Diversity of arbuscular mycorrhizal fungi in pine forest of Meghalaya, North East India

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Species composition and diversity of arbuscular mycorrhizal fungi (AMF) were investigated in three common and widely distributed plant species (*Crotalaria anagyroides*, *Eupatorium adenophorum* and *Hedychium coronarium*) from subtropical pine forest of Meghalaya, North East India. AMF colonization ranged from 66–71%. Colonization of dark septate endophyte was also evaluated and ranged from 0.17–0.85%. AMF spore densities with a range of 319 to 661 in 25 g^{-1} rhizosphere soils were detected. Based on morphological characteristics, 23 AMF species belonging to two genera (*Acaulospora* and *Glomus*) were isolated and identified, plus one unidentified AMF species. *E. adenophorum* harbours the highest number of AMF species (15), followed by *C. anagyroides* (14) and *H. coronarium* (11). Species of *Acaulospora* were dominant in all three plant species. *Acaulospora koskei*, *A. laevis*, *A. mellea*, *A. morrowiae* and *Glomus geosporum* were isolated from all plant species. Diversity indices showed little difference between the three plant species. It is concluded that the three plant species from sub-tropical pine forest are well-colonized by AMF and host many AMF species.

Key words – colonization – diversity – species richness – spore density

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Introduction

Arbuscular mycorrhizal fungi (AMF) are soil microorganisms known to establish a universally distributed mutual symbiosis with most higher plants (Ferrol et al. 2004) and, in natural ecosystems, plant roots are almost entirely mycorrhizal (Muthukumar et al. 2004). AMF colonize root tissues biotrophically and form an extensive network of extra-radical mycelium, providing a direct physical link between soil and plant roots (Smith & Read 1997). AM fungi are known to benefit plant establishment by increasing resistance to environmental stresses, enhancing plant nutrient acquisition, water relations, disease resistance and improving soil quality (Smith & Read 2008).

Mycorrhizal fungi are an essential component for ecosystem functioning, particularly due to their effect on plant diversity and productivity (van der Heijden et al. 1998). Naturally occurring plant-AMF combinations may indicate functional relationships, and different plant species are colonized by different AMF communities. The identity and diversity of AMF have a great impact on plant community structure (Scheublin et al. 2004) and, therefore, it is important to investigate AMF community composition in different plant species.

Plant roots are also colonized by numerous species of fungi as symptomless endophytes or biotrophs (Parbery 1996). Among these fungi, dark septate endophyte (DSE) that belongs to a ubiquitous group of ascomycetous

fungi is well recognized and forms a mutualistic association similar to mycorrhizas. They are observed most frequently growing inter- and intracellularly within the cortex, epidermis and on root surfaces (Barrow 2003). Several studies reported that some host plants of AMF were co-colonized by DSE (Jumpponen & Trappe 1998). DSE can be easily distinguished from AM fungal hyphae by their dark red-brown to dark brown colour, thicker lateral wall and frequent septa (Kai & Zhiwei 2006).

The aim of the present work was to analyse the species composition and diversity of AM fungal species in three common and widely distributed plant species of pine forest: *Crotalaria anagyroides* (Fabaceae), *Eupatorium adenophorum* (Asteraceae) and *Hedychium coronarium* (Zingiberaceae) in Meghalaya, North East India. AMF and DSE colonization as well as the physico-chemical parameters of rhizosphere soils were also estimated.

Materials and methods

Site description and field sampling

Sampling was done from the subtropical pine forest of North Eastern Hill University campus, Meghalaya, India, located at 25° 36'40"N, 091°53'57.4"E, elevation 1424 m a.s.l. The roots and rhizosphere soil of three different plant species (five replicates for each plants species) were collected and merged into one composite sample. The composite root samples were kept in a sterilized plastic bag and transported to the laboratory for analysis.

Analysis of soil physicochemical properties

Soil moisture was determined by drying 10 g fresh soil at 105°C for 24 hours in hot-air oven. Soil pH was determined using a digital pH meter. Organic carbon was analysed by colorimetric method (Anderson & Ingram 1993) and available phosphorus by molybdenum blue method (Allen et al. 1974). Soil texture was determined using the bouyoucos method (Allen et al. 1974).

