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Arbuscular mycorrhizal and dark septate endophyte fungal association in some plants of Tripura, North-East India

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Abstract

Mycorrhizal fungi are ecologically significant because they form relationships with the host plants and provide a better knowledge about the nutrition and growth of the plants. The present investigation was carried out in three sites to examine mycorrhizal colonization in twenty plants. Among the twenty plants, arbuscular mycorrhizal (AM) fungal and dark septate endophytes (DSE) colonization was found in 18 and 11 species, respectively. Dual association of AM fungi and DSE were found in 10 plants. The presence of only vesicles and aseptate hyphae were observed in *Alternanthera dentata* and *Bambusa vulgaris.* The arbuscular mycorrhizal colonization (%) was highest recorded in *Eupatorium odoratum*. Root length with DSE fungal structures (%) was maximum in *A. dentata*. A total of 16 AM fungal species was isolated from the three soil samples. There were five, eleven and eight species of AM fungi were found from three sites belonging to the spore of *Acaulospora*, *Ambispora*, *Diversispora*, *Funneliformis*, *Glomus*, *Paraglomus*, *Rhizophagus* and *Sclerocystis*. This study revealed the wide spread occurrence of AM fungi and DSE fungal association in the studied ecosystem.

Keywords – arbuscular mycorrhizal colonization **–** dark septate endophyte colonization **–** plants

Introduction

Arbuscular mycorrhizal (AM) fungi are considered to be formed with the majority of plants growing under natural conditions (Smith & Read 1997). AM fungi are known to improve the nutritional status, growth and development of plants, protect plants against root pathogens and also offer resistance to drought and salinity (Jeffries 1987). They play a fundamental role in soil fertility and in the maintenance of stability and biodiversity within plant communities (Giovannetti & Avio 2002). Studies on the distribution and activity of AM fungi can help in the understanding of the ecological significance of AM fungal associations (Sanders 1990). Moreover, there is growing evidence that the diversity and distribution of AM fungi is related to plant community structure and ecosystem function (van der Heijden & Sanders 2002).

In addition to the widely studied AM fungi, increased attention has recently been given to ubiquitous group of miscellaneous fungi designated as dark septate endophytes (DSE) and characterized by melanized septate hyphae and microsclerotia (Barrow 2003). An analysis of the role of

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DSE in ecosystems (Mandyam & Jumpponen 2005) indicated facilitation of nutrient uptake of the host plant, alterations in host water uptake, stress tolerance and utilization of wider nutrient pools by the host through DSE. These fungi are frequent root colonizers of trees, shrubs, terrestrial orchids, and a broad range of plants in temperate and tropical habitats (Jumpponen & Trappe 1998).

There is meager report on mycorrhizal assessments from northeast India; moreover, there is also dearth of report on mycorrhizal status of bamboos. The specific goals of this study are: (i) to evaluate AM fungi and DSE colonization in 20 plants comprising six herbs, four shrubs and ten bamboos (ii) to quantify the numbers of AM fungal spores (iii) to estimate the AM fungal composition from the rhizospheric soil of the study sites.

Materials & Methods

Root and soil Sampling

Roots were collected from dominant plants from three locations of Tripura i.e., Suryamaninagar (23°45'44.00"N; 91°15'48.43"E), Nalchar (23°32'24.58"N; 91°23'38.48"E) and Salbagan (23°52'58.80"N; 91°17'18.50"E) during the period of January to March, 2013 (Fig. 1). The list of collected plants along with their families and collection sites are presented in Table 1.

The root and soil samples were collected from three plants of each species in case of herbs and shrubs and made into composite samples. The plants were sampled by collecting each plant of single species lying at a distance of 10 m apart. Moreover, one bamboo clump was considered while collecting root and soil samples from each bamboo species and from three sides around the clump, samples were collected and made into composite samples. The soil samples were collected at 0–20 cm depth around species and a sample of approximately 200 g soil per plant was collected. All the soil samples from each location were combined and collected in polythene bags, labelled and were brought to the laboratory for analysis.

