Colonization of arbuscular mycorrhizal fungi in moderately degraded sub-tropical forest stands of Meghalaya, Northeast India

Songachan, L.S., Lyngdoh, I. and Highland, K.*

Microbial Ecology Laboratory, Department of Botany, North Eastern Hill University, Shillong-793 022, India

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The roots of eight plants species in six families from moderately degraded sub-tropical forest stands of Meghalaya, Northeast India were examined for arbuscular mycorrhizal fungi (AMF). All the surveyed plant species had AMF colonization in the form of arbuscules, vesicles and hyphae. The result of the investigation reveals that the percentage of AMF colonization varied in different plant species. The intensity of colonization ranges from 38.98% to 87.77%, with the lowest in *Cinnamomum tamala* and highest in *Ophiopogon intermedius*. Analysis of the rhizosphere soils showed that mycorrhizal spores were present in all locations from where the plant roots were collected. The mean spore density per 25g of soil ranged between 38.67 and 89. Correlation analyses demonstrated that total AMF colonization shows a positive correlation (p < 0.05) with spore density and also with hyphae and vesicles. The hyphal colonization had a negative correlation (p < 0.05) with arbuscules and positive correlation (p < 0.05) with vesicles. The present study indicated that AMF was formed in the roots of various plants in moderately degraded sub-tropical forest of Northeast India with varied rate of colonization and spore density.

Key words: Arbuscular mycorrhizal fungi, colonization, spore density, plant species.

Introduction

AMF are a major component of rhizosphere soils, forming mutualistic symbiotic association (Closa and Goicoeche, 2011) with 80% of all terrestrial plant (Smith and Read, 2008). AMF benefit from this association by obtaining carbon compounds which are necessary for their growth and in return, they have diverse, beneficial impacts on plants and soils (Brundrett *et al.*, 1999; Li *et al.*, 2006). Mycorrhiza is of basic importance for both individual plants and for entire plant community as it influences the plant diversity, productivity, community structure, ecosystem processes and improves the growth of young

^{*} Corresponding author: Highland, K; e-mail address: hkayang@yahoo.com

seedlings (van der Heijden *et al.*, 1998). Mycorrhizal symbioses play a key role in nutrient cycling in the ecosystem and also protect plants against environmental stress (Barea and Jeffries, 1995).

Distribution of AMF in different plant species of a particular ecological zone is important in order to evaluate the natural status of AM fungi in that region. However, selection of the most specific and appropriate plant-fungus association for each specific environmental and ecological situation is one of the main challenges in current research on AMF (Khanam *et al.*, 2006). AMF shows different responses on root colonization intensity and spore population depending on the host species or environmental factors (Likar *et al.*, 2008; Li *et al.*, 2005; Muthukumar and Udaiyan, 2002).

In many parts of the world, natural vegetation has been disturbed as a consequence of management practices. Moreover, an increasing demand for forest products may endanger many important plant species and their habitats (Fuchs and Haselwandter, 2008). Considerable amount of research has done, which aims at understanding the effects of human-induced environmental change (Oechel *et al.*, 2000). Unfortunately, much of the work focuses on the aboveground rather than belowground level despite these two subsystems being closely interlinked (Wardle *et al.*, 2004). The restoration and re-establishment of degraded ecosystems should include not only the aboveground systems but also the below-ground microorganisms which are associated functionally with plants (Li *et al.*, 2007a). It has been suggested that the success of any ecosystem reforestation efforts are likely to depend on the establishment of mycorrhizas, and AM should receive special attention in restoration of degraded ecosystem (Wubet *et al.*, 2003).

Owing to the multiple beneficial effects on plant performance and soil health, AMF are crucial for the restoration and re-establishment of the vegetation in degraded ecosystems (Cuenca *et al.*, 1998; Dhillion and Gardsjord, 2004). Therefore, mycorrhizal technology, which is cost effective and simple, can be use to improve forest products and environmental quality in different production systems. Although, many different types of plant species are found in Meghalaya, Northeast India, very little work has been done on AMF association with different plants species from this region. Moreover, most of the studies with AMF are confined to agricultural soils (Grayston *et al.*, 2001) and in forest ecosystem such studies are rather limited. Due to the importance of AMF for vegetation re-establishment in degraded ecosystems, we investigate the AM colonization and its fungal spore density from eight plant species (Table. 1) belonging to six families growing in a moderately degraded tropical forest stands of Meghalaya.

