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# Diversity and species composition of arbuscular mycorrhizal fungi in *Flemingia vestita* under shifting and continuous cropping system

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ABSTRACT

Diversity and species composition of arbuscular mycorrhizal fungi (AMF) was investigated in the rhizosphere soils of *Flemingia vestita* Benth. ex Baker (Fabaceae) under shifting plantation (SP) and continuous cropping plantation (CCP). AMF colonization, spore density, species richness and diversity were higher in SP when compared to that of CCP, except for dark septate endophyte (DSE) that was higher in CCP. A total of 68 AMF morphotypes (57 from SP and 53 from CCP) were identified comprising of six genera viz., *Acaulospora, Ambispora, Gigaspora, Glomus, Pacispora* and *Scutellospora*. *Glomus* species are dominant in SP whereas *Acaulospora* species in CCP. Our result suggested that shifting plantation which is characterized by slash-and-burn does not affect on AMF community while on the other hand continuous cropping system of plantation that involves intensive land-use, crop rotation and tillage affect the development of AMF.

Keywords: arbuscular mycorrhizal fungi, colonization, diversity, *Flemingia vestita*

Main components of the soil microbiota in most agro-ecosystems are the AMF **(**Oehl *et al*. 2003), an ancient group of fungi that establishes mutualistic symbiosis with a majority of plant species (Leake *et al*. 2004). It is an interaction where both partners benefited primarily from the exchange of nutrients i.e., mycorrhizal fungi gets a carbon substrates from plants and in turn the plants are provided with nutrients. They can improve plant establishment and survival, enhance plant nutrient uptake, reduce the negative effects of various biotic or abiotic stresses, and improve soil structure (Smith and Read, 2008). In addition to mycorrhizal associations, plants are also associated with 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 which co-colonized with AMF in some host plants (Jumpponen & Trappe, 1998).

*Flemingia vestita* Benth. ex Baker is an indigenous plant of Meghalaya, Northeast India. It is a weak climber that 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 use as curative 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). *F. vestita* is commonly planted in shifting as well as continuous cropping system. The slash-and-burn or shifting system of cultivation in Northeast India is a unique feature of the region, which is a predominant practice in the majority of tropical hilly tracts (Karthik *et al*. 2009).

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In Meghalaya, plants have been investigated for their botanical aspects and medicinal values. However, mycorrhizal investigations have been rarely conducted and data on the occurrence and diversity of AMF and DSE association in *F. vestita* are virtually, non-existent. Therefore, in view of this, the present work was carried out to understand the association of AMF in *Flemingia vestita* under slash-and-burn and continuous cropping system with a possibility of AMF biotechnological application in improving the cultivation of the plant.

## Materials and methods

### Site description and field sampling

Sampling of *F. vestita* were done at monthly intervals for one crop cycle (March to December, 2009) from two different sites, shifting system of plantation located at Mawprem (25°32´33.2´´N, 091°45´05.5´´E) and continuous cropping system of plantation located at Thangsning (25°28´57.1´´ N, 091°55´00.9´´E), East Khasi Hills, Meghalaya, Northeast India. In SP, slash-and-burn was done in the month of February-March, after which *F. vestita* was planted without tillage. After every crop cycle, the land was left fallowed for at least one year. In case of CCP, intensive agricultural practice was done, where potato was used as a crop rotational plant with *F. vestita*. During the sampling periods, root and soil samples (five replicates per site) were collected, kept in a sterilized plastic bag 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 roots were then cleared in 10% (w/v) KOH by heating at 90ºC for 1 to 2 hr, depending on the degree of lignifications of the roots. It is then washed and stained with Trypan blue (Phillips & Hayman, 1970). The stained root samples were mounted on microscope slides and examined for AM fungal structure under light microscope. The colonization of root length with arbuscules (RLA), vesicles (RLV), hyphae (RLH) and dark septate endophytes (RLDSE) per sample were quantified by the magnified intersections method of McGonigle *et al*. (1990) and expressed it in percentage.

