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Arbuscular mycorrhizal fungal colonization in three different age groups of rubber plantations in Tripura, North-East India

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Abstract

Soil chemical properties, arbuscular mycorrhizal (AM) fungal colonization, AM fungal morphology in root and diversity of AM fungi in rubber plantations of three different age groups of 5, 10 and 30 years old of Tripura, North-East India were investigated. The result exhibited positive correlation (p<0.05) between AM colonization and spore density with available nitrogen. There was a positive significant correlation between AM colonization with spore density. AM fungal colonization was significantly higher in 5 and 10 than 30 years old plantation. *Arum* type of AM fungal morphology was observed in the root. A total of 12 morphotypes were isolated belonging to the genera *Acaulospora*, *Ambispora* and *Glomus*. Out of which 5, 9 and 9 AM fungal species were isolated from the rubber plantations of 30, 10 and 5 years old, respectively. Shannon diversity index was highest in 5 years old and lowest in 30 years old plantation. Evenness was highest in 30 years old and lowest in 10 years old plantation. The relation of soil chemical properties with AM fungal colonization and AM fungal species composition is discussed.

Key words – AM fungal morphology – diversity – rubber tree – soil properties

Introduction

Rubber tree (*Hevea brasiliensis* Muell. Arg.) is one of the perennial crop species which are grown in large scale plantations in Tripura, North-East India. In Tripura, rubber plantation was introduced in 1963 to check soil degradation due to slash and burn agriculture practiced by the local tribal people. Rubber is an important cash crop in the economy of Tripura where it is cultivated in more than 40,000 ha area over hill slopes, hillocks and plains (Chaudhuri et al. 2013).

Arbuscular mycorrhizal (AM) fungi are found in 70–90% of land plant species (Smith & Read 2008) with fungi that belong to Glomeromycota (Schübler et al. 2001). The main benefit of mycorrhiza is its greater soil exploration and increasing uptake of P, N, K, Zn, Cu, S, Fe, Mg, Ca and Mn by the plants (Javot et al. 2007). AM fungal community investigation using the diversity and relative abundance of AM fungal spores has been a common practice in studies of AM fungal ecology (Oehl et al. 2005, Lovera & Cuenca 2007). The special importance of the mycorrhizal symbiosis for the rubber tree was pointed out by different groups all over the world with respect to

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its influence on growth parameters (Ikram & Mahmud 1984, Ikram et al. 1992, Schwob et al. 1998), use in agroforestry plant production (Feldmann & Lieberei 1994), comparison of diversity in open land with rubber plantation (Debnath et al. 2014) and colonization by AM fungi of rubber trees in different age group of plantations was examined (Herrmann et al. 2016).

However, there is no study related to soil chemical properties and its relation with AM fungal colonization in the plantations of rubber in Tripura. Moreover, there is meager study confining to the diversity of AM fungi from the rubber plantations. In this connection, the present study is focused on the analysis of soil chemical properties, arbuscular mycorrhizal (AM) fungal colonization, AM fungal morphology in root and diversity of AM fungi in three different age groups of rubber plantations in Tripura, North-East India.

Materials & Methods

Study sites

The samples were collected from three different rubber plantation sites of Tripura, India, i.e., 5 years old and 10 years old plantations are at Pathaliaghat and both located in the same site (23º24'06.23" N 91º33'04.54" E at 35 m.a.s.l.) and 30 years old is located at Takmachara (23º36'14.88" N 91º23'04.32" E at 41 m.a.s.l.). The rubber plantations harbour several understorey plant communities (Debnath et al. 2014, Talapatra et al. 2015). There is no known history of using external fertilizers in these selected rubber plantations.

Root and soil sampling

The roots of five rubber trees were collected from four points around each tree from each plantation site. All the roots from each site were mixed and transferred to the laboratory for root processing and estimation of AM fungal colonization. The soil samples of approximately 500 g at depths of approximately 0−20 cm around four points of plants were mixed, labeled and zipped in polythene bags from each site. After transferring to the laboratory, the soil samples were divided into two where one fresh sample was kept for analysis of spore density and diversity. The other was then air dried in a shady place, crushed into powder form and sieved for soil properties analysis.

