

# Diversity and Distribution of Endomycorrhizae and Dark Septate Endophytes of some Economically Important Bamboos of Assam, India

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## Abstract

The genus *Bambusa* Schreb. which belongs to the Poaceae family is commonly present in north-eastern region of India. A survey was undertaken in three villages viz. *Tilikiaam*, *Maoutgaon* and *Nathgaon* of Jorhat district, Assam, North-east India, where natural populations of *Bambusa* species were observed. Four bamboo species i.e. *Bambusa bambos* (L.) Voss, *B. tulda* Roxb., *B. pallida* Munro and *B. nutans* Wall. ex. Munro were found locally economically important and the rhizospheric soil and root samples were collected for screening of dark septate endophytes (DSE) as well as arbuscular mycorrhizal (AM) colonization. Quantitative analysis of root samples showed the presences of all the three types of endomycorrhizal root infection/colonization namely hyphal, vesicular and arbuscular. Beside this, the dark septate endophytic infections were also observed in all the bamboo species. The cent-percent endomycorrhizal (hyphal and vesicular) and DSE hyphal infections were reported in roots of all the bamboo species respectively. There were variations observed in arbuscular infection in *B. nutans* and *B. bambos* (100%), *B. pallida* (90%) and least in *B. tulda* (70%). Qualitative analysis revealed that the Endomycorrhizae found in the rhizospheric soil predominantly belongs to five genera viz. *Glomus*, *Acaulospora*, *Gigaspora*, *Scutellospora* and *Entrophospora*. The genus *Glomus*, is the most dominant, with 17 species (61%), *Acaulospora* with 7 species (25%), *Entrophospora* with 2 species (7%), *Scutellospora* (3.5%) and *Gigaspora* (3.5%) with 1 species each. Distribution of AM fungi were highest in *B. bambos* (67.7%) followed by *B. pallida* (19.4%), *B. tulda* (11.1%) and least in *B. nutans* (2.8%). Bamboo resources have been considerably declining due to exploitation, shifting cultivation, gregarious flowering and extensive forest fires from the natural habitats. Therefore, further microbial based applied researches should be undertaken to protect the dwindling natural bamboo resources and considering AMF bioinoculants in future management practices in order to maintain diminishing ecosystems.

**Keywords:** bambusicolous fungi; endophytic fungi; rhizosphere; root colonization; VAM fungi

## Introduction

In addition to being susceptible to soil-borne pathogens, plant roots are also colonised by non-pathogenic or mutualistic fungi like arbuscular mycorrhizae (AM), ectomycorrhizae (EM) and dark septate endophytes (DSE). The majority (probably 70-80%) of terrestrial plants are capable of interacting with arbuscular mycorrhizal (AM) fungi in nature (Jiang *et al.*, 2013). Mycorrhizas form a symbiotic association with the roots of host plants, where carbon flows to the fungus and inorganic nutrients move to plant. These fungi develop external hyphae approximately 3 m in diameter (Simard *et al.*, 2002). The hyphae produce arbuscules and in most fungal genera, vesicles act as a bridge connecting the root with the surrounding soil (Allen, 1992). The AM fungi are characterized by the formation of

an extraradical mycelium and branched haustorial structures within the cortical cells, termed arbuscules (Hock and Varma, 1995). The symbiotic nature of VAM fungi with higher plants plays an important role in plant nutrition cycle (Harley and Smith, 1983). In recent years, it has been well established that the VAM (Vesicular Arbuscular Mycorrhizal) fungi influence plant growth and biomass production (Hall, 1988). This is brought out by alterations in host physiology. Like photosynthesis, root exudation, carbohydrate and the amino acid status (Nemec and Meredith, 1981; Nemec and Guy, 1982; Harries *et al.*, 1985). They may be involved in improving uptake of macro- and micronutrient, increasing plant resistance against biotic and abiotic stress, and beneficial alternations of plant growth regulators (Smith, 1996; Liu and Li, 2000; Govindarajulu *et al.*, 2005; Cruz and Husain, 2008; Smith and Read, 2008). Nowadays, mycorrhizal fungi have been

widely used in agriculture, horticulture, and forestry programs, as well as for environmental reclamation, to increase crop yield and health and to limit the application of agrochemicals (Johansson *et al.*, 2004).

