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RESEARCH PAPER

# Mycorrhizal colonization and distribution of arbuscular mycorrhizal fungi associated with *Michelia champaca* L. under plantation system in northeast India

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**Abstract**: Arbuscular mycorrhizal fungi (AMF) and dark septate endophyte (DSE) colonization were investigated in three different plantation sites (Umdihar, Umsaw and Mawlein) of Meghalaya, northeast India. Isolation and identification of the AMF spore were conducted to evaluate the AMF diversity and host preference in terms of AMF species distribution and abundance in the plantation sites. Results showed that AMF colonization was significantly higher than dark septate endophyte colonization ($p$>0.05). AMF and DSE colonization had a narrow range of colonization, varying from 50.91%–58.95% and 1.84%–4.11%, respectively. Spore density varied significantly in all the sites ($p$>0.05). Out of 29 species identified from 7 genera, the species from *Glomus* was found to be highly abundant. Sorenson coefficient (Cs) ranged from 0.35–7.0. Species richness varied from 2.0–2.9 in the sites. Total species richness was significantly correlated with total relative abundance ($p$=0.001). The distribution, abundance and principal component analysis plot suggest that *Glomus macrocarpum*, *G. multicaulis*, *G. constrictum* and *Acaulospora* sp 1 were the most host preferred species which possibly may favour the host with proper nutrient acquisition and growth.

**Keywords**: arbuscular mycorrhizal colonization; dark septate endophyte colonization; *Glomus; Michelia champaca*

## Introduction

*Michelia champaca* Linnaeus (Magnoliaceae) is famous for its striking appearance with large, very aromatic yellow blossoms, smooth trunk, and large ovate, glossy leaves. The species has highly economic value for perfume and timber industries in India. Flower buds of *M. champaca* are used in most of the herbal preparations for several diseases and possess active constituents (Jarald et al. 2008).

Mycorrhiza is widespread in natural ecosystems, and plays a crucial role in the uptake of mineral nutrition of forest trees, which is one of important nutrient acquiring mechanisms (Pate 1994). Arbuscular mycorrhizal fungi (AMF) form associations with the majority of terrestrial plant species (Smith and Read 1997). AMF stimulate plant uptake of nutrients such as P, Zn, Cu, and Fe in deficient soils and mycorrhizal hyphae can significantly improve $^{15}$N, P, and K uptake (Chen and Zhao 2009). It can play an important role in ecological system protection, restoration, and reconstruction (Wu et al. 2009). Moreover, AMF are now well practiced in the forestry management (Mridha and Dhar 2007). They belong to four orders: Glomerales, Archaeosporales, Paraglomales and Diversisporales in the division Glomeromycota (Schubler et al. 2001). Another type of mycorrhizal association is dark septate endophyte (DSE). It comprises of miscellaneous group of root inhabiting conidial sterile ascomycetous fungi that colonize living plant roots without causing noticeable harmful effects to the host (Jumpponen 2001).

The coarse structure of the root typified by the order Magnoliales are especially dependent on AMF for mineral uptake (Baylis 1975). Moreover, occurrence of DSE and *Paris* type of AMF colonization in *M. champaca* was reported earlier (Muthukumar et al. 2006). However, there is no detailed study of AMF diversity of this extremely important multipurpose tree. Composition of indigenous AMF spores in the plantation sites may be helpful in indicating the preference of AMF spores in inoculation program for seedling production in nursery. Therefore, the study was undertaken to evaluate (1) the status of AMF and DSE colonization in the three plantation sites, (2) the biodiversity of AMF in the plantation sites, and (3) whether some level of host preference in terms of frequency and abundance exist among AMF species in the plantation sites.

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## Materials and methods

### Study sites and sampling

Three plantation sites were selected in Ribhoi District of Meghalaya, Northeast India. They were located in Umdihar (N25º51.156'; E91º52.699'; with an elevation of 544.0 m.a.s.l.), Umsaw (N25º49.051'; E91º52.642'; 553.5 m.a.s.l.), and Mawlein (N25º42.642'; E91º53.55'; 828.5 m.a.s.l.), respectively. The plantations in Umdihar and Mawlein are privately managed sites, while the plantation in Umsaw is under State Forestry Department, Meghalaya.

