

## **Mycorrhizal Associations of Bamboo Species of FRI Bambusetum, India**

**S.M. Salunkhe<sup>1\*</sup>, S. Rawat<sup>2</sup>, N. Singh<sup>2</sup>, N. Mehta<sup>2</sup>, S. Das<sup>2</sup> and Y.P. Singh<sup>2</sup>**

<sup>1</sup>Department of Botany, Pune University, Pune 411 007, India

<sup>2</sup>Forest Pathology Division, Forest Research Institute, Dehradun-248 006, Uttarakhand

**Abstract:** A survey was conducted for evaluating the mycorrhizal status of seven bamboo species, namely, *Bambusa polymorpha*, *B. multiplex*, *B. nutans*, *B. tulda*, *Cephalostachyum pergracile*, *Dendrocalamus giganteus* and *Melocanna baccifera* planted in Bambusetum of Forest Research Institute, Dehradun. Composite samples of root and soil from each species were analyzed for mycorrhizal types, root colonization and spore population, and physico-chemical properties respectively. All the species, barring *B. multiplex* and *M. baccifera*, reported the low presence of arbuscules, scanty internal- and external-mycelium and had a good number of chlamydospores. Root colonization showed a diverse pattern ranging from moderate colonization [50-60%, *C. pergracile* (50.6 %), and *B. polymorpha* (63.4%)] to high colonization [> 70%, *M. baccifera* (79.5 %) and *B. tulda* (80.8 %)]. Spore population, in general, was high (14.1 spores/ml of root zone soil) and it was maximum in *M. baccifera* (21.9 spores/ml) and minimum in *C. pergracile* (5.0 spores/ml). Physical aspects of the mycorrhizal relationship has been defined in the ambit of phenology of the host.

*Key words:* Bamboo, Mycorrhizal structure, Phenology.

### **INTRODUCTION**

Bamboo, a giant grass, is a member of the family Poaceae and subfamily Bambusoideae. India is the second richest country in bamboo genetic resources following China (Bystriakova *et al.*, 2003). Bamboo has age-old connection with the material needs of rural people (Mukherjee *et al.*, 2010). It is fast growing plant and widely recognized as cost effective investment due to its very short harvestable maturity (Tewari, 1992). Bamboo forests have ecological and environmental functions in terms of control of soil erosion, land rehabilitation, water conservation and carbon sequestration. Bamboos also play an important role in biodiversity conservation (Forest Survey of India, 2011). Bamboos may be able to increase the bio-capacity in the future by simultaneously increasing the area of fertile global hectares that is able to supply resources (Mishra *et al.*, 2014). The root system of bamboos, in general, is superficial and does not grow to more than a meter deep (Deogun, 1937).

\* To whom correspondence should be addressed: singh\_yp@rediffmail.com

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs, which can form mutualistic symbioses with the roots of around 80% of plant species (Giovannetti, 2008). They are reported to be found in diverse land areas such as calcareous grasslands, arid/semi arid grasslands, several temperate forests, tropical rain forests, shrub and degraded lands in different parts of the world (Muthukumar and Udaiyan, 2002; Oehl *et al.*, 2003; Renker *et al.*, 2005). The essence of mycorrhizal relation has perhaps been best captured in the surveys of AMF for grass and shrub lands (Miller, 1987) and for humid tropical ecosystem (Janos, 1987). The arid and semi-arid regions of the world, where soil organic matter is low, also reported to have AM associations (Read 1993; Allen *et al.*, 1995; Muthukumar and Udaiyan, 2002). While, the tundra region with high organic matter has virtually no AM relationship with plants (Bledsoe *et al.*, 1990).

The information on bamboo mycorrhiza is very limited from India as well as outside. It includes survey on the AM associations of bamboo in Western Ghats of Kerala (Appaswamy and Ganapati, 1992), in semiarid tropical grassland (Muthukumar *et al.*, 1996; Muthukumar and Udaiyan, 2002) and field mycorrhizal status of bamboos in Kerala (Mohanan and Sebastian, 1999). Rawat (2005) surveyed different growth forms of *D. strictus* growing in three locations for field populations of indigenous mycorrhizae. She also studied the root colonization and spore populations in different seasons. Rawat reported highest root colonization during July (monsoon) and lowest in January (winter). To address the paucity of the mycorrhizal studies in bamboo, Das (2010) 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.