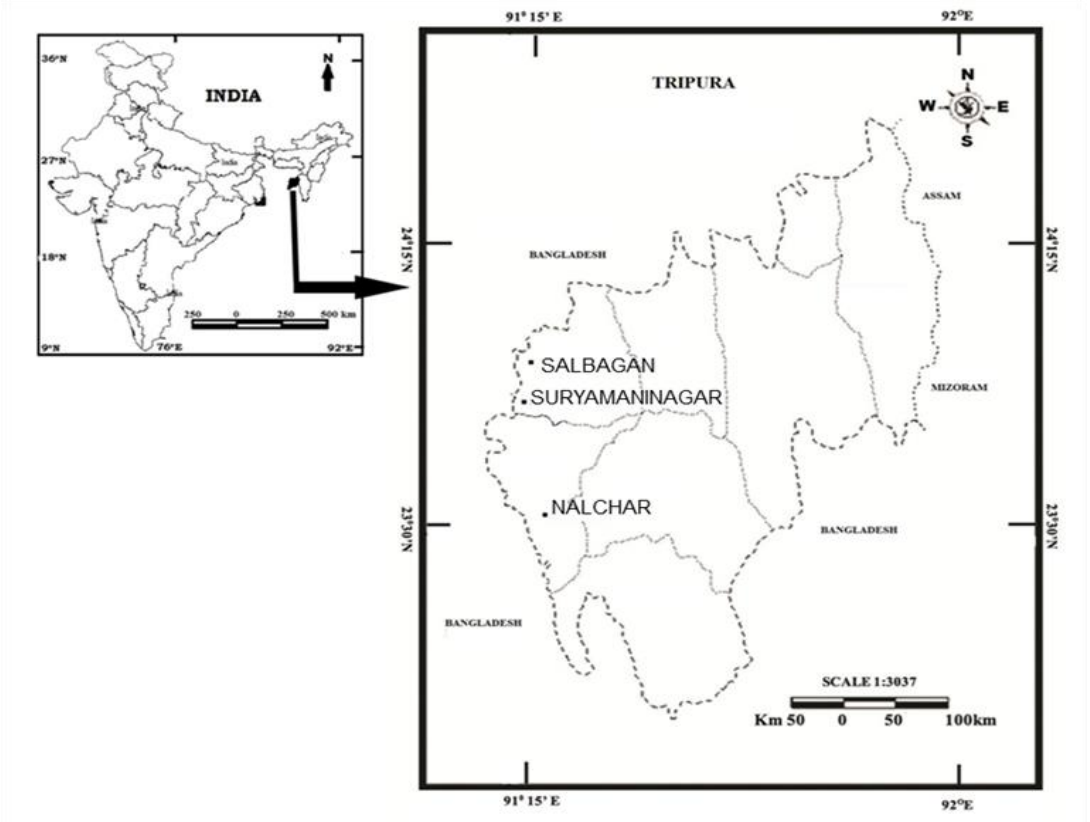

Fig. 1 – Location of map of Tripura showing study sites.

Table 1 Family and collection site of plants

Plants name	Family	Collection site
Eupatorium odoratum L.	Asteraceae	Suryamaninagar
Sida cordifolia L.	Malvaceae	Suryamaninagar
Melastoma malabathricum L.	Melastomacee	Suryamaninagar
Sida cordata (Burm.f.) Borss.Waalk.	Malvaceae	Suryamaninagar
Solanum nigrum L.	Solanaceae	Suryamaninagar
Alternanthera dentata (Moench) Stuchlik ex R.E.Fr.	Amaranthaceae	Salbagan
Andrographis paniculata (Burm.f.) Wall ex Ness	Acanthaceae	Salbagan
Vernonia cinerea (L.) Less.	Asteraceae	Suryamaninagar
Tabernaemontana divaricata (L.) R.Br. ex. Roem & Schult	Apocynaceae	Suryamaninagar
Lantana camara L.	Verbenaceae	Suryamaninagar
Bambusa balcooa Roxb.	Poaceae	Suryamaninagar
Bambusa tulda Roxb.	Poaceae	Suryamaninagar
Bambusa bambos (L.) Voss	Poaceae	Suryamaninagar
Dendrocalamus hamiltonii Nees & Arn. ex Munro	Poaceae	Suryamaninagar
Bambusa vulgaris Schrad. ex Wendl.	Poaceae	Nalchar
Bambusa polymorpha Munro	Poaceae	Nalchar
Bambusa cacharensis Majumdar	Poaceae	Suryamaninagar
Oxytenanthera nigrociliata (Buse) Munro	Poaceae	Suryamaninagar
Dendrocalamus asper (Schult. & Schult. f.)	Poaceae	Suryamaninagar
Bambusa tuldoides Munro	Poaceae	Suryamaninagar

