J Mycopathol, Res, **50(2)** : 279-284 (2012)

Comparison of mycorrhizal association in *Michelia champaca, Solanum tuberosum and Phyllostachys manii*

PANNA DAS[1] AND AJAY KRISHNA SAHA[2]
Botany Department, Tripura University, Suryamaninagar 799 022 Tripura

Received : 25.11.2011 Accepted : 12.07.2012 Published : 29.10.2012

A comparative account of mycorrhizal association was investigated from the roots of *Michelia champaca* L., *Solanum tuberosum* L. and *Phyllostachys manii* Gamble and diversity of arbuscular mycorrhizal (AM) fungi from the plantation of *M. champaca*, field of *S. tuberosum* and from the runner of *P. manii.* was evaluated. Highest AM fungal colonization was observed in *M. champaca* and lowest was observed in *S. tuberosum*. However, maximum level of dark septate endophyte colonization was observed in *S. tuberosum*. Twenty eight taxa of AM fungi were isolated from the rhizosphere of three plant species. Of which, 18 were isolated from *M. champaca*, 12 from *P. manii* and 15 from *S. tuberosum*. *Glomus tortuosum* and *G. macrocarpum* were the most abundant species. Shannon diversity was high in *P. manii* with maximum evenness. The similarity in terms of species composition exhibited between *M. champaca* and *P. manii*. The results revealed similarity in mycorrhizal colonization between tree species and bamboo species. Nevertheless, mycorrhizal colonization in potato followed different pattern and diversity of AM fungi demonstrated high amount of dominance by a few species.

Key words : *Michelia champaca*, *Solanum tuberosum*, *Phyllostachys manii*, arbuscular mycorrhizal fungi, dark septate endophyte

INTRODUCTION

The benefits of mycorrhizal associations to plants include enhanced nutrient and water uptake, protection against pathogens, improved resistance to drought, higher tolerance to heavy metals, and increased root surface area (Smith and Read, 1997). In species-rich natural communities, colonization of individual plants by mycorrhizal fungi occurs within a few days of emergence of radicle (Read and Birch, 1988), and can be important for establishment and survival of compatible plant species (Francis and Read, 1994). Because of their effects on individual plant performance, mycorrhizas influence the productivity of plant communities and can affect plant community composition, succession, and species diversity (Janos, 1996).

Arbuscular mycorrhizal (AM) fungi are common soil fungi associating with roots of plants in natural and agro-ecosystems. AM fungi can significantly improve the mineral nutrition of plants (especially P) by effectively enlarging the rhizosphere of the plants with which they associate (Muthukumar and Vediyappan, 2009). Like mycorrhizal fungi, another group of root fungal symbionts, dark septate endophytic (DSE) fungi, have been characterized as producing a range of effects on their host (Jumpponen, 2001).

Mycorrhizal colonization has been compared in the plantations of *Michelia champaca* L. (Das and Kayang 2010a). Recently, there has been a study in the DSE fungi and arbuscular mycorrhizal colonization in *Solanum tuberosum* L. (Das and Kayang 2010b). Moreover, there has been also a study of arbuscular mycorrhizal colonization and DSE fungi in *Phyllostachys manii* Gamble (Das and Kayang, 2010c). However, there is no report on comparison of mycorrhizal colonization in a tree species, a crop plant and a bamboo species.

MATERIALS AND METHODS

Site description

The plantation site of *M. champaca* was selected

Email : [1]panna11d@yahoo.com, [2]aksaha.58@gmail.com

in Mawlein (N25°42 and E91°53; 828.5 m.a.s.l.), in Ribhoi District of Meghalaya, northeast India. *P. manii* Gamble were selected for mycorrhizal studies in the month of October 2008 from plantations of Meghalaya, northeast India. The bamboo species was located at N 25°36 and E 91°53 at 1537 m.a.s.l. Two potato plots, each 100x 50 m^2 in area, were selected for root and soil sampling in Swer village (N 25°25 and E 91°47 at 1910 m.a.s.l) in the East Khasi Hills District of Meghalaya in northeast India.

Sample collection

The rhizospheric soil and roots at depths of 0-20 cm around each species, at four different points for each plant were collected. The soil samples of each collection were combined to approximately 500 g soil per plant and was collected. The soil samples were placed in polythene bags, labeled and transported for further analysis in the laboratory. Then leaf litters were removed, ground, sieved with a 2-mm sieve, stored at refrigerator and processed for spore analysis. The roots were fixed in FAA.