Dark septate endophytes (DSE) comprise a miscellaneous group of root-inhabiting fungi. A recent review (Jumpponen and Trappe, 1998) defined DSE as conidial or sterile ascomycetous fungi that colonize living plant roots without causing apparent negative effects such as tissue disorganization. DSE have been reported to possess a range of enzymatic capabilities (Currah and Tsuneda, 1993; Fernando and Currah, 1995; Haselwandter and Read, 1980; Caldwell *et al.*, 2000). Caldwell *et al.* (2000), for example, concluded that DSE are able to utilize some of the major organic detrital nutrient pools (Jumpponen, 2001). DSE are found worldwide and coexist often with different mycorrhizal fungi. They have been reported from 600 plant species including plants that have been considered non-mycorrhizal (Jumpponen and Trappe, 1998).

In India, bamboo encompasses about 8.96 million hectares of forest area and its present usage is to the tune of Rs. 2,043 crores. Bamboo, being an important forest turn out, plays a vital role in the rural financial system of our country. In order to meet the future demand of bamboo, its growth and yield has to be hastened from the nursery stage onwards. Inoculations of different bamboo species with bio-fertilizers including arbuscular mycorrhizal (AM) fungi have been shown to significantly increase the plant growth and yield under variable conditions (Jha *et al.*, 2012).

The occurrence and distribution of AMF in the forestry species including bamboo and association of AMF and dependency of bamboo species on them have been reported (Khan and Uniyal, 1999). Dash *et al.* (2008) have reported higher growth and biomass production of *D. strictus*, *Bambusa bambos* (L.) Voss, and *B. vulgaris* Wendl. Ex Nees, after inoculation with an unidentified AM species. However so far, there are no DSE studies on bamboos. Moreover, till date, a very few reports are available on the endomycorrhizal diversity on the bamboos of North East India. Thus, the present study was undertaken to enumerate the endomycorrhizal diversity associated with some of the economically important bamboos of Assam in North East India viz. *Bambusa bambos* (L.) Voss, *Bambusa tulda* Roxb., *Bambusa pallida* Munro and *Bambusa nutans* Wall. ex. Munro (Table 1).

## Materials and Methods

### Soil sample collection

The plant species were collected from three sites viz. *Tilikiaam*, *Maoutgaon* and *Nathgaon* of Jorhat district, Assam in North East India (Fig. 1) (26.75° N - 94.22° E)

and the morphology of the target species was studied on spot. Rhizospheric soil samples (at least three samples) were taken by digging out a small amount of soil (500 g) close to plant roots up to the depth of 15-30 cm and these samples were kept in sterilized polythene bags at 10 °C for further processing in the laboratory for analyses of endomycorrhizal micro-symbiotic associations.

### Isolation of AM spores

Isolation of AM spores was done by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Approximately 100 g of soil was suspended in 1 litre or more of water. Heavier particles were allowed to 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 which removes 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 different Petri dishes then the trapped spores were transferred to Whatman filter paper no. 1 by repeating washing with water. The spores were picked by hypodermic needle under stereo- binocular microscope.

### Mycorrhizal quantification

For quantitative estimation of AM spores, Gaur and Adholeya (1994) modified method was used. The filter paper was divided into many small sectors by marking with a ball 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 $\approx$ AM fungi

For identification of AM spores the following criteria were used like conventional morphological character *i.e.* 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 AM 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 Phillips and Hayman (1970) method. Roots were first cleared with 10% (w/v) KOH at 98 °C for one hour. After clearing, roots were bleached with a 5% H<sub>2</sub>O<sub>2</sub> (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.