In the bamboo forests, a total of 34 species belonging to 21 families of ground covers including bamboo (*Phyllostachys pubescens*) were investigated and 28 were found to be VA mycorrhizal. Fourteen species of VAM fungi were recovered including five newly recorded species from Taiwan and two of *P. pubescens* (Das, 2010). China has about 30,000 plant species, along with a total of 104 AMF species belonging to nine genera and 12 new species. These have been reported in croplands, grasslands, forests and numerous disturbed environments (Mohanan and Sebastian, 1999).

Basically, the present investigation addresses the physical expression of bamboo-mycorrhiza symbiosis in terms of mycorrhizal infection types, root colonization of the host by the fungus and quantification of fungal spores in the root zone of various bamboo species. The variations, if any, have been linked to the edaphic factors of the FRI Bambusetum and host phenology.

## MATERIALS AND METHODS

The study area includes Compartment No. 3 of the Bambusetum of Forest Research Institute, Dehradun, where all the seven bamboo species, viz., *Bambusa multiplex* (Lour) Schult., *Bambusa nutans* Wall. Ex Munro, *Bambusa polymorpha* Munro,

*Bambusa tulda* Roxb., *Cephalostachyum pergracile* Munro, *Dendrocalamus giganteus* Munro and *Melocanna baccifera* (Roxb.) Kurz are located. It lies between the latitude of 30°-30° 32'N and longitude of 77° 43'-78° 24'E. The site receives annual rainfall of about 2,000 mm and most of which is concentrated during the monsoon season (June- September). Temperature ranges 0.2°-36.9°C for minimum and maximum, respectively. Samplings were done in the months of late June and early July 2005.

After selecting the plants, a small amount of soil preferably close to the plant roots was dug out with a spade or trowel/hand shovel to a depth of 3-15 cm after avoiding the top one cm or two. Roots sampled by avoiding pulling out of the soil, to reduce the root cortex damage. The samples were placed in labeled polythene bags and stored at 4°C until they processed. Sugar gradient centrifugation method (Gerdemann and Nicholson, 1963; Daniel's and Skipper, 1982) was employed for collection of spores from soil. Spore population of root zone soil was quantified with the help of Doncaster's Counting Disc.

For enumeration of fungi, staining of roots was done using Phillips and Hayman (1970) method and mycorrhizal root colonization was quantified by the Grid line - Intersect method (Giovanetti and Mosse, 1980). The physico- chemical characteristics of the field soil under clumps of different species are detailed in Table1. Data of different characters of field survey were analyzed by one-way model using Microsoft Excel. The difference between the treatment means was tested at 5 percent level of significance

**Table 1:** Physico-chemical characteristics of the soil under clumps of different bamboo species

Sand (%)	Silt (%)	Clay (%)	Textural class	Soil reaction (pH)	Electrical conductivity (desi simon/m)	W.H.C. (%)	Organic matter (%)	Total nitrogen (%)	Available P (ppm)	Available K (ppm)
71.3	15.5	13.3	loam	6.2	0.097	43.9	2.19	0.24	16.6	149.4