Preparation of roots and assessment of AM fungal colonization

The collected roots were thoroughly washed with tap water several times and cut into approximately 1 cm in size. Then the roots were cleaned with 10% NaOH at 90°C for 2−3 h, depending on the clearance of root samples. The cleared roots were washed again with tap water 4−5 times and bleached in 2 drops of alkaline H_2O_2. After acidifying with 1% HCl, roots were stained with Black Faber Castell stamp pad ink (Das & Kayang 2008). After a while the roots were mounted on a slide and observed using a compound microscope for arbuscular mycorrhizal fungal structures such as arbuscules, vesicles and hyphae. The quantification of AM fungal colonization was done by the magnified intersection method (McGonigle et al. 1990).

Soil analysis

For pH and electrical conductivity, 10 g of soil was dissolved in 50 ml of distilled water and stirred for 20 min. This solution was kept overnight and then the soil pH and electrical conductivity was measured using a digital pH meter. The organic carbon was determined by using Walkley & Black (1934) method. The available soil nitrogen was estimated following Black (1982) method. Available phosphorus and potassium of soil were determined using the method of Jackson (1978).

Spore analysis

Spores of AM fungi were extracted from 25 g of soil samples by modified wet sieving and decanting method (Muthukumar et al. 2006). The retained spores after passing through a 35 µm sieve were picked up with a needle from filter paper in 1–2 drops of polyvinyl alcohol-lactoglycerol under a dissecting microscope (Koske & Tessier 1983) for counting and

identification. The identification of spores was based on morphological characteristics using descriptions provided from the websites (http://www.invam.caf.wvu.edu and http://www.lrzmuenchen.de/~schuessler/ amphylo).

Data analysis

Spore density (SD) was calculated from the number of spores in 25 g of soil. The correlation coefficient was employed to determine the relationships between AM fungal colonization, spore density and soil physico-chemical properties. Means were separated by Duncan test and all the statistics was performed using the software (Statistica 9.0). Species richness (SR) was calculated from the number of identified AM fungal species per soil sample. Relative abundance (RA) was measured by the following formula:

$$RA = \frac{\text{Spore numbers of a species (genus)}}{\text{Total number of identified spore samples}} \times 100$$

Shannon–Wiener index of diversity (H') was calculated from the following formula:

$$H' = -\sum Pi \ln Pi$$

Evenness (E) was measured by the following formula:

$$E = \frac{H'}{H'max}$$

Simpson's index of dominance (D) was calculated from the following formula:

$$D = -\sum \left[\frac{ni(ni-1)}{N(N-1)}\right]$$

Sorenson's coefficient (Cs) was measured by the following formula:

$$Cs = \frac{2j}{(a+b)}$$

Pi is the relative abundance of each identified species per sampling site and calculated by the following formula: Pi = ni/N, where ni is the spore numbers of a species, and N is the total number of identified spore samples. H' max is the maximal H' and calculated by the following formula: H' = ln S, where S is the total number of identified species per sampling site. a or b was the total number of identified species per sampling site, and j was the number of identified species common to both sites (Dandan & Zhiwei 2007).

Results

Soil properties

The chemical properties of soil showed organic carbon highest in 5 years old and lowest in 10 years old plantation, available nitrogen was also highest in 5 years old and lowest in 30 years old. The available phosphorus was high in 30 years old than 5 years old and 10 years old plantation. The 5 years old showed a lower amount of available potassium than other two sites. The soil properties are presented in Table 1.

Table 1 Soil chemical properties of soils and spore density of AM fungi from three rubber plantations of Tripura

Age (Years)	pH	Electrical conductivity (cS cm-1)	Organic carbon %	Available nitrogen (Kg/ha)	Available phosphorus (Kg/ha)	Available potassium (Kg/ha)	Spore density/25 g soil
5	4.44	131	1.56	376.06	2.03	3.11	392
10	4.32	137	1.19	366.74	3.66	320.79	316
30	4.52	128	1.40	349.09	14.77	160.395	195

