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RESEARCH ARTICLE

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Arbuscular mycorrhizal fungi and dark septate endophyte colonization in bamboo from Northeast India

Abstract To address paucity of the mycorrhizal studies in bamboo, we have investigated arbuscular mycorrhizal fungal (AMF) distribution and dark septate endophyte (DSE) colonization on four species of bamboo from Northeast India. *Bambusa tulda* exhibits *Arum* type of AMF morphology, and other bamboo species have *Paris* type. AMF colonization was significantly higher than DSE colonization ($P < 0.05$). Vesicular and DSE colonization exhibit a significant positive correlation with organic carbon and available phosphorus, respectively ($P < 0.05$). Of 17 species isolated from *Acaulospora*, *Ambispora* and *Glomus*, 12 were isolated from *Phyllostachys manii*. *Acaulospora tuberculata*, *A. rehmii*, *Glomus intraradices* and *G. tortuosum* were the most frequently distributed species. Shannon diversity index was the highest in *P. manii*. Principal component analysis (PCA) plots and cluster analysis suggest that *P. manii* was the most dissimilar bamboo species in terms of mycorrhizal colonization and soil properties.

Keywords arbuscular mycorrhizal fungi, dark septate endophyte, bamboo, Northeast India

1 Introduction

Bamboo is a vernacular term for members of subfamily Bambusoideae of family Poaceae. Almost all of the 75 genera and 1250 species of bamboo are woody and fast growing. Bamboo is the most versatile forest produce, and it is one of the most important renewable natural resources which have capability to produce maximum biomass compared to other forest plants. Bamboo is extensively propagated in natural afforestation programmes to meet industrial and rural requirements for wood products and to check erosion and conserve soil. Bamboo resources have considerably dwindled from the natural habitats due to exploitation, shifting cultivation, gregarious flowering and extensive forest fires. Therefore, system cultivation and their scientific management can ensure sustainable production (Tewari, 1992).

Arbuscular mycorrhizal fungi (AMF) form the most frequent type of symbiosis between plants and fungi in terrestrial ecosystems (Smith and Read, 1997). AMF develop two morphological types of colonization, which were first described (Gallaud, 1905). *Arum*-type of AMF morphology is common in cultivated plants, characterized by intercellular hyphae and intracellular arbuscules, whereas many plants in natural ecosystems have the *Paris*-type morphology, with intracellular hyphal coils and arbuscular coils (Muthukumar and Prakash, 2009). Another group of fungi with dematiaceous septate hyphae, termed dark septate endophytes (DSE), are the frequent colonist of plant roots at high latitudes and altitudes (Upson et al., 2009). In addition, DSE are abundant in many plant genera (Jumpponen and Trappe, 1998) may be as ubiquitous chiefly under harsh climatic conditions (Addy et al., 2005).

The occurrence and distribution of AMF in forestry species including bamboo and association of AMF and the dependency of bamboo species on them have been reported (Khan and Uniyal, 1999). In addition, differences in growth responses of bamboo to mycorrhizal inoculation have been demonstrated. The synergistic effect of AMF and plant growth promoting rhizobacteria in bamboo was reported (Muthukumar and Udaiyan, 2006).

However, there are no DSE studies on bamboo. Therefore, we have undertaken the present work to examine AMF morphology, diversity of AMF and DSE

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colonization in four species of bamboo and, in addition, to analyze presence of any relation of mycorrhizal structural colonization with properties of rhizosphere soil of bamboo species.