Analysis of AMF and DSE colonization

Roots were washed thoroughly in tap water and cut into approximately 1 cm long segments. The roots were then cleared in 10% (w/v) KOH by heating at 90°C for 1 to 2 hours, depending on the degree of lignifications of the roots. They were then washed and stained with stamp pad ink (Das & Kayang 2008). The stained root samples were mounted on microscope slides and examined for AM fungal structures under a 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 (McGonigle et al. 1990) and expressed as a percentage.

AMF spore isolation, enumeration and identification

AM fungal spores were extracted from 25 g subsamples from each soil sample by wet-sieving and decanting (Gerdemann & Nicolson 1963), through a series of 710 to 37µm sieves. The residues in the sieves were washed into a beaker the sievates were dispersed in water and filtered through filter paper, and spores were counted using a dissection microscope at 40× magnification. Sporocarps and spore clusters were considered as one unit. AMF spores were mounted in PVLG with Meltzer's reagent and identified based on morphological descriptions published by INVAM (http://invam.caf.wvu.edu) and AMF phylogeny (www.amf-phylogeny.com).

Statistical analysis

Spore density and species richness were expressed as number of AM fungal spores and numbers of AM fungal species in 25 g^{-1} soil sample. Relative abundance, isolation frequency, Shannon-Wiener index of diversity (H'), Simpson's index of dominance (D), evenness (E) and Sorenson's coefficient (Cs) were calculated (Dandan & Zhiwei 2007). Relationships between AMF and DSE colonization, spore density, species richness and soil physico-chemical properties were computed using Pearson's correlation coefficient. Standard errors of means were calculated.

Result

Soil characteristics

Soil texture was silty sand, soils were acidic to near neutral with moisture content of 28% to 36%. The soil was low in nutrients,

especially phosphorus. Organic carbon content ranged from 1% to 2%. The soil properties are depicted in Table 1.

AMF and DSE colonization

AMF and DSE colonization was observed in the three plant species studied with different rates of colonization. AMF colonization was lowest in *Hedychium coronarium* (66%), followed by *Eupatorium adenophorum* (69%) and highest in *Crotalaria anagyroides* (71%) (Fig. 1). Colonization in the form of arbuscule, vesicle, hyphae and occasionally intraradical spores were observed (Fig. 2). Root length colonization of arbuscules was high as compared to vesicles and hyphae. The root length colonization of AMF structures and DSE are given in Table 2.

AM fungal composition and diversity

The mean AMF spore density ($25g^{-1}$ soil) in the three plant species was 546. Spore density of AM fungi (Fig. 3) was *H. coronarium* (319), *C. anagyroides* (659) and *E. adenophorum* (661). Based on morphological characteristics, 23 AMF species belonging to two genera viz., *Acaulospora* (12 species) and *Glomus* (10 species) were isolated and identified (plus one unidentified AMF species). AMF species richness and composition are given in Table 3. *Acaulospora capsiculata*, *A. denticulata*, *Glomus caledonium, G. luteum* and one unidentified species were restricted to *C. anagyroides*. Four species (*Acaulospora delicata*, *Glomus clavisporum*, *G. fistulosum* and *G. viscosum*) were restricted to *E. adenophorum*. Only two species (*Acaulospora spinosa* and *Glomus* sp.) were restricted to *H. coronarium*.

Isolated AMF species with their relative abundance and isolation frequency are presented in Table 4 and some of the species are shown in Fig. 4. *Acaulospora koskei* and *A. mellea* were the dominant species in *C. anagyroides*. In *E. adenophorum,* the dominant species was *A. mellea* and in *H. coronarium* the dominant species were *A. mellea* and *A. spinosa*. *Acaulospora koskei, A. laevis, A. mellea, A. morrowiae* and *Glomus geosporum* were frequently isolated from all plant species.

