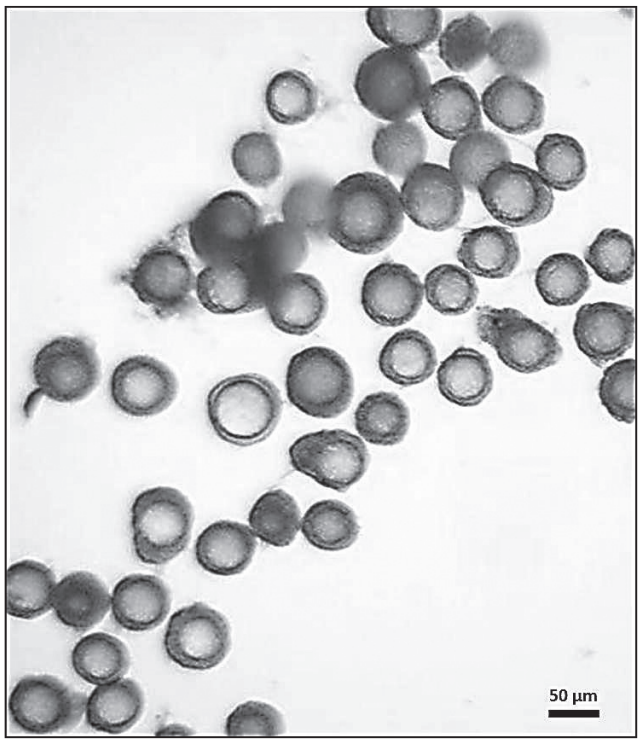
Figure 1: Spores isolated from monosporal culture of CMCC/AM–1806



was hyaline while young and yellowish when matured. Often these were found with short single subtending hyphae. The shape of the spores varied from globose to sub-globose. The average spore diameter ranged from 95.34–(119.52)–178.88 µm. Scanning electron micrograph of the spore surface revealed an outer warty wall surface. The outer wall is mucilaginous and tends to attract a lot of organic material due to which the outer wall surface appears warty or rough (Figures 2 and 3).

Subcellular Structure of Spore

Mature spores are composed of the following sub-cellular structures and wall layers:

Layer 1 (L1): The outermost layer known as L1 is mucilaginous and hyaline. This outer layer attracts a lot of organic debris and appears warty and ultimately sloughs off with age. This layer is granular and often adheres to the organic debris. This layer generally detaches itself with Layer 2 with a slight mechanical pressure (Figure 4).

Layer 2 (L2): This layer is a laminated layer consisting of many thin hyaline to sub-hyaline sub-layers known as laminae. This layer is highly elastic and tends to spread with slight pressure. The innermost sub-layer produces fine wrinkles which appear as another wall layer. Both the layers show very less or no reaction towards Melzer's reagent (Figures 4 and 5).

Figure 2: Compound and scanning electron micrograph of spores of CMCC/AM–1806 showing globose spore structure and a single spore with subtending hyphae