Preparation of roots and assessment of AM fungi and DSE

The collected roots were thoroughly washed with tap water several times and cut into approximately 1cm. Then the roots were cleaned with 10% NaOH at 90°C for 24 hrs depending on the root characteristics. The cleared roots were washed again with tap water for 4-5 times and bleached in 2 drops of alkaline H_2O_2 before acidification for 2-3 mins. After acidifying with 1% HCl, roots were stained with Black Faber Castell stamp pad ink (Das & Kayang 2008). After a while the roots were mounted on slide and observed under compound microscope for AM fungal structures such as arbuscules, vesicles and hyphae and DSE fungal structures such as dark septate hyphae and microsclerotia. The estimation of AM and DSE fungal colonization were done by the magnified intersection method (McGonigle et al. 1990).

Spore analysis

For spore analysis, 25 g of soil was taken and extracted by modified wet sieving and decanting method (Muthukumar et al. 2006). The isolated spores were picked up with needle in 1–2 drops of polyvinyl alcohol-lactoglycerol under a dissecting microscope (Koske & Tessier 1983) for identification. Under a compound microscope the intact and broken spores were identified. The taxonomic identification of spores to species level was based on sporocarpic size, colour, ornamentation and wall characteristics by matching original descriptions (http://www.invam.caf.wvu.edu and http://www.lrzmuenchen.de/~schuessler/ amphylo).

Determination of soil characters

The pH and electrical conductivity were determined by taking 10 g of soil dissolved in 50 ml distilled water and stirred for 20 mins and kept it for overnight. Measurement of the soil pH and electrical conductivity were determined using a digital pH meter and conductivity meter. Soil moisture was determined. The Organic Carbon was estimated by using Walkley-Black method (Walkley & Black 1934). The soil available Nitrogen was estimated by Black (1982). Available Phosphorus of soil was determined using Jackson (1978) method.

Data analysis

Standard errors of means were calculated. ANOVA was done and means were separated by Tukey test to analyse AM and DSE fungal colonization. (Statistica 9.0).

Results

Moisture content of Salbagan soil was significantly higher than other two sites. The pH of all the samples was acidic and significantly higher in soils from Suryamaninagar. The electrical conductivity was significantly higher in Salbagan soil. The organic Carbon (%) was significantly higher in Salbagan. Available Nitrogen was higher in Nalchar soil and available Phosphorus was highest in Suryamaninagar soil. However, there was no significant difference in available Nitrogen and available Phosphorus in between the three sites (Table 2).

Table 2 Soil properties of soils collected from three locations

Soil samples	pH	Electrical conductivity (cS cm^{-1})	Moisture Content (%)	Organic Carbon (%)	Available Nitrogen (Kg/ha)	Available Phosphorus (Kg/ha)
Nalchar	5.37 ±0.03 a	20.33 ±0.88 a	12.15 ±0.08 a	0.26 ±0.04 a	327.37 ±13.59a	26.38 ±0.99a
Suryamaninagar	6.85 ±0.02 b	8.00 ±1.15 b	14.4 ±0.17 b	0.35 ±0.03b	312.87 ±25.46a	28.64 ±0.33a
Salbagan	6.24 ±0.02 c	41.00 ±1.00 c	15.9 ±0.06 c	0.41 ±0.02 b	298.36 ±25.12a	26.68 ±1.04a

Different alphabets differ significantly (p<0.05)