### AMF spore isolation, enumeration and identification

AMF spores were extracted by wet-sieving and decanting method of Gerdemann and Nicolson (1963). Suspension of 25g soil sample in water was decanted through a series of 710 to 37µm sieves. The residues in the sieves were washed into beaker and the sievates were dispersed in water and filtered through filter papers and spores were counted using a dissection microscope at 40X magnification. Sporocarps and spore clusters were considered as one unit. AMF spores were picked up using a needle and mounted in PVLG with Meltzer's reagent on a glass slide for identification based on morphological descriptions published by International Culture Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu), AMF phylogeny (www.amf-phylogeny.com), Oehl and Sieverding (2004) and Goto *et al*. (2008).

### Analysis of soil physicochemical properties

Soil temperature was recorded in the field at the time of sampling. Soil texture was determined using the bouyoucos method of Allen *et al*. (1974). Soil moisture was determined by drying 10g fresh soil at 105ºC for 24 hr in a hot-air oven. Soil pH was determined using a digital pH meter. Organic carbon (colorimetric method) and available nitrogen (Kjaldahl method) were determined following the methods outline in Anderson and Ingram (1993). Available phosphorus was determined by Molybdenum blue method (Allen *et al*. 1974). Potassium was determined using flame photometer (Jackson, 1973).

### Statistical analysis

Spore density and species richness were expressed as number of AM fungal spores and numbers of AM fungal species in 25g soil samples. Relative abundance, isolation frequency, Shannon-Wiener index of diversity (H') and Simpson's index of dominance (D) were calculated (Dandan and Zhiwei, 2007). Data were statistically analyzed using one-way ANOVA. Pearson correlation coefficient was employed to determine the relationships between mycorrhizal structural colonization and soil physico-chemical properties. Standard errors of means were calculated.

## Result

The soil physico-chemical properties of the study sites are presented in Table 1. The soils were sandy and slightly acidic in nature. Soil organic carbon content was similar in both the sites; available nitrogen and phosphorus were higher in CCP than in

SP. However, exchangeable potassium was higher in SP.

**Table 1.** Soil physico-chemical properties of *F. vestita* at two plantation sites (Mean ± SE)

| Soil physico-chemical properties | SP | CCP |
|---|---|---|
| pH | 5.89 ± 0.08 | 5.66 ± 0.11 |
| Moisture content (%) | 26.68 ± 3.20 | 30.61 ± 1.87 |
| Temperature (°C) | 18.02 ± 1.68 | 17.68 ± 1.22 |
| Organic carbon (%) | 2.16 ± 0.03 | 2.16 ± 0.03 |
| Available nitrogen (%) | 0.26 ± 0.02 | 0.32 ± 0.01 |
| Available phosphorus (%) | 0.15 ± 0.01 | 0.47 ± 0.02 |
| Exchangeable potassium (%) | 0.13 ± 0.01 | 0.07 ± 0.00 |

Roots of *F. vestita* showed high degree of AMF colonization in both plantation sites (Table 2). AMF colonization in the form of arbuscules, vesicles and hyphae, and occasionally, intraradical spores were observed (Figure 1). ANOVA shows no significant variation ($P < 0.05$) between AMF colonization in SP and CCP. No significant variation ($P < 0.05$) was observed between DSE colonization of SP and CCP. AMF colonization was significantly higher ($P < 0.05$) than DSE colonization in both the study sites. Mycorrhizal structural colonization shows a significant positive correlation ($P < 0.05$) with pH, soil temperature and N in SP. No such correlation was seen in CCP.

**Table 2.** Mean mycorrhizal colonization (percentage) in roots of *F. vestita*

| | Mycorrhizal structure | | | | |
|---|---|---|---|---|---|
| Site | RLA | RLV | RLH | Total AMF | RLDSE |
| SP | 30.11 ± 6.39 | 6.12 ± 1.74 | 43.77 ± 7.66 | 79.99 ± 8.43 | 1.09 ± 0.74 |
| CCP | 35.21 ± 10.89 | 0.83 ± 0.18 | 29.51 ± 6.58 | 65.55 ± 8.04 | 1.70 ± 0.43 |

Note: RLA= root length with arbuscules, RLV= root length with vesicles, RLH= root length with hyphae and RLDSE= root length with dark septate endophytes; Mean ± SE

