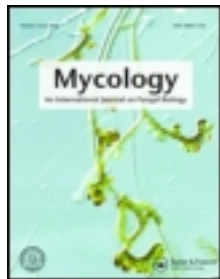


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## Diversity of arbuscular mycorrhizal fungi associated with *Flemingia vestita* Benth. ex Baker

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Diversity of arbuscular mycorrhizal fungi (AMF) was investigated from natural (MF) and cultivated sites (TF) of *Flemingia vestita* Benth. ex Baker. Samplings were done at monthly intervals for one crop cycle. Root samples were evaluated for AMF colonization. Trap cultures were also established with four different host plants (*Oryza sativa* L., *Paspalum notatum* Flügge, *Trifolium repens* L. and *Zea mays* L.) using rhizosphere soils from natural and cultivated sites of *F. vestita* as the source of AMF inoculum. A total of 61 AMF species (51 from natural site and 46 from cultivated site) belonging to six genera (*Acaulospora*, *Ambispora*, *Gigaspora*, *Glomus*, *Pacispora* and *Scutellospora*) could be distinguished from *F. vestita* rhizosphere soil. 33 AMF species belonging to five genera (*Acaulospora*, *Gigaspora*, *Glomus*, *Paraglomus* and *Scutellospora*) were isolated from trap cultures (25 AMF species from trap plants with MF derived inoculum and 18 AMF species from trap plants with TF derived inoculum). Three additional AMF species were isolated in trap cultures that were not detected in field soils, indicating that trap culture allows development and sporulation of AMF species which otherwise were not recovered from field soil. Higher AMF diversity was observed in natural site than in cultivated site of *F. vestita*.

**Keywords:** arbuscular mycorrhizal fungi; *Flemingia vestita*; natural site; cultivated site; trap cultures

### Introduction

Arbuscular mycorrhizal fungi (AMF) are one of the most important and widespread components of the soil biota in natural and agricultural ecosystems. They are obligate biotrophic symbionts associated with roots of over 90% terrestrial plant species (Smith & Read 1997). AMF benefit from this association by obtaining carbon compounds which are necessary for their growth and in return, they have diverse, beneficial impact on plants and soils (Brundrett et al. 1999; Li et al. 2006). It is a beneficial microbe fundamental for soil fertility in natural and agricultural ecosystems. It also helps in increasing resistance to environmental stresses, enhances plant nutrient acquisition, water relations, disease resistance and improves soil quality (Smith & Read 2008). In addition to mycorrhizal associations, plants are also associated with dark septate endophytes (DSE), which are a miscellaneous group of ascomycetous anamorphic fungi that colonize root tissues intracellularly and intercellularly (Jumpponen 2001). They are characterized by melanized septate hyphae and microsclerotia (Peterson et al. 2004). Their widespread occurrence and their potential to function as mycorrhizal fungi suggest that these endophytes are significant components of natural ecosystems (Jumpponen & Trappe 1998).

The species richness and diversity of AMF differ among ecosystems, which usually is greater in natural ecosystems compared to agricultural ecosystems (Wang

et al. 2003). Mycorrhizal communities are site specific and may prefer certain habitats; earlier studies demonstrated the role of environmental factors, plant communities (Brundrett 1991; Read et al. 1992) and abiotic factors on AMF community composition (Hijri et al. 2006; Schalamuk et al. 2006). Moreover, plant diversity and productivity in ecosystems are influenced significantly by the AMF diversity in the soil (van der Heijden et al. 1998). In fact plants and AMF communities influence each other profoundly.

Most studies addressing AMF diversity rely on the morphological identification of AMF spores obtained either directly from the field (Landis et al. 2004; Gai et al. 2006) or from trap cultures (Bever et al. 1996). Field collected spores, however, in some circumstances, lack informative taxonomic characteristics impairing a more accurate identification. Establishment of trap cultures using bulk soil or by mixing rhizosphere soil and root fragments from field with sterilized diluents and growing with suitable hosts, represents a strategy to yield healthy spores which can be readily identified and supplements the assessment of AMF species diversity (Leal et al. 2009).

*Flemingia vestita* Benth. ex Baker (Fabaceae) is an indigenous plant of Meghalaya, Northeast India. It produces an edible root tuber, which is somewhat juicy, sweet and nut like flavor, is eaten raw and has a high local market value. In addition, its root-tuber peel is used as curative

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against worm infection in traditional medicine among the natives of Meghalaya. Anthelmintic efficacy of this plant derived materials has provided evidences that support and authenticate the usage of the tuberous root of this plant as vermifuge and vermicide (Das et al. 2004). In Meghalaya, many plants have been investigated for their botanical aspects and medicinal values. However, mycorrhizal investigations have been rarely conducted. Therefore, in view of this, the present work was carried out to understand AMF diversity of *F. vestita* under field and trap conditions.

## Materials and methods

### Site description and field sampling

Samplings were done at monthly intervals from natural and cultivated sites of *F. vestita*. Natural site was located at Mawprem, MF (25° 32' 33.2" N, 91° 45' 05.5" E) and cultivated site at Thangsning, TF (25° 28' 57.1" N, 91° 55' 00.9" E) in East Khasi Hills, Meghalaya, Northeast India. In natural site *F. vestita* were found growing along with some plants such as *Eupatorium adenophorum*, *Ageratum conyzoides*, *Trifolium repens*, *Osbeckia stellata*, *Plantago erosa*, *Artemisia nilagirica*, etc. In cultivated site, *F. vestita* was crop rotated with *Solanum tuberosum* (potato). *F. vestita*, being a seasonal plant, develops its root in the month of April–May, and therefore, root samplings were done from May to October 2010. Soil samplings were done from March to December 2010 including pre-plantation and post-plantation periods. From each sampling site, roots and rhizosphere soils of ten plants were collected monthly with sampling points approximately 5 m apart, and mixed to obtain a representative sample. The collected samples were then kept in sterilized plastic bags and transported to the laboratory for analysis.