Diversity indices (H', D and E) of AMF show little difference among the three plant species (Table 5). Cs value of AMF was 0.62 between *E. adenophorum* and *H. coronarium* (E.a × H.c), 0.55 between *C. anagyroides* and *E. adenophorum* (C.a × E.a) and 0.48 between *C. anagyroides* and *H. coronarium* (C.a × H.c) (Fig. 5).

Discussion

The plant species studied are high colonizers of AMF with different percentages among them (66%–71%). A similar observation of AMF colonization (21%–78%) was reported by Li et al (2007) from the natural vegetation of Southwest China. However, AMF root infection of only 1% to 7% in terrestrial plant species was reported from Costa Rica (Maffia et al. 1993). Uma et al (2010) also reported AMF colonization in *H. coronarium* (69.7%) from South India, which is slightly higher than our finding, suggesting that the intensity of AMF colonization could be influenced by specific habitat conditions. DSE colonization was observed to be lower than AMF colonization. This is due to the ability of AM fungi to colonize a wide range of plants in various environmental conditions. However, DSE is more common in cold, nutrient-stressed environments where AM fungi do not proliferate (Kohn & Stasovski 1990).

Correlation analysis revealed that AMF colonization and spore density are positively correlated, which is consistent with the finding of Nadarajah & Nawawi (1990) and Muthukumar et al (2003a). Negative correlation obtained between P and AMF colonization is in accordance with the finding of Li et al (2005). P is also negatively correlated with AMF species richness and spore density. AMF could enhance plant uptake of P and other nutrients, especially in a nutrient deficient environment. Increasing soil P is known to reduce or suppress AMF formation, which may either be due to the direct effect of P on the external hyphal growth or an indirect effect associated with the P status of the plant (Muthukumar & Udaiyan 2002). AMF species richness was significantly correlated with spore density, which is consistent with the finding of Dandan & Zhiwei (2007).The lack of correlation between AM and DSE fungal colonization levels suggests that they do not influence each other within roots, which is in agreement with Sathiyadash et al (2010). Positive correlation observed

Table 1 Soil physico-chemical parameters under three plant species.

Plants	MC (%)	pH	P (%)	OC (%)
Crotalaria anagyroides	35.75 ± 0.28	5.81 ± 0.01	0.01 ± 0.00	2.43 ± 0.03
Eupatorium adenophorum	27.60 ± 0.19	6.08 ± 0.02	0.01 ± 0.01	2.36 ± 0.04
Hedychium coronarium	32.07 ± 0.06	6.20 ± 0.01	0.03 ± 0.00	1.38 ± 0.01

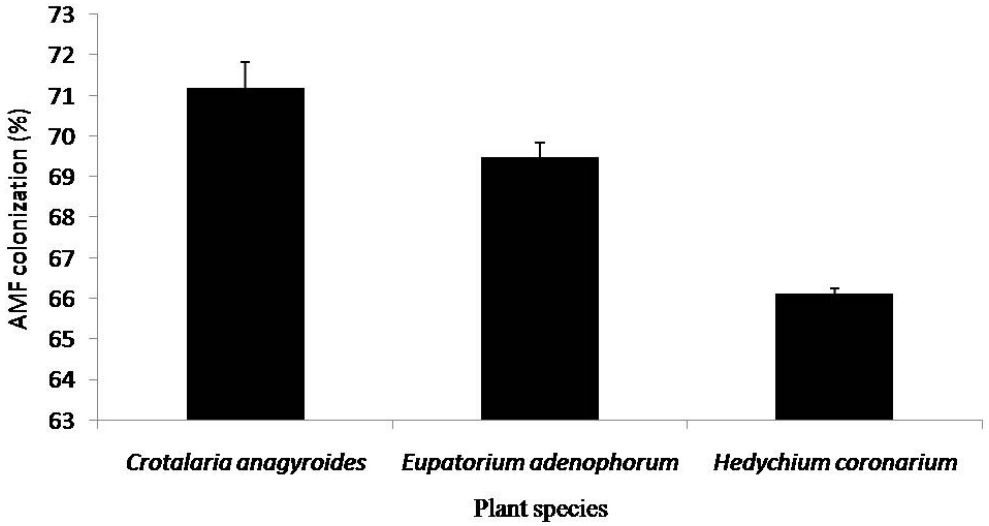

Fig. 1 – Percentage of mycorrhizal colonization in three plant species.