Among the 20 plant species, AM fungal colonization (%) was observed in 18 plants and DSE colonization (%) was observed in 11 species. AM fungi and DSE were absent in two and nine species of plants, respectively (Table 3). Dual association between AM fungi and DSE were found in 10 plants. The different fungal structures observed included intra-radical hyphae, hyphal coils, arbuscules, vesicles and DSE (Fig. 2). Root length with arbuscules (RLA) ranged from 0.42% (*Sida cordata*) to 21.49% (*Bambusa tuldoides*). Root length with vesicles (RLV) ranged from 0.42% (*B. tuldoides*) to 19.55% (*Eupatorium odoratum*) and hyphae (RLH) ranged from 4.46% (*B. vulgaris*) to 55.80% (*E. odoratum*). Root length with DSE fungal structures (%) was ranged from 0.76% (*Vernonia cinerea*) to 38.95% (*Alternanthera dentata*). The presence of only vesicles and aseptate hyphae were observed in *A. dentata* and *B. vulgaris.* There is significant differences (p<0.05) in AM fungal and DSE colonization (Table 3). However, in *A. dentata* aseptate hyphal colonization does not differs significantly with DSE fungal colonization.

AM fungal spore density in the soil samples was 43.67 ±6.94 spores/25 g, 33.67 ±3.71 spores/25 g and 55.33 ±6.39 spores/25 g of soil from Nalchar, Salbagan and Suryamaninagar, respectively (Table 4). There is significant differences (p<0.05) in spore density between sites. Spore morphotypes belonging to *Acaulospora, Ambispora, Diversispora, Funneliformis, Glomus, Paraglomus, Rhizophagus* and *Sclerocystis* were isolated from three sites i.e., Nalchar, Suryamaninagar and Salbagan (Fig. 3). Total 16 species were isolated from the three soil samples (Table 4). In this investigation, 5 species of AM fungi were found from Nalchar, 11 from Suryamaninagar and 8 from Salbagan. *Glomus* sp 1 and *Glomus* sp 5 were common to all the three sites. *Funneliformis mosseae* was common in Nalchar and Suryamaninagar soil samples. Three species namely *Glomus* sp 2, *Glomus* sp 3 and *Paraglomus brasilianum* were common in 2 sites i.e., Suryamaninagar and Salbagan.

Discussion

This study is the first report on AM fungal colonization in *Sida cordata, Bambusa balcooa, B. cacharensis, B. tuldoides* and *Oxytenanthera nigrociliata*. Furthermore, it is also the first report on DSE colonization in *E. odoratum, S. cordifolia, Melastoma malabathricum, S. cordata, Solanum nigrum, A. dentata, Andrographis paniculata, V. cinerea, Lantana camara,* and *B. cacharensis.* DSE

Table 3 Arbuscular mycorrhizal and dark septate endophyte fungal colonization (%) in plants collected from three different sites.

Name of the plants	AM fungi			DSE	Ref.[b]	Mycorrhizal status[c]
	% RLA[a]	% RLV[a]	% RLH[a]	%RLDSE[a]		
[*]Eupatorium odoratum	5.48±1.70a	19.55±4.19a	55.80±6.19b	6.58±1.76a	Muthukumar et al. (2003)	AM fungi & DSE
[*]Sida cordifolia	5.53±1.53a	18.68±3.44a	49.50±5.77b	7.53±2.02a	Tarafdar & Rao (1997)	AM fungi & DSE
[*]Melastoma malabathricum	4.86±1.37a	9.65±2.87a	31.96±4.02b	9.46±2.81a	Tawaraya et al. (2003)	AM fungi & DSE
[#]Sida cordata*	0.42±0.42a	8.17±2.63a	37.48±3.56b	1.92±0.90a	-	AM fungi & DSE
[*]Solanum nigrum	5.79±1.90a	2.05±1.12a	38.38±4.95b	3.65±2.13a	Harley & Harley (1987)	AM fungi & DSE
[*]Alternanthera dentata	0.00	16.93±3.39a	40.81±6.52b	38.95±6.45b	-	Endophyte &DSE
[*]Andrographis paniculata	1.81±1.34a	7.14±2.08a	22.19±3.88b	2.43±1.44a	Muthukumar et al. (2006)	AM fungi & DSE
[*]Vernonia cinerea	10.56±1.97a	7.98±1.69a	55.11±4.23b	0.76±0.55a	Muthukumar et al. (2006)	AM fungi & DSE
Tabernaemontana divericata	7.50±2.38a	16.93±2.85a	48.09±4.76b	0.00	Muthukumar & Udayan (1994)	AM fungi
[*]Lantana camara	3.35±1.22a	14.64±3.10a	52.37±4.58b	0.83±0.83a	Koske *et al*. (1992)	AM fungi & DSE
[#]Bambusa balcooa	6.07±1.61a	11.27±3.00a	32.48±4.14b	0.00	-	AM fungi
Bambusa tulda	12.74±3.94a	6.58±1.78a	28.72±3.61b	0.00	Das and Kayang (2010)	AM fungi
Bambusa bambos	14.37±3.19a	7.67±2.73a	27.95±5.33b	0.00	Jha et al. (2012)	AM fungi
Dendrocalamus hamiltonii	7.92±2.33a	4.58±1.76a	24.29±3.88b	2.42±0.91a	Das & Kayang (2010)	AM fungi & DSE
Bambusa vulgaris	0.00	2.55±1.34a	4.46±1.63a	0.00	Verma & Soni (2008)	Endophyte
Bambusa polymorpha	1.22±0.90a	1.27±0.88a	10.93±3.41a	0.00	Jamaluddin et al. (1997)	AM fungi
[#]Bambusa cacharensis*	19.53±4.78a	6.77±2.62a	36.28±3.89b	5.92±2.14a	-	AM fungi & DSE
[#]Oxytenanthera nigrociliata	10.55±1.46a	6.86±1.91a	32.36±3.04b	0.00	-	AM fungi
[#]Bambusa tuldoides	21.49±3.48a	0.42±0.42b	32.33±4.06a	0.00	-	AM fungi
Dendrocalamus asper	17.77±2.27a	5.76±1.95a	38.57±3.07b	0.00	Verma & Soni (2008)	AM fungi