Plant spp	Family	Growth form		
Disporum cantoniense	Colchicaceae	Herb		
Hedera helix	Araliaceae	Herb		
Ophiopogon intermedius	Ruscaceae	Herb		
Sarcandra glabra	Chloranthaceae	Shrub		
Smilax rotundifolia	Smilacaceae	Herb		
Cinnamomum tamala	Lauraceae	Shrub		
Neolitsea sericea	Lauraceae	Tree		
Persea odoratissima	Lauraceae	Tree		

Table 1. The plant species studied for AMF colonization

Materials and methods

Study site and field sampling

The roots and rhizosphere soil of eight different plant species were collected from Upper Shillong, Meghalaya, North East India, located at 25°33'38'' N and 91°51'2''E, with an elevation of 1900msl. Three subsamples each of roots and rhizosphere soil were collected randomly, which were then, merge into one composite sample. The samples were kept in a sterilized plastic bag and transported in the laboratory for analysis.

Root treatment and AMF colonization assessment

Roots were washed thoroughly with 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. It is then washed and stained stamp pad ink (Das and Kayang, 2008). AMF colonization was determined by the magnified intersection method of McGonigle *et al.* (1990) and expressed it in percentage.

AMF spore extraction and density

AMF spore extraction was done by following the method of INVAM (www.invam.caf.wvu.edu). Spore density was expressed as number of AM fungal spores per 25g soil sample.

Analysis of soil physicochemical properties

Soil moisture was determined by drying 10 g fresh soil at 105°C for 24 h in a hot-air oven. Soil pH was determined using a digital pH meter. Available phosphorus and organic carbon were determined by following the methods of 1675

Allen *et al.* (1974) and Anderson and Ingram (1993) respectively. Soil texture was determined using the bouyoucos method of Allen *et al.* (1974).

Statistical analysis

Standard errors of means were calculated. Relationship between mycorrhizal structural colonization and soil physico-chemical properties were computed using Pearson's correlation coefficient.

Results

The soil physico-chemical properties are presented in Table 2. The soil pH was acidic for all plant species (4.7 to 5.1). There was a large difference in soil moisture content, organic carbon and available phosphorus among plant species. Texture of the soil was sandy loam. All of the surveyed plants formed mycorrhizal association. Arbuscules, vesicles and hyphae (Fig.1 and Fig.2) were observed, and occasionally intra-radical spores were also observed. AMF colonization and spore density of 8 plant species are given in Fig.3a and 3b. Vesicular colonization is comparatively lesser than arbuscular and hyphal colonization. Wide variation was observed in the percentage of AMF colonization among plant species. The percentage of AMF colonization was 57.87 in *Disporum cantoniense*, 63.56 in *Hedera helix*, 87.77 in *Ophiopogon intermedius*, 75.29 in *Sarcandra glabra*, 49.37 in *Smilax rotundifolia*, 38.98 in *Cinnamomum tamala*, 52.96 in *Neolitsea sericea* and 71.02 in *Persea odoratissima*. It shows that colonization was lowest in *Cinnamomum tamala* and highest in *Ophiopogon intermedius*.

Plant species	pH	MC (%)	OC (%)	AP(µg)	Clay%	Sand%	Silt%
Disporum cantoniense	4.74 ± 0.01	23.42 ± 0.14	2.07 ± 0.09	2.13 ± 0.13	2.78	95.33	1.89
Hedera helix	4.90 ± 0.07	15.88 ± 0.05	1.95 ± 0.07	1.70 ± 0.30	2.85	95.22	1.93
Ophiopogon intermedius	4.86 ± 0.02	21.88 ± 0.15	2.14 ± 0.06	2.40 ± 0.40	4.69	93.42	1.89
Sarcandra glabra	4.65 ± 0.01	26.30 ± 0.14	2.50 ± 0.01	2.73 ± 0.07	4.65	93.47	1.88
Smilax rotundifolia	4.88 ± 0.03	14.07 ± 0.09	1.19 ± 0.19	2.87 ± 0.13	2.88	95.17	1.95
Cinnamomum tamala	5.16 ± 0.02	42.40 ± 0.10	2.74 ± 0.05	2.31 ± 0.16	4.90	93.67	1.43
Neolitsea sericea	4.87 ± 0.01	39.70±0.06	2.45±0.21	$2.39{\pm}0.09$	3.36	94.54	2.10
Persea odoratissima	5.03±0.01	39.40±0.06	2.61±0.10	5.04 ± 0.08	3.66	94.11	2.23

	Table 2. Selected	soil physico	-chemical	characteristics	of eight plan	t species
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Note: MC = Soil moisture content, OC = Organic carbon, AP = Available phosphorus and \pm indicates standard error.