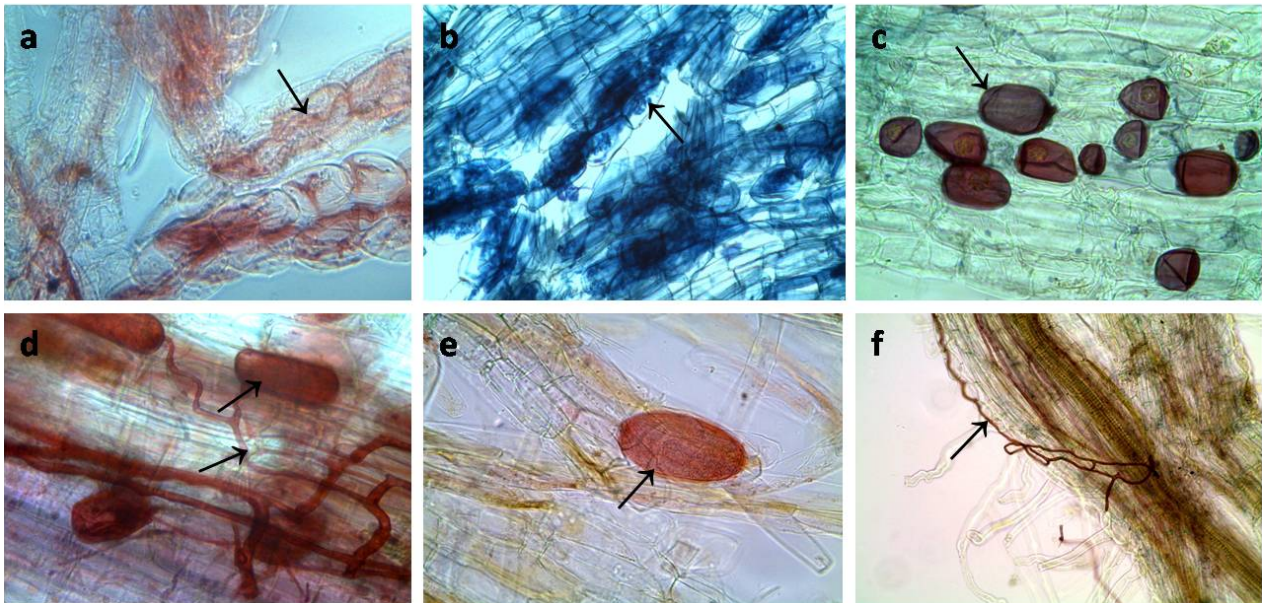

Fig. 2 – Mycorrhizal structural colonization in three plant species. **a, b** arbuscules in *H. coronarium* and *C. anagyroides*, **c**–**e** vesicles in *E. adenophorum*, *C. anagyroides* and *H. coronarium*, and **f** hyphae in *E. adenophorum*.

Table 2 Percentage of mycorrhizal structural colonization in three plant species.

Plants	RLA	RLV	RLH	RLDSE
Crotalaria anagyroides	42.27 ± 16.90	0.14 ±0.26	11.61 ±13.18	0.17 ± 0.40
Eupatorium adenophorum	29.86 ±22.49	0.36 ±0.48	16.27 ±14.19	0.85 ± 1.99
Hedychium coronarium	36.19 ±16.82	0.39 ±0.50	12.21 ±9.08	0.46 ± 0.74