Table 1. Selected bamboo species along with their local name and economic uses in Assam

Botanical names	Local name	Importance
<i>Bambusa bambos</i> (L.) Voss	Kotoha	Live hedge, paper and pulp, scaffolding and construction, rafts, shoots and seed are edible
<i>Bambusa tulda</i> Roxb.	Jati	Construction, scaffolding, basketry, handicraft and woven application
<i>Bambusa pallida</i> Munro	Bijuli	Basket making and mats and artefacts
<i>Bambusa nutans</i> Wall. ex. Munro	Mokal	Construction, scaffolding, basketry and handicraft, artisans

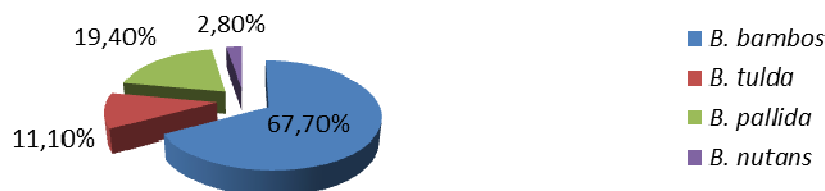


Fig. 1. Pie chart showing the distribution of AM fungi

#### 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 mycorrhizas or other root associated fungi were documented and micro-photographed with 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 AM was the presence of vesicles, arbuscules and mycelium at least in one individual root segment and the occurrence of the rest of typical AM structures in the samples (intra or intercellular hyphae, vesicles, coils and arbuscules). The intensity of length colonized by AM fungi was estimated according to the following formula.

Intensity of length colonized % = (total length of mycorrhizal infection/total length of root segment) × 100

#### Results and Discussion

The rhizospheric soil sample of four species of *Bambusa* i.e. *B. bambos*, *B. tulda*, *B. pallida* and *B. nutans* was collected during November 2013 from three villages viz. *Tilikiaam*, *Maoutgaon* and *Nathgaon* of Jorhat district, Assam, where natural populations of *Bambusa* species were observed. The collected rhizospheric soil and root samples were screened for Dark Septate Endophytes (DSE) as well as Arbuscular mycorrhizal (AM) infection/colonization.

Quantitative analysis of root samples showed the presence of all the three types of endomycorrhizal root infection namely hyphal, vesicular and arbuscular. Beside

this, the Dark Septate Endophytic infections were also observed in all the bamboo species. 100% endomycorrhizal (hyphal and vesicular) and DSE hyphal infections were reported in roots of all the bamboo species. However, Das and Kayang (2010), reported the AMF infection were significantly higher than DSE colonization. In our study, variations were observed in arbuscular infection i.e. *B. nutans* (Plate 4) and *B. bambos* (100%) (Plate 1), *B. pallida* (90%) (Plate 3) and least in *B. tulda* (70%) (see Plate 2 and Table 2). In studies conducted at the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal, *Dendrocalamus strictus* showed above 40% VAM dependency for shoot dry matter production (Singh, 2001). Contradictorily, Jamalludin *et al.* (2001) noticed maximum root colonization of VAM in *Bambusa nana* followed by *Bambusa vulgaris* (green) out of all species of bamboos. The minimum root colonization was noticed in *Bambusa bamboos* (33.33%). The root colonization percent was varied considerably among species to species. These variations in root colonization of bamboos species by VAM might be due to development of VAM in roots which are controlled by host nature and fungus root symbiosis (Gianinazzi Pearson *et al.*, 1991) and Phosphorus acquisition of the host. Host reaction to penetration and proliferation of the fungus in roots is influenced by the presence of intracellular haustoria that the host cell develops the most specific modifications (Serrigny, 1982; Bonfante-Fasolo, 1984). The occurrence of heavy mycorrhizal infection could be attributed to nutrient stress and intense competition between surrounding plant species. The fibrous root systems of the early colonizing herbaceous species that occupied the site may also have contributed to the high level of infection, allowing better contact between the roots of different plant species and thus helping to spread the infection.