2 Materials and methods

2.1 Sampling

Bambusa tulda Roxb., *Dendrocalamus hookerii* Munro, *Dendrocalamus hamiltonii* Nees et Arn. ex Munro and *Phyllostachys manii* Gamble were selected for mycorrhizal studies in the month of October 2008 from plantations of Meghalaya, Northeast India. All the bamboo species were relatively located in 25°36.735′ N, 091°53.880′ E at 1537 m.a.s.l. The patches of *D. hookerii* were 200 m apart from *D. hamiltonii*. *B. tulda* was situated in between *D. hamiltonii* and *Phyllostachys manii* at a distance of 100 m. 1st order roots of four species along with attached subsequent order roots which are generally thin and small comparatively in size were collected from five rhizomes at several points for each bamboo species and merged to one sample to quantify mycorrhizal colonization. The soil samples of each species were collected at 0–20 cm depth around from five different rhizomes of each species, and a combined sample of approximately 500 g soil per plant was collected. The soil samples were placed in polythene bags, labeled and transported for further analysis in the laboratory. The soil samples were air-dried after analysis of pH and moisture content. Then leaf litters were removed, ground, sieved with a 2-mm sieve, stored at refrigerator and processed for further soil physical and chemical properties as well as for spore analysis.

2.2 Root processing and spore analysis

For the determination of percent root colonization, root samples collected from rhizome were washed in tap water and cut into 1-cm-long pieces for further processing to investigate hyphal, arbuscular, vesicular and dark septate hyphal structures and stained with black Faber Castell stamp pad ink (Das and Kayang, 2008). Fifty segments of approximately stained samples were mounted on slides in lactoglycerol and examined for AMF and DSE structures under light microscope (Olympus 41209). The estimation of AMF and DSE colonization was estimated by the magnified intersection method (Mcgonigle et al., 1990) and converted into percentage root length.

The spores were isolated from 25 g soil (Muthukumar et al., 2006). The isolated spores were picked up with needle in 1–2 drops of polyvinyl alcohol-lactoglycerol under a dissecting microscope and also in mixed polyvinyl alcohol-lactoglycerol: Meltzer's reagent (1∶1, v∶v) for identification. The complete and broken spores were examined using a compound microscope Olympus. Taxonomic identification of spores to species level was based on sporocarpic size, color, and ornamentation and wall characteristics by matching original descriptions (http://www.invam.caf.wvu.edu & http://www.lrz-muenchen.de/~schuessler/amphylo). The photography of the root segments colonized by fungi and spores of AMF were done with the help of Leica EC 3 camera attached in Leica DM 1000 microscope (Switzerland). Spore density (SD), relative abundance (RA) and isolation frequency (IF) were determined (ZHAO Dandan and ZHAO Zhiwei, 2007).

2.3 Soil analysis

Soil texture was analyzed using a sodium hexametaphosphate method (Allen et al., 1974). For moisture content (%), 10 g sub sample of three soil replicates was oven-dried, and weight was determined from each site. Measurement of pH was done using microprocessor-based Pocket pH Tester 2 (Eutech Instruments). Available soil phosphorus was determined following molybdenum-blue method (Allen et al., 1974). The soil organic carbon was estimated using colorimetric method (Anderson and Ingram, 1993).

2.4 Data analysis

Standard errors of means were calculated, analysis of variance (ANOVA) was carried out and means were separated by Tukey test. Pearson correlation coefficients were computed for soil physicochemical properties and mycorrhizal structural colonization. The mycorrhizal, physical, and chemical variables of soil with higher influence on the variability between bamboo species were determined by principal component analysis (PCA). Furthermore, PCA was used to determine variation in AMF abundance. Soil physico-chemical properties, mycorrhizal colonization and AMF abundance were scored for cluster analysis to determine the similarity between bamboo species. Diversity indices, cluster analysis and PCA analysis were done with the help of PAST (Hammer et al., 2001).

3 Results

The soil physical and chemical characteristics are presented in Table 1. All the bamboo species were colonized by AMF and DSE (Fig. 1 and Table 2). AMF colonization is significantly higher ($P > 0.05$) than DSE colonization (Fig. 2). Spore density count reveals that 166, 481, 103 and 714 spores isolated from 25 g rhizosphere soil of *B. tulda*, *D. hookerii*, *D. hamiltonii* and *P. manii*, respectively. Correlation between soil properties and mycorrhizal structural colonization is presented in Table 3.