The plant individuals with circumference breast height (CBH) >5 cm were considered for sampling. Tree height (H) was measured with the help of a Clinometer (Suunto pm-5/1520). From each sampling site, five trees were randomly selected with sampling points approximately 5 m apart. *M. champaca* from the order Magnoliales is distinguishable by its coarse root structure (Baylis 1975) and characteristic interesting aroma. The rhizospheric soil and roots at depths of 0–20 cm around each tree, at four different points for each plant were collected. Combined samples of approximately 500 g of soil per plant were placed in 3-kg polythene bags, labeled and transported for further analysis in the laboratory.

### Root processing

Electron microscopy of the root sample was carried out to confirm the arbuscular mycorrhizal association in the root. The transverse sections of fresh root samples were fixed in 3% glutaraldehyde for 24 h at 4°C. The root sections were washed thrice in 0.1 M sodium cacodylate buffer. The roots were then dehydrated with acetone series. Final dry treatment was given with the tetra methyl silane. The specimens were mounted in brass stubs. Coating of the root sections with gold were done for final examination in the microscope. The root segments were examined under scanning electron microscope (Jeol, JSM 6360).

To determine percent root colonization, the root samples were washed in tap water, processed and stained with black Faber Castell stamp pad ink (Das and Kayang 2008). Root segments of approximately 1-cm long stained samples were mounted on slides in lactoglycerol and examined for mycorrhizal structures under light microscope (Olympus 41209) to investigate different colonization patterns (the structures of hyphal, arbuscular, vesicular, and dark septate hyphal). The estimation of AMF and DSE colonization were done by magnified intersection method (McGonigle et al. 1990).

### Spore analysis

The spores were extracted by modified wet sieving and decanting method (Muthukumar et al. 2006). The isolated spores were picked up with needle in polyvinyl alcohol-lactoglycerol under a dissecting microscope (Koske and Tessier 1983) 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, colour, 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), isolation frequency (IF), species richness (SR), evenness (E), Simpson's diversity index (D), Shannon-Wiener index of diversity (H') and Sorenson's coefficient (Cs) were calculated (Zhao and Zhao 2007).

### Soil analysis

The soil samples were air dried after analysis of pH and moisture content. They were cleaned, ground, sieved with a 2-mm sieve, stored at 4°C, and processed for further soil analysis. Soil texture was analyzed using sodium hexametaphosphate method (Allen et al. 1974). For moisture content (%), 10 g sub sample of soil was oven dried and weight was determined. Measurement of the soil pH was done using microprocessor-based pocket pH tester 2 (Eutech Instruments). Available phosphorus of soil was determined following molybdenum-blue method (Allen et al. 1974). The soil organic carbon was estimated using colorimetric method (Anderson and Ingram 1993).

### Data analysis

Standard errors of means were calculated. Analysis of variance (ANOVA) was carried out and the means were separated by Tukey test. Pearson correlation coefficients were computed between soil physico-chemical properties, mycorrhizal colonization, CBH and H of tree species and between relative abundance and species richness of AMF species. Principal component analysis (PCA) was used to determine variation in AMF abundance. PCA analysis was done with the help of software, PAST (Hammer et al. 2001).

## Results

### Tree and soil characteristics

The circumference breast height and height of *M. champaca* in the plantations of Umdihar were 169.92±2.53 cm and 2293.33±7.87 cm, respectively, 35.2±0.9 cm and 1486.92±4.72 cm in Umsaw, and 32.4±2.02 cm and 947.14±3.09 cm in Mawlein, respectively. The soil physical and chemical properties were presented in Table 1.

### Mycorrhizal colonization

Scanning electron microscopy (SEM) reveals arbuscular mycorrhizal structures likely arbuscules and hyphal coils (Fig. 1a & b). The light microscopy also reveals various structures of dark

septate endophyte, including arbuscules, vesicles, hyphal coils and hyphae of AMF (Fig. 1c-f). The mycorrhizal structural colonization was presented in Table 2. *Paris* type of AMF morphology existed in the roots. There was no significant difference in total AMF and DSE colonization between the sites. However, AMF colonization was significantly higher ($p$>0.05) than DSE (Fig. 2). Spore density differed statistically in all the sites ($p$>0.05). No significant correlation was found between mycorrhizal colonization, soil properties, CBH and H ($p$>0.05).