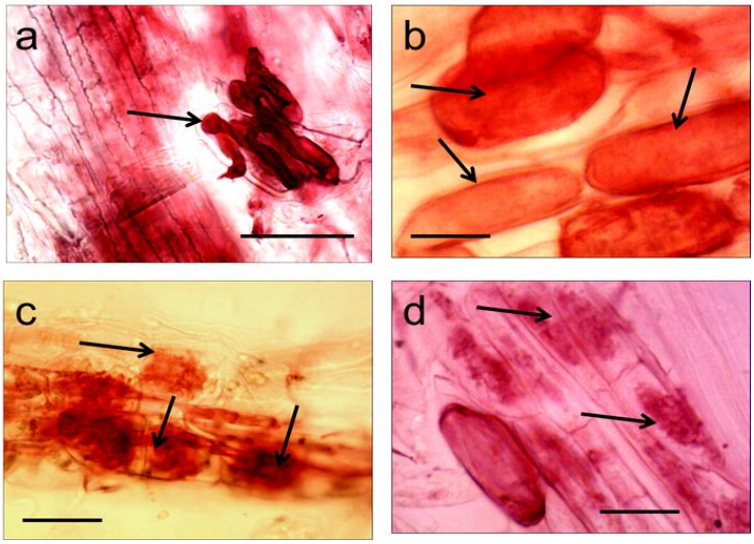

Fig 1 – Mycorrhizal colonization in *Hevea brasiliensis*. (a) Appressoria formation in the epidermal layer of root. (b) Vesicles. (c, d) Arbuscules exhibiting *Arum* type of AM morphology. Scale bars: a = 200 µm, b–d = 100 µm.

Mycorrhizal colonization

The mycorrhiza-forming structures were observed in the roots of rubber plant from all the plantations. The structures such as appressoria forming in the epidermal layers, vesicles in the cortex and arbuscules in the cortex were observed (Fig. 1). The colonization of arbuscules, vesicles and hyphae were higher in the 5 years old plantation than the other two (Table 2). *Arum* type of AM fungal morphology was observed. The presence of intercellular fungal hyphal growth in the root cortex and development of arbuscules on intracellular hyphal branches is shown (Fig. 1c, d).

Table 2 Mycorrhizal colonization in three age groups of rubber plants

Age (Years)	% Arbuscules	% Vesicles	% Hyphae
5	13.56±1.33a	21.93±1.63a	59.05±1.82a
10	11.12±1.37ab	18.61±1.92ab	54.25±2.35a
30	8.77±1.07b	15.57±1.02b	43.99±1.77b

Different alphabets differ significantly at $p<0.05$

Correlation of age, soil properties and spore density of AM fungi

The obtained data exhibited a significant positive correlation between the AM fungal colonization and spore densities with available nitrogen. There was a significant positive correlation between AM colonization with spore density. The results also describe the negative correlation between pH and electrical conductivity and between available potassium with organic carbon (Table 3).

Distribution of AM fungal spores

The spore density of 30 years old was lower than the other two plantation sites (Table 1). A total of 12 AM fungal morphospecies (Fig. 2) were distinguished by morphological criteria, of which 5, 9 and 9 AM fungal species were isolated from rubber plantations of 30, 5 and 10 years old plantation, respectively (Table 4). The most common genus was *Glomus*. It was represented by 8 species followed by *Acaulospora* by 2 species and *Ambispora* by 1 species. One morphospecies remained unidentified. *G. multiculae*, *Glomus* sp. 1, *Glomus* sp. 2 and *Glomus* sp. 3 were found in all three plantation sites. Among the isolated species, *Glomus* sp. 1 showed highest relative abundance. The lowest abundance in 30 years old was showed by *Glomus* sp. 5, in 5 years old by 3 species i.e., *Acaulospora* sp. 1, *Ambispora* sp. 1, unidentified sp. 1 and in 10 years old plantation by *Acaulospora* sp. 2 and *G. clavisporum*.

AM fungal diversity

The diversity of AM fungi reveals Shannon-Wiener diversity index was highest in 5 years old and lowest in 30 years old. Simpson index was highest in 10 years and lowest in 5 years old plantation. Evenness was highest in 30 years old and lowest in 10 years old plantation. The diversity indices are depicted in Table 5. Sorenson coefficient of 5 and 10 years old, 5 and 30 years old, 10 and 30 years old are 0.67, 0.57 and 0.71, respectively.