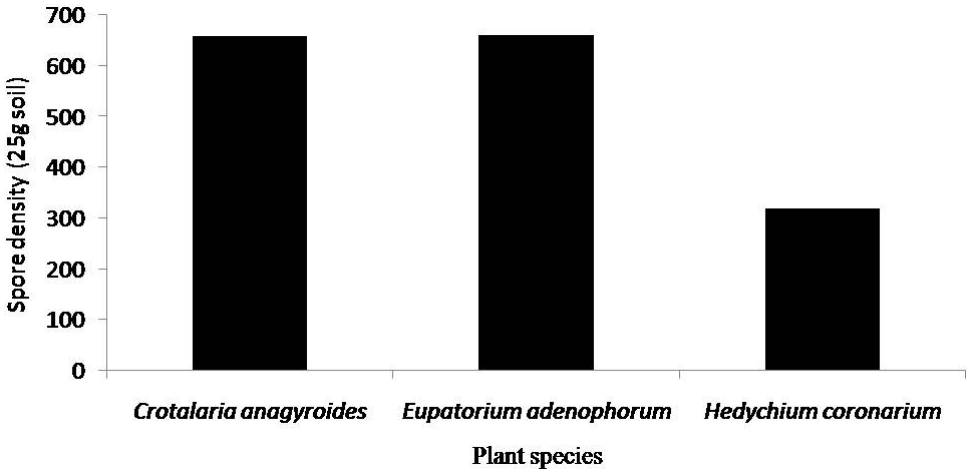

Fig. 3 – AMF spore density of three plant species.

Table 3 AMF species richness (SR) and species composition of three plant species.

Plants	Species Richness	*Acaulospora* sp.	*Glomus* sp.	Unidentified sp.
Crotalaria anagyroides	14	7	6	1
Eupatorium adenophorum	15	9	6	—
Hedychium coronarium	11	8	3	—

Table 4 AMF species relative abundance and isolation frequency (IF) from three plant species.

AMF species	IF	Relative abundance (%)		
		C. anagyroides	*E. adenophorum*	*H. coronarium*
Acaulospora capsiculata Blaszk.	33.33	4.26	—	—
Acaulospora delicata Walker, Pfeiffer & Bloss	33.33	—	6.25	—
Acaulospora denticulata Sieverding & Toro	33.33	2.13	—	—
Acaulospora dilatata Morton	66.67	—	6.25	11.11
Acaulospora koskei Blaszk.	100.00	17.02	9.38	3.70
Acaulospora lacunosa Morton	66.67	—	3.13	7.41
Acaulospora laevis Gerdemann & Trappe	100.00	12.77	3.13	3.70
Acaulospora mellea Spain & Schenck	100.00	25.53	25.00	18.52
Acaulospora morrowiae Spain & Schenck	100.00	4.26	6.25	7.41
Acaulospora scrobiculata Trappe	66.67	2.13	9.38	—
Acaulospora spinosa Walker & Trappe	33.33	—	—	22.22
Acaulospora tuberculata Janos & Trappe	66.67	—	3.13	3.70
Glomus caledonium Nicolson & Gerdemann	33.33	2.13	—	—
Glomus clavisporum (Trappe) Almeida & Schenck	33.33	—	3.13	—
Glomus etunicatum Becker & Gerdemann	66.67	8.51	9.38	—
Glomus fistulosum Skuo & Jakobsen	33.33	—	3.13	—
Glomus geosporum (Nicol. & Gerd.) Walker	100.00	2.13	6.25	14.81
Glomus intraradices Schenck & Smith	66.67	2.13	3.13	—
Glomus luteum Kenn., Stutz & Morton	33.33	12.77	—	—
Glomus sp.	33.33	—	—	3.70
Glomus verrucolusum Blaszkowski & Tadych	66.67	2.13	—	3.70
Glomus viscosum Nicolson	33.33	—	3.13	—
Unidentified	33.33	2.13	—	—

'—' not found.

between OC and spore density is in accordance with Singh et al (2003) who reported it from natural forest of North East India. Species richness was also positively correlated with spore density, although Yang et al (2011) reported no significant positive correlation between these two variables from a subtropical forest of China.