**Table 1. Physical and chemical properties of soil collected from the three different sites of *Michelia champaca***

| Sites | Moisture (%) | Texture (%) | | | pH | Organic carbon (%) | Available P (μg·g$^{-1}$) |
|---|---|---|---|---|---|---|---|
| | | Silt | Sand | Clay | | | |
| Umdihar | 15.3±0.5 | 12.20 | 83.77 | 4.03 | 6.13±0.09 | 0.63±0.023 | 166.67±3.33 |
| Umsaw | 15.5±1.4 | 14.21 | 69.61 | 16.19 | 6.13±0.03 | 0.59±0.012 | 163.33±3.33 |
| Mawlein | 27.2±0.4 | 19.52 | 75.19 | 5.29 | 6.07±0.12 | 0.57±0.005 | 176.67±3.33 |

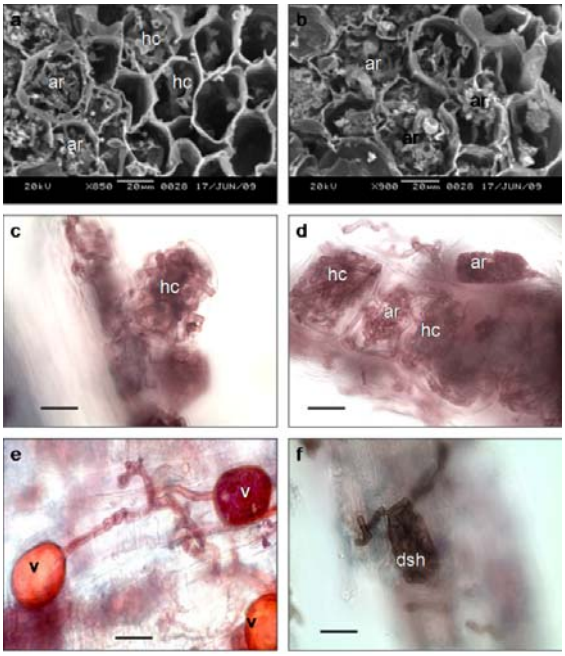


**Fig. 1 (a-f) Mycorrhizal structure in the roots of *M. champaca*.** (a & b) Scanning electron microscopy of transverse section of the roots showed the structures of hyphal coils (hc) and arbuscules (ar). (c-f) Light microscopy of the root segments showed the structures of hyphal coils, arbuscules, vesicles (v) and dark septate hyphae (dsh). Bar scale = 100 μm

**Table 2. Mycorrhizal structural colonization in the roots of *Michelia champaca***

| Sites | Arbuscules | Vesicles | Hyphae | Dark septate hyphae | Spore density / 50 g soil |
|---|---|---|---|---|---|
| Umdihar | 9.91±1.23b | 4.24±0.87b | 52.49±2.84c | 4.11±1.11b | 537.66±65.83 d |
| Umsaw | 21.99±1.57a | 8.46±0.95b | 58.95±2.01c | 1.84±0.97b | 1939.66±58.37f |
| Mawlein | 17.42±1.31a | 9.30±1.14b | 50.91±2.84c | 1.99±0.40b | 1163.00±177.88g |

Tukey test showing different alphabetical letters varies significantly ($p$ > 0.05)

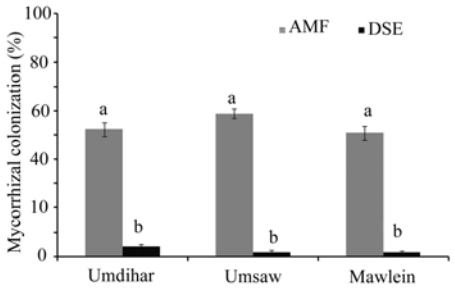


**Fig. 2 Mycorrhizal colonization in the roots of *M. champaca* from three sites**

Arbuscular mycorrhizal spore distribution

*Acaulospora*, *Ambispora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Pacispora*, and *Paraglomus* were extracted from three plantation sites of *M. champaca* (Fig. 3). A total 29 species were identified from all the soil samples (Table 3).