Table 3 Correlation matrix of age of rubber plantations and soil chemical properties of soils from three rubber plantations of Tripura

	Age	pH	EC	OC	N	P	K	Ca	SD	Col
Age	1.00	0.67	-0.62	-0.11	-0.99	0.99*	0.18	-0.78	-0.98	-0.99
pH		1.00	-0.99*	0.66	-0.55	0.73	-0.60	-0.99	-0.51	-0.58
EC			1.00	-0.71	0.49	-0.67	0.66	0.97	0.45	0.51
OC				1.00	0.27	-0.04	-0.99*	-0.53	0.31	0.24
N					1.00	-0.97	-0.33	0.67	0.99*	0.99*
P						1.00	0.11	-0.82	-0.96	-0.98
K							1.00	0.47	-0.38	-0.31
Ca								1.00	0.64	0.69
SD									1.00	0.99*
Col										1.00

*Correlation are significant at $p<0.05$

Age-Age (Years), EC- Electrical conductivity (cS cm-1), OC- Organic carbon %, N- Available nitrogen (Kg/ha), P- Available phosphorus (Kg/ha), K- Available potassium (Kg/ha), Ca- Available calcium (Kg/ha), SD- Spore density/25 g soil, Col- AM fungal colonization (%)

Discussion

This is one of few studies of AM fungal colonization in rubber plantations and it is the first study of AM fungal morphology and comparison of AM fungal diversity in rubber plantations considering the importance of the host species worldwide.

Rubber plantations, being a closed ecosystem, recycles enormous biomass through litter decomposition which takes place rapidly thus producing considerable organic carbon in surface

layers (Krishnakumar et al. 1991). The high contribution of organic carbon in 5 years old plantation may be due to more litter accumulation by annuals and herbaceous plant communities present in it.

There was a positive correlation between AM fungal colonization with spore density, in accord with an earlier report (Songachan et al. 2011). There is no correlation found between age and spore density. The obtained data exhibited a positive correlation between spore density with available nitrogen. Nitrogen plays an important role in influencing the mycorrhizal formation and functions mainly through changes in soil pH and thereby suppressing or stimulating root colonization and spore production of AM fungi (Syliva & Neal 1990). It is assumed that under such conditions differences in spore community composition is not due to site-specific sporulation behaviour of the AM fungi but reflects the presence or absence of sporulating AM fungi at the specific site (Feldmann et al. 1995). AM fungi species composition and spore density are highly variable and influenced by plant characteristics and some environmental factors (Boddington & Dodd 1999). Agricultural management practices, environmental conditions might affect AM fungal communities both qualitatively and quantitatively (Sieverding 1990, Miller et al. 1995).

Table 4 Relative abundance of arbuscular mycorrhizal fungi isolated from rubber plantations.

AM fungi	5 years old	10 years old	30 years old
Acaulospora sp. 1	1.02	3.16	0.00
Acaulospora sp. 2	0.00	1.05	0.00
Ambispora sp. 1	1.02	0.00	0.00
Glomus clavisporum	3.06	1.05	0.00
G. multiculae	23.47	13.68	30.77
G. macrocarpum	0.00	3.16	0.00
Glomus sp. 1	31.63	43.16	34.62
Glomus sp. 2	22.45	24.21	14.10
Glomus sp. 3	11.22	8.42	16.67
Glomus sp. 4	5.10	0.00	0.00
Glomus sp. 5	0.00	2.11	3.85
Unidentified species	1.02	0.00	0.00
	100.00	100.00	100.00

Table 5 Diversity indices of AM fungi isolated from rubber plantations.

Years	Shannon-Wiener	Evenness	Simpson's index
5	1.68	0.77	0.21
10	1.58	0.72	0.27
30	1.43	0.89	0.25

Work on AM fungi of rubber plant of Tripura was done earlier by Deka et al. (1998) but the site of their study (Mohanpur, Agartala) was different. The results of their study showed the AM fungal spore population at horizontal, and vertical distribution from the base of the tree, and they

isolated *Glomus* spp. and *Gigaspora* sp. In contrast, *Gigaspora* sp. was not found in the present study. Ikram & Mahmud (1984) identified different spore types of *Glomus* sp., *Acaulospora* sp. and *Sclerocystis coremiodes* in rubber growing soils of Malaysia. Similarly, Jayaratne (1982) identified eleven species of AM fungi from rubber growing soils of Sri Lanka. The genus *Glomus* was highly abundant in all the plantation sites. The reports of earlier workers also revealed that *Glomus* is the dominant genus occurring in Indian soil (Muthukumar & Udaiyan 2000, Bhattacharya & Bagyaraj 2002). Das & Kayang (2009) also reported the dominance of the *Glomus* from North-East region of India.