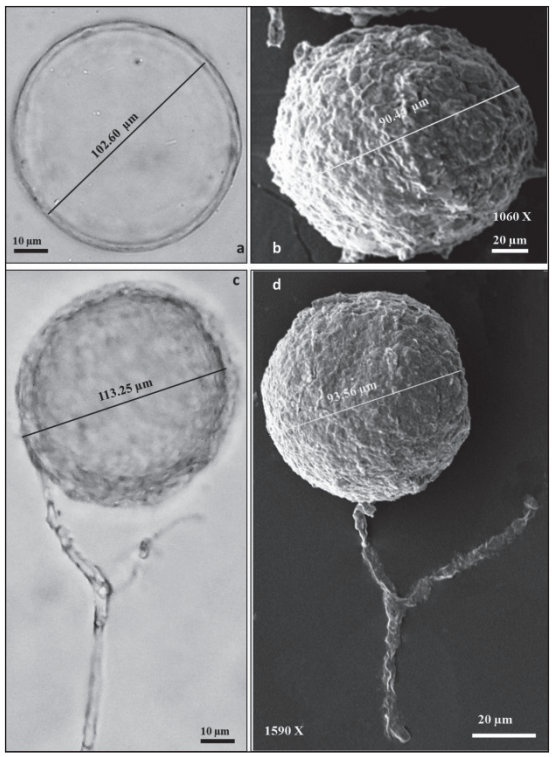
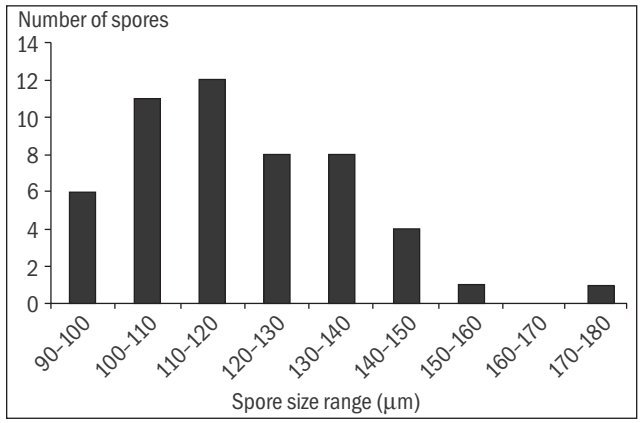


Figure 3: Analysis of spore diameters of 50 healthy spores obtained from one-year old monosporal culture



Subtending Hyphae

Spores are attached to cylindrical or slightly flared subtending hyphae that generally have a width of 4.5–7.5 µm (Figure 6). The L1 and L2 of the spore extend to the hyphal wall of subtending hyphae but L2 gets discontinued in the region of attachment and does not form the part of the hyphal wall structure. Therefore, L1 is the only layer that forms the hyphal wall layer, which is thin and often found to break off or get folded that makes it hard to detect.

Figure 4: Compound microscopic images of spore wall layers of CMCC/AM–1806 after mounting in PVLG (a and b) and PVLG: Melzer's reagent (c and d); both the layers are designated as outer mucilaginous layer L1 and inner laminated layer L2, respectively

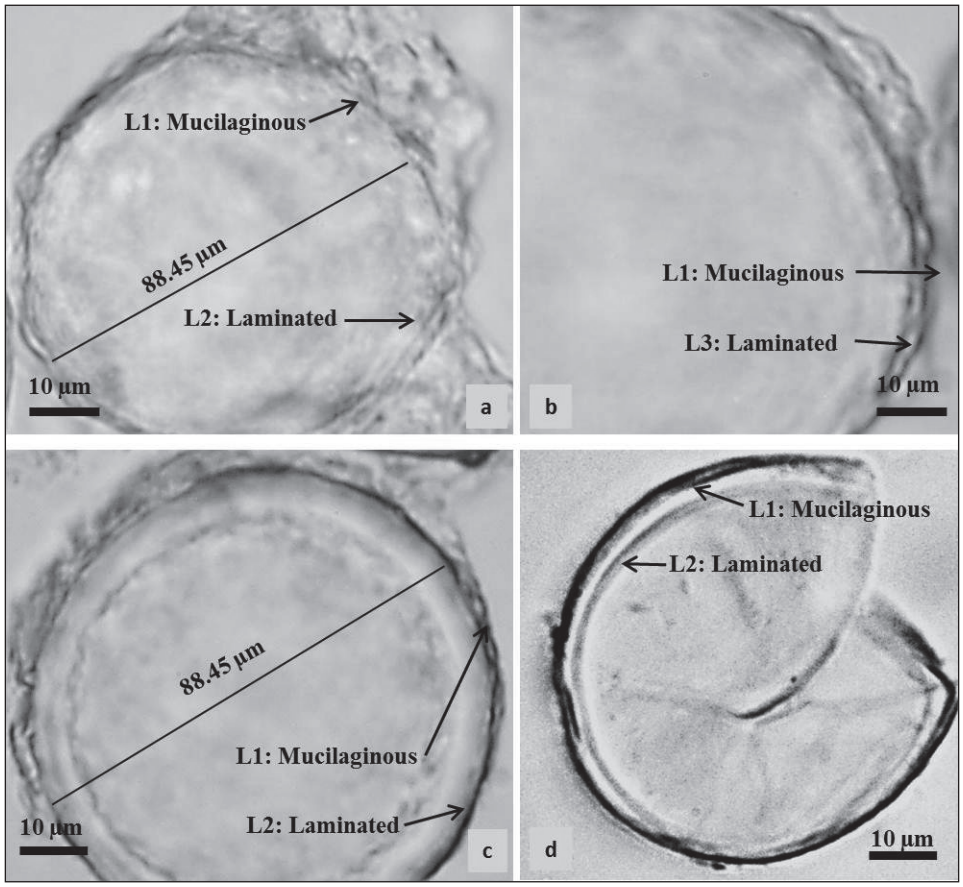
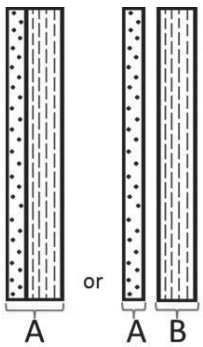