### Analysis of AMF and DSE colonization

Roots were washed thoroughly in tap water and cut into approximately 1 cm segments. The root segments were then cleared in 10% (w/v) KOH by heating at 90°C for 1 to 2 h, depending on the degree of lignification of the roots. It was then washed and stained with trypan blue (Philips & Hayman 1970). The stained root segments were mounted on microscopic slides and examined for AMF structure under light microscope. The root segments of *F. vestita* were also examined under scanning electron microscope and transmission electron microscope. Root lengths with mycorrhizal colonization in the form of arbuscules, vesicles, hyphae and DSE in 100 root segments per sample were estimated using the magnified intersections method of McGonigle et al. (1990) and converted to percentage.

### AMF spore isolation, enumeration and identification

AMF spores were extracted by wet sieving and decanting method of Gerdemann and Nicolson (1963). Suspension

of 25 g rhizosphere soil sample in water was decanted through a series of 710 to 37 µm sieves. The materials collected on the 37 µm sieve were washed into a beaker with water and filtered through filter papers. Each filter paper was spread on Petri dish and spores were counted using a dissecting microscope at 40× magnification. Sporocarps and spore clusters were considered as one unit. AMF spores were picked up using a needle, mounted in polyvinyl alcohol-lactoglycerol with Meltzer's reagent and identified based on morphological characteristics using identification keys from an internet-published reference culture database established by Morton ([http://invam.caf.wvu.edu/Myc\\_Info/Taxonomy/species.htm](http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm)), AMF phylogeny ([www.amf-phylogeny.com](http://www.amf-phylogeny.com)), Oehl and Sieverding (2004) and Goto et al. (2008).

### Trap culture establishment

Trap cultures were established from fresh field soil collected from natural and cultivated sites. The soil from each site was mixed with autoclaved coarse sand (1:1 v/v) which serves as a substrate. Each pots were planted with one of the four host plants; *Oryza sativa* L. (upland varieties), *Paspalum notatum* Flügge, *T. repens* L. and *Zea mays* L. Seeds of each plant species were evenly sown on 10 plastic pots each containing 1.5 kg substrate and monitored in greenhouse condition for 5 months. It was watered whenever required. After 5 months, the roots of the trap plants were evaluated for AMF colonization and spores were isolated and analyzed as described above.

### Soil physico-chemical analysis

Rhizosphere soil of *F. vestita* were analyzed for its physico-chemical properties with three replicates each. Soil moisture content was determined by drying 10 g fresh soil at 105°C for 24 h in a hot-air oven. Soil pH was read using a digital pH meter. Organic carbon and total nitrogen of the soil were determined using methods outlined in Anderson and Ingram (1993). Available phosphorus of soil was estimated by molybdenum blue method of Allen et al. (1974). Soil exchangeable potassium was estimated using flame photometer method of Jackson (1973). The soil physico-chemical properties are presented in Table 1.

### Calculation and statistical analysis

Spore density and species richness were expressed as total number of spores and number of species occurring in 25 g of soil. Relative abundance, isolation frequency, Shannon–Wiener index of diversity, Simpson index of dominance, Evenness and Sorenson's coefficient of AMF were calculated (Dandan & Zhiwei 2007). Relationships between AMF, DSE colonization, spore density and soil physico-chemical properties were computed

Table 1. Soil physico-chemical properties of *F. vestita* rhizosphere soil.

Site	Soil properties						
	MC (%)	Temp	pH	OC (%)	N (%)	P (%)	K (%)
MF	25.93 ± 3.17	22.04 ± 1.61	6.28 ± 0.17	2.36 ± 0.05	0.25 ± 0.01	0.12 ± 0.00	0.14 ± 0.02
TF	30.09 ± 1.87	19.48 ± 1.77	5.83 ± 0.14	2.38 ± 0.02	0.34 ± 0.01	0.38 ± 0.01	0.13 ± 0.02

Note: MC = Moisture content; Temp = Temperature; OC = Organic carbon; N = Nitrogen; P = Phosphorus; K = Potassium.

using Pearson's correlation coefficient. Data were statistically analyzed using one-way ANOVA. Standard errors of means were calculated.

## Results

### AMF and DSE colonization

Mycorrhizal and DSE colonization were higher in natural site than in cultivated site. AMF structures, i.e. arbuscules, vesicles and hyphae, and occasionally, intraradical spores were present in the root samples throughout the crop cycle (Figure 1 and 2). Total AMF colonization ranged from 27.13% to 83.72% in natural site and 11.38% to 96.76% in cultivated site (Figure 3). The mean AMF and DSE colonization are given in Table 2. In natural site, AMF colonization had a significant positive correlation with organic carbon ( $r = 0.923$ ,  $p < 0.01$ ), and a negative correlation with P ( $r = -0.821$ ,  $p < 0.05$ ) and K ( $r = -0.951$ ,  $p < 0.01$ ). Spore density showed a negative correlation with moisture content ( $r = -0.944$ ,  $p < 0.01$ ) and a positive correlation with pH ( $r = 0.978$ ,  $p < 0.01$ ). In case of cultivated site, AMF colonization had a significant positive correlation with organic carbon ( $r = 0.993$ ,  $p < 0.01$ ) and P ( $r = 0.797$ ,  $p < 0.05$ ), and a negative correlation with K ( $r = -0.778$ ,  $p < 0.05$ ). N had a positive correlation with arbuscular colonization ( $r = 0.791$ ,

$p < 0.05$ ) and a negative colonization with DSE ( $r = -0.900$ ,  $p < 0.01$ ). ANOVA did not show any significant variation in mycorrhizal and DSE colonization among the two sites.