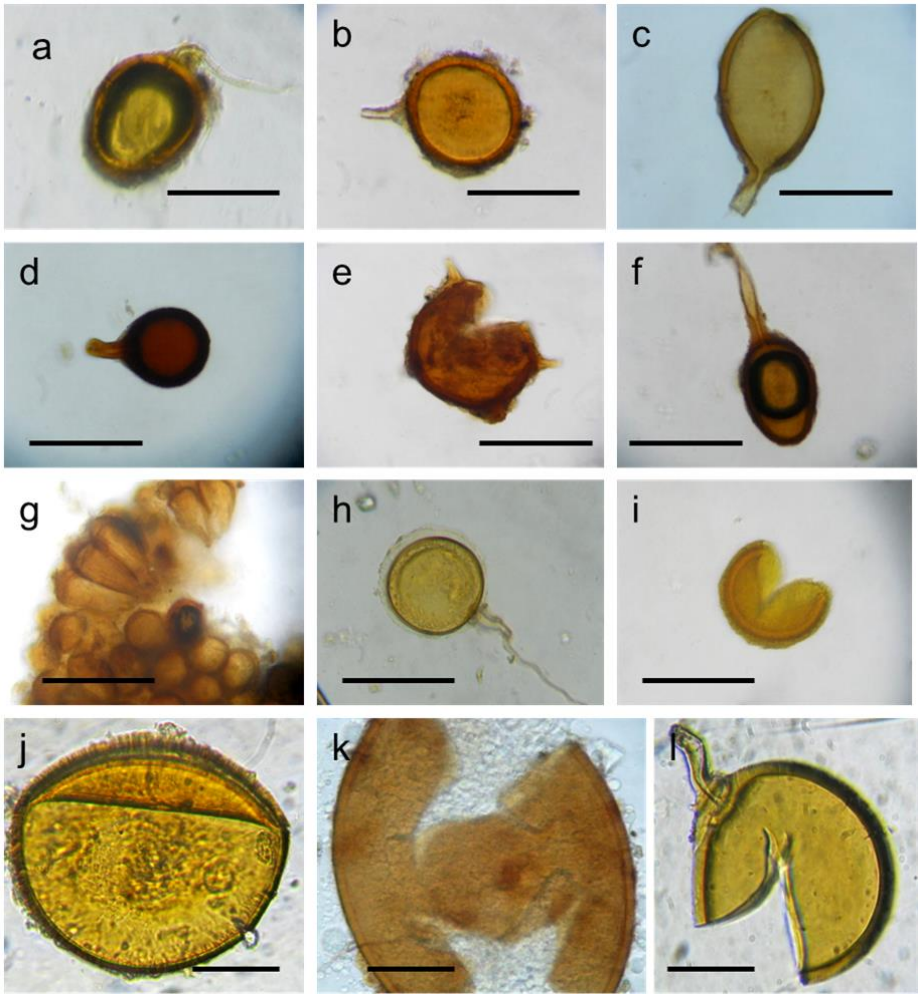

Fig 2 – Arbuscular mycorrhizal fungi isolated from rubber plantations. (a) *Glomus* sp. 1 (b) *Glomus* sp. 2 (c) *Glomus* sp. 3 (d) *Glomus* sp. 4 (e) *Glomus multiculae* (f) *Glomus* sp. 5 (g) *G. clavisporum*, (h) Unidentified species, (i) *Ambispora* sp. 1, (j) *Acaulospora* sp. 1, (k) *Acaulospora* sp. 2 and (l) *Glomus macrocarpum*. Scale bars: a–f , h, I = 50 µm, g, j–l = 100 µm.

The diversity of AM fungi observed in the present study was slightly decreasing with the age of the rubber plantations. The diversity of AM fungi in 30 year old rubber plantation reported earlier (Debnath et al. 2014) was found to be almost similar to our findings. The diversity of AM fungi was similar in most of the coffee plantation systems (Arias et al. 2012). Helgason et al. (1998) found that woodlands had a much higher AM fungal species richness and diversity compared to agricultural land. Chifflot et al. (2009) found that AM fungal diversity was higher and spatial distribution of spores was significantly different in a tree-based intercropping site compared to a monoculture forest plantation system. Tree-based intercropping may have a positive effect on increased abundance and diversity of AM fungi (Lacombe et al. 2009, Chifflot et al. 2009). However, intercropping is not practiced in rubber plantations of Tripura. Talapatra et al. (2015)

reported the mycorrhizal colonization of understorey plant communities in rubber plantations of Tripura. The understorey plant communities may have a role in influencing the diversity of AM fungi in the present study.

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