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Seasonal Dynamics of Arbuscular Mycorrhizal Fungi (AMF) in Forest Trees of Chittagong University Campus in Bangladesh

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Abstract

Status of Arbuscular Mycorrhizal (AM) colonization in seven tree species (*Albizia saman, Acacia auriculiformis* A. Cunn. ex Benth., *Albizia lebbeck, Chickrassia tabularis* A. Juss., *Eucalyptus camaldulensis* Dehnn., *Gmelina arborea* (Roxb) DC, *Swietenia macrophylla* King.) collected from the hilly areas of Chittagong University (CU) was investigated. Roots and rhizosphere soil samples were collected in different seasons (pre-monsoon, monsoon and post monsoon). Percentage of AM colonization in root and number of spores/100 gm dry soil were assessed. The result of the investigation reveals that the intensity and percentage of AM colonization varied in different forest tree species in different seasons. In this study, maximum AM colonization and spore population were found in pre-monsoon and minimum were in monsoon season. The intensity of colonization was maximum in *C. tabularis* (74.43%) in pre-monsoon, *A. lebbeck* (69.45%) in monsoon and *S. macrophylla* (67.8%) in post monsoon seasons and minimum in *A. auriculiformis* (53.75%) during pre-monsoon, *A. saman* (24.4%) in monsoon and *A. saman* (19.36%) in post monsoon. The number of spores found per 100 g dry soil ranged between 164-376 during pre-monsoon, 27-310 during monsoon and 194-299 in post monsoon season. Out of six recognized genera of AM fungi, *Glomus, Sclerocystis, Entrophospora, Scutellospora, Acaulospora* and other unidentified spores were observed.

Key Words: Arbuscular Mycorrhizal fungi, colonization, rhizosphere, spore population, tree species

Introduction

Bangladesh is predominantly an agrarian country, depending mainly on agricultural crops and forest products for its economic development. It is one of the densely populated, developing countries in the world. The country has 2.60 million hectares (17.62%) of forest land of which 1.53 million hectares (10.37%) of land is under the purview of the Government's Forest Department. Forestry is one of the major sectors of renewable resources in Bangladesh, which contributes to the economic and ecological stability (Mridha and Xu 2001). The contribution of the forestry sector to the GDP is about 4 percent. Low tree coverage and the high population density are continually increasing the demand for tree products, such as timber, fuel wood, leaves, twigs, fruits and other non-wood products, leading to serious pressure on the forests and planted trees. We have to update techniques in order to maximize our forest production in minimum land. Because of economic and environmental constraints, it is necessary to develop least ex-

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pensive and technologically simple methodologies for immediate benefit. Mycorrhizal technology can be one of the alternatives to improve forest products, farm profitability and environmental quality in different production systems in Bangladesh (Auge et al. 1987a).

Arbuscular mycorrhizal technology, the most advanced, well balanced, and eco-friendly biotechnology has been considered worldwide for better management, survival and sustainability of the forest tree seedlings in the nutrient deficient soils of the tropical and subtropical countries (Klironomos et al. 2000; Bever et al. 2001; Burrows and Pfleger 2002a, 2002b; O'Connor et al. 2002). Arbuscular mycorrhizal fungi (AMF), their genetical and functional diversity in the forest ecosystems are of vital importance. Biodiversity of AMF can be decisive for both plant community structures and productivity (Klironomos et al. 2000; Bever et al. 2001; Burrows and Pfleger 2002a, 2002b; O'Connor et al. 2002). Different AM fungal species associated with the roots of different host species are important in influencing regeneration, diversity and the distribution of plant communities (Van der Haijden et al. 1998). Moreover, differences in spore production exhibited by different AM fungal species coupled with differential growth promotion and host association might increase the plant diversity through both niche diversification and negative feedback (Van der Haijden et al. 1998; Kiers et al. 2000; Bever 2002; Casteli and Casper 2003). Growth increases are usually accompanied by improved plant nutrient content and are attributed to enhanced mineral nutrition (Marschner and Dell 1994). They reduce the soil borne diseases or the effects of disease caused by fungal pathogens (Jalali and Jalali 1991; Singh et al. 2000). Some free-living nitrogen fixing bacterial genera *Azotobacteria, Beijerinkia, Clostridium, Pseudomonas* and *Azospirillum* have been reported to be more active in presence of AM fungi (Brown and Carr 1984; Mohandas 1987). Colonization of AM fungi can improve the drought resistance (Bethlenfalvay et al. 1988), change the elasticity; improve leaf water potentials and maintenance of stomatal openings and transpiration (Auge et al. 1987a, 1987b). Extra radical hyphae of AM fungi play an important role in creating stable soil aggregate structure (Tisdall and Oades 1982; Bethlenfalvay et al. 1988; Miller and Jastrow 1990).There are important interactions between mycorrhizal fungi and free-living microorganisms and specific microbial communities on rhizosphere and rhizoplane regions. Different microorganisms like fungi, plant growth promoting rhizobacteria (PGPRs) etc have been reported to affect the mycorrhizal fungi and vice versa (Kumar et al. 1995). Besides, mycorrhizal fungi stimulate the effects of microbes and plants retrieve the positive effects.

Although in Bangladesh there are different types of forest tree species, very little work about the status of Arbuscular Mycorrhizal Fungi (AMF) in forest tree species during pre-monsoon, monsoon and post monsoon season has been done in our country especially at the hilly areas of the Chittagong University Campus, Bangladesh. Also reports on mycorrhizal colonization of the different forest tree species of Bangladesh are very limited (Dhar and Mridha 2003; Rahman et al. 2003). Therefore the purpose of this study is to assess the occurrence of Arbuscular Mycorrhizal colonization in the roots and rhizosphere soils of different forest tree species in pre-monsoon, monsoon and post monsoon season at the hills of Chittagong University Campus, Bangladesh.

Materials and Methods

Study site

The study was conducted at the hilly areas of Chittagong University, Bangladesh. The area lies between about 22°27'30" and 22°29'0" North latitudes and 91°46'30" and 91°47'45" East longitudes (Anon 1989). The hills are low to medium high and slope ranges from gentle to steep (Anon 1979). Soils are yellowish brown to yellowish red loamy sand and weak to strong blocky. The sandy loam soil had moisture content around 25 percent and pH 5.6. The area is characterized by hot humid summer receiving about 90% rainfall of the year from May to October and dry season extending from November to next April, with mean annual rainfall of 287 cm and mean annual temperature of 26°C.

Sample collection and analysis

Fine roots and rhizosphere soils of different forest tree species *Acacia auriculiformis, Gmelina arborea, Eucalyptus camaldulensis, Chickrassia tabularis, Albizia lebbeck, Albizia saman* and *Swietenia macrophylla* from hills of Chittagong

University Campus in the pre-monsoon (March 2009-May 2009), monsoon (June 2009-Sep 2009), and post-monsoon (Oct 2009-Nov 2009) seasons with a soil corer from 0-15 cm depth. In order to confirm the fine root collection from the sample species, fine roots were collected by removing some portion of coarse roots from the upper surface of the same tree. Fine roots were gently separated from soils in the laboratory to avoid damaging the fine roots and gently washed to remove soil particles and soaked in distilled water. Then the roots were preserved in 5% formalin for future use. For spore extraction from the soil, the collected soils were sieved with 2 mm sieve to remove the gravels and other particles. To avoid the damage or desiccation of the spores, soils were assessed immediately after collection. From each sample, 100 gm of soils were taken in a bucket of 5-litre capacity and 1 litre of water was mixed with the soils. The soils were mixed well with water by the soft pressure of the thumb and index fingers and a soil-water suspension was made. The suspension was left for five minutes for settle down of insoluble and heavy particles. The suspension was passed through the 400μm, 240 μm, 100 μm and 60 μm. sieves gradually to extract the spores following by wet sieving and decanting method (Gerdemann and Nicolson 1963). The supernatants on the sieves were taken separately in wash glasses and observed under stereo-binocular microscope at 10x2.5 magnifications. Root and soil sample of each species were collected and analysed separately in three seasons. Larger spores, sporocarps and any structures resembling AM spores were separated from the supernatants of the sieves of 400 μm and 240 μm by soft forceps. Relatively smaller spores were collected in a wash glass from the 100 μm and 60 μm sieves with water. The suspension of water and spores were filtrated by the Whatman filter paper. Squares were drawn formerly on the filter paper by intersecting lines for easy counting of spores. Total number of spores for each species were counted 100 gm dry soil basis, as the sum of larger and smaller spores and sporocarps if found. Spores were identified according to Morton and Benny (1990) and Schenck and Perez (1990) after mounting on Melzer's reagent and PVLG. Roots preserved in 5% formalin were washed well to remove the formalin and chopped into 1-cm pieces. Clean root samples were cleared in 10% KOH solution for 10 min at 85-90°C and deeply pigmented roots were treated in 10% H2O2 at room temperature for 10 min, stained with 0.05% aniline blue solution at 90°C for 90 min, and then stored in glycerol solution (Phillips and Hayman 1970) with some modifications. A total of 20 segments from each tree species (total 140 segments for seven tree species in each season) were examined in each season. Roots segments were observed by a compound microscope at 10x10 magnification. The assessment of mycorrhizal infection was done by the slide method. Root segments were selected randomly from the stained samples and observed for the presence or absence of functional structures (Mycelium, Arbuscules and Vesicles) of AM fungi. Percent root colonization was calculated (Dhar and Mridha 2003). Presence of mycelium was regarded as the AM positive and total mycelial colonization was treated as the total AM colonization. The intensity of mycelial, vesicular and arbuscular colonization were recorded as poor, moderate and abundant (Dhar and Mridha 2003). The colonization by AM fungi was calculated using the following formula:

$$\% \text{ colonization} = \frac{\text{Total no. of root segments colonized}}{\text{Total no. of root segments studied}} \times 100$$

Total percent AM colonization, percent colonization of different AM fungal structures (mycelium, vesicles and arbuscules) and intensity of AM structural colonization were calculated.

Mycelial colonization was considered as total AM colonization and the intensity of structural colonization was recorded as poor, moderate and abundant (Dhar and Mridha 2003).

Results

Arbuscular mycorrhizal colonization

AM mycorrhizal root infection in tree species in different seasons is shown in Table 1. Seven forest tree species were collected and their AM root colonization was studied. Table 1 indicates the prevalence of AM fungal association in all the tree species and they were not found to form same status of mycorrhizal colonization. The range of colonization varied from species to species and season to season. AM infection was found less during monsoon compared to pre-monsoon and post monsoon season. The highest aver-

age infection was 74.43% in *C. tabularis* and the lowest infection was 53.75% in *A. auriculiformis* in pre-monsoon season. The range of colonization was 53-75 % in pre-monsoon season. In the case of colonization in monsoon season, *A. lebbeck* showed the maximum AM colonization 69.45% and *A. saman* showed the minimum AM colonization 24.4%. The range of colonization was 24-69% during monsoon season. But the highest average infection was observed in *S. macrophylla* (67.8%) and the lowest average infection was 19.36% in *A. saman*. The range of colonization was 19-67% in post monsoon season. The highest average AM root infection (65%) was observed in pre-monsoon season.

AM structural colonization was also different in different forest tree species in pre-monsoon season (Table 2). Poor, moderate and abundant intensity were recorded 44.31-68.1%, 12-28.2% and 1-19.4% for mycelia colonization; 6.1-27.1%, 0.5-3% and 0% for vesicular colonization and 1.8-5%, 0% and 0% for arbuscular colonization respectively.

Table 1. % of VA mycorrhizal root infection in different seasons in different tree species collected from Chittagong University campus

Species	Total % of AMF infection		
	(Pre-monsoon)	(Monsoon)	(Post monsoon)
Albizia saman	57.31	24.4	19.36
Acacia auriculiformis	53.75	35.54	39.4
Albizia lebbeck	73.34	69.45	66.12
Chickrassia tabularis	74.43	53.32	57.78
Eucalyptus camaldulensis	57.7	33.2	56.60
Gmelina arborea	69.3	48.18	40.9
Swietenia macrophylla	69.3	27.6	67.8

Table 2. Intensity of arbuscular mycorrhizal structural colonization in different forest trees in pre-monsoon season

Species	Intensity of colonization (%)								
	Mycelium			Vesicle			Arbuscule		
	P	M	A	P	M	A	P	M	A
Albizia saman	44.31	12	1	6.1	0	0	0	0	0
Acacia auriculiformis	63.2	28.2	4.3	27.1	0	0	2.5	0	0
Albizia lebbeck	47	21.5	5.4	20	1	0	0	0	0
Chickrassia tabularis	57.11	14.3	2.7	9.2	0	0	5	0	0
Eucalyptus camaldulensis	52.16	25.4	19