Root processing

The fixed roots were washed in tap water, cleared in 10% KOH at 90°C and stained with black Faber Castell stamp pad ink (Das and Kayang, 2008). One cm long stained root samples were mounted on slides in lactoglycerol and examined for AMF and DSE structures under a light microscope (Olympus 41209). Estimations of AMF and DSE colonization were done by the magnified intersection method (McGonigle *et al.*, 1990).

AMF spore diversity

The spores were extracted by a modified wet sieving and decanting method (Muthukumar *et al.* 2006). The soil weighing 100 g was dispersed in 1 L of water and decanted through a series of 710- to 38-µm sieves. The residues were filtered through gridded filter papers and all whole spores were counted using a light microscope at 40x magnification. Sporocarps and spore clusters were considered as one unit. The isolated spores were picked up with a needle in polyvinyl alcohol/lactoglycerol under a microscope (Koske and Tessier, 1983) and also in mixed polyvinyl alcohol/lactoglycerol–Meltzer's reagent (1:1, v/v) for identification. Complete and broken spores were examined using a light microscope. 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; Koske *et al.* 1986; Blaszkowski 1989; Almeida and Schenck 1990; Wu *et al.* 1995; Oehl and Sieverding, 2004). Photography of the root segments colonized by fungi and spores of AMF was via a Leica EC 3 camera attached in a Leica dm 1000 microscope. Spore density, relative abundance (RA), species richness (SR) and diversity indices were calculated.

Data analysis

All colonization variables were submitted to one-way ANOVA and Fischer's LSD test was used for comparison of means. The data were analyzed with Statistica 9.0 software.

RESULTS

Mycorrhizal colonization

The AM fungal structural colonization revealed that arbuscules were significantly different in each species. There was no significant difference in vesicular colonization in between *S. tuberosum* and *P. manii*. Moreover, there was no significant difference in hyphal colonization between *M. champaca* and *P. manii* (Table 1). However, DSE colonization was slightly high in *S. tuberosum* The spore density was found to be 712/100 g of soil in *M. champaca*, 137/100 g of soil in *P. manii* and 261/100 g of soil in *S. tuberosum*.

Table 1 : Comparison of mycorrhizal colonization (%) in three species of plants

Plant species	Arbuscules (%)	Vesicles (%)	Hyphae (%)	DSE (%)
Michelia champaca	26.70± 2.34a	14.46± 1.86a	71.84± 4.17a	2.90± 0.59a
Solanum tuberosum	8.72± 2.64b	0.44± 0.22b	11.96± 3.28b	13.220± 1.65b
Phyllostachys manii	47.89± 2.29c	0.27± 0.11b	65.26± 2.46a	0.44± 0.22a

Different alphabetical letters denotes different significance level ($p < 0.05$)

Table 2 : Comparison of species of AM fungi in the rhizosphere of three species of plant

Species	*Michelia champaca*	*Phyllostachys manii*	*Solanum tuberosum*
Acaulospora cavernata	0.30	0.00	2.99
A. foveata	0.00	1.69	0.00
A. rehmii	0.30	4.24	0.43
A. scrobiculata	0.00	3.39	0.00
A. tuberculata	2.07	1.69	1.28
Acaulospora sp 1	5.47	0.00	0.00
Acaulospora sp 2	0.00	0.00	0.85
Ambispora sp 1	0.15	0.85	0.00
Glomus aggregatum	0.00	0.00	0.43
G. ambisporum	0.00	0.85	0.43
G. clavisporum	0.00	2.54	0.43
G. constrictum	14.03	4.24	0.00
G. fueganium	0.15	0.00	0.00
G. glomeratum	0.00	0.00	2.14
G. intraradices	1.33	10.17	0.00
G. macrocarpum	32.50	27.97	0.00
G. microaggregatum	0.15	0.00	0.00
G. multiculae	26.88	0.00	0.00
G. rubiforme	0.00	8.47	0.43
G. tawanense	1.62	0.00	0.00
G. tortuosum	10.93	33.90	47.44
Glomus sp 2	0.44	0.00	0.00
Glomus sp 3	0.00	0.00	0.43
Gigaspora sp 1	1.03	0.00	19.23
Pacispora boliviana	0.44	0.00	21.37
P. chimono bambusae	1.62	0.00	1.28
Paraglomus occultum	0.59	0.00	0.00
Scutellospora fulgida	0.00	0.00	0.85
Total	100.00	100.00	100.00

AM fungal diversity

Twenty eight species of AM fungi were isolated from the rhizosphere of *M. champaca*, *P. manii* and *S. tuberosum* (Table 2). Of the total 28 species, 18 were found from *M. champaca*, 12 from *P. manii* and 15 from *S. tuberosum*. *Glomus tortuosum, Acaulospora rehmii* and *A. tuberculata* were the most frequent species encountered from all the sites.