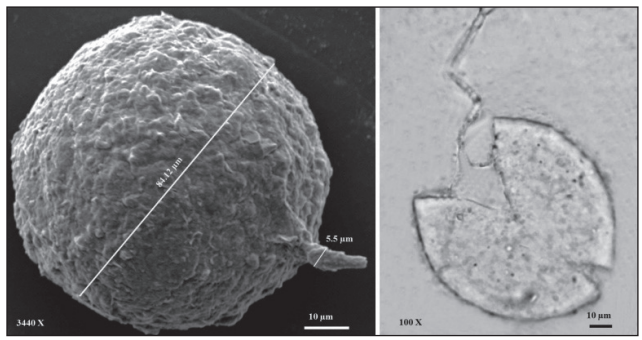
Figure 5: Murograph (Walker 1983) of the mature spore showing outer mucilaginous layer L1 and inner laminated layer L2



Occlusion

Many workers have reported the presence of an occlusion near the point of attachment of subtending hyphae and spore. Though it is very hard to see because of the fragile nature of hyphae, in few cases it has been observed that the L2, which continues from the spore, discontinues by forming a thickened structure that divides the subtending hyphae from the spore.

Figure 6: Scanning electron micrograph and compound microscopic images of CMCC/AM–1806 showing thin subtending hyphae attached to globose spore