High AMF spore numbers were observed in rhizosphere soil of *F. vestita* in both plantations sites. A mean spore density of 1275 and 1261 in 25g soils recorded in SP and CCP respectively. Morphotypes of five AMF genera viz., (*Acaulospora*, *Ambispora*, *Gigaspora*, *Glomus* and *Scutellospora*) and six viz., (*Acaulospora*, *Ambispora*, *Gigaspora*, *Glomus*, *Pacispora* and *Scutellospora*) were isolated from SP and CCP respectively. A total of 68 AMF species; 57 from SP and 53 from CCP were distinguished based on morphological characteristics (Table 3). Out of these, 42 species were common to both, while, 16 species were restricted to SP and 11 to CCP. A list of AMF morphotypes with their relative abundance and isolation frequency are presented in Table 4 and some of the isolated morphotypes are depicted in Figure 2. *Glomus aggregatum*, *G. etunicatum*, *G. luteum* and *G. verruculosum* were dominant in SP, whereas *Acaulospora delicata*, *A. morrowiae* and *A. scrobiculata* were dominant in CCP. Shannon and Simpson index was evaluated for the diversity of AMF in *F. vestita* plantation. H' value was higher in SP (2.68) than in CCP (2.37). D value was lower at SP (0.08) than at CCP (0.11).

## Discussion

### AMF and DSE colonization

This is the first report of survey of mycorrhizal and DSE fungi in *F. vestita*. The plant is co-colonized by AMF and DSE, where AMF colonization was comparatively higher to DSE. Kohn & Stasovski (1990) reported that DSE are found extensively in cold, nutrient-stressed environments where AM fungi do not proliferate. Thus, the paucity of DSE is due to its more prevailing condition in extreme environments.

*F. vestita* were highly colonized by AMF throughout the crop cycle, where colonization percentage was more in SP than in CCP. Likewise, spore density and species richness were also higher in SP. Our result indicates that shifting plantation (slash-and-burn) does not affect much on AMF communities which was also reported by Aguilar-Fernández *et al*. (2009) that fire did not damage mycorrhizal fungi directly, likely due to low heat conductivity of soil. Bellgard *et al*. (1994) suggested that moderate fires had no significant impact on the infectivity of AMF. However, Adeniyi (2010) suggested that only those species that are resistance to burning of soil survived the effects of burning. Dodd (2000) suggested that no-tillage conditions stimulate mycorrhizal activity in soil. However, crop rotation and tillage influence the composition and diversity of AMF communities as well as spore and mycelium densities in temperate and tropical agro-ecosystems (Oehl *et al*. 2003), indicating that agricultural practice is an important factor affecting AMF community and diversity.

No correlation between AMF colonization and P which is in agreement with Singh *et al*. (2003). Generally, AMF colonization potential is higher in soils where the P concentration is low (Galvez *et al*. 2001). Significant positive correlation was observed between mycorrhizal structural colonization and pH. Wang (1993) suggested that pH is an important factor influencing AM fungal species composition.

**Table 3.** AMF species composition and species richness (SR) of *F. vestita*.

| | AMF species | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Site | *Acaulospora* | *Ambispora* | *Gigaspora* | *Glomus* | *Pacispora* | *Scutellospora* | Unidentified | SR |
| SP | 11 | 1 | 2 | 34 | — | 8 | 1 | 57 |
| CCP | 12 | 1 | 4 | 26 | 1 | 8 | 1 | 53 |

"—" indicates the absence of a species.

**Table 4.** Isolated AMF species with relative abundance and isolation frequency (IF) from rhizosphere soil of *F. vestita*