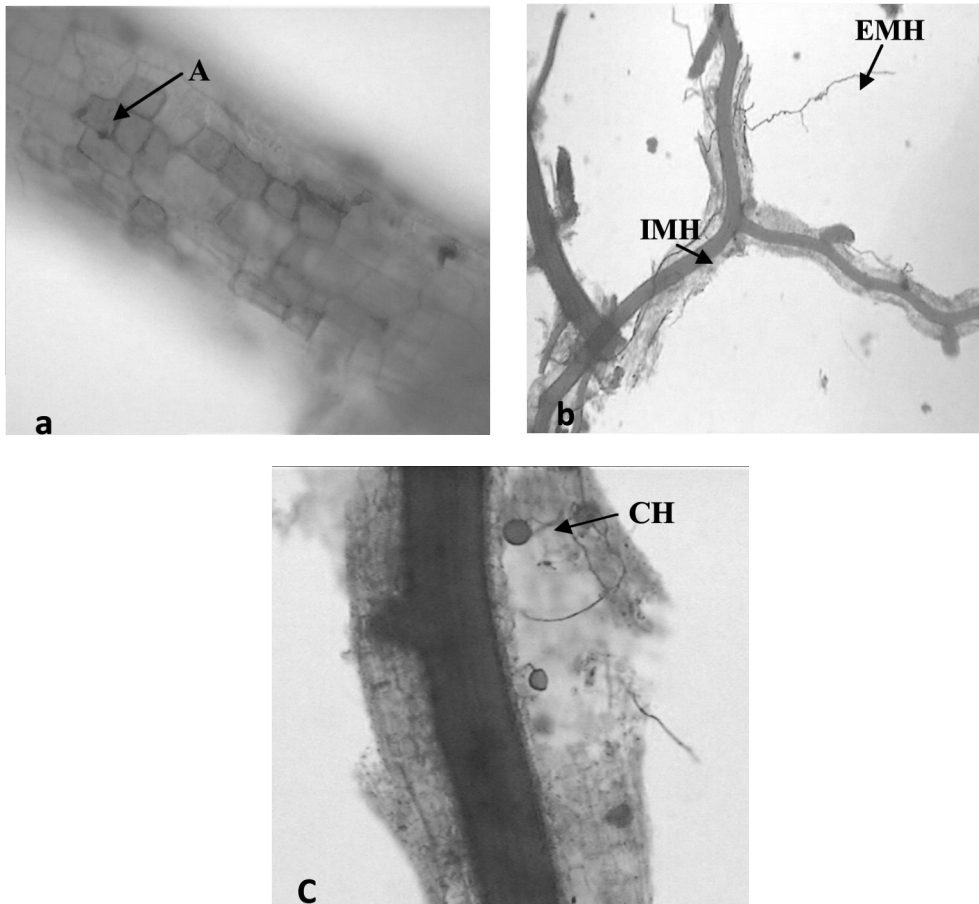
## RESULTS AND DISCUSSION

It is apparent from Table2 that all the seven species of bamboo were having arbuscules (Figure 1A), vesicles and intra- and extra – matrical hyphae (Figure 1B.) in their roots. The presence of arbuscules in root cortex qualifies them to be mycorrhizal. However, invariably arbuscules showed their moderate presence in the root cortex of all the bamboo species. Moreover, vesicles were scanty and mycelium was common but not profuse. Chlamydo spores were also encountered quite frequently (Figure 1C).

**Table 2:** Arbuscular mycorrhizal infection types of different bamboo species

Sl. No.	Species	AM infection type		
		AR*	VC	EMH
1.	<i>B. multiplex</i>	+	+	+
2.	<i>B. nutans</i>	+	+	+
3.	<i>B. polymorpha</i>	+	+	+
4.	<i>B. tulda</i>	+	+	+
5.	<i>C. pergracile</i>	+	+	+
6.	<i>D. giganteus</i>	+	+	+
7.	<i>M. baccifera</i>	+	+	+

\*AR= arbuscules, VC= vesicles, EMH= extra-matrical hyphae; + = present, -= absent.



**Figure 1:** Showing mycorrhizal structures in the roots of different bamboo species. (a) Arbuscules (A) in the root of *D. giganteus* (400X), (b) intra – and extra-matrical hyphae in (IMH and EMH) and (c) chlamydospores (CH) at the root surface of *C. pergracile* (100X).