### AMF species composition and diversity

High AMF spore density was observed in rhizosphere soil of *F. vestita*. Spore numbers with a range of 574 to 3074 in 25 g of rhizosphere soil was recorded from natural site. In cultivated site, it ranged from 552 to 1854 in 25 g of the soil. The monthly variations of AMF spore density are given in Figure 4. A total of 61 AMF species (51 from natural site and 46 from cultivated site) could be distinguished on the basis of morphological characteristics; out of which, 36 species were common to both sites, 15 species were restricted in natural site and 10 species in cultivated site. These AMF species belonged to six genera namely, *Acaulospora*, *Ambispora*, *Gigaspora*, *Glomus*, *Pacispora* and *Scutellospora*. A total of 35 species belonging to genus *Glomus*, 12 to *Acaulospora*, 9 to in *Scutellospora*, 3 to *Gigaspora* and one each to *Ambispora* and *Pacispora*. Micrographs of some of the isolated species are given in Figure 5. The isolated AMF species with their relative abundance and isolation frequency are presented in Table 3. The natural site was dominated by *Acaulospora delicata*,

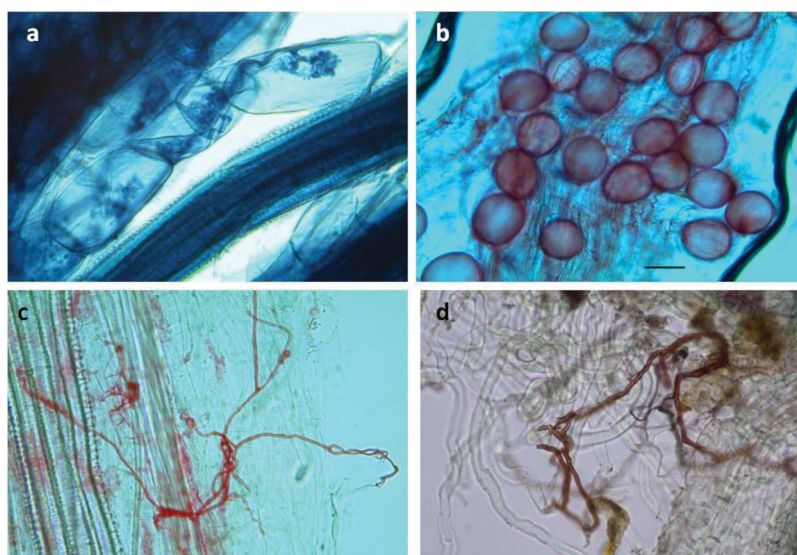


Figure 1. Mycorrhizal colonization in the roots of *F. vestita*; (a) arbuscules, (b) vesicles, (c) hyphae and (d) DSE.

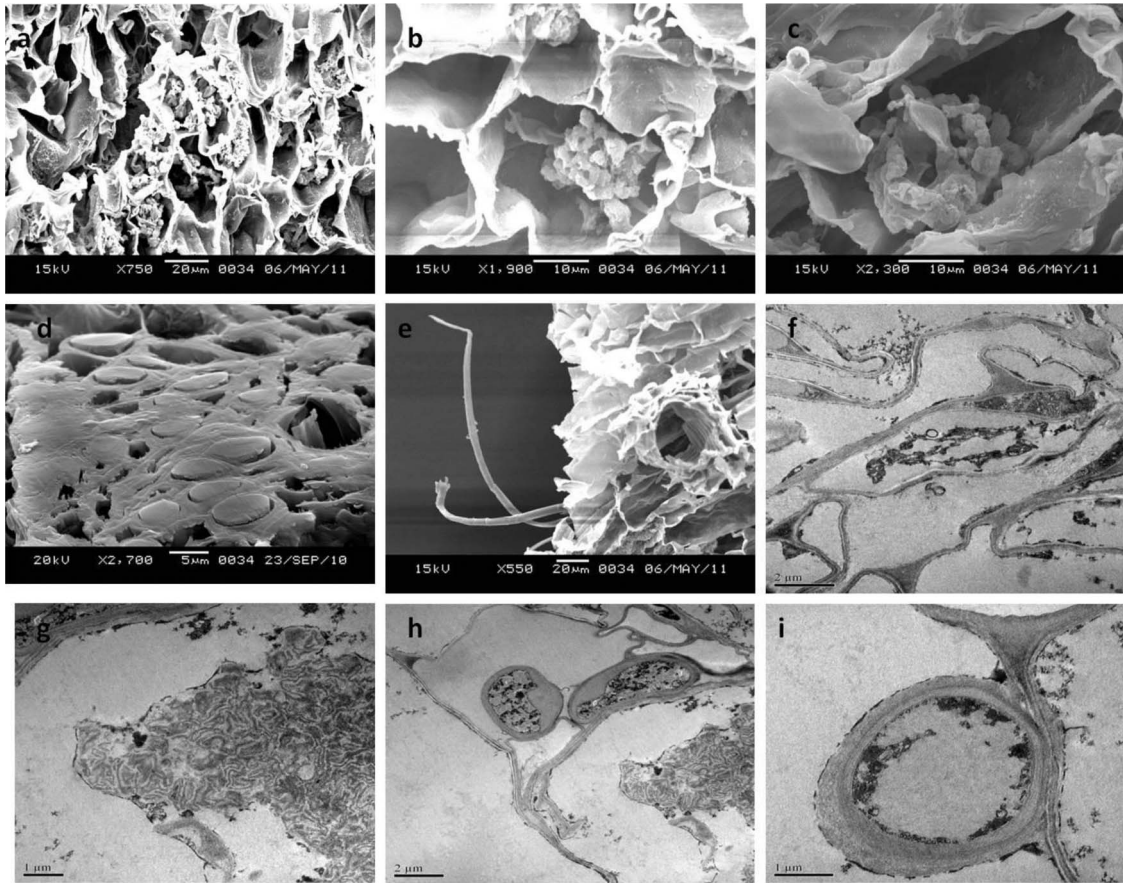


Figure 2. Electron micrograph of the roots of *F. vestita*; (a–e) scanning electron micrograph of arbuscules, vesicles and hyphae, (f–i) transmission electron micrograph of arbuscules and vesicles.