| | Relative abundance (%) | | |
|---|---|---|---|
| AMF species | SP | CCP | IF (%) |
| *Acaulospora alpina* Oehl, Sykorova & Sieverd. | 0.27 | 1.63 | 100 |
| *Acaulospora capsiculata* Blaszk. | 1.10 | 1.63 | 100 |
| *Acaulospora delicata* Walker, Pfeiffer & Bloss | 1.92 | 11.84 | 100 |
| *Acaulospora denticulata* Sieverding & Toro | 0.82 | 2.45 | 100 |
| *Acaulospora dilatata* Morton | — | 0.41 | 50 |
| *Acaulospora foveata* Trappe & Janos | 0.55 | — | 50 |
| *Acaulospora koskei* Blaszk. | 2.74 | 1.22 | 100 |
| *Acaulospora lacunosa* Morton | 0.27 | 0.82 | 100 |
| *Acaulospora mellea* Spain & Schenck | 1.64 | 1.22 | 100 |
| *Acaulospora morrowiae* Spain & Schenck | 3.29 | 6.12 | 100 |
| *Acaulospora rehmii* Sieverding & Toro | — | 4.08 | 50 |
| *Acaulospora scrobiculata* Trappe | 1.92 | 17.96 | 100 |
| *Acaulospora spinosa* Walker & Trappe | 0.27 | 0.41 | 100 |
| *Ambispora brasiliensis* Goto, Maia & Oehl | 0.27 | 0.41 | 100 |
| *Gigaspora albida* Schenck & Smith | 0.27 | 0.82 | 100 |
| *Gigaspora decipiens* Hall & Abbott | — | 1.22 | 50 |
| *Gigaspora margarita* Becker & Hall | — | 0.41 | 50 |
| *Gigaspora rosea* Nicolson & Schenck | 0.82 | 0.82 | 100 |
| *Glomus aggregatum* Schenck & Smith | 7.40 | 1.63 | 100 |
| *Glomus ambisporum* Smith & Schenck | 1.92 | 0.82 | 100 |
| *Glomus aurantium* Błaszk., Blanke,Renker & Buscot | 0.55 | 0.41 | 100 |
| *Glomus badium* sp. nov. Oehl, Redecker & Sieverd. | 3.84 | 0.41 | 100 |
| *Glomus caledonium* Nicolson & Gerdemann | 1.37 | — | 50 |
| *Glomus claroideum* (Schenck & Smith emend. Walker & Vestberg) | — | 0.41 | 50 |
| *Glomus clavisporum* (Trappe) Almeida & Schenck | 0.82 | — | 50 |
| *Glomus convolutum* Gerdemann & Trappe | 0.82 | — | 50 |
| *Glomus coronatum* Giovann. | 1.10 | 0.82 | 100 |
| *Glomus corymbiforme* Blaszkowski | 1.64 | 2.86 | 100 |
| *Glomus eburneum* Kenn., Stutz & Morton | 1.37 | 1.63 | 100 |
| *Glomus etunicatum* Becker & Gerdemann | 6.03 | 1.63 | 100 |
| *Glomus fasciculatum* (Thaxter) Gerdemann & Trappe | 4.38 | 1.63 | 100 |
| *Glomus fistulosum* Skuo and Jakobsen | 1.37 | 1.22 | 100 |
| *Glomus fuegianum* (Spegazzini) Trappe & Gerdemann | 2.47 | — | 50 |
| *Glomus geosporum* (Nicol. & Gerd.) Walker | 5.21 | 1.22 | 100 |
| *Glomus gibbosum* Błaszk. | — | 0.41 | 50 |
| *Glomus glomerulatum* Sieverding | 1.37 | — | 50 |
| *Glomus heterosporum* Smith & Schenck | — | 1.22 | 50 |
| *Glomus hoi* Berch & Trappe | 0.27 | 0.82 | 100 |
| *Glomus intraradices* Schenck & Smith | 4.93 | 2.45 | 100 |
| *Glomus lamellosum* Dalpe, Koske & Tews | 0.55 | — | 50 |
| *Glomus luteum* Kenn., Stutz & Morton | 9.04 | 3.27 | 100 |
| *Glomus macrocarpum* Tul. & Tul. | 4.93 | 0.82 | 100 |
| *Glomus manihotis* Howeler, Sieverding & Schenck | 0.27 | — | 50 |
| *Glomus melanosporus* Gerd. & Trappe | — | 0.41 | 50 |
| *Glomus microaggregatum* Koske, Gemma & Olexia | 0.55 | — | 50 |
| *Glomus microcarpum* Tul. & Tul. | 1.10 | 1.63 | 100 |
| *Glomus minutum* Blaszkowski, Tadych et Madej, sp. Nov. | 0.27 | — | 50 |
| *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe | 1.64 | — | 50 |
| *Glomus rubiforme* Gerdemann & Trappe | 2.47 | 2.04 | 100 |
| *Glomus sinuosum* (Gerd. & Bakshi) Almeida & Schenck | 0.27 | 1.22 | 100 |
| *Glomus spinosum* Hu | 0.27 | — | 50 |
| *Glomus tenebrosum* (Thaxter) Berch | 0.27 | — | 50 |
| *Glomus tortuosum* Schenck & Smith | 0.27 | 0.41 | 100 |
| *Glomus verruculosum* Blaszkowski & Tadych | 7.95 | 1.22 | 100 |
| *Glomus versiforme* (Karsten) Berch | 1.37 | 0.41 | 100 |
| *Glomus viscosum* Nicolson | 0.27 | 1.63 | 100 |
| *Pacispora robigina* Oehl & Sieverd. | — | 2.04 | 50 |
| *Scutellospora calospora* Walker & Sanders | 0.55 | — | 50 |
| *Scutellospora cerradensis* Spain & Miranda | 0.27 | 2.86 | 100 |
| *Scutellospora coralloidea* Koske and Walker | — | 1.22 | 50 |
| *Scutellospora erythropa* (Koske & Walker) Walker & Sanders | — | 0.41 | 50 |
| *Scutellospora fulgida* Koske & Walker | 0.27 | — | 50 |
| *Scutellospora heterogama* (Nicolson & Gerd.) Walker & Sanders | 1.64 | 0.41 | 100 |
| *Scutellospora pellucida* (Nicolson & Schenck) Walker & Sanders | 0.55 | 3.67 | 100 |
| *Scutellospora pernambucana* Oehl, Silva, Freitas & Maia | 0.27 | 0.41 | 100 |
| *Scutellospora rubra* Stu$rmer & Morton | 0.27 | 0.41 | 100 |
| *Scutellospora scutata* Walker et Diederichs | 1.10 | 2.04 | 100 |
| Unidentified species | 0.55 | 0.41 | 100 |