G. tortuosum G. macrocarpum, G. multiculae Pacispora boliviana, G. constrictum and *Gigaspora* sp 1 were the most abundant genera (Fig. 1). Fifteen species of *Glomus* were found, 7 from *Acaulospora,* 2 from *Pacispora* and 1 each from *Ambispora*, *Gigaspora*, *Paraglomus* and *Scutellospora*.

Nine species of *Glomus* were found from the rhizosphere of *M. champaca,* 4 species of *Acaulospora*, 2 from *Pacispora*, and 1 each from *Ambispora*, *Gigaspora* and *Paraglomus*. The number of species from the rhizosphere of *P. manii* was 7 from *Glomus*, 4 from *Acaulospora*, and 1 from *Ambispora*. From the rhizosphere of *S. tuberosum*, AM fungal species isolated were 7 species from *Glomus*, 4 from *Acaulospora*, 2 from *Pacispora* and 1 each from *Gigaspora* and *Scutellospora*. *G. macrocarpum* was the most abundant species isolated from the rhizosphere of *M. champaca*. *G. tortuosum* was the most abundant genera isolated from the rhizosphere of *P. manii* and *S. tuberosum* (Fig. 2).

The diversity indices revealed the maximum dominance of a few AM fungi in *S. tuberosum*. Shannon diversity index was highest in *P. manii* and Simpson diversity index was high in *M. champaca*. In *P. manii*, evenness was maximum (Table 3).

The similarity in terms of abundance in between the sites revealed that *M. champaca* and *P. manii* exhibited similarity in species distribution. However, the species composition in potato field displayed dissimilarity from the other two species.

Table 3 : Diversity indices of AM fungi in three plant species

	Michelia champaca	*Phyllostachys manii*	*Solanum tuberosum*
Taxa_S	18	12	15
Dominance_D	0.2472	0.2506	0.358
Shannon_H	1.917	1.922	1.557
Simpson_1-D	0.7528	0.7494	0.642
Evenness_e^H/S	0.3778	0.5693	0.3164