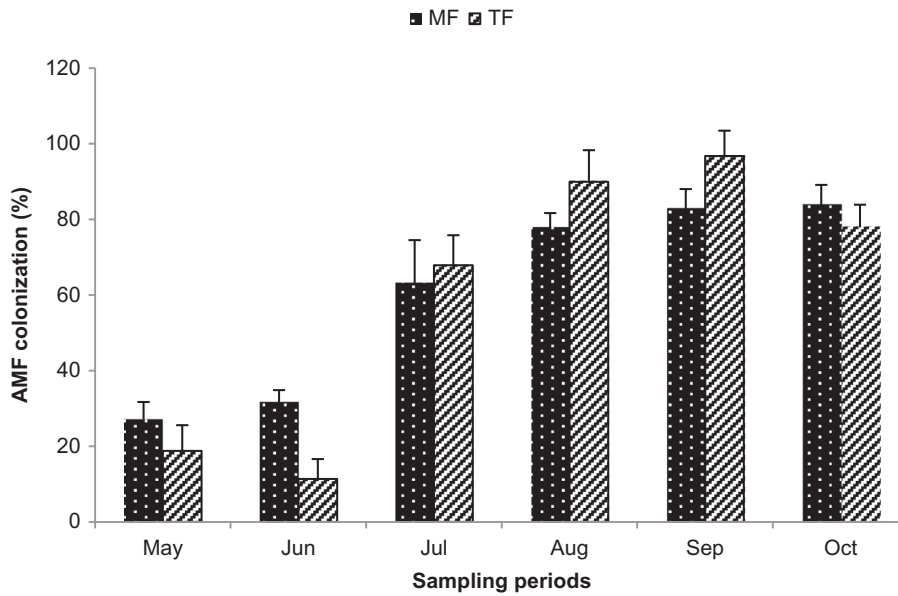
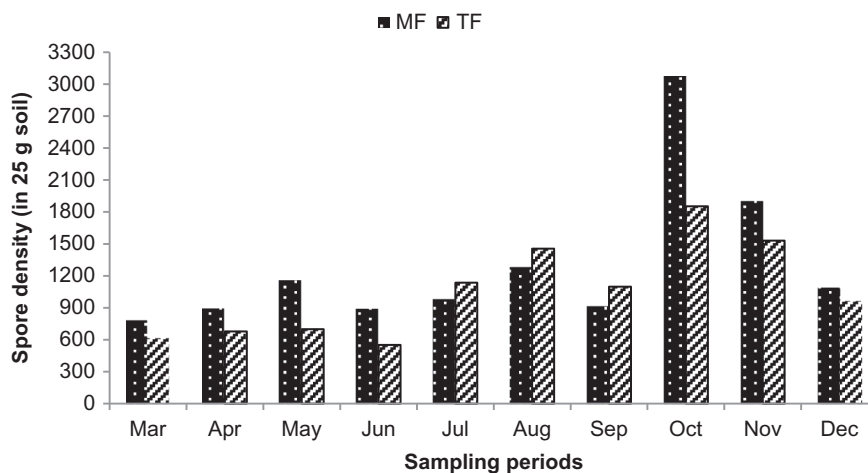


Figure 3. AMF colonization in the roots of *F. vestita*.

Table 2. Mean AMF and DSE colonization (%) in the roots of *F. vestita*.

Site	Mycorrhizal structural colonization				
	Arbuscules	Vesicles	Hyphae	AMF	DSE
MF	26.20 ± 7.75	3.74 ± 2.72	31.08 ± 7.53	61.02 ± 5.62	6.08 ± 1.76
TF	27.73 ± 7.12	3.86 ± 1.91	28.84 ± 9.92	60.43 ± 6.83	3.12 ± 1.82

Figure 4. AMF spore density in the rhizosphere soils of *F. vestita*.

*Glomus geosporum*, *G. intraradices* and *G. luteum*, while in cultivated site it was dominated by *Acaulospora scrobiculata*.

Shannon–Wiener index of diversity and Simpson’s index of dominance were calculated to evaluate the diversity of AMF in both natural and cultivated *F. vestita* rhizosphere soil. Results showed higher values of the Shannon–Wiener index and lower values of the Simpson’s index of AMF species at the rhizosphere of natural site as compared to the cultivated site. The value of Shannon–Wiener index of diversity ranged from 2.43 to 3.07 in natural site, and 2.21 to 2.69 in cultivated site. Simpson’s index of dominance ranged from 0.05 to 0.10 in natural site whereas it ranged from 0.07 to 0.13 in cultivated site. The value of evenness of AMF species ranged from 0.94 to 0.97 in natural sites whereas in cultivated site it ranged from 0.91 to 0.97. Sorenson’s coefficient of AMF species between natural and cultivated sites ranged from 0.15 to 0.81.

#### AMF from trap cultures

Among the four trap plants, AMF colonization was lowest in *P. notatum* (26.54% in MF derived inoculum and 27.22% in TF derived inoculum) and highest in *T. repens* (96.32% in MF derived inoculum and 97.07% in TF derived inoculum). It was found that in all plant species, colonization was higher in TF derived inoculum as compared to MF derived inoculum (Figure 6). Colonization in the form

of arbuscules was comparatively higher than vesicles and hyphae in all plant species except in *T. repens* growing in MF derived inoculum.

AMF spore density in trap culture are presented in Figure 7. It was lowest in *O. sativa* (93 and 68 spores per 25 g soil in MF and TF derived inoculum) and it was highest in *P. notatum* in MF derived inoculum (218 spores) and *T. repens* in TF derived inoculum (150 spores). Altogether 33 AMF species were isolated from trap cultures of MF and TF derived inoculum, out of which 10 AMF species were common to both MF and TF trap cultures. In trap plants with MF derived inoculum, a total of 25 AMF species were isolated belonging to 5 genera (5 species of *Acaulospora*, 1 of *Gigaspora*, 15 of *Glomus*, 1 of *Paraglomus* and 3 of *Scutellospora*). AMF species isolated from trap cultures (MF derived inoculum) with their relative abundance and frequency are given in Table 4. All the AMF species from MF derived trap cultures were also isolated from the field soil of *F. vestita* with the exception of *Paraglomus brasilianum* which was isolated only from *P. notatum*. In TF derived inoculum, 18 AMF species were isolated belonging to three genera (4 species of *Acaulospora*, 2 species of *Gigaspora* and 12 species of *Glomus*). Isolated AMF species from trap cultures (TF derived inoculum) with their relative abundance and isolation frequency are given in Table 5. All the AMF species from TF derived trap cultures were isolated from the field soil of *F. vestita* with the exception of *G. caledonium* isolated from *T. repens* and *G. glomerulatum* isolated from *P. notatum* and *T. repens*.