[a] %RLA, %RLV, % RLH and %RLDSE percent root length with arbuscules, vesicles, hyphae and dark septate endophyte, respectively.
[b]Earlier references showing mycorrhizal status of plant species.
[c]Presence of arbuscules exhibit AM fungal status, dark septate hyphae and microsclerotia indicates DSE status and endophyte status was given for the presence of vesicles and aseptate hyphae only.
Asterisks indicate the species with the first report of DSE status in this work.
Hash indicates the species with the first report of AM fungal status in this work.
Different alphabets differ significantly (p<0.05)

was absent in *T. divericata, B. balcooa, B. tulda, B. bambos, B. vulgaris, B. polymorpha, Oxytenanthera nigrociliata, B. tuldoides, D. asper*. AM fungal and DSE colonization were found in 18 and 11 plant species, respectively. Hence, AM fungal colonization is more prevalent than DSE colonization in such ecosystem. However, of the 20 species *A. dentata* belongs to the family Amaranthaceae which is thought to be non-mycorrhizal and possessed only vesicles and aseptate

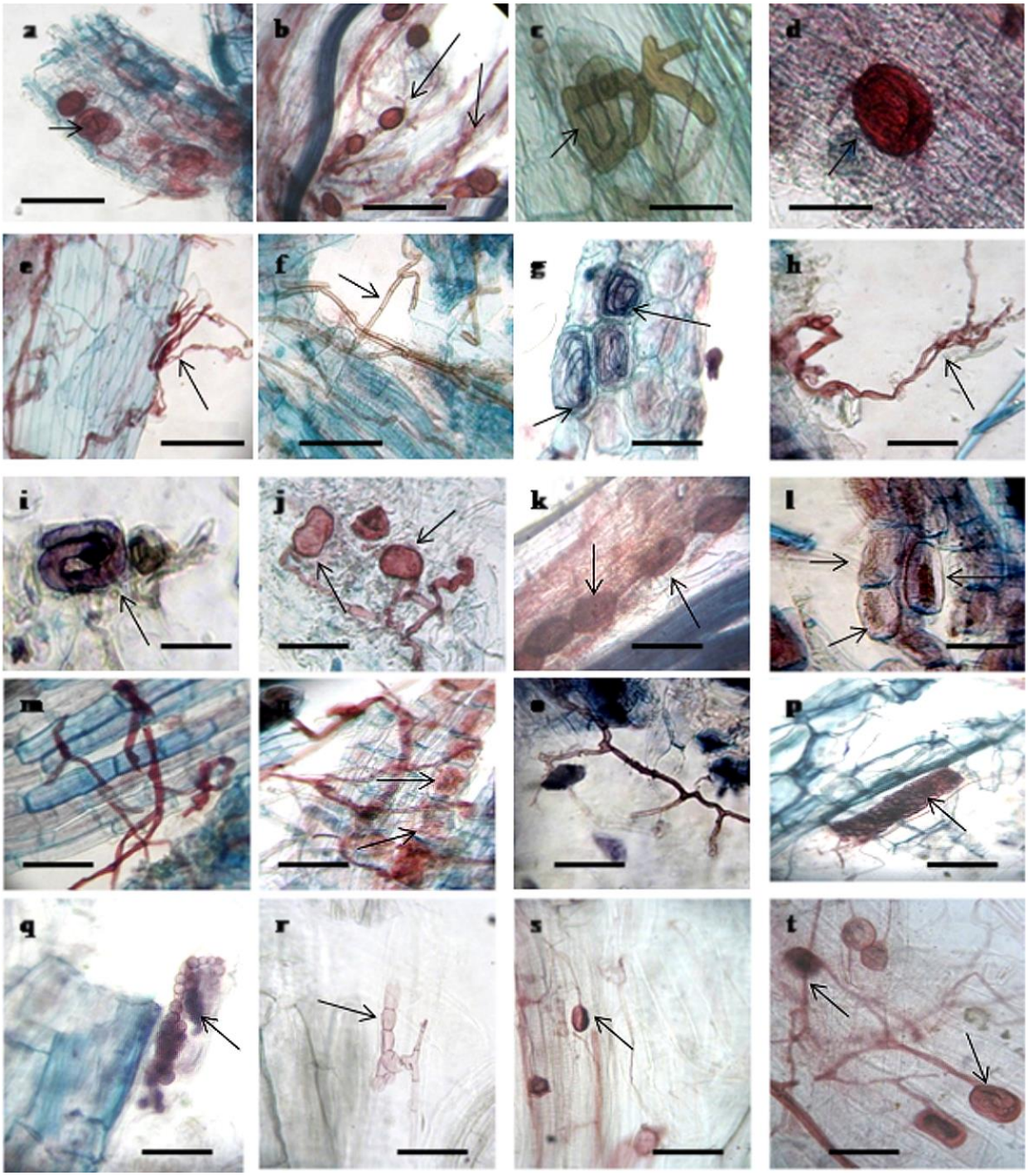