'—` indicates the absence of a species

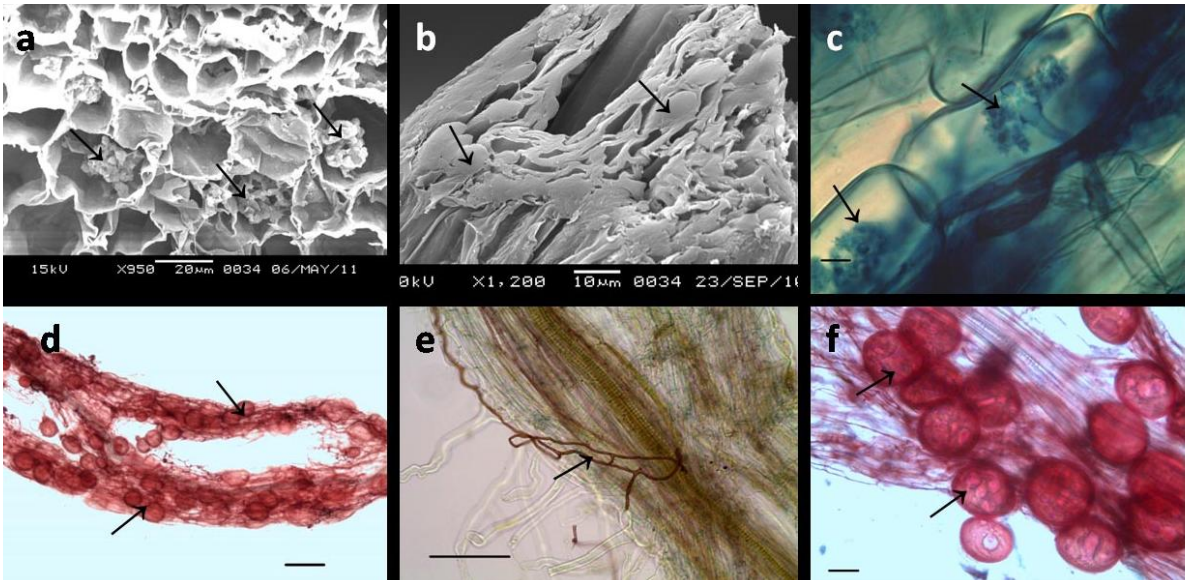

**Figure 1.** Mycorrhizal and DSE colonization in roots of *F. vestita*: (a & b) Scanning electron micrograph of arbuscules and vesicles, (c-f) arbuscules, vesicles, DSE and intraradical spores in light microscope.