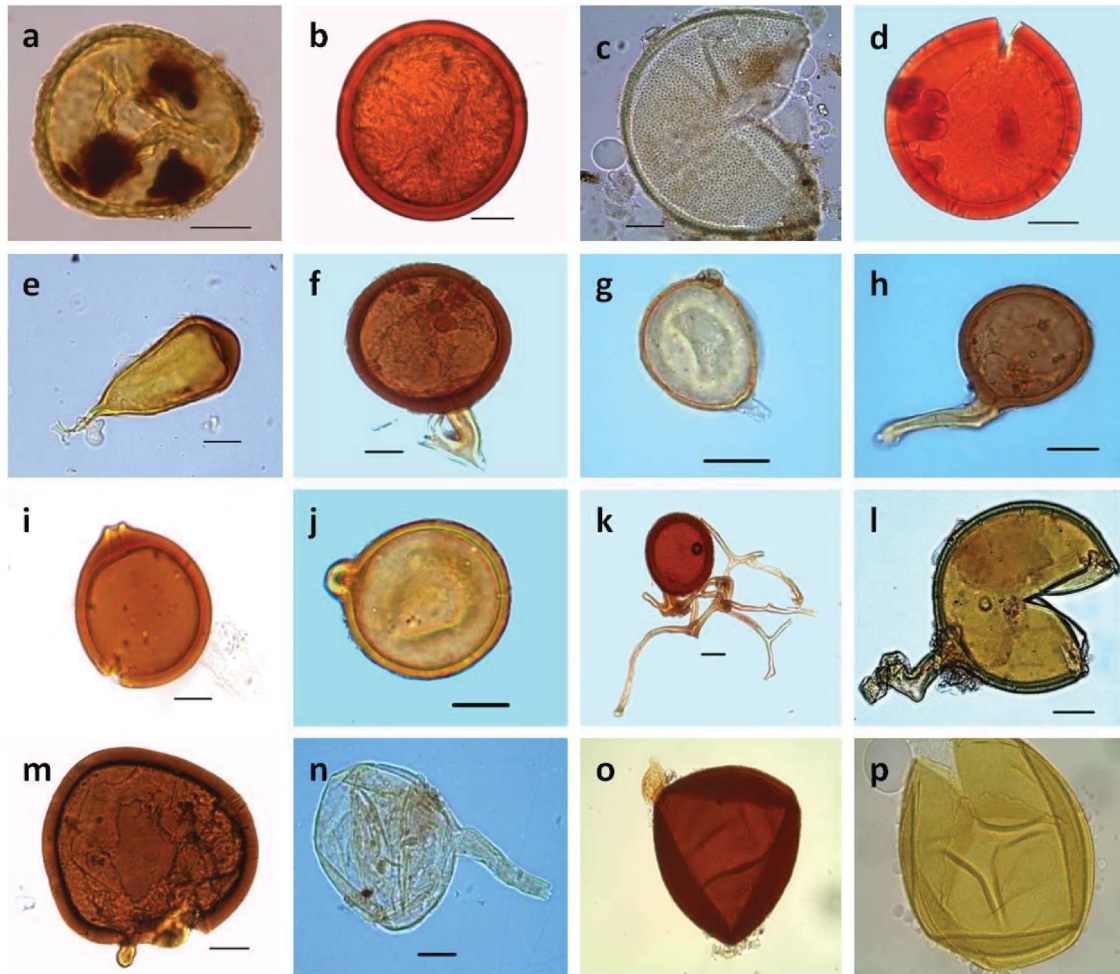


Figure 5. Isolated AMF species from *F. vestita* rhizosphere soils; (a) *Acaulospora foveata*, (b) *A. mellea*, (c) *A. spinosa*, (d) *Glomus badium*, (e) *G. claviforme*, (f) *G. convolutes*, (g) *G. glomerulatum*, (h) *G. hoi*, (i) *G. macrocarpum*, (j & k) *G. microcarpum*, (l) *G. coronatum*, (m) *G. verruculosum*, (n) *G. viscosum*, (o) *Scutellospora heterogama*, (p) *S. scutata*. Scale bars = 40  $\mu\text{m}$ .

Shannon–Wiener index of AMF diversity in trap cultures set up with MF derived inoculum ranged from 1.96 to 2.42 while in set up with TF derived inoculum, it ranged from 1.50 to 1.99. Simpson’s dominance index of AMF ranged from 0.10 to 0.16 in trap plants from MF derived inoculum, while it ranged from 0.15 to 0.24 in trap plants from TF derived inoculum. Sorenson’s coefficient of AMF species among the trap plants of MT derived inoculum ranged from 0.21 to 0.44 whereas among the trap plants of TF derived inoculum, it ranged from 0.31 to 0.40. The value of evenness of AMF species ranged from 0.94 to 0.96 in trap plants from MF derived inoculum while it ranged from 0.88 to 0.93 in trap plants from TF derived inoculum.

## Discussion

*Flemingia vestita* roots were simultaneously colonized by AMF and DSE throughout the plant growth. This type of dual colonization by different root-associated fungi reflects

a dynamic nature of the root-colonizing fungal community. DSE colonization was comparatively lesser than that of AMF colonization. Kohn and Stasovski (1990) reported that DSE are found extensively in cold, nutrient-stressed environments where AMF do not proliferate. Thus, the paucity of DSE in this study is due to its more prevailing condition in extreme environments. AMF colonization in roots changes across different seasons and phenological stages of plants (Schalamuk et al. 2004; Singh et al. 2008). The percent colonization was lowest during the initial stage of plant development that increases gradually and it was highest during the flowering season of *F. vestita*, i.e. September in both natural and cultivated sites.

Each AMF species and its colonization rate could be affected in several ways by adopting different agricultural management practices. Even though AMF colonization rate did not differ much between the two sites, lower colonization in cultivated site could be due to the use of agricultural machineries and conventional tillage which might have reduced the hyphal network in the soil. This

Table 3. Isolated AMF species with their relative abundance (RA) and isolation frequency (IF) from the rhizosphere soil of *F. vestita*.