Fig. 2 – Mycorrhizal colonization in the plant roots. (a) Root segment of *Tabernaemontana divericata* showing vesicles. (b) Root segment of *Eupatorium odoratum* showing hyphae and vesicles. (c) *Melastoma malabathricum* root segment showing hyphal coil. (d), (e) & (f) root segment of *Sida cordata* showing vesicle, hyphae and DSE. (g) Root segment of *Dendrocalamus asper* showing hyphal coils. (h) Root segment of *Dendrocalamus hamiltonii* showing intraradical hyphae. (i) Root segment of *Bambusa cacharensis* showing hyphal coil. (j) Segment of *Bambusa teres* root showing vesicles with hyphae. (k) *Bambusa balcooa* root segment showing vesicles. (l) Root segment of *Bambusa nigrociliata* showing arbuscules. (m) Root segment of *Bambusa polymorpha* showing intraradical hyphae. (n) Segment of *Bambusa tulda* root showing intraradical hyphae with arbuscules. (o) Root segment of *Bambusa tuldoides* showing intraradical hyphae. (p) & (q) Root segments showing microsclerotia in *Alternanthera dentata*. (r) Portion of root colonized by DSE in *A. dentata.* (s) & (t) Portion of root colonized by vesicles like structures in *A. dentata* – Bars (a-f) = 150 µm; Bars (g-t) =200µm.