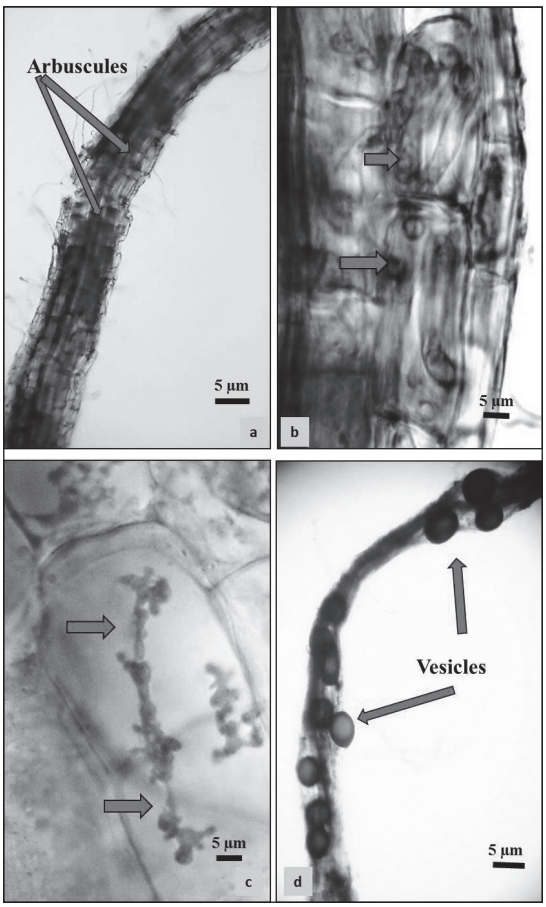


Mycorrhiza

Mycorrhiza structures observed in *Sorghum bicolor* roots are deeply stained in ink vinegar. Root colonization assays showed the presence of both extraradical and intraradical hyphae. The hyphae are coiled intracellularly forming Paris-type. The intracellular hyphae are dense and coiled, and form unique super-coiled structures. Arbuscules are

numerous and finely branched. They are formed within the hyphal coils (Figure 7).

Figure 7: Compound microscopic images of roots of *Sorghum bicolor* stained in ink vinegar observed for root colonization by CMCC/AM–1806 showing extraradical hypha and abundant arbuscules in the cortical cells (a), extremely coiled hyphal structures inside the cortical cell (b). Magnified view (100×) of finely branched Arbuscules (c) and intercalary vesicles (d)



Conclusion and Classification level

On the basis of above morphotaxonomic analysis of the accession CMCC/AM–1806, many distinguishing features regarding the family, genera, and the species could be derived. The following features were taken into consideration for characterization:

- Glomoid spores with spore wall structures consisting of a thin outer wall and inner laminated and structured wall. In some cases, the laminated wall is superseded by a flexible inner wall. All the wall layers do not react with Melzer's reagent.
- Spore germination is not accompanied by the formation of germination shield.

All these features suggest that the culture CMCC/AM–1206 belongs to the family Diversisporaceae (Walker and Schüßler 2004).

Some of the unique morphotaxonomic features of the accession are as follows:

- Glomoid spores are formed singly. The spore shape varies from globose to sub-globose. Spore diameter ranges from 95.34–(119.52)–178.88 µm.
- The spore contains two wall layers L1 and L2. L1 is thin outer wall which is mucilaginous and hyaline. The inner layer, L2, is laminated and structured wall. All the wall layers do not react with Melzer's reagent.
- The inner wall is highly flexible, tends to extend in slight pressure, and swells in lactic acid mountants.
- Spore germination is not accompanied by the formation of germination shield.
- Spores are attached to cylindrical or slightly flared subtending hyphae that generally have a width of 4.5–7.5 µm. L1 is the only layer that forms the hyphal wall layer, which is thin and often found to break off or get folded that makes it hard to detect.

The morphotaxonomic characters of the accession CMCC/AM–1806 match the description of a new genus designated as *Diversispora spurca* by Walker and Schüßler (2004). Formerly, this genus was known as *Glomus spurca*. Walker and Schüßler (2004) transferred *Glomus spurcum* to the new genus *Diversispora* (*Diversispora spurcum*) on the basis of inner flexible wall as a distinguishing character. *D. spurca* is the type and so far the only species of the genus *Diversispora* in the family Diversisporaceae.

Systematic Classification

Glomeromycota
Glomeromycetes
Diversisporales
Diversisporaceae
Diversispora spurca

Morphotaxonomic characterization of closely related species or genera species identification has got its own limitations. This limitation has been successfully overcome with the advent of molecular methods for identification of organisms. Sequence analyses of rDNA regions have often confirmed the morphologically defined species and the molecular data have characterized new genera and families in AM taxonomy. Moreover, general molecular phylogenies have shown that glomeromycotan diversity at the phylum and genus level is much higher than expected through microscopic observation of spore morphology. Some of the morphological characters that were used previously to delimit genera and families probably have evolved multiple times independently. It is therefore advised to our distinguished readers to kindly correlate

their morphotaxonomic studies with molecular phylogenetic results.

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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 Soil Ecology*
- *Chemosphere*
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Agnieszka Szuba. 2015	**Ectomycorrhiza of Populus** *Forest Ecology and Management* **347**: 156–169 [Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62–035 Kórnik, Poland]
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