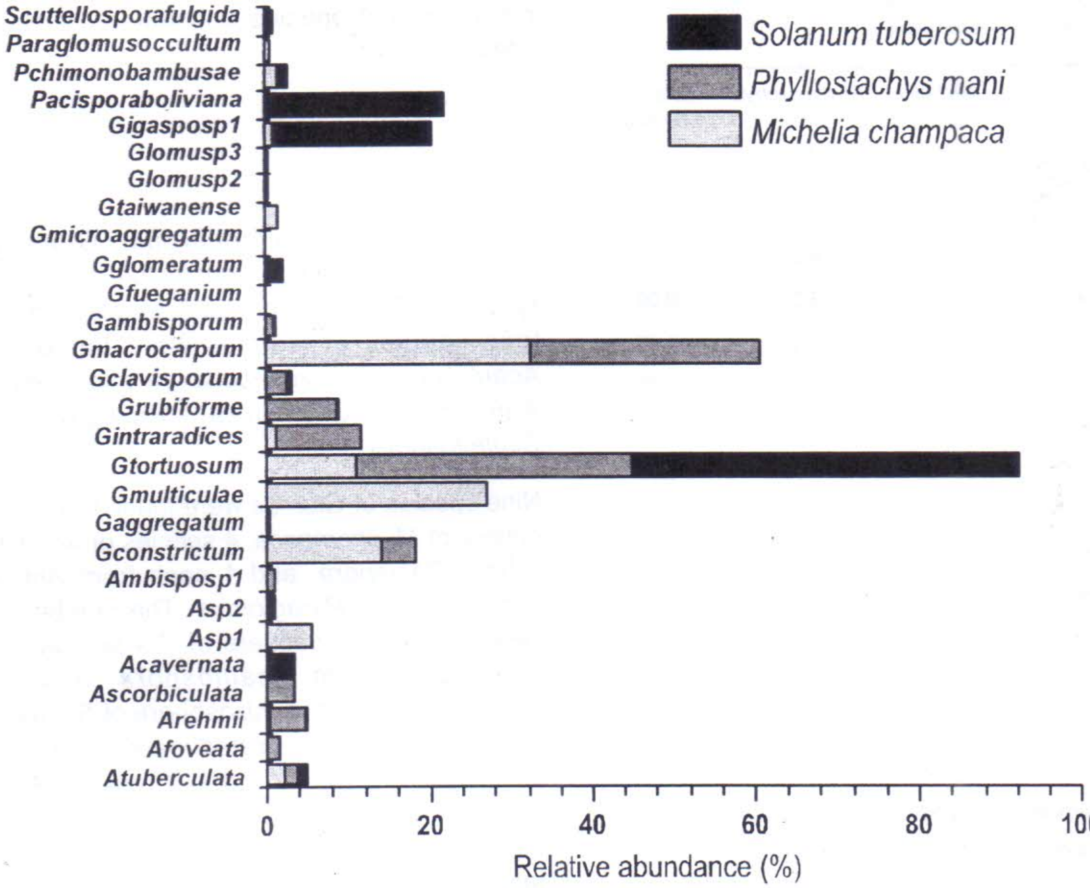

Fig. 1 : Relative abundance of arbuscular mycorrhizal spores in the rhizosphere of three plant species.

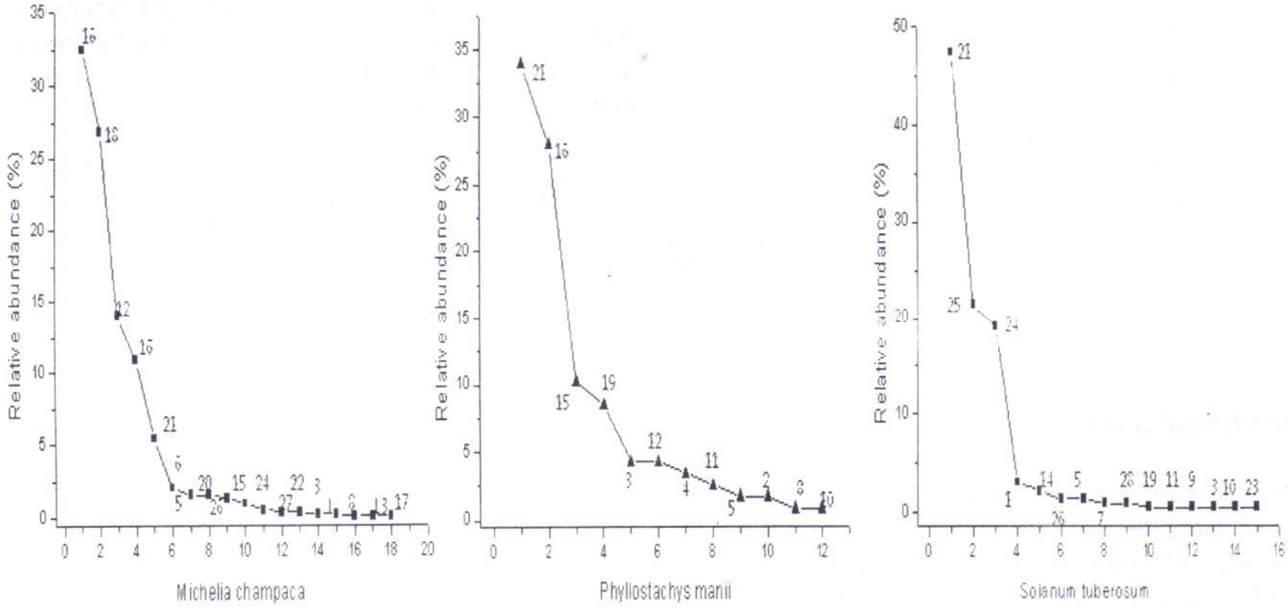

Fig. 2 : Rank/abundance plots illustrating the richness and abundance of the AM fungal spore morphospecies detected in the study sites.