Apparently, soil pH and P do influence certain fungal species and their ability to colonize roots.

**AMF spore density and species composition**

The mean spore density of AMF was higher than that reported by others in agricultural soil systems (Akond *et al*. 2008; Li *et al*. 2007). However, Kurle and Pfleger (1996) reported a high number of AMF spores (up to 300 $g^{-1}$ soil) in intensively used agricultural fields in the United States.

In the present study, *Glomus* were the most common species followed by *Acaulospora*, *Scutellospora* and *Gigaspora* in both plantation sites, which is consistent with the study of Li *et al*. (2007) who recorded *Glomus* and *Acaulospora* as most frequently occurred AMF species, followed by *Gigaspora* and *Scutellospora* in a hot and arid ecosystem. Charoenpakdee *et al*. (2010) also reported that *Glomus* and *Acaulospora* occurred most frequently in *Jatropha curcas* plantation. Species of *Glomus* were dominant in shifting system and *Acaulospora* species in continuous system of plantation. Castillo *et al*. (2006) reported that in agricultural soils, *Glomus* is the most prevailing AM species which is similar to our finding. *Acaulospora* species were also frequently encountered in our study which is known to favor more acid soils (Abbott and Robson, 1991; Stürmer, 1998). The relative abundance and the dominant characteristics of *Acaulospora* species under continuous cropping is interesting, and it agrees with the finding of Castillo *et al*. (2006) that tillage had a significant influence on the sporulation of non- *Glomus* AMF species. Tchabi *et al*. (2009) reported that *G. etunicatum* and *A. scrobiculata* which are two dominant species isolated in our study tend to be a dominant species in soil samples from the yam field sites.

Biermann & Linderman (1983) suggested that the *Scutellospora* species (Gigasporaceae) are capable of propagation only with viable spores or from an intact mycelium whereas Glomeraceae are capable of colonizing even with fragments of mycelium. Moreover, they are much more frequently associated with wild plants than with field crops (Gai *et al*., 2006). Due to these, low occurrence of *Scutellospora* and *Gigaspora* were observed in our study. 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. Individual AMF species compete for resources through a combination of strategies resulting in the maintenance of a diverse AMF community (Koske, 1987) and thus, AMF species having high competitive interaction and adaptability likely dominates in the rhizosphere of *F. vestita*.