Sl. No.	AMF species	RA (%)		IF (%)
		MF	TF	
1.	<i>Acaulospora alpina</i> Oehl, Sykorova & Sieverd	0.55	1.35	100
2.	<i>A. capsiculata</i> Blaszk.	1.10	1.79	100
3.	<i>A. delicata</i> Walker, Pfeiffer & Bloss	4.67	6.73	100
4.	<i>A. denticulata</i> Sieverding & Toro	0.82	3.59	100
5.	<i>A. foveata</i> Trappe & Janos	0.27	0.90	100
6.	<i>A. koskei</i> Blaszk.	2.20	1.35	100
7.	<i>A. lacunosa</i> Morton	0.27	–	50
8.	<i>A. mellea</i> Spain & Schenck	3.02	2.24	100
9.	<i>A. morrowae</i> Spain & Schenck	3.57	6.73	100
10.	<i>A. rehmii</i> Sieverding & Toro	–	3.59	50
11.	<i>A. scrobiculata</i> Trappe	3.85	18.39	100
12.	<i>A. spinosa</i> Walker & Trappe	–	1.35	50
13.	<i>Ambispora brasiliensis</i> Goto, Maia & Oehl.	–	3.14	50
14.	<i>Gigaspora albida</i> Schenck & Smith	0.55	0.90	100
15.	<i>Gi. decipiens</i> Hall & Abbott	–	0.90	50
16.	<i>Gi. rosea</i> Nicolson & Schenck	2.20	1.79	100
17.	<i>Glomus aggregatum</i> Schenck & Smith	3.85	2.24	100
18.	<i>G. ambisporum</i> Smith & Schenck	3.30	–	50
19.	<i>G. aurantium</i> Blaszk., Blanke, Renker & Buscot	0.27	0.90	100
20.	<i>G. badium</i> sp. nov. Oehl, Redecker & Sieverd.	2.75	0.45	100
21.	<i>G. caledonium</i> Nicolson & Gerdemann	1.65	–	50
22.	<i>G. claroideum</i> (Schenck & Smith emend. Walker & Vestberg)	–	0.45	50
23.	<i>G. clavisorum</i> (Trappe) Almeida & Schenck	1.92	–	50
24.	<i>G. convolutes</i> Gerdemann & Trappe	1.37	–	50
25.	<i>G. coronatum</i> Giovann.	1.92	0.45	100
26.	<i>G. corymbiforme</i> Blaszkowski	3.02	0.90	100
27.	<i>G. eburneum</i> Kenn., Stutz & Morton	0.82	4.93	100
28.	<i>G. etunicatum</i> Becker & Gerdemann	3.30	0.90	100
29.	<i>G. fasciculatum</i> (Thaxter) Gerdemann & Trappe	4.40	2.24	100
30.	<i>G. fistulosum</i> Skuo and Jakobsen	1.10	1.79	100
31.	<i>G. fuegianum</i> (Spegazzini) Trappe & Gerdemann	2.20	–	50
32.	<i>G. geosporum</i> (Nicol. & Gerd.) Walker	4.67	0.90	100
33.	<i>G. glomerulatum</i> Sieverding	2.47	–	50
34.	<i>G. heterosporum</i> Smith & Schenck	–	3.59	50
35.	<i>G. hoi</i> Berch & Trappe	0.55	0.45	100
36.	<i>G. intraradices</i> Schenck & Smith	4.67	2.69	100
37.	<i>G. lamellosum</i> Dalpe, Koske & Tews	0.27	–	50
38.	<i>G. luteum</i> Kenn., Stutz & Morton	4.67	1.79	100
39.	<i>G. macrocarpum</i> Tul. & Tul.	3.30	1.35	100
40.	<i>G. manihotis</i> Howeler, Sieverding & Schenck	0.82	–	50
41.	<i>G. melanosporus</i> Gerd. & Trappe	–	0.45	50
42.	<i>G. microaggregatum</i> Koske, Gemma & Olexia	0.27	–	50
43.	<i>G. microcarpum</i> Tul. & Tul.	1.65	0.90	100
44.	<i>G. mosseae</i> (Nicol. & Gerd.) Gerdemann & Trappe	1.10	1.35	100
45.	<i>G. rubiforme</i> Gerdemann & Trappe	3.02	0.90	100
46.	<i>G. sinuosum</i> (Gerd. & Bakshi) Almeida & Schenck	1.65	1.35	100
47.	<i>G. tenebrosus</i> (Thaxter) Berch	1.10	–	50
48.	<i>G. tortuosum</i> Schenck & Smith	0.27	2.24	100
49.	<i>G. verruculosum</i> Blaszkowski & Tadych	3.57	1.79	100
50.	<i>G. versiforme</i> (Karsten) Berch	1.65	0.90	100
51.	<i>G. viscosum</i> Nicolson	–	0.45	50
52.	<i>Pacispora robigina</i> Oehl & Sieverd.	–	1.79	50
53.	<i>Scutellospora calospora</i> Walker & Sanders	1.37	–	50
54.	<i>S. cerradensis</i> Spain & Miranda	0.82	2.24	100
55.	<i>S. coralloidea</i> Koske and Walker	–	0.45	50
56.	<i>S. fulgida</i> Koske & Walker	0.27	–	50
57.	<i>S. heterogama</i> (Nicol. & Gerd.) Walker & Sanders	2.20	2.69	100
58.	<i>S. pellucida</i> (Nicol. & Schenck) Walker & Sanders	1.65	0.90	100
59.	<i>S. pernambucana</i> Oehl, Silva, Freitas, Maia	0.27	–	50
60.	<i>S. rubra</i> Stutzmer & Morton	0.55	–	50
61.	<i>S. scutata</i> Walker et Diederichs	2.20	0.90	100

Note: '–' indicates absence of species.

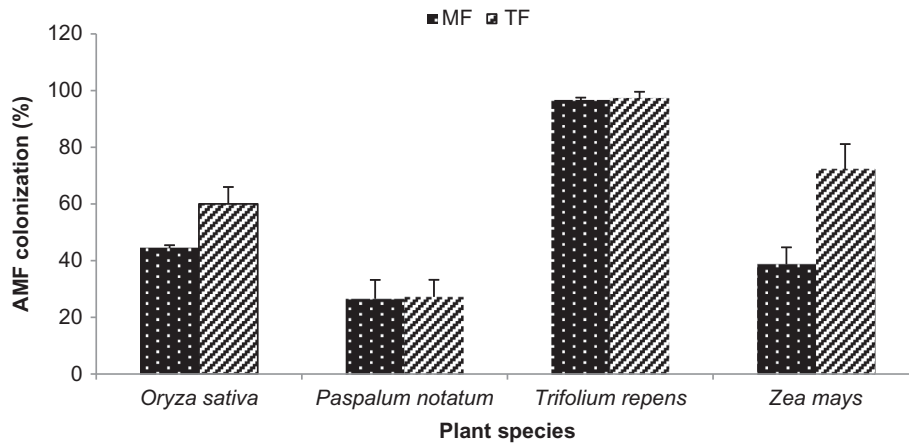


Figure 6. AMF colonization in four trap plants set up with natural (MF) and cultivated site (TF) derived inoculum.