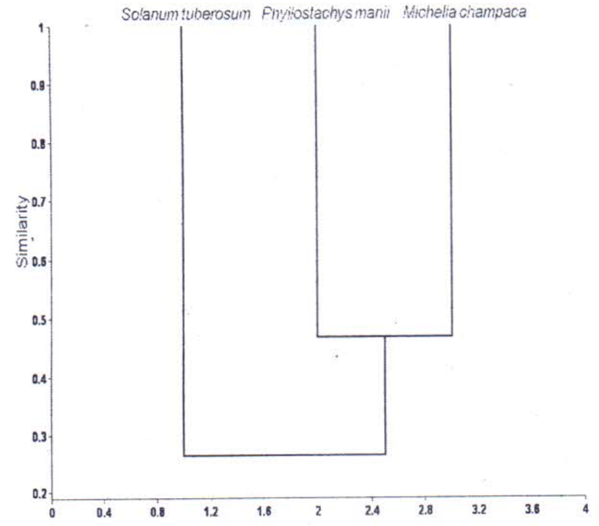

Fig. 3 : Cluster analysis showing similarity between three species of plants

DISCUSSION

All the three species exhibited both AM fungal and DSE colonization. However, there is significant difference in colonization in *S. tuberosum* compared to the other two species. Different edapho-climatic factors and environmental factors like soil type, soil quality, low nutrient status of soil, high aeration, soil pH, organic matter, soil moisture, rainfall, temperature, etc. might be responsible to the variation in root colonization (Sharma *et al.* 1986). The occurrence of DSE fungi in >90% of the plant species corroborates the fact that DSE fungi are ubiquitous (Jumpponen and Trappe, 1998). Ruotsalainen *et al.* (2007) reported a DSE fungal colonization levels of >40% in *D. flexuosa* on an industrial barren. We did not observe any high levels DSE colonization. The existence of a significant inverse relationship between the root length colonized by AM and DSE fungi do suggest that these variables are influenced by different factors (Muthukumar and Vediyappan, 2009). However, this observation corroborates the suggestion that that increasing stress or site characteristics may favour DSE over AM fungi (Christie and Kilpatrick, 1992).

Out of seven established genera, *Glomus* was the most distributed genera. *Glomus* sporulated abundantly regardless of tree species and sites selected. Sharma *et al.* (1986) also reported the dominance of the *Glomus*. They described the wider adaptation of the taxon in varied soil conditions. *Glomus* and *Acaulospora* were common and widely distributed genera among the samples. Dominancy of *Glomus* in the present study is in agreement with the reports of Sharma *et al.* (1986) and Pande and Tarafder (2004). The predominance of *Glomus* under varying soil conditions may be due to the fact that they are widely adaptable to the varied soil conditions and can survive in acidic as well as in alkaline soils (Pande and Tarafder, 2004). The sporulation pattern of *Glomus* might bring about the dominance of the taxon. Spores of *Glomus* are grown in cluster and sporulate more frequently while other like *Gigaspora, Scutellospora* etc sporulated singly. Thus, less population of Gigasporineae might be quite expectable.

The distribution of AM fungi can be measured in terms of fungal species occurring under certain conditions (Sieverding, 1991). Variation of diversity indices was observed in the present study. Variations of diversity indices observed in the present study might be due to the climatic factors, different life durations of the host plants, different disturbing agents etc which might be responsible for spore abundance and distribution of AM fungi (Chaurasia *et al.* 2005; Muthukumar and Udaiyan, 2000). Diversity of mycorrhizal fungi might often be variable with the same plant (Allen and Boosalis, 1983). Different life duration of the different host plants had been reported to be the controlling factors of the species composition of AM fungi (Muthukumar and Udaiyan, 2000), thus the variation of diversity indices might be resulted in.

The results indicated that *M. champaca* harbours maximum number of species in the plantations with high spore density. Although, similarity exists between *P. manii* and *M. champaca*. Furthermore, the presence of diverse community of AM fungi should be tested to evaluate the growth efficacy of the various isolates.

REFERENCES

Allen, M.F., and Boosalis, M.G. 1983. Effects of two species of vesicular arbuscular mycorrhizal fungi on drought tolerance of winter wheat. *New Phytologist* **93**: 67-76

Almeida, R.T., and Schenck, N.C. 1990. A revision of the genus *Sclerocystis* (Glomaceae, Glomales). *Mycologia* **82**: 703–714.

Blaszkowski, J. 1989. *Acaulospora cavernata* (Endogonaceae): a new species from Poland with pitted spores. *Cryptogamic Botany* **1:** 204–207.

Chaurasia, B., Pandey, A., and Palni, L.M.S. 2005. Distribution, colonization, and diversity of arbuscular mycorrhizal fungi associated with central Himalayan rhododendrons. *Forest Ecology and Management* **207:** 315-325.