AMF species from *Acaulospora*, *Ambispora* and

Table 1 Selected soil physical and chemical characteristics of bamboo species

bamboo species	moisture content /%	texture /%				organic carbon /%	available phosphorus /(μg·g^{-1})
		sand	silt	clay	pH		
Bambusa tulda	28.60	75.06	11.79	13.15	5.33	0.44	1.73
Dendrocalamus hookerii	24.10	93.73	3.63	2.64	5.90	0.42	1.43
Dendrocalamus hamiltonii	19.77	88.34	3.63	5.33	5.87	0.45	1.50
Phyllostachys manii	14.77	75.36	2.56	22.08	5.60	0.54	1.57

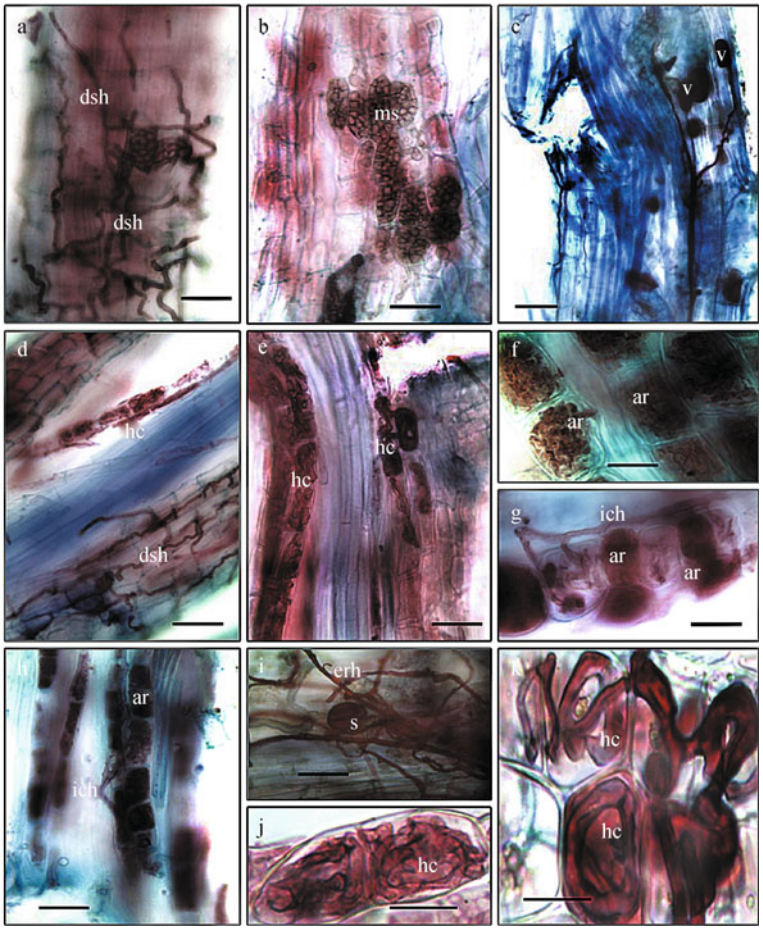

Fig. 1 Mycorrhizal colonization in the root of four bamboo species
Note: (a–e) are root segment of *Dendrocalamus hamiltonii* showing dark septate hyphae (dsh), microsclerotia (ms), vesicles (v) and hyphal coil (hc). Scale bar = 150, 200, 400, 300 and 150 μm, respectively. (f) is arbuscules (ar) in *Phyllostachys manii*. Scale bar = 100 μm. (g–i) are section of root of *Bambusa tulda* showing intercellular hyphae (ich), arbuscules , extra radical hyphae (erh) and spore (s). Scale bar = 100 μm all. (j–k) are root portion of *Dendrocalamus hookerii* showing hyphal coils. Scale bar = 50 μm all.