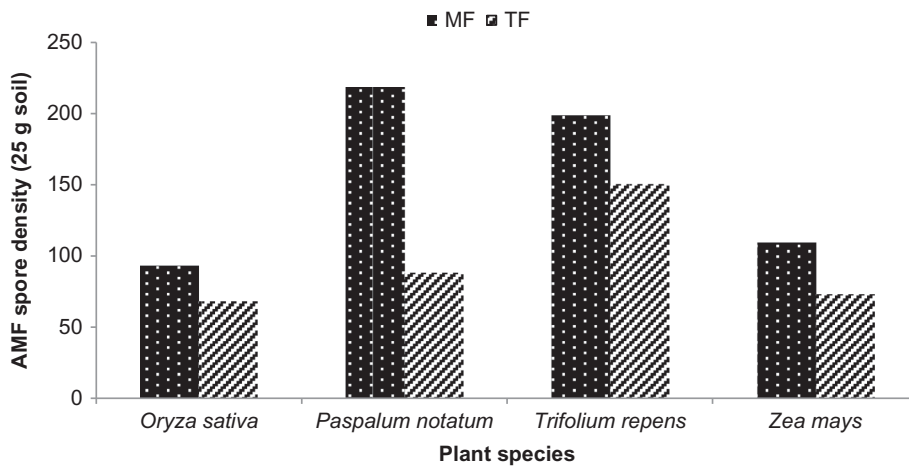


Figure 7. AMF spore density in four trap plants set up with natural (MF) and cultivated site (TF) derived inoculum.

finding is in accordance with Kabir et al. (1998) who reported more hyphal densities of AMF in no-tilled than in tilled conditions. Kurlle and Pflieger (1994) hypothesized that in intensive tillage systems, AMF species which sporulate more heavily would be favored.

Despite different soil management, our study revealed that *F. vestita* rhizosphere soils had high number of AMF spores as well as high AMF species diversity in both the sites. The high spore density obtained in this study does not, however, represent the actual numbers of infective propagules in the soil. This is because field collected spores might be a victim of parasitism, or it may not be viable, and some of it also present in clusters which would function as one or multiple infective propagule. A detailed investigation of AMF association with *F. vestita* in natural and cultivated sites helped in isolation and identification of 61 AMF species belonging to 6 genera. This is a fairly good number, as altogether, only 228 AMF species have been described worldwide till date ([www.amf-phylogeny.com](http://www.amf-phylogeny.com)). In natural site, we found that *Glomus* species were dominating, and

such kinds of observations have also been made by other workers in different ecosystems (Muthukumar & Udaiyan 2000). Brundrett et al. (1999) and Klironomos and Hart (2002) found differences among AMF genera in their life history characteristics and suggested that the mycelium is of major importance as propagule for some *Glomus* species. Taking these affirmations into consideration we hypothesize that the lack of hyphal network disruption in natural site could have favored *Glomus* species. Species of *Acaulospora* dominated in cultivated site. A similar finding was reported from rhizosphere soil of crop farm in northern Thailand (Nandakwang et al. 2008). Plant cultivation has significant influence on the sporulation of non-*Glomus* AMF species (Castillo et al. 2006), and thus it reflects the relative abundance and the dominant characteristics of *Acaulospora* species under cultivated site.

Occurrence of other AMF genera was lower in the present study. Biermann and Linderman (1983) suggested that Gigasporaceae are capable of propagation only with viable spores or from an intact mycelium. Due to these,

Table 4. AMF species isolated from trap cultures (MF derived inoculum) with their relative abundance and isolation frequency (IF).

Sl. No.	AMF species	Relative abundance (%)				IF (%)
		<i>O.s</i>	<i>P.n</i>	<i>T.r</i>	<i>Z.m</i>	
1.	<i>Acaulospora capsiculata</i> Blaszk.	–	–	–	10.00	25
2.	<i>A. delicata</i> Walker, Pfeiffer & Bloss	20.00	10.00	6.67	5.00	100
3.	<i>A. koskei</i> Blaszk.	–	–	–	15.00	25
4.	<i>A. mellea</i> Spain & Schenck	6.67	3.33	–	–	50
5.	<i>A. scrobiculata</i> Trappe	–	10.00	6.67	–	50
6.	<i>Gigaspora rosea</i> Nicolson & Schenck	–	–	–	5.00	25
7.	<i>Glomus aggregatum</i> Schenck & Smith	20.00	–	–	–	25
8.	<i>G. badium</i> sp. nov. Oehl, Redecker & Sieverd.	–	–	13.33	–	25
9.	<i>G. clavispurum</i> (Trappe) Almeida & Schenck	6.67	–	–	5.00	50
10.	<i>G. etunicatum</i> Becker & Gerdemann	13.33	–	–	15.00	50
11.	<i>G. fasciculatum</i> (Thaxter) Gerdemann & Trappe	6.67	16.67	–	15.00	75
12.	<i>G. fuegianum</i> (Spegazzini) Trappe & Gerdemann	–	3.33	–	–	25
13.	<i>G. geosporum</i> (Nicol. & Gerd.) Walker	–	3.33	20.00	10.00	75
14.	<i>G. glomerulatum</i> Sieverding	–	3.33	–	–	25
15.	<i>G. intraradices</i> Schenck & Smith	–	6.67	13.33	–	50
16.	<i>G. luteum</i> Kenn., Stutz & Morton	20.00	6.67	–	–	50
17.	<i>G. macrocarpum</i> Tul. & Tul.	–	–	13.33	–	25
18.	<i>G. microcarpum</i> Tul. & Tul.	6.67	–	6.67	–	50
19.	<i>G. tortuosum</i> Schenck & Smith	–	10.00	–	–	25
20.	<i>G. verruculosum</i> Blaszkowski & Tadych	–	13.33	–	–	25
21.	<i>G. versiforme</i> (Karsten) Berch	–	–	13.33	–	25
22.	<i>Paraglomus brasilianum</i> Spain & J. Miranda	–	3.33	–	–	25
23.	<i>Scutellospora calospora</i> Walker & Sanders	–	10.00	–	5.00	50
24.	<i>S. fulgida</i> Koske & Walker	–	–	6.67	–	25
25.	<i>S. scutata</i> Walker et Diederichs	–	–	–	15.00	25

Note: *O.s* = *Oryza sativa*; *P.n* = *Paspalum notatum*; *T.r* = *Trifolium repens*; *Z.m* = *Zea mays*; ‘–’ indicates absence of species.