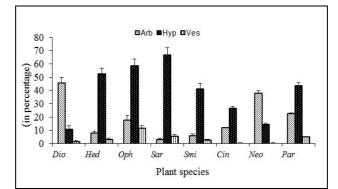


Fig 1. Mycorrhizal colonization in eight different plant species. Note: *Dio = Disporum cantoniense, Hed = Hedera helix, Oph = Ophiopogon intermedius, Sar = Sarcandra glabra, Smi = Smilax rotundifolia, Cin = Cinnamomum tamala, Neo = Neolitsea sericea and Par = Persea odoratissima*

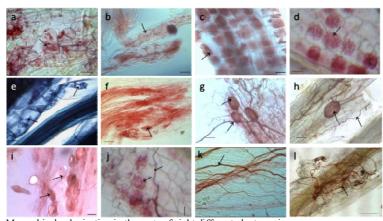


Fig 2. Mycorrhizal colonization in the roots of eight different plant species. Note: (a-e) Arbuscules in *Disporum cantoniense*, *Hedera helix*, *Cinnamonum tamala*, *Neolitsea sericea* and *Sarcandra glabra*. Scale bar = 65, 60, 60, 40 and 80 μm, respectively. (f) Vesicles in *Hedera helix*. Scale bar = 170 μm. (g-j) Vesicles and hyphae in *Smilax rotundifolia*, *Persea odoratissima*, *Ophiopogon intermedius and Neolitsea sericea*. Scale bar = 40, 40, 60 and 40 μm, respectively. (k & l) Hyphae in *Hedera helix* and *Disporum cantoniense*. Scale bar = 40 and 170 μm.

Similar to AMF colonization, spore density also varied greatly among plant species. AMF spore density was 71 in *Disporum cantoniense*, 89 in *Hedera helix*, 63 in *Ophiopogon intermedius*, 77.33 *Sarcandra glabra*, 38.67 in *Smilax rotundifolia*, 45 in *Cinnamomum tamala*, 48 in *Neolitsea sericea* and 78.33 in *Persea odoratissima*. Correlation between mycorrhizal structural colonization, AMF colonization, spore density and soil physico-chemical properties are presented in Table 3. Correlation analysis demonstrated that total AMF colonization shows a positive correlation (p < 0.05) with spore density and also with hyphae and vesicles. The hyphal colonization had a negative correlation (p < 0.05) with arbuscules and positive correlation (p < 0.05) with vesicles. Total AMF colonization shows a negative correlation with soil pH.

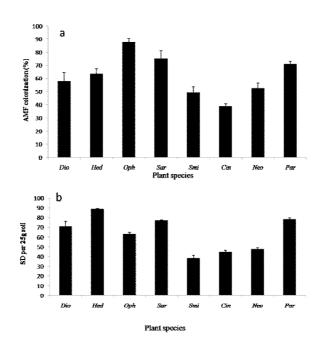


Fig.3. (a) Percentage of AMF colonization in the roots of eight plant species and (b) AMF spore density (SD) of eight plant species. Note: Dio = Disportum cantoniense, Hed = Hedera helix, Oph = Ophiopogon intermedius, Sar = Sarcandra glabra, Smi = Smilax rotundifolia, Cin = Cinnamomum tamala, Neo = Neolitsea sericea and Par = Persea odoratissima

Table 3. Pearson's correlation coefficient between mycorrhizal structural colonization, spore density and selected physico-chemical properties of soil

All New	Arb	Нур	Ves	AMF	SD	pН	MC	OC	AP	Clay	Sand	Silt
Arb	1.00	-0.79*	-0.27	-0.11	0.05	-0.11	0.32	0.19	-0.01	-0.37	0.32	0.29
Нур		1.00	0.72*	0.69*	0.38	-0.23	-0.41	-0.08	0.15	0.40	-0.47*	0.08
Ves			1.00	0.90*	0.31	-0.22	-0.28	-0.01	0.17	0.46*	-0.56*	0.18
AMF				1.00	0.62*	-0.46*	-0.28	0.08	0.22	0.26	-0.42	0.43
SD					1.00	-0.37	-0.23	0.21	0.02	-0.12	0.05	0.30
pН						1.00	0.53*	0.28	0.26	0.21	-0.14	-0.32
MC							1.00	0.85*	0.40	0.44	-0.47*	-0.09
OC								1.00	0.24	0.62*	-0.64*	-0.17
AP									1.00	0.08	-0.24	0.54*
Clay										1.00	-0.97*	-0.50*
Sand											1.00	0.28
silt												1.00

Note: *Correlation is significant at p < 0.05. Arb = Arbuscules, Hyp = Hyphae, Ves = Vesicles, AMF = Total AMF, SD = Spore density, MC = Moisture content, OC = Organic carbon, AP = Available phosphorous.