Glomus were recovered from the bamboo species. Relative abundance and isolation frequency of AMF species isolated from bamboo species are available in Table 4. *Acaulospora foveata* was the highest abundant species extracted from the clump of *B. tulda*. *A. rehmii* and *Glomus* sp. 1 were the least abundant species isolated from the clump of *D. hookerii*. Of 17 species isolated, *A. tuberculata, A. rehmii*, *G. intraradices* and *G. tortuosum* were extracted from the rhizosphere of three species. Diversity indices are presented in Table 5. Diversity indices such as Shannon, Simpson, Menhinick and Margalef were high in *P. manii*. However, evenness and equitability were high in the clump of *B. tulda*.

PCA plotted with soil properties and mycorrhizal structural colonization shows variation of 50.18% and 40.86% in principal components (PC) 1 and 2, respectively (Fig. 3). Clay, organic carbon, phosphorus, arbuscules, vesicles and dark septate hyphae relate with axis 1. Moisture, silt and hyphae relate with axis 2. Among the bamboo species, *P. manii* and *B. tulda* correlate with axes 1

Table 2 Mycorrhizal structural colonization (%) in the roots of bamboo species

bamboo species	AMF morphology	AMF /%		DSE /%	
		arbuscules	vesicles	hyphae	dark septate hyphae
Bambusa tulda	*Arum*	21.58±1.83a	1.49±0.34c	53.51±3.62d	1.85±0.72c
Dendrocalamus hookerii	*Paris*	19.97±2.18a	1.11±0.4c	29.9±1.83b	1.84±0.77c
Dendrocalamus hamiltonii	*Paris*	21.04±2.56 a	1.24±0.36 c	35.94±2.29b	6.58±1.52c
Phyllostachys manii	*Paris*	31.77±1.17b	0.22±0.1c	44.41±1.44e	0.25±0.14c

Note: Tukey test showing different alphabetical letters significantly varies ($P < 0.05$).

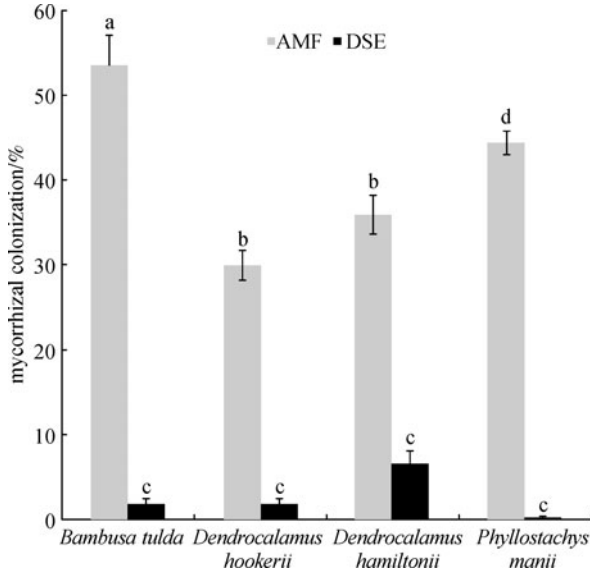

Fig. 2 Mycorrhizal colonization in the root of four species of bamboo
Note: Tukey test showing different alphabetical letters significantly varies ($P < 0.05$).

and 2, respectively. AMF species abundance PCA ordination explains 48.21% and 34.85% variation in PC 1 and PC 2, respectively (Fig. 4). *A. rehmii*, *A. scrobiculata*, *G. tortuosum* and *G. macrocarpum* relate with axis 1. *G. mosseae*, *G. aggregatum*, *G. constrictum* and *A. tuberculata* relate with axis 2. *P. manii* and *D. hookerii* were related with axes 1 and 2, respectively. Cluster analysis suggests that *P. manii* and *B. tulda* were the most dissimilar sites in terms of soil properties and mycorrhizal structural colonization as well as in AMF species abundance (Fig. 5).