Table 2. Percentage infection and colonization of AM fungi

Botanical name	Accession No.	Hyphal infection		Vesicular infection		Arbuscular infection		Presence of DSE*	AM Spore count (50 g of soil)
		Colonization %	Colonization Intensity	Colonization %	Colonization Intensity	Colonization %	Colonization Intensity		
<i>B. bambos</i> (L.) Voss	JRT/AYUR/BB-01	100	50	100	68	100	44	+	104.7 ± 8.45
<i>B. tulda</i> Roxb.	JRT/AYUR/BT-02	100	30	100	78	70	70	+	110.0 ± 4.56
<i>B. pallida</i> Munro	JRT/MAG/BP-03	100	50	100	148	90	27	+	192.7 ± 6.78
<i>B. nutans</i> Wall. ex Munro	JRT/NAG/BN-04	100	50	100	85	100	100	+	52 ± 8.42

\* Dark Septate Endophytes, ± SEM- Standard error of mean

Other possible causes of a high level of mycorrhizal infection include dominance of the disturbed site by mycorrhizal weeds (Miller, 1979), plant age (Martin, 1971), and deficiencies of N and P in the soil (Hayman, 1975). According to Miller (1979), occurrence of vesicular-arbuscular mycorrhizae (VAM) is controlled by the degree of disturbance and harshness of site. These could be another reason as reported by Miller (1979) explaining the abundance of mycorrhizal fungi in the study site. Jha *et al.* (1992) also explained the similar factor for disturbance of mycorrhizal fungi in degraded forest soil.

Qualitative analysis revealed that the endomycorrhizae found in the rhizospheric soil predominantly belongs to five genera *viz.* *Glomus*, *Acaulospora*, *Gigaspora*, *Scutellospora* and *Entrophospora*. *Glomus* with 17 species (61%) is the most dominant genus followed by *Acaulospora* with 7 species (25%), *Entrophospora* with 2 species (7%) and least with 1 species (3.5%) each of *Scutellospora* and *Gigaspora* respectively (Plate 5). Distribution of AM fungi were highest in *B. bambos* (67.7%) followed by *B. pallida* (19.4%), *B. tulda* (11.1%) and least in *B. nutans* (2.8%) (Fig. 1).

About fifteen AM species, five belonging to genus *Acaulospora* (*Acaulospora laevis*, *A. scrobiculata*, *A. lacunose*, *A. mellea*, *Acaulospora* species) seven belonging to *Glomus* (*Glomus clavispurum*, *G. reticulatum*, *G. macrocarpum*, *G. clariodeum*, *G. pansihalos*, *G. geosporum*) two belonging to

genus *Gigaspora* (*Gigaspora giganteus*, *Gigaspora* species) and one *Entrophospora* species were recorded and isolated from the rhizosphere of naturally growing populations of *B. bambos*. Jha *et al.* (2012) reported eight AM inoculants (*G. aggregatum*, *G. arborensis*, *G. cerebriforme*, *G. fasciculatum*, *G. hoi*, *G. intraradix*, *G. occultum* and an unidentified *Glomus* species) significantly increased shoot length in *B. bambos*.

In *Bambusa tulda*, altogether four species belonging to three genera of AM were isolated *viz.* *Acaulospora foveata*, *Glomus clavispurum*, *G. albidum* and *Scutellospora* sp. Das and Kayang (2010) reported *Bambusa tulda* exhibited arum type of AMF morphology. In *Bambusa pallida*, altogether eight AM species were isolated out of which two belonging to *Acaulospora* (*Acaulospora laevis*, unidentified *Acaulospora* species) and rest of the six species belonging to genus *Glomus* were recorded *viz.* *Glomus macrocarpum*, *G. monosporum*, *G. geosporum*, *G. epigaeum*, *G. fasciculatum* and *Glomus* species. While only one species of AM fungi belonging to genus *Glomus* (*G. epigaeum*) were isolated from *B. nutans*. However, Jamaluddin *et al.* (2001) have reported significant increase in growth and biomass of *B. nutans* G. C. Wall. ex Munro after inoculation with AM inoculum obtained from rhizosphere of field grown plants of *B. nutans*, which contained *G. mosseae*, *G. intraradix* Schenck & Smith and an unidentified species of *Gigaspora*.