Frequencies of different mycorrhizal structures indicate that bamboo roots were not in quite active state metabolically; otherwise, arbuscules distribution might have been wide and frequent. The sample timing, i.e., June represents the hottest part of the year besides, most of the species, barring *B. multiplex* and *M. baccifera*, were in the leaf shedding stage. In such situations, metabolic activity of bamboo is at its lowest which may manifest in low frequency of arbuscules, with scanty internal and external mycelium and a good number of chlamydospores. Probably, a lag phase existed in the present situation that may reflect the availability of small number of propagules and/or unfavourable conditions for mycorrhiza formation (Sutton, 1973). The latter condition seems to be more applicable in the present case owing to high summer temperature and the practically leafless stage of the majority of the bamboo species leading to low food requirements as well as synthesis by the host. A stage of the host that supports low mycorrhization of, otherwise, highly mycotropic bamboo. It has earlier been reported that endomycorrhizal colonization and sporulation may vary in different seasons in a year (Hayman, 1970; Saif and Khan, 1975; Nicolson and Jhonson, 1979).

Roots of *B. tulda* were colonized to the maximum (80.8%) and it was closely followed by that of *M. baccifera* (79.5%) and both were statistically at par (Table 2). The minimum root colonization was found in *C. pergracile* (50.6%). The general colonization of bamboo has been moderate ranging from 50.6 (*C. pergracile*; Table 3.) to 80.8 per cent (*B. tulda*). Four out of seven species, viz., *C. pergracile* (50.6%), *D. giganteus* (56.4%), *B. multiplex* (59.3%) and *B. polymorpha* (63.4%) were having moderate root colonization between 50 to 60 per cent. While, remaining three species, namely, *B. nutans* (69.3%), *M. baccifera* (79.5%) and *B. tulda* (80.8%) had moderately high root infection of 70 per cent or above.

**Table 3:** Extent of colonization and spore population in the roots of different bamboo species

Sl. No.	Species	Root colonization (%)	Spore population (No./ml of soil)
1.	<i>B. multiplex</i>	59.3	10.4
2.	<i>B. nutans</i>	69.3	16.8
3.	<i>B. polymorpha</i>	63.4	7.8
4.	<i>B. tulda</i>	80.8	21.0
5.	<i>C. pergracile</i>	50.6	5.0
6.	<i>D. giganteus</i>	56.4	15.9
7.	<i>M. baccifera</i>	79.5	21.9
	S.E.M	9.5	3.7
	C.D. (5%)	5.2	2.0

The percent colonization of the roots of the bamboo species, generally, is not so high as reported by earlier workers (Rawat, 2005) for *D. strictus* (80.9%). However, it has shades of colonization from moderate (50-60%) to high (more than 70%; Table 3.). Nevertheless, a majority of the species (4) fall in the former category. Bamboos, in general, have shallow root system and high growth rate which make them more dependent on mycorrhiza for their normal growth. Moderate value of percent root colonization may represent a growth stage of bamboos (leaf fall), when their nutrient requirements and photosynthetic ability go down making them, comparatively, less dependent on mycorrhizal associations. Low frequency of arbuscules in root cortex is also a pointer towards this.

Spores in the root zone of different bamboo species were quite high (Table3). For example, minimum spores could be recovered from the soil under *C. pergracile* (5.0 spores/ml of soil; Table 3.) while, maximum were quantified in the root zone of *M. baccifera* (21.9 spores/ml of soil) this was closely followed by *B. tulda* with 21.0 spores/ml of root zone soil and was statistically as par with the former. Second to the maximum were two species, namely, *B. nutans* (16.8 spores/ml) and *D. giganteus* (15.9 spores/ml) that were mutually at par. *B. multiplex* had 10.4 spores per ml in its root zone that were significantly more than 7.8 spores of *B. polymorpha*.