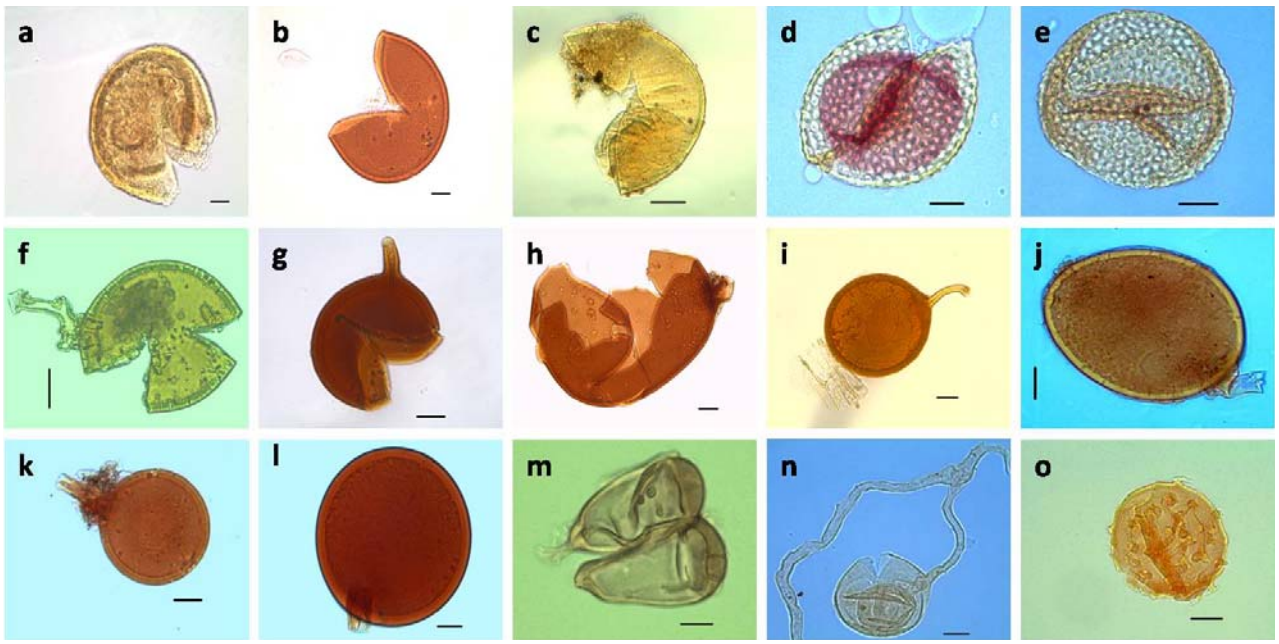

Fig. 4 – AMF spores isolated from rhizosphere soil of three plant species. **a–e** *Acaulospora* species: *A. koskei*, *A. laevis*, *A. delicata*, *A. denticulata*, *A. scrobiculata*, **f–n** *Glomus* species: *G. fistulosum*, *G. intraradices*, *G. verrucolusum*, *G. caledonium*, *G. luteum*, *G. etunicatum*, *G. geosporum*, *G. clavisporum*, *G. viscosum*, and **o** unidentified species. Scale basrs: a, b = 50µm, c–e = 40 µm, f = 45 µm, g = 40 µm, h = 30 µm, i = 30 µm, j–o = 40 µm.

Table 5 AMF diversity indices in three plant species.

Diversity index	***C. anagyroides***	***E. adenophorum***	***H. coronarium***
Shannon	2.23	2.52	2.17
Simpson	0.14	0.11	0.14
Evenness	0.84	0.93	0.90