Table 3. Occurrence of AM fungi among the bamboo species

Sl. no.	Strains of AM fungi	<i>B. bambos</i>	<i>B. tulda</i>	<i>B. pallida</i>	<i>B. nutans</i>
1	<i>Acaulospora laevis</i> Gerd.& Trappe	+	-	+	-
2	<i>A. scrobiculata</i> Trappe	+	-	-	-
3	<i>A. lacunose</i> Morton	+	-	-	-
4	<i>A. mellea</i> Spain and Schenck	+	-	-	-
5	<i>A. foveata</i> Trappe & Janos	-	+	-	-
6	<i>Acaulospora</i> sp.	+	-	+	-
7	<i>Glomus clavispurum</i> Almeida & Schenck	+	+	-	-
8	<i>G. reticulatum</i> Bhattacharjee & Mukerji	+	-	-	-
9	<i>G. macrocarpum</i> Tul. & C. Tul	+	-	+	-
10	<i>G. monosporum</i> Gerd. & Trappe	-	-	+	-
11	<i>G. clariodeum</i> Schenck & Smith	+	-	-	-
12	<i>G. pansihalos</i> Berch & Koske	+	-	-	-
13	<i>G. albidum</i> Walker & Rhodes	-	+	-	-
14	<i>G. geosporum</i> Nicolson & Gerd.	+	-	+	-
15	<i>G. epigaeum</i> Daniels & Trappe	-	-	+	+
16	<i>G. fasciculatum</i> Gerd. & Trappe	-	-	+	-
17	<i>Glomus</i> sp.	+	-	+	-
18	<i>Gigaspora gigantea</i> Gerd. & Trappe	+	-	-	-
19	<i>Gigaspora</i> sp.	+	-	-	-
20	<i>Entrophospora</i> sp.	+	-	-	-
21	<i>Scutellospora</i> sp.	-	+	-	-

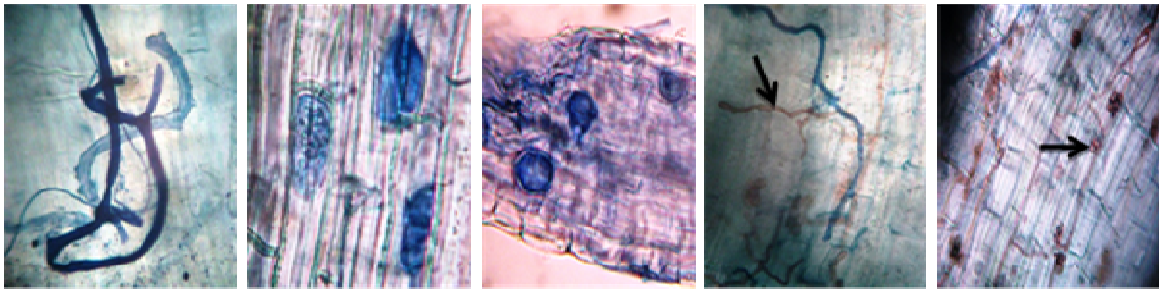


Plate 1. Hyphal, vesicular, arbuscular infection and DSE colonization in *B. bambos*