The populations of the mycorrhizal spores (14.1 spores/ml of soil) in the root zones of different bamboo species seem quite high in comparison to the other study carried out on similar lines (Rawat, 2005, 2.9 spores/ml of soil of *D. strictus*). Three soil factors i.e. acidic or low pH (6.2), high organic carbon (2.19%) and medium phosphorus (16.6 ppm, Table 1.) may be contributing to the higher populations of mycorrhizal spores individually or in combination (St John, 1980; Hepper and Warner, 1983). As discussed in the previous paragraphs, the growth stage of the hosts (leaf fall) leading to dearth of food availability must also be triggering the soil fungi to be in resting stage (chlamydozoetes) as supported by their high recovery from the soil as well as the presence on the roots. In case of *D. strictus* growth forms, Rawat (2005) reported highest root colonization during July (monsoon) and lowest in January (winter). However, spore recovery from soil followed a just reverse trend reflecting growth linked mycorrhization. Garg *et al.* (2013) recorded substantially low root colonization and higher spore population when they conducted a survey in all these bamboo species used in the present study in the month of April, the growth initiation phase of bamboos in north India.

The present study indicates the variations in root colonization as well as soil spore populations among the seven species of bamboo. These variations in mycorrhization seem to have direct linkage with season, soil and host phenology. Still, due to lack of precise estimation of comparative growth rates of hosts and phosphorus content of their healthy foliage, it will be premature to draw a relationship between their biomass and physical expression of symbiosis (root colonization and soil spore population). The prospects for further investigation into these aspects are, therefore, clear.

## ACKNOWLEDGEMENTS

We would like to thank Dr. S.S. Negi, Director, Forest Research Institute, Dehradun for granting permission for carrying out the project work. Also, thanks are due to Directorate of Statistics, Indian Council of Forestry Research and Education, Dehradun for help with statistical analysis.

## REFERENCES

- Allen, E.B., Allen, M.F., Helm, D.J., Trappe, J.M., Molina, R. and Ricon, E. 1995. Patterns and regulation of mycorrhizal plants and fungal diversity. *Plant Soil*. 170: 47–62.
- Appasamy, T. and Ganapati, A. 1992. Preliminary survey of vesicular-arbuscular mycorrhiza (VAM) association with bamboos in Western Ghats. *BIC-India Bull.* 2: 13–16.
- Bledsoe, C., Klier, P. and Bliss, L.C. 1990. A survey of plants on Truelove Lowlands, Devon Island, N.W.J. Canada. *Can. J. Bot.* 68: 1848–1856.
- Bystriakova, N., Kapos, V., Lysenko, I. and Stapleton, C. 2003. Distribution and conservation status of forest bamboo biodiversity in the Asia-Pacific region. *Biodiversity Conserv.* 12: 1833–1841.
- Daniels, B.A. and Skipper, H.D. 1982. Methods for the recovery and quantitative estimation of propagules from soil. In: N. C. Schenck (Ed.). *Methods and principles of mycorrhizal research*. St. Paul, MN, The American Phytopathological Society: 29–36
- Das, P. 2010. Arbuscular mycorrhizal fungi and dark septate endophyte colonization in bamboo from northeast India. *Front. Agric. China*. 3: 375–382.
- Deogun, P.N. 1937. The silviculture and management of the bamboo *Dendrocalamus strictus* Nees. *Indian Forest Records, New Sec., Silviculture* 2 (4): 75–173.
- Forest Survey of India. 2011. State of forest report. Dehra Dun, FSI. ([www.fsi.nic.in](http://www.fsi.nic.in)), 210p.
- Garg, A., Das, S., Singh, Y.P. and Sharma, P. 2013. Exploring the microbial activities in the rhizospheres of exotic bamboos. *Octa J. Environ. Res.* 1 (1): 30–38.
- Gerdemann, J.W. and Nicolson, T.H. 1963. Spores of mycorrhizal Endogone extracted from soil by wet sieving and decanting. *Trans. Brit. Mycol. Soc.* 46: 235–244.
- Giovannetti, M. 2008. Structure, exten and functional significance of belowground arbuscular mycorrhizal networks. In: Varma, A. (Ed.). *Mycorrhiza: State of the art, genetics and molecular biology, eco-function, biotechnology, eco-physiology, structure and systematics*. Berlin Heidelberg, Springer-Verlag: 59–72.
- Giovannetti, M. and Mosse, B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* 84: 489–500.
- Hayman, D.S. 1970. Endogone spore number in soil and vesicular-arbuscular