Table 4 Spore density and occurrence of AM fungal spores from three sites

AM Fungal species	Nalchar	Suryamaninagar	Salbagan
Acaulospora rehmii Sieverd. & Toro	-	-	+
Ambispora appendicula (Spain, Sieverd. & Schenck) Walker	+	-	-
Diversispora spurca (Pfeiff., Walker & Bloss) Walker & Schüßler	-	+	-
Funneliformis badium (Oehl, Redecker & Sieverd.) Walker & Schüßler	-	+	-
Funneliformis mosseae (Nicolson & Gerd.) Walker & Schüßler	+	+	-
Glomus aureum Oehl & Sieverd.	-	+	-
Glomus macrocarpum Tul. & Tul.	+	-	-
Glomus sp 1	+	+	+
Glomus sp 2	-	+	+
Glomus sp 3	-	+	+
Glomus sp 4	-	+	-
Glomus sp 5	+	+	+
Paraglomus brasilianum (Spain & Miranda) Morton & Redecker	-	+	+
Rhizophagus irregulare (Błaszk., Wubet, Renker & Buscot) Walker & Schüßler	-	-	+
Sclerocystis rubiformis Gerd. & Trappe	-	-	+
Sclerocystis taiwanensis Wu & Chen	-	+	-
No. of Species	5.0	11.0	8.0
Spore density/ 25g soil	43.67 ±6.94	55.33 ±6.39	33.67 ±3.71

Presence (+) or absence (–) of AM fungal species

hyphae which is in accord with the earlier findings (Neeraj 1991) where only vesicles and hyphae have been reported in most members of Amaranthaceae. Moreover, *B. vulgaris* which has been reported to be colonized by AM fungi (Verma & Soni 2008) possessed only vesicles and hyphae in the present study.

In this study, DSE colonization was very low in *S. nigrum* but DSE colonization was absent in this plant as studied earlier (Muthukumar et al. 2006). Deka et al. (1990) reported AM fungal colonization in *Dendrocalamus hamiltonii* ranged between 38% and 100% in seasonal studies. But it had been reported less than 36% colonization in *D. hamiltonii* (Das & Kayang 2010). However, our result reveals more than 24% colonization in *D. hamiltonii*. Das and Kayang (2010) also observed 1.85 % DSE colonization in *B. tulda* and 6.58 % DSE colonization in *D. hamiltonii*. In contrast, in this study DSE colonization was absent in *B. tulda.*

Before attaining the true management of soil microbes, it is essential to understand better the interactions between plants and microbes to the soil around the roots (Tacon et al. 1971). Edaphic factors or soil nutrient status are claimed to be implicated in the patterns and timing of the development of AM fungi (Sanders 1990; Mullen & Schmidt 1993). Here, in this study we found acidic soils from the three sites. In general, slightly acidic soils (pH 6.0 to 6.3) had significantly greater number of AM propagules, whereas the soils with pH 5.3 - 5.7 had fewer propagules (Rajeshkumar et al. 2013).

The average AM fungal spore number of this study was within the range of 54–3920 spores per 100 g soil reported for tropical soils (Valsalakumar et al. 2007). Prasad (1998) also reported a range of 5-370 spores/100 g dried soil in India. In case of grasses, AM fungal spore number was lower than the range of 272–348 spores per 100 g soil in mycorrhizal association of *Ochlandra travancorica* in Kerala, India reported recently (Rajeshkumar et al. 2013). Das and Kayang (2010) also reported higher number of spores isolated from the rhizospheric soil of four bamboo species than this study.

Out of 8 genera, *Glomus* was the predominant one represented by 7 species followed by *Funneliformis* and *Sclerocystis* represented by 2 species each, and 1 species each of *Acaulospora, Ambispora, Diversispora, Paraglomus and Rhizophagus*. Certain species of Glomales are adapted to acidic soils and generally, dominate the AM fungal community (Sieverding 1991). The dominance of *Glomus* from North-East region of India was also reported earlier (Das & Kayang 2009).