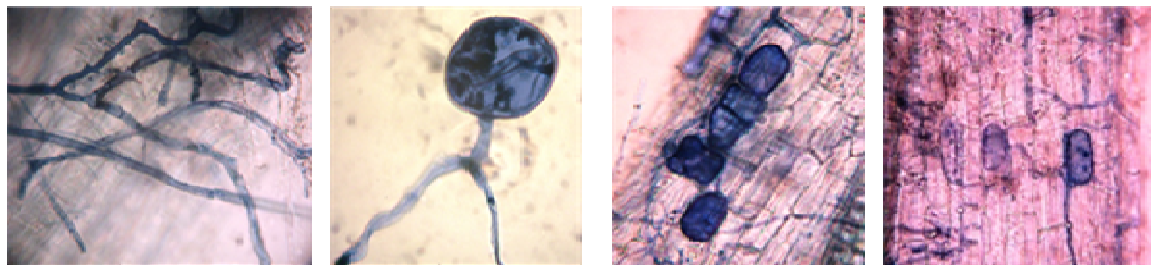


Plate 2. Hyphal, vesicular, arbuscular infection in *B. tulda*

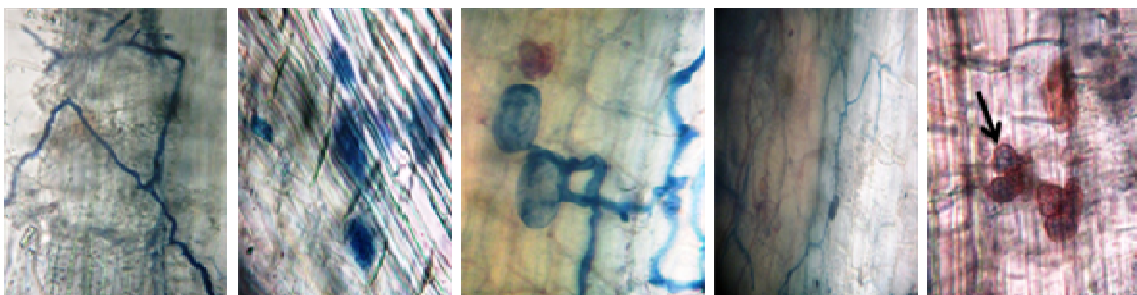
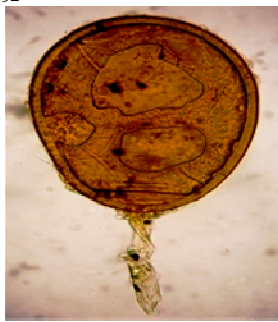


Plate 3. Hyphal, vesicular, arbuscular infection and DSE colonization in *B. pallida*



Plate 4. Hyphal, vesicular, arbuscular infection in *B. nutans*





*Glomus geosporum*



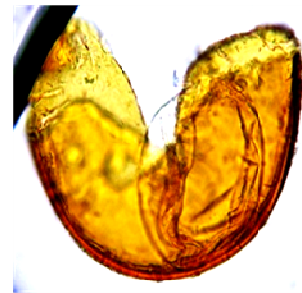
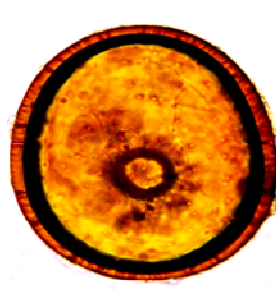
*G. monosporum*



*Acaulospora foveata*



*A. lacunosa*



*Scutellospora* sp.



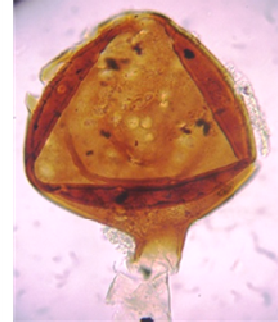
*Glomus* sp.



*G. fasciculatum*



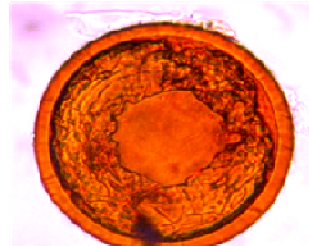
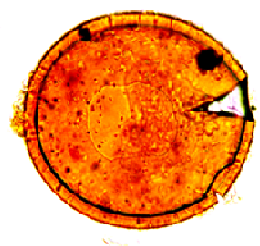
*Gigaspora gigantea*



*Gigaspora* sp.



*G. geosporum*



*A. lacunosa*



## Conclusions

Generally, better nutrient (especially P) and water uptake leads to increase in bamboo biomass. Bamboo species were benefited by VA-mycorrhizal fungi which was evident from this study that all bamboos were infected by endomycorrhizae under natural condition. However, their population and root infection varied considerably among species to species. Bamboo, being a fast growing plant, requires more nutrients during the initial stage of seedling establishment. During this period, the root system is not well developed and the AM fungal symbiosis might play a vital role by supplying the nutrients to the host plant. The results of present study showed that all the target plant species are highly dependent on mycorrhizal associations for its survival, growth and development. Therefore, the inoculation of VA mycorrhizal fungi at the time of plantation may be beneficial as the phosphorus requirement of bamboo can be optimized through AM inoculation.

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## Conflict of interest

The author(s) declares that there are no conflicts of interest related to this article.

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