**Table 3. Relative abundance and isolation frequency of arbuscular mycorrhizal fungi from the three plantation sites.**

| AMF Species | Species abbreviation | Relative abundance (%) | | | Isolation frequency (%) |
|---|---|---|---|---|---|
| | | Umdihar | Umsaw | Mawlein | |
| *Acaulospora* sp 1 | A1 | 27.91 | 0.46 | 5.39 | 100.00 |
| *A. bireticulata* | Ab | 0.20 | - | - | 33.33 |
| *A. rehmii* | Ar | - | 0.26 | 0.29 | 66.67 |
| *A. foveata* | Af | 0.40 | 0.07 | - | 66.67 |
| *A. lacunose* | Al | 10.04 | - | - | 33.33 |
| *A. tuberculata* | At | - | 0.13 | 2.04 | 66.67 |
| *A. cavernata* | Ac | - | - | 0.29 | 33.33 |
| *Ambispora* sp 1 | Am1 | - | - | 0.15 | 33.33 |
| *Entrophospora colombiana* | Ec | - | 0.13 | - | 33.33 |
| *Gigaspora* sp 1 | Gi1 | - | 0.07 | 1.02 | 66.67 |
| *Glomus* sp 1 | G1 | 0.4 | 0.40 | - | 66.67 |
| *Glomus* sp 2 | G2 | - | - | 0.44 | 33.33 |
| *Glomus* sp 3 | G3 | 23.49 | - | - | 33.33 |
| *G. aggregatum* | Gag | 0.20 | - | - | 33.33 |
| *G. ambisporum* | Gam | 6.02 | - | - | 33.33 |
| *G. aureum* | Gau | - | 0.07 | 0.15 | 66.67 |
| *G. constrictum* | Gcon | 9.04 | 9.26 | 13.83 | 100.00 |
| *G. fuegianum* | Gfu | - | - | 0.15 | 33.33 |
| *G. glomeratum* | Gglo | - | 2.67 | 1.6 | 66.67 |
| *G. intraradices* | Gin | - | 0.07 | 1.31 | 66.67 |
| *G. macrocarpum* | Gmac | 13.05 | 45.11 | 32.02 | 100.00 |
| *G. microaggregatum* | Gmic | - | - | 0.58 | 33.33 |
| *G. mosseae* | Gmos | 0.40 | 0.13 | - | 66.67 |
| *G. multicaulis* | Gm | 8.43 | 32.79 | 26.49 | 100.00 |
| *G. taiwanense* | Gtaw | - | 2.80 | 0.44 | 66.67 |
| *G. tortuosum* | Gto | - | 5.93 | 10.77 | 66.67 |
| *Pacispora boliviana* | Pb | - | - | 1.60 | 33.33 |
| *P. chimonobambusae* | Pc | 0.20 | 0.07 | 0.58 | 100.00 |
| *Paraglomus occultum* | Po | 0.20 | - | 0.87 | 66.67 |
| Total | | 100 | 100 | 100 | |

Out of seven genera, four were isolated from Umdihar, five from Umsaw, and six from Mawlein. *Glomus macrocarpum*, *G. multicaulis*, *G. constrictum, Acaulospora* sp 1 and *Pacispora chimonobambusae* were present in all the sites. Among all the 29 species, there were five species with the isolation frequency of 100%, 12 species with the frequency of 66.67%, and the rest 12 species having the frequency of 33.33%. *Acaulospora* sp 1 was relatively more abundant in Umdihar than other species, and *G. macrocarpum* was comparatively abundant than other species in Umsaw and Mawlein. *Ambispora* sp 1, *Entrophospora colombiana* and *G. fuegianum* were lower in relative abundance. Significant positive correlation ($p$ = 0.001) was found between relative abundance and species richness of AMF spores (Fig. 4). *Glomus* exhibited high relative abundance and high species richness, whereas *Entrophospora and Ambispora* were the lowest in terms of abundance and species richness (Table 4). In addition, species richness increased with the increase in relative abundance as depicted in the plot (Fig. 4). The highest species richness was observed in the plantations of Mawlein (Table 5). Sorenson coefficient varied between the sites. Moreover, high Cs was observed between nearest sites and the lowest value between distant sites. The dissimilar number of AMF species between Umdihar X Umsaw, Umsaw X Mawlein and Umdihar X Mawlein were 15, 11 and 22, respectively. The similar number of species between Umdihar X Umsaw, Umsaw X Mawlein and Umdihar X Mawlein were eight, 13 and six, respectively (Fig. 5).