Christie, P. and Kilpatrick, D.J. 1992 Vesicular-arbuscular mycorrhiza infection in cut grassland following long-term slurry application. *Soil Biology and Biochemistry* **24:** 325-330.

Das, P., and Kayang, H. 2008. Stamp pad ink, an effective stain for observing arbuscular mycorrhizal structure in roots. *World Journal of Agricultural Science* **4:** 58–60.

Das, P., and Kayang, H. 2010a. Mycorrhizal colonization and distribution of arbuscular mycorrhizal fungi associated with *Michelia champaca* L. under plantation system in northeast India. *Journal of forestry research.* **21:** 137-142.

Das, P., and Kayang, H. 2010b Arbuscular mycorrhizal fungi and dark septate endophyte colonization in bamboo from northeast India. *Frontiers of agriculture in China.* **4:** 375–382.

Das, P., and Kayang, H. 2010c Association of dark septate endophytes and arbuscular mycorrhizal fungi in potato under field conditions in the northeast region of India. *Mycology.* **3:** 171–178.

Francis, R., and Read, D.J. 1994, The contributions of mycorrhizal fungi to the determination of plant community structure. *Plant and Soil* **159:** 11–25.

Janos, D.P. 1996 Mycorrhizas, succession and the rehabilitation of deforested lands in the humid tropics. In: Frankland JC, Magan N, Gadd GM (eds) *Fungi and environmental change.* Cambridge University Press, Cambridge, pp 129–162.

Jumpponen, A., and Trappe, J.M. 1998 Dark septate endophytes: a review of facultative biotrophic root colonizing fungi. *New Phytologist* **140:** 295-310.

Jumpponen, A. 2001 Dark septate endophytes, are they mycorrhizal *Mycorrhiza* **11:** 207-211

Koske, R.E. and Tessier, B. 1983. A convenient, permanent slide mounting medium. *Mycol Soc Am News.* **34:** 59.

Koske, R.E., Gemma, J.N., and Olexia, P.D. 1986. *Glomus microaggregatum*, a new species in the Endogonaceae. *Mycotaxon* **26:** 125–132.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., and Swan, J.A. 1990 A method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. *New Phytologist* **115:** 495-501.

Muthukumar, T. and Udaiyan, K. 2000 Arbuscular mycorrhizas of plants growing in the Western Ghats region, Southern India *Mycorrhiza* **9:** 297-313.

Muthukumar, T., and Vediyappan, S. 2009 Comparison of arbuscular mycorrhizal and dark septate endophyte fungal associations in soils irrigated with pulp and paper mill effluent and well-water. *European Journal of Soil Biology* **46:** 157-167.

Oehl, F., and Sieverding, E. 2004. *Pacispora*, a new vesicular–arbuscular mycorrhizal fungal genus in the Glomeromycetes. *Journal of Applied Botany.* **78:** 72–82.

Pande, M., and Tarafder, J.C. 2004. Arbuscular mycorrhizal fungal diversity in neem based agroforestry systems in Rajasthan *Applied Soil Ecology*, **26:** 233-241.

Read, D.J., and Birch, C.P.D. 1988 The effects and implications of distribution of mycorrhizal mycelial systems. *Proc R Soc Edinburgh* **94:**13–24.

Ruotsalainen, A.L., Makkola, A., and Kozlov, M.V. 2007 Root fungal colonization in *Deschampsia flexuosa*: effects of pollution and neighbouring trees. *Environmental Pollution* **147:** 723-728.

Sharma, S.K., Sharma, G.D. and Mishra, R.R. 1986. Status of mycorrhizae in subtropical forest ecosystems of Meghalaya. *Acta Botanica India* **14:** 87-92.

Sieverding, E. 1991. Vesicular arbuscular mycorrhizae management in tropical agrosystems. Deutsche Gesellschaft fur technische Zusammenarbeit (GTZ) GmBH, Eschborn, p.371.

Smith, S.E. and Read, D.J. 1997 *Mycorrhizal symbiosis.* Academic, London

Wu, C.G., Liu, Y.S., Hwuang, Y.L., Wang, Y.P., and Chao, C.C. 1995. Glomales of Taiwan: V. *Glomus chimonobambusae* and *Entrophospora kentinensis*, spp. nov. *Mycotaxon*, **53:** 283–294.