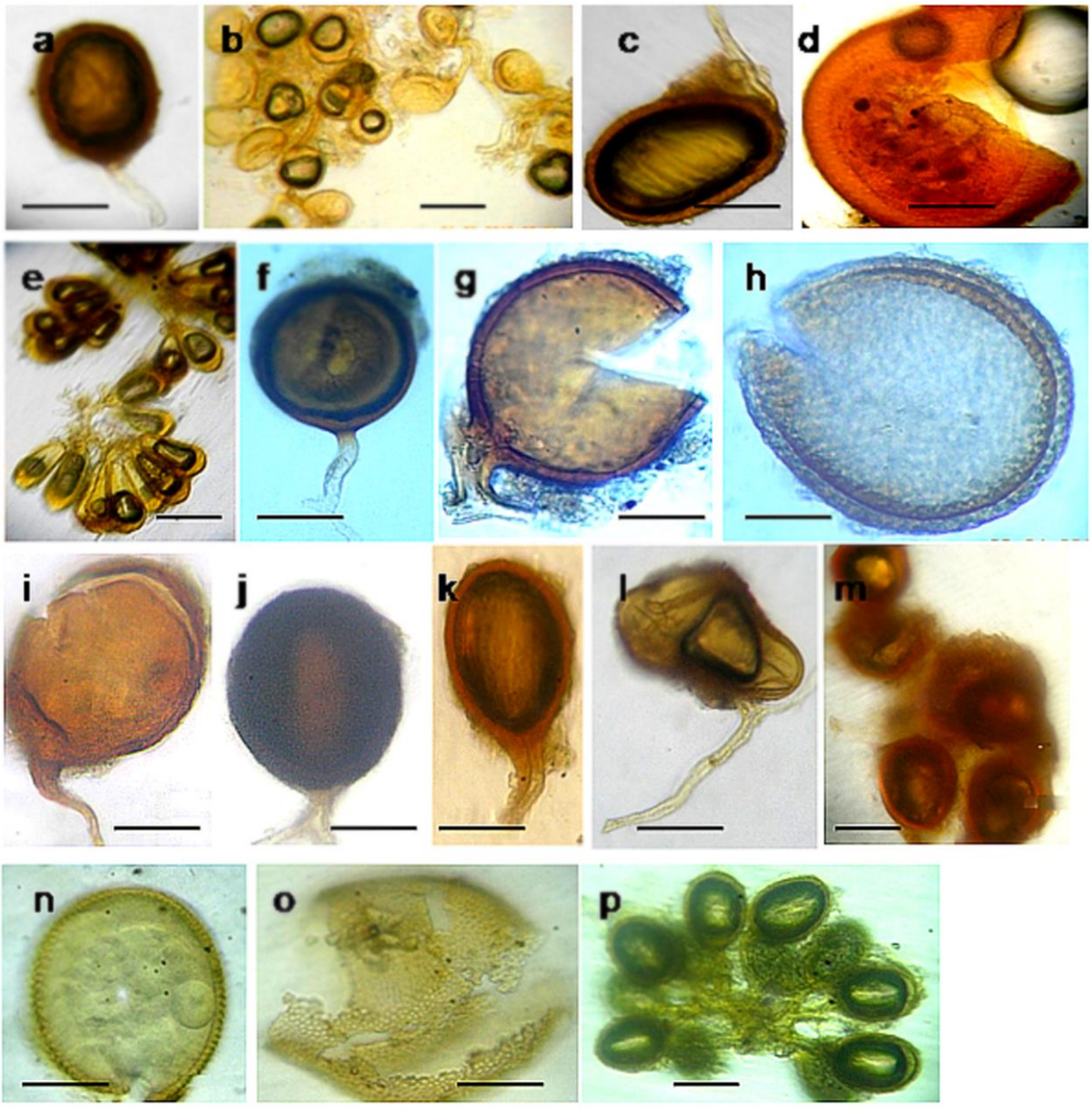

Fig. 3 – AM fungal spores. (a) *Glomus* sp 1. (b) *Glomus aureum*. (c) *Glomus* sp 2. (d) *Diversispora spurca*. (e) *Sclerocystis taiwanensis*. (f) *Glomus* sp 3. (g) *G. macrocarpum*. (h) *Ambispora appendicula*. (i) *Funneliformis mosseae*. (j) *Glomus* sp 4. (k) *Glomus* sp 5. (l) *Rhizophagus irregulare*. (m) *F. badium*. (n) *Paraglomus brasilianum*. (o) *Acaulospora rehmii*. (p) *S. rubiformis*. – Bar (acd, f-l, n & o) =50 µm; Bar (b, e, m & p) =150 µm).

Conclusion

This study revealed the wide spread occurrence of AM fungi and DSE fungal association in herbs, shrubs and bamboo of Tripura. Moreover, highest abundance of *Glomus* revealed its suitability with the plants in natural environment of Tripura. Thus, AM and DSE fungi may play important role in improving growth of plants in such ecosystem.

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