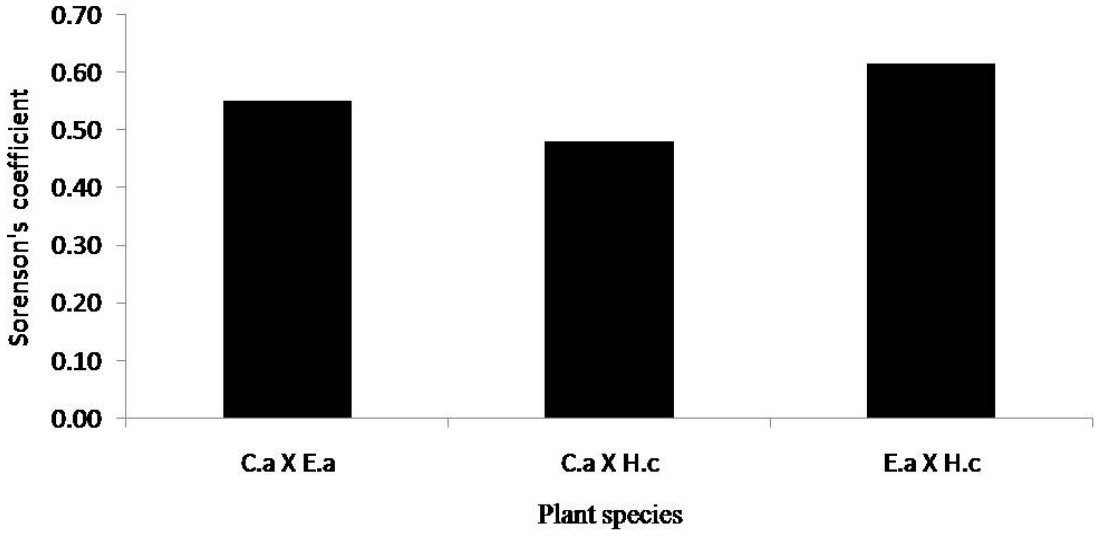

Fig. 5 – Sorenson's index of three plant species.

Twenty-three AM species were isolated, which is fewer than that reported by Zhang et al (2004) and Yang et al (2011) in subtropical forests of China. Our investigation indicated higher number of *Acaulospora* species than in other studies (Oehl et al. 2003, Castillo et al. 2006). *Acaulospora* species were dominant and widespread in all the plant species followed by

Glomus species similar to the finding of Love lock et al (2003), Tchabi et al (2008) and Gao & Guo (2010) in different forest types. *Acaulospora* species are often associated with acidic soil (Abbott & Robson 1991) and this could explain our finding of frequent detection of *Acaulospora* species. Furthermore, species of *Acaulospora* are adapted to a wide array of soil and host species, appearing in soils of widely different nutrient availability (Straker et al. 2010).

Occurrence of only *Glomus* and *Acaulospora* species 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). Furthermore, it is highly probable that the different host species may support a suite of AM fungal species for colonization and sporulation (Öpik et al 2008).

The AMF spore density in our observation is higher than those reported by Visalakshi (1997) and Singh et al (2003) in different forest soil of India. Spore production of AM fungi is known to vary greatly in different ecosystems, and it is thus influenced by an array of factors such as environment, host, and fungus, and spore density, which tends to decrease during root growth but increases during root inactivity or senescence (Muthukumar et al 2003b). Zhao et al (2001) suggested that the uneven spatial distribution of AM fungal spores and the complex structure of the underground root component could be major factors affecting spore density of AMF.

The mean Shannon-Wiener index of 2.3 was similar to that reported by Husband et al (2002) from subtropical forest. *C. anagyroides* and *H. coronarium* have a lower Shannon-Wei ner index value, which may be due to relatively fewer AMF species. Higher value of D (0.14) indicates dominance by a few species of AMF fungi in *C. anagyroides* and *H. coronarium* while the lower index of dominance (0.11) indicates shared dominance of many AMF fungal species in *E. adenophorum*. There was a different AM fungal community composition (Cs: 0.55–0.62) among the three plant species, and a higher degree of overlap in fungal species composition was observed between *E. adenophorum* and *H. coronarium* when compared between *C. anagyroides* and *E. adenophorum*, and between *C. anagyroides* and *H. coronarium*. Evenness values obtained indicate that distribution of AMF species was more uniform in *E. adenophorum* than in the other two plant species. Furthermore, different AM species composition and disparity in AM fungal relative abundance also cause the differences between diversity indices (Yang et al. 2011).

Conclusions

The present study reveals the status of AMF in three studied plant species from subtropical pine forest of North East India. The plant species are good colonizers of AMF and support many AMF species. The variation in AMF communities, with different AMF diversity, spore density and colonization associated with different host plant species may be generated by a variety of potential mechanisms, including biological characteristics, variation in host species, mycorrhizal dependency and host plant-mediated alteration of the soil microenvironment. Further research is required for a better understanding of AM fungal species composition, diversity and its influence on plant growth and community stability to understand the overall status of AMF in this forest.

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