- mycorrhizae in wheat as influenced by season and soil treatment. *Trans. Br. Mycol. Soc.* 54: 53-63.
- Hepper, C.M. and Warner, A. 1983. Role of organic matter in growth of a vesicular arbuscular mycorrhizal fungus in soil. *Trans. Br. Mycol. Soc.* 8: 155-156.
- Janos, D.P. 1987. VA mycorrhizas in humid tropical ecosystem. In: G.R. Safir (Ed.). *Ecophysiology of VA mycorrhizal plants*. Boca Raton, FA, CRC Press: 107–134.
- Miller, R. M. 1987. The ecology of vesicular arbuscular mycorrhizae in grass and shrub lands. In: G. R. Safir, (Ed.). *Ecophysiology of VA mycorrhizal plants*. Boca Raton, FA, CRC Press: 135–170
- Mishra, G., Giri, K., Panday, S., Kumar R. and Bisht N. S. 2014. Bamboo: potential resource for eco-restoration of degraded lands. *J Biol Earth Sci.* 4(2): B130-B136.
- Mohanam, C. and Sebastian, M. 1999. Mycorrhizal status of nineteen species of bamboos in Kerala, India. In: Proceedings of the National Conference on Mycorrhiza, 5-7 March 1999, Institute of Microbiology and Biotechnology, Barkatullah University, Bhopal, pp. 4–9.
- Mukherjee, A.K., Ratha, S., Dhar, S., Debata, A.K., Acharya, P.K., Mandal, S., Panda, P.C. and Mahapatra, A.K. 2010. Genetic relationships among 22 taxa of bamboo revealed by ISSR and EST-based random primers. *Biochem Genet.* 48: 1015–1025.
- Muthukumar, T., Udaiyan, K. and Manians, S. 1996. Vesicular-arbuscular mycorrhizae in tropical sedges of southern India. *Bio. Fertil. Soil.* 22: 96–100.
- Muthukumar, T and Udaiyan, K. 2002. Arbuscular mycorrhizal fungal composition in semi- arid soils of Western Ghats, Southern India. *Curr. Sci.* 82: 624–628.
- Nicholson, T. H. and Johnston, C. 1979. Mycorrhiza in the Graminae-III, *Glomus fasciculatum* as the endophyte of pioneer grasses in a maritime and dune, *Trans, Brit, Mycol. Soc.* 72: 261-268.
- Oehl, F., Sieverding, E., Ineichen, K., Mader, P., Boller, T. and Wiemken, A. 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agro- ecosystems of central Europe. *Appl. Environ. Microbiol.* 69 (5): 2816–2824.
- Phillips, J.M. and Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.* 55: 158–161.
- Rawat, S. 2005. Bamboo-endomycorrhiza: Ecology, growth and macroproliferation, Ph.D. thesis, Forest Research Institute (Deemed) University, Dehradun: 200p.
- Read, D.J. 1993. Mycorrhizas in plant communities. *Adv. Plant Pathol.* 9: 1–31.
- Renker, C., Blanke, V. and Buscot, F. 2005. Diversity of arbuscular mycorrhizal fungi in grassland spontaneously developed on area polluted by a fertilizer plant. *Environ. Pollut.* 135: 255–266.
- Saif, S.R. and Khan, A.G. 1975. The influence of season and stage of development of



- plant on Endogone mycorrhiza of field grown wheat. *Can. J. Microbiol.* 21(7): 1020-1024.
- St. John, T.V. 1980. A survey on mycorrhizal infection in an Amazonian rain forest. *Acta Amazonica.* 10: 527.
- Sutton, J.C. 1973. Development of vesicular- arbuscular mycorrhizae in crop plants. *Can. J. Bot.* 51: 2487-2493.
- Tewari, D.N. 1992. Bamboo, Dehra Dun International Book Distributors : 22p.