Table 5. AMF species isolated from trap cultures (TF derived inoculum) with their relative abundance and isolation frequency (IF).

Sl. No.	AMF species	Relative abundance (%)				IF (%)
		<i>O.s</i>	<i>P.n</i>	<i>T.r</i>	<i>Z.m</i>	
1.	<i>Acaulospora delicata</i> Walker, Pfeiffer & Bloss	–	6.25	–	11.76	50
2.	<i>A. morrowiae</i> Spain & Schenck	–	–	18.75	11.76	50
3.	<i>A. rehmi</i> Sieverding & Toro	–	–	25.00	17.65	50
4.	<i>Acaulospora scrobiculata</i> Trappe	20.00	31.25	18.75	23.53	100
5.	<i>Gigaspora margarita</i> Becker & Hall	–	–	–	5.88	25
6.	<i>Gi. rosea</i> Nicolson & Schenck	–	–	–	11.76	25
7.	<i>Glomus aggregatum</i> Schenck & Smith	–	6.25	12.50	–	50
8.	<i>G. badium</i> sp. nov. Oehl, Redecker & Sieverd.	10.00	–	–	–	25
9.	<i>G. caledonium</i> Nicolson & Gerdemann	–	–	6.25	–	25
10.	<i>G. eburneum</i> Kenn., Stutz & Morton	–	6.25	–	–	25
11.	<i>G. fasciculatum</i> (Thaxter) Gerdemann & Trappe	–	18.75	–	–	25
12.	<i>G. fistulosum</i> Skuo and Jakobsen	–	–	6.25	–	25
13.	<i>G. geosporum</i> (Nicol. & Gerd.) Walker	–	–	–	11.76	25
14.	<i>G. glomerulatum</i> Sieverding	–	6.25	6.25	–	50
15.	<i>G. intraradices</i> Schenck & Smith	30.00	–	–	–	25
16.	<i>G. luteum</i> Kenn., Stutz & Morton	30.00	25.00	–	5.88	75
17.	<i>G. rubiforme</i> Gerdemann & Trappe	10.00	–	–	–	25
18.	<i>G. viscosum</i> Nicolson	–	–	6.25	–	25

Note: *O.s* = *Oryza sativa*; *P.n* = *Paspalum notatum*; *T.r* = *Trifolium repens*; *Z.m* = *Zea mays*; ‘–’ indicates absence of species.

low occurrence of *Scutellospora* and *Gigaspora* were observed in our study. Furthermore, both *Scutellospora* and *Gigaspora* produced large spores and these require a longer period to develop as compared to the small-spore species (Hepper 1984). One species each of *Ambispora*

and *Pacispora* were also detected in our study. It appears that these species might be a poor competitor in colonizing plant roots, and thus, their rate of occurrence is less. Occurrence of only six genera may be related to their high competitive interaction and adaptability, thus allowing

them to establish better than the others, supporting the view of Singh et al. (2008). Individual AMF species compete for resources through a combination of strategies resulting in the maintenance of a diverse AMF community.

In trap cultures, AMF colonization was higher in plants with TF derived inoculum than those of MF derived inoculum. However, spore density was higher in plants with MF derived inoculum than those of TF derived inoculum. This indicates that spore density does not exactly reflect the AMF community that is actually colonizing the plant roots, and thus, variation in spore production could not be explained by mycorrhizal colonization level (Brundrett et al. 1999). Evaluation of AMF spores as well as colonization is therefore important to know the level of its association. In our study, among all trap plants, *T. repens* showed highest colonization rate and spore density indicating that it had the capability to develop a maximum interaction with MF and TF derived AMF inoculum.

Thirty three AMF species were recovered from trap cultures with four different host plants. Trap culture does not necessarily allow identification of all species present in the original field soil, because sporulation of the fungal community may be affected by the host plant through their effects upon propagule activation, hyphal development and sporulation (Siqueira et al. 1985; Bever et al. 1996) whereas in some cases it can promote the sporulation of cryptic AMF species that were not sporulating at the sampling time or field conditions (Stürmer 2004). It is noteworthy to mention that one additional AMF species was isolated from MF derived trap cultures and two additional AMF species from TF derived trap cultures. Jansa et al. (2002) and Oehl et al. (2004) reported a similar finding where high proportions of additional species appeared exclusively in the trap cultures and not in the original field soil. Occurrence of additional AMF species in the traps is a well documented phenomenon, justifying the use of trap cultures in addition to direct isolation of spores from the field soils for more complete AMF analysis. It explains that trap cultures might act as a filter allowing sporulation of only part of the indigenous AMF species which are aggressive enough to colonize and sporulate in a fast growing host under greenhouse conditions in short time span. Trap culture also produces healthy spores and can be used to establish monospecific cultures that can be used for inoculum conservation.

The role of AMF in natural and agricultural ecosystems is increasingly being recognized. The important observation of the present investigation is that *F. vestita* harbors a relatively high AMF community, supporting the view that the representatives of Fabaceae have a high mycorrhizal dependency (Duponnois et al. 2001). The present study also suggests that AMF diversity is higher in natural site as compared to the cultivated site of *F. vestita* as it could be affected by different agricultural management practices. Many plant species are in high demand for their

medicinal properties, for use as food crops, and for other various purposes. Considering the importance of AMF, its proper management has the potential to improve the quality and quantity of plant yields in natural and agricultural ecosystems.

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