**Table 4. Species richness and relative abundance of arbuscular mycorrhizal fungi associated with *Michelia champaca***

| AMF species | Species richness | Relative abundance (%) |
|---|---|---|
| *Glomus* | 9.7 | 89.14 |
| *Acaulospora* | 4.0 | 9.60 |
| *Pacispora* | 1.3 | 0.63 |
| *Gigaspora* | 0.7 | 0.26 |
| *Paraglomus* | 0.7 | 0.26 |
| *Ambispora* | 0.3 | 0.04 |
| *Entrophospora* | 0.3 | 0.07 |
| Total | 17 | 100 |

**Table 5. Diversity index of arbuscular mycorrhizal fungi associ ated with *Michelia champaca***

| Sites | Species richness | Shannon - Wiener index of diversity (H') | Simpson's index of diversity (D) | Evenness (E) |
|---|---|---|---|---|
| Umdihar | 2.0 | 1.9 | 0.18 | 0.38 |
| Umsaw | 2.4 | 1.4 | 0.32 | 0.20 |
| Mawlein | 2.9 | 1.9 | 0.21 | 0.43 |

Diversity index reveals that H' and E were high in Umdihar and Mawlein, however, D was high in Umsaw (Table 5). PCA plot showed the distribution of AMF species and host preference (Fig. 6). The variability in the distribution existed between species, where *G. macrocarpum*, *G. multicaulis, G. constrictum* and *Acaulospora* sp 1 accounted for 78.57% of the total variation. The other species were clumped together in one spot, indicating no variation. PCA plot showed close relation of the four species to the sites. *G. macrocarpum* and *G. multicaulis* were closely related to Mawlein and Umsaw. *Acaulospora* sp 1 was closely correlated with Umdihar, while *G. constrictum* was relatively associated with Mawlein and Umsaw. Moreover, the entire three axes showed that these four species were distributed in all the sites.