Discussion

The variation in AMF colonization associated with different host plant species may be generated by a variety of potential mechanisms, including biological characteristics of rhizosphere under host species, mycorrhizal dependency, host plant-mediated alteration of the soil microenvironment (Wu *et al.* (2009), specific habitat conditions (Štajerová *et al.*, 2009), AMF diversity and species composition (Gange *et al.*, 1990), or seasonal and ontogenetic variations (Jakobsen *et al.*, 2002) and nutrient demands of the host (Muthukumar and Udaiyan, 2002). Our studies suggested that the incidence of AMF colonization was moderate to high in moderately degraded sub-tropical forest. These results in contrast with the study of Gehring and Connell (2006) who found low occurrence of AMF in tropical forest but are consistent with the results of several studies that observed high occurrence of AMF in such ecosystem (Allen *et al.*, 1998; Onguene and Kuyper, 2002).

The mean AMF spore density $(25g \text{ soil})^{-1}$ ranges from 38.67 to 89 which was lower than that of other undisturbed environments. Gehring *et al.* (2002) reported 54 spores per g soil. Whereas, Johnson and Wedin (1997) reported 15,531 AMF spores per 25 g soil in tropical ecosystem. However, the threatened semiarid Mediterranean ecosystem is indicated. Ferrol *et al.* (2004) reported a relatively low AM fungal spores (2 to 55 per 100 g of soil). Enkhtuya *et al.* (2000) reported that the spore density in undisturbed land was significantly higher than that in cropped land, which further supported the view that disturbances reduce AMF root colonization and spore density (Helgason *et al.*, 1998; Oehl *et al.*, 2003).

Large difference in spore density might be influenced by an array of factors which come from environment, host and fungus, and spore density tend to decrease during root growth but increase during root inactivity or senescence (Muthukumar *et al.* 2003a). Moreover, fluctuations in the number of AMF fungal spores would be expected to occur if they were lost during periods of mycorrhizal formation, or as a result of predation by soil organisms (Brundrett, 1991). Availability and activity of AMF spores can be affected by vegetation removal (Boddington and Dodd, 2000) leading to a significant decrease in spore density and colonization, as well as a loss in infectivity (Jasper *et al.*, 1989. According to Troeh and Loynachan (2003) stated that AMF survival in soil may be affected by the presence or absence of host plants and by the plant species being grown.

In our present study, we observed that total AMF colonization shows a positive correlation with spore density, which is in accordance with the study of Sigüenza *et al.* (1996). However, some researchers suggested that no significant correlation between AM colonization and spore density were observed (Camargo-Raicalde and Dhillion, 2003; Li *et al.*, 2007b) whereas, Fontenla *et al.*, (1998) found a negative relationship between AMF colonization and spore density. Generally, AM colonization is influenced by spore availability

(Muthukumar *et al.*, 2003b). Zhao *et al.* (2001) suggested that the uneven spatial distribution of AMF spores and the complex structure of the underground root component should be considered as major factors affecting AMF spore density which could contribute to variable rates of AMF colonization among plants (Lovelock and Miller, 2002). Gai *et al.* (2006) reported that mycorrhizal incidence among wild herbaceous is consistently high, with over 90% of the species examined exhibiting AMF colonization. Correlation analysis demonstrated that total colonization was significantly and positively correlated with hyphal and vesicular colonization. Wu *et al.* (2009) also reported a similar finding and suggested that it is due to the hyphae and vesicles, which are the primary structures of AMF, exist for months or years. However, arbuscules begin to form approximately two days after root penetration and begin to collapse after a few days (Smith, 1995).

Soil phosphorus does not show correlation with total colonization which is in agreement with the study by Ruotsalainen *et al.* (2002). The low phosphorus content of the soil could be ecologically significant for AMF in phosphorus uptake. Many studies have reported that the colonization potential was higher in soils where the P concentration was lower (Harley and Smith, 1983; Galvez *et al.*, 2001) and vice versa (Nogueira and Cardoso, 2006). Sylvia *et al.* (1993) observed a decrease in all AMF parameters by different AMF from a wide array of habitats, when P exceeded 10 mg kg⁻¹ soil. Total AMF colonization shows a negative correlation (p < 0.05) with soil pH. Wang, (1993) suggested that pH is an important factor influencing AM fungal species composition. AMF respond the most at pH range of 5.6 to 7.1 (Friberg, 2001). Apparently, soil pH and P do influence certain fungal species and their ability to colonize roots. (Johnson *et al.*, 1991) observed that overall AMF production in the form of spore propagules may increase with soil pH.

AMF plays an important role in plant survival and community stability of vegetation in natural ecosystems (Hartnett and Wilson, 2002; Moraes *et al.*, 2004). AM symbiosis is the most effective among the biotic factors that could favor rapid plant re-establishment and fasten plant growth (Duponnois *et al.*, 2001). The present study indicated that AMF was formed in the roots of various plants in moderately degraded forest, which demonstrated that these associations might be one of the effective methods of acclimatizing plants to the degraded environments.

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