4 Discussion

There are no previous reports on AMF morphology and DSE studies in bamboo to the best of our knowledge. Moreover, AMF colonization exceeds DSE colonization while Li et al. (2005) found DSE colonization has less variation than that of AMF in a grassland ecosystem. Our study reveals that bamboo species prefer AMF colonization to DSE colonization. Occurrence of both *Arum* and *Paris* morphology in bamboo species was in accord with the study of Muthukumar and Prakash (2009) which suggests that there was a lack of relationship between plant classifications and AMF morphological type. Deka et al. (1990) reported that in *D. hamiltonii* AMF colonization ranged between 38% and 100% in seasonal studies. However, our result reveals less than 36% colonization.

The AMF colonization parameters did not correlate with

Table 3 Correlation between mycorrhizal colonization and soil properties

item	arbuscule	vesicle	hyphae	dark septate hyphae	spore density/ 25 g soil
moisture content (Mc) /%	−0.59	−0.76	0.87	0.25	0.03
sand (Sa) /%	−0.15	−0.64	0.29	−0.94	0.48
silt (Si) /%	−0.55	−0.36	0.66	0.75	−0.11
clay (Cl) /%	0.53	0.91	−0.68	0.69	−0.61
pH(pH)	0.08	−0.28	−0.09	−0.98*	0.47
organic carbon (C) /%	0.68	0.99**	−0.91	0.29	−0.43
available phosphorus (P) /($\mu g \cdot g^{-1}$)	−0.28	0.15	0.24	0.98*	−0.27
arbuscules (Ar)	1.0	0.75	−0.89	−0.14	−0.77
vesicles (V)		1.0	−0.92	0.33	−0.56
hyphae (Hy)			1.0	0.06	0.5
dark septate hyphae (Dsh)				1.0	−0.34
spore density/25 g soil (Sd)					1.0

Note: * means significant correlation at $P < 0.05$ and ** means significant correlation at $P < 0.01$.

Table 4 Relative abundance and isolation frequency of arbuscular mycorrhizal fungi of bamboo species from 25 g of soil

fungi species	abbreviation	relative abundance /%				isolation frequency
		Bambusa tulda	*Dendrocalamus hookerii*	*Dendrocalamus hamiltonii*	*Phyllostachys manii*	
Acaulospora tuberculata Janos & Trappe	*At*	21.05	31.86	0.00	1.69	75
A. foveata Janos & Trappe	*Af*	50.88	0.00	0.00	1.69	50
A. rehmii Sieverd. & Toro	*Ar*	0.00	0.49	1.43	4.24	75
A. scrobiculata Trappe	*As*	0.00	0.00	0.00	3.39	25
A. cavernata Blaszk.	*Ac*	0.00	0.00	7.14	0.00	25
Acaulospora sp. 1	*A1*	0.00	0.00	14.29	0.00	25
Glomus aggregatum Schenck & Smith emend. Koske	*Ga*	0.00	36.27	0.00	0.00	25
G. ambisporum Smith & Schenck	*Gam*	0.00	0.00	0.00	0.85	25
G. clavisporum (Trappe) Almeida & Schenck	*Gc*	0.00	0.00	0.00	2.54	25
G. constrictum Trappe	*Gco*	0.00	26.96	0.00	4.24	50
G. intraradices Schenck & Smith	*Gi*	24.56	0.00	45.71	10.17	75
G. macrocarpum Tulasne & Tulasne	*Gmac*	0.00	0.00	0.00	27.97	25
G. mosseae (Nicol. & Gerd.) Gerd. & Trappe	*Gmos*	0.00	2.94	1.43	0.00	50
G. tortuosum Schenck & Smith	*Gt*	3.51	0.00	15.71	33.90	75
G. rubiforme (Gerd. & Trappe) Almeida & Schenck	*Gr*	0.00	0.00	0.00	8.47	25
Glomus sp. 1	*G1*	0.00	0.49	14.29	0.00	50
Ambispora leptoticha (Schenck & Smith) C. Walker, Vestberg & Schuessler	*Am*	0.00	0.98	0.00	0.85	50
total		100.00	100.00	100.00	100.00	

soil P, which is in accord with the study by Ruotsalainen et al. (2002). However, the DSE colonization was significantly correlated with available phosphorus, whereas Upson et al. (2009) reported a negative correlation between shoot and root phosphorus content and DSE colonization. Insignificant negative correlation was observed between dark septate hyphal colonization and spore density and arbuscules of AMF, while Wu et al. (2009) noticed a negative significant correlation with hyphal colonization and DSE; however, they suggested that there may be presence of AMF competition with DSE for the econiche. Vesicular colonization was significantly correlated with organic carbon, which is in accord with the finding of Panwar and Tarafdar (2006).