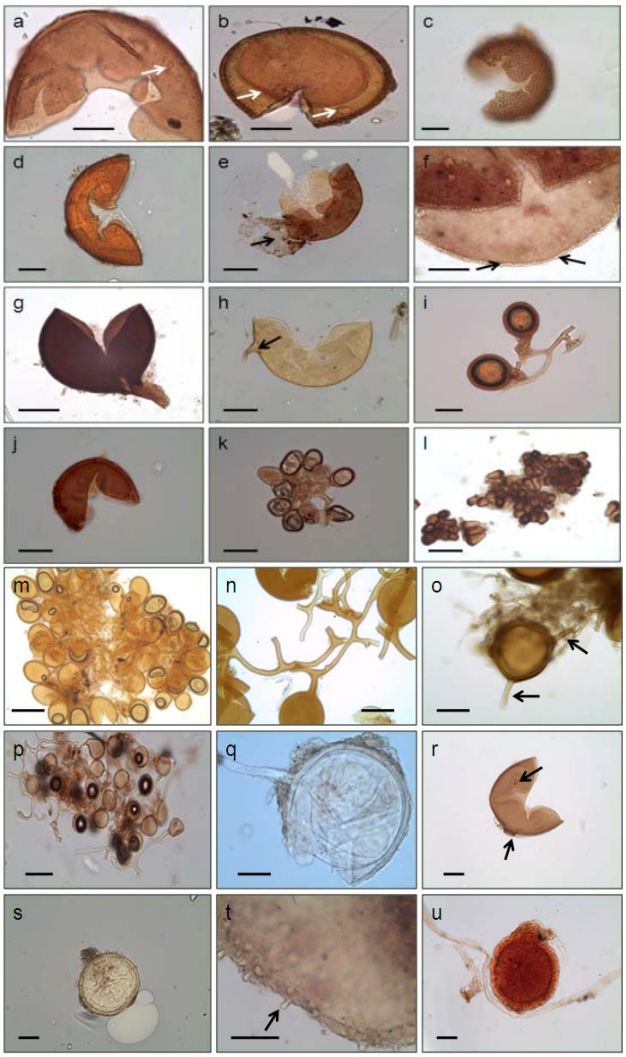


**Fig. 3 (a-u) Arbuscular mycorrhizal spores.** (a) *Acaulospora tuberculata* with cicatrix. Bar scale = 200 µm. (b) *A. lacunosa* with cicatrix and wall layers. Bar scale = 150 µm. (c) *A. rehmii* with cicatrix. Bar scale = 100 µm. (d) *A. foveata*. Bar scale = 150 µm. (e) *A. cavernata* with soporiferous saccule. Bar scale = 200 µm. (f) *A. bireticulata*. Bar scale = 50 µm. (g) *Glomus constrictum* Bar scale = 150 µm. (h) *G. mosseae*. Bar scale = 200 µm. (i) *G. multicaulis*. Bar scale = 150 µm. (j) *Glomus* sp 1. Bar scale = 100 µm. (k) *G. feugianum*. Bar scale = 100 µm. (l) *G. taiwanense*. Bar scale = 200 µm. (m) *G. aureum*. Bar scale = 100 µm. (n) *G. macrocarpum*. Bar scale = 100 µm. (o) *G. glomeratum*. Bar scale = 50 µm. (p) *G. microaggregatum*. Bar scale = 250 µm. (q) *Paraglomus occultum*. Bar scale = 100 µm. (r) *Entrophospora colombiana*. Bar scale = 150 µm. (s) *Ambispora* sp 1. Bar scale = 100 µm. (t) *Pacispora chimonobambusae*. Bar scale = 50 µm. (u) Unidentified Bar scale =100 µm.

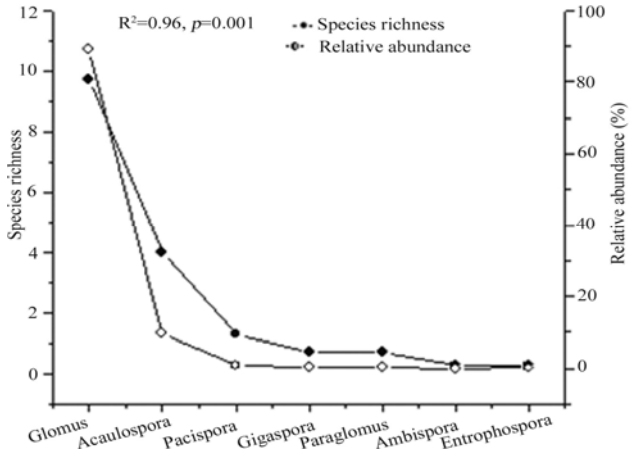


**Fig. 4 Relation between species richness and relative abundance of arbuscular mycorrhizal fungi**

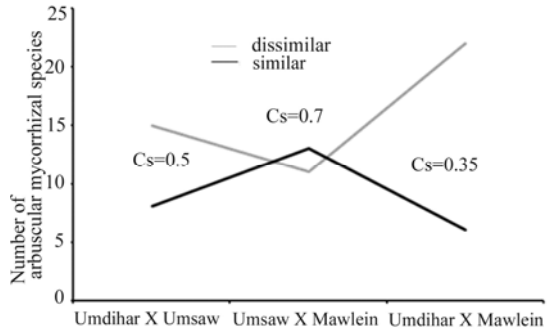


**Fig. 5 Sorenson coefficient (Cs) of arbuscular mycorrhizal fungi in the three plantations**

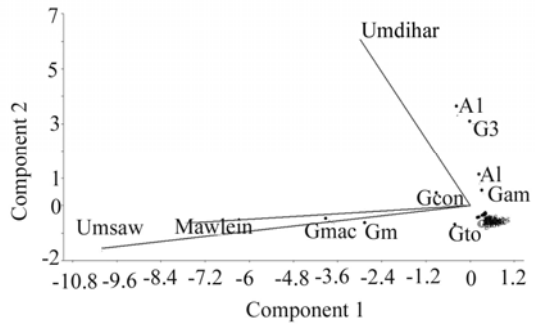


**Fig. 6 Array of the relative abundance of AMF species distribution in the three plantation sites, which were determined by PCA biplot.** For other abbreviations, see Tab. 3