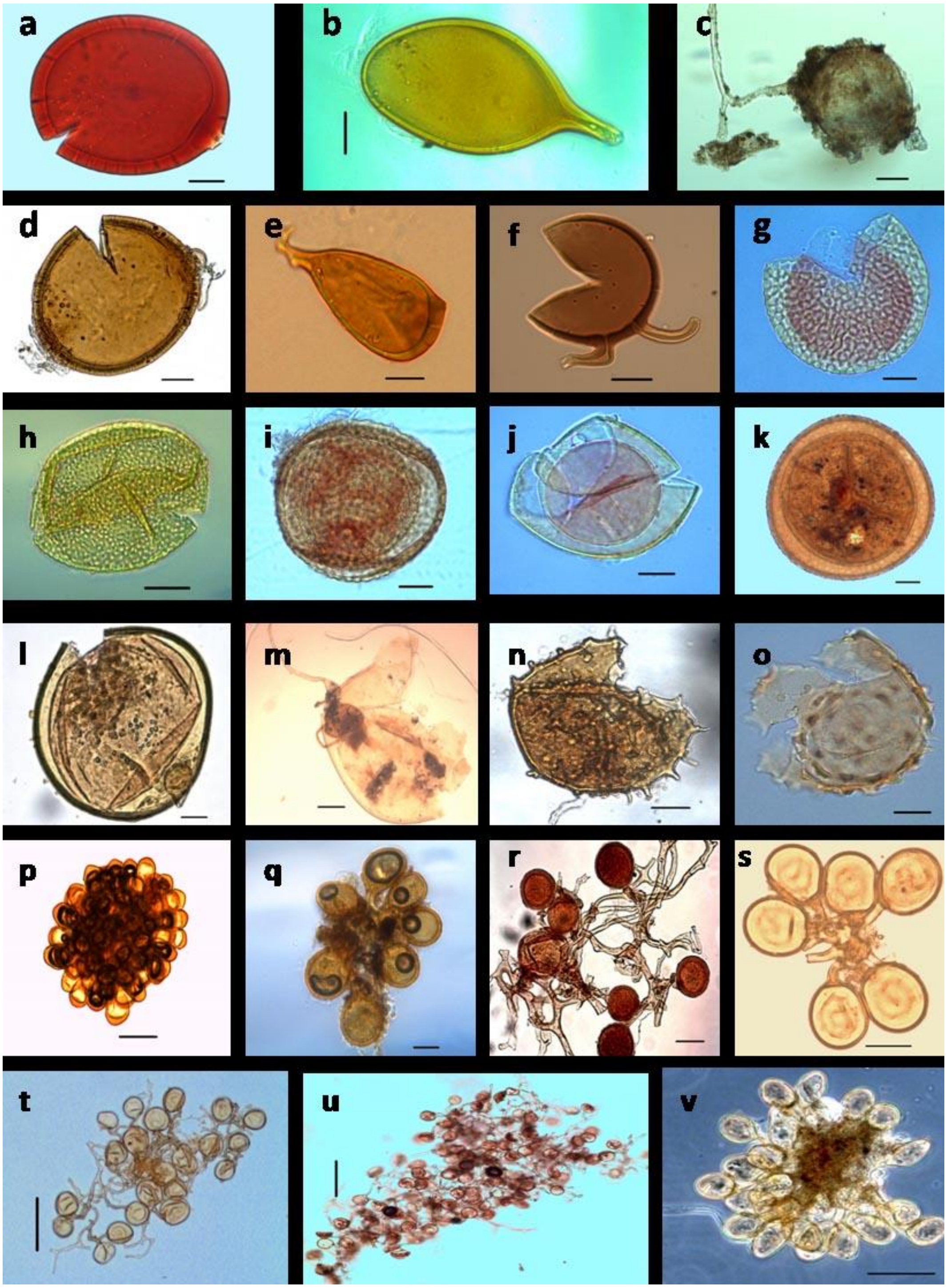

**Figure 2** AMF spores and sporocarps isolated from rhizosphere soil of *F. vestita*: (a-f) *Glomus* species- *G. badium*, *G. indraradices*, *G.tortuosum*, *G. indraradices*, *G. clavisporum and G. glomerulatum*, (g-k) *Acaulospora* species- *A. scrobiculata*, *A. denticulata*, *A. delicata* and *A. foveata*, (l-m) *Scutellospora* species, (n-o) Unidentified species and (p-v) Sporocarpic species. Scale bar (a-o) = 50µm, p =200 µm, (q-r) = 100µm, (s-u) =100 µm, v =60 µm.

## AMF species richness and diversity

A total of 68 species of Glomeromycota were obtained from the two plantation sites of *F. vestita*, which was greater than those of different agricultural land in temperate climatic zone (61 AMF species) of Central Europe (Oehl *et al*. 2010) and Sichuan Province (30 AMF species) of mainland China (Wang *et al*. 2008). Youpensuk *et al*. (2004) reported 29 AMF species associated with *Macaranga denticulata* in upland shifting agriculture in the North of Thailand. The higher number of AMF species in *F. vestita* rhizosphere soil is likely because we sampled for one complete crop cycle, allowing the fast as well as slow sporulating AMF species to develop. In our study, higher diversity of AMF was obtained in SP than in CCP. The hyphal network which may act as propagule for some AMF species remains undisturbed and favored its sporulation in SP.

## Conclusion

Nowadays, the role of AMF in agricultural ecosystems is increasingly being recognized. The present study indicates 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). In general, AMF communities are reduced by soil disturbance resulting from agricultural activities. Considering the importance of AMF, its proper management has the potential to improve the quality and quantity of crop yields in agricultural systems. Therefore, appropriate management of ecosystem services rendered by AMF will have an impact on the net gain for human society.

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