The distribution of AMF was found to be relatively uneven as suggested from the isolation frequency and evenness. The distribution of 13 AMF fungi was restricted to only one or two bamboo species. Eight and four AMF species were isolated from *D. hamiltonii* and *B. tulda*, respectively, while Khan and Uniyal (1999) reported 10 and seven species, respectively. *Ambispora* was recorded for the first time from bamboo, whereas *Entrophospora* and *Scutellospora* were not observed in our study as reported by Khan and Uniyal (1999). *G. macrocarpum* and *G. mossae* were seen to be common with the studies (Khan and Uniyal, 1999). Moreover, 14 species were recorded in the rhizosphere of plants in a bamboo forest in Taiwan (Wu and Chen, 1986).

The bamboo species *Chusquea simpliciflora* resulted in the prolific sporulation of a wider range of AMF species,

Table 5 Diversity index of arbuscular mycorrhizal fungi associated with bamboo

diversity index	*Bambusa tulda*	*Dendrocalamus hookerii*	*Dendrocalamus hamiltonii*	*Phyllostachys manii*
Taxa_S	4	7	7	12
Dominance_D	0.38	0.34	0.29	0.25
Shannon_H	1.14	1.3	1.53	1.92
Simpson_1-D	0.62	0.66	0.7	0.75
Evenness_e^H/S	0.78	0.52	0.66	0.57
Menhinick	0.4	0.72	0.71	1.24
Margalef	0.65	1.32	1.31	2.43
Equitability_J	0.82	0.67	0.79	0.77
Fisher alpha	0.84	1.74	1.73	3.67
Berger-Parker	0.51	0.38	0.47	0.35

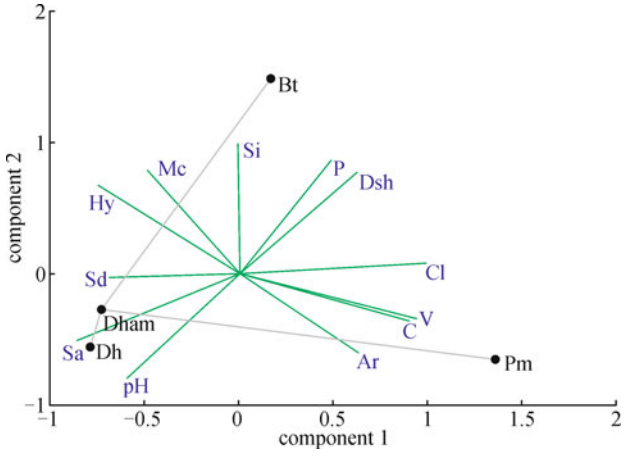

Fig. 3 PCA ordination showing variation in soil properties and mycorrhizal structural colonization
Note: Bt, Dh, Dham and Pm represent *Bambusa tulda*, *Dendrocalamus hookerii*, *Dendrocalamus hamiltonii* and *Phyllostachys manii*, respectively. For other abbreviations, see Table 3.

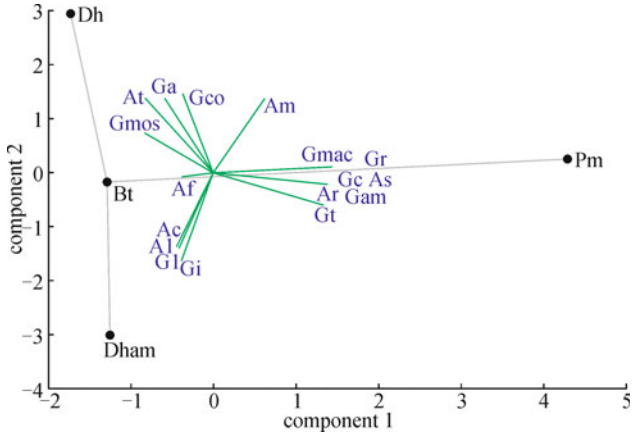

Fig. 4 PCA biplot showing variation in AMF abundance
Note: Bt, Dh, Dham and Pm represent *Bambusa tulda*, *Dendrocalamus hookerii*, *Dendrocalamus hamiltonii* and *Phyllostachys manii*, respectively. For other abbreviations, see Table 4.

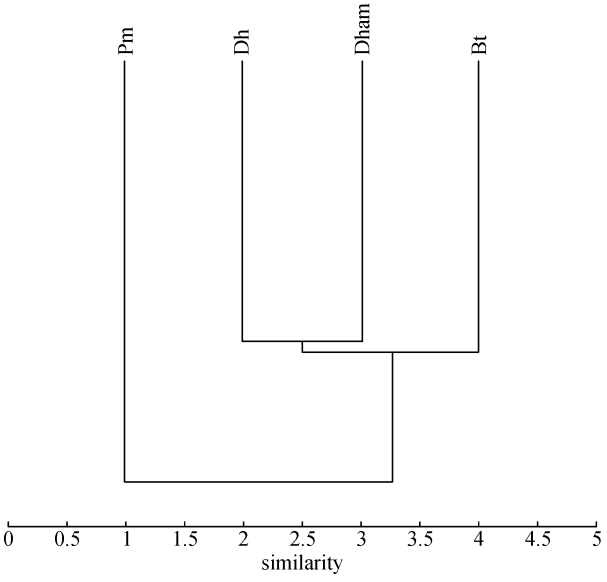

Fig. 5 Cluster analysis plotted with soil properties, mycorrhizal structural colonization and AMF abundance showing similarity measurement in bamboo species

and the high dominance of this grass most likely contributed to the high species richness and notable differences in composition of AMF spores (Mangan et al., 2004). *P. manii* was the most dissimilar as expressed from mycorrhizal colonization and soil properties. In addition, *P. manii* harbors 12 AMF species that were most likely influenced by its runner habit than the clump habit of other bamboo species.

With over-exploitation and decline of their habitats, the bamboo resource is being depleted at an alarming rate. Sustained availability can be ensured only by raising bamboo plantations. Therefore, it was suggested to evolve technology to combat exploitation of bamboo resources (Tewari, 1992). With the proper knowledge of mycorrhizal diversity in the rhizosphere of bamboo species, we can ensure better mycorrhizal inoculation programmes for sustainable availability of bamboo resources.

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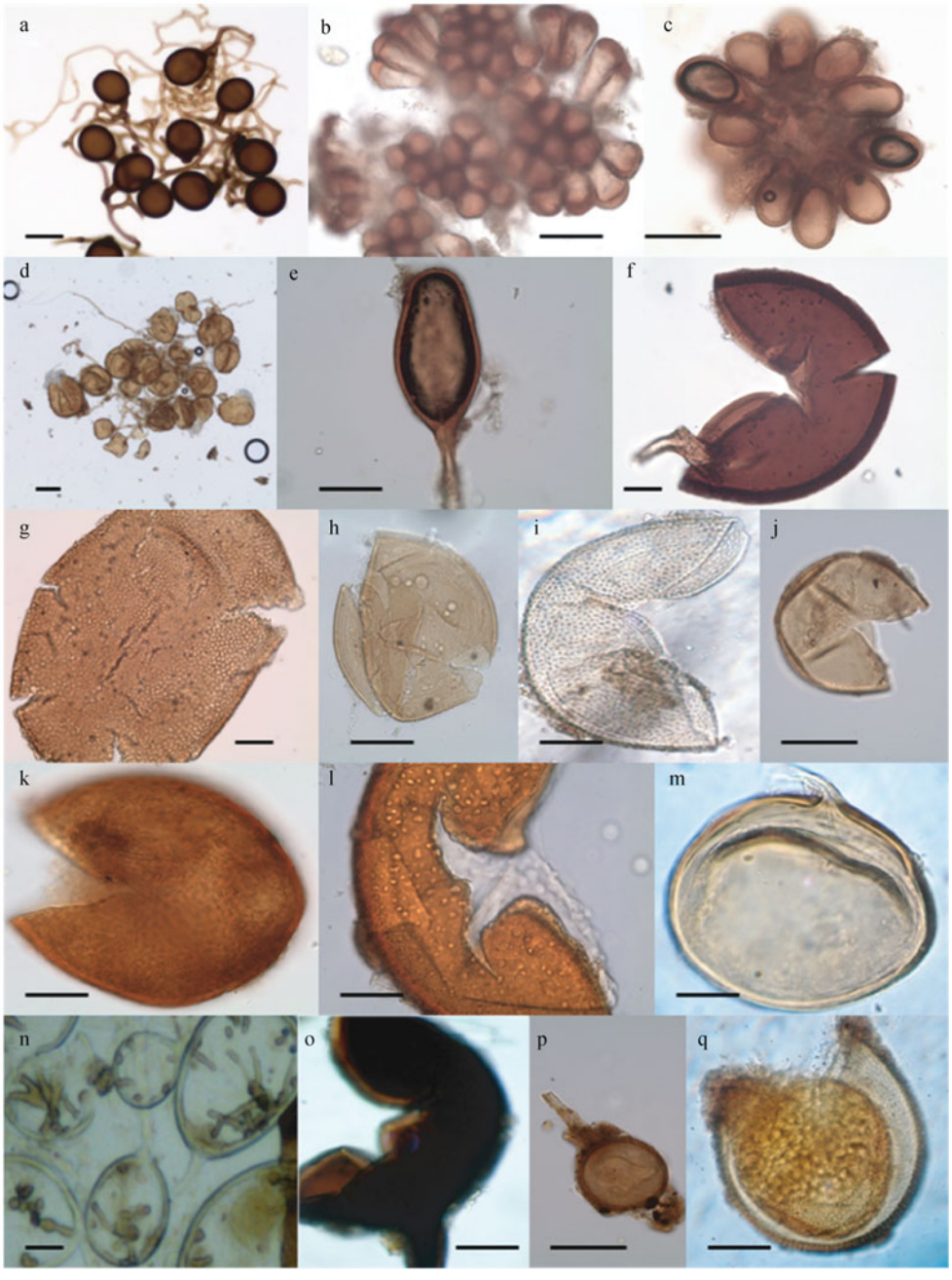

Supplementary Fig. 1 Arbuscular mycorrhizal spores associated with bamboo species

Note: (a) is spore cluster of *Glomus macrocarpum* with scale bar = 250 μm. (b) is crushed sporocarp of *G. clavisporum* with scale bar = 100 μm. (c) is sporocarp of *G. rubiforme* with scale bar = 75 μm. (d) is spore cluster of *G. intraradices* with scale bar = 500 μm. (e) is *Glomus* sp 1with scale bar = 100 μm. (f) is *G. ambisporum* with scale bar = 200 μm. (g) is *Acaulospora cavernata* with scale bar = 200 μm. (h) is *A. tuberculata* with scale bar = 100 μm. (i) is *A. scrobiculata* with scale bar = 100 μm. (j) is *Acaulospora* sp. 1with scale bar = 50 μm. (k) is *A. rehmii* with scale bar = 100 μm. (l) is *A. foveata* with scale bar = 100 μm. (m) is *G. mosseae* with scale bar = 100 μm. (n) is *G. aggregatum* with scale bar = 200 μm. (o) is *G. constrictum* with scale bar = 100 μm. (p) is *G. tortuosum* with scale bar = 50 μm. (q) is Acaulosporoid spore of *Ambispora leptoticha* with scale bar = 100 μm.