## Discussion

*M. champaca* was colonized by AMF and DSE in all the sites. Presence of DSE and AMF confirms the findings of Muthukumar et al. (2006). Mycorrhizal colonization recorded in the present study was less than the earlier report (Muthukumar et al. 2006). Moreover, our results showed that the AMF colonization was significantly higher than DSE colonization. This may be due to the presence of coarse structure of the root, the characteristic feature of Magnoliales, which favors mycorrhizal infection (Baylis 1975). The results suggest that mycorrhizal colonization of *M. champaca* in different plantations recorded in the investigation had a narrow range i.e., intermediate AMF colonization (50.91%−58.95%) and low in DSE (1.84%−4.1%).

No correlation was found between mycorrhizal colonization, soil properties, CBH and H. It implies that AMF colonization may be affected by the comprehensive interactions of several factors, such as the factors inherent to the host plant, climatic and edaphic factors, and effects of the soil community (Moreira et al. 2006).

The spore number varied significantly between the sites. This might be due to production of AMF spores in the rhizosphere vicinity of surrounding herbaceous species (Kruckelmann 1975). Presence of herbaceous community in Umsaw and Mawlein were observed but in Umdihar spore density and herbaceous community were comparatively lower. The herbaceous community was removed or not frequent in Umdihar, however, the spore density could be disturbed as the site was located very near to national highway No. 44. Moreover, in an undisturbed ecosystem, higher spore population was quite natural as the number of AMF spores and the population diversity were higher in native undisturbed forests than the disturbed and replanted areas (Moreira-Souza et al. 2003). Spore population is affected by a wide range of soil, climatic, fungal and host factors (Anderson et al. 1983; Howeler et al. 1987). Plant phenology and root production are closely related to the patterns of spore production and spore quantity (Brundrett 1991).

Out of seven genera, *Glomus* was the most widely distributed genera, followed by *Acaulospora* and *Pacispora*. *Glomus* sporulated abundantly regardless of the sites selected. Das and Kayang (2009) also reported the dominance of the *Glomus* from this region. They described the wider adaptation of the taxon in varied soil conditions. The sporulation pattern of *Glomus* might bring about the dominance of the taxon. Spores of *Glomus* grow in cluster and sporulate more frequently while the spores of other genera like *Gigaspora* sporulated singly (Dhar and Mridha 2006).

The altitudinal variation and distances of plantation sites could play an important role in the similarities of AMF species. Higher similarity coefficient (Cs) was found between the nearest sites (Umsaw and Umdihar) with a relatively low altitude, and lower between the distant sites (Umdihar and Mawlein) with a relatively high altitude. In contrast, the results of An et al. (2008) showed that highest similarity index was observed between the most distant sites and the lowest value was observed between the nearest sites.

In the present study, diversity attribute of *M. champaca* plants such as H' and E were higher in Mawlein and Umdihar than in Umsaw. The variation in the diversity attribute can be substantiated to the study of Allen and Boosalis (1983) where the diversity of mycorrhizal fungi associated with the same plants was found to vary.

*G. macrocarpum*, *G. multicaulis, G. constrictum, Acaulospora* sp 1 and *P. chimonobambusae* were most frequently distributed in the three sites, of which *G. macrocarpum*, *G. multicaulis, G. constrictum* and *Acaulospora* sp 1 were relatively abundant. However, *P. chimonobambusae* was less abundant than other four species. PCA plot with relative abundance of AMF species in all three sites showed that there was close relation between the sites and the highly abundant species. The distribution and relative abundance indicate that these four species may favour *M. champaca*.

## Conclusion

Relative abundance, species distribution and PCA plot suggest that *G. macrocarpum*, *G. multicaulis*, *G. constrictum* and *Acaulospora* sp 1 were most preferred by the host plants, which may possibly favour host nutrition and growth. Furthermore, the investigation was emphasized to select the suitable indigenous AMF species for sustainable management of tree plantations, and to create consciousness among foresters and local folks about the significance of mycorrhiza as a tool to maintain such ecosystem environment friendly. In addition, this work provides a platform for analysis the role of AMF and DSE in several tree species that are used in northeast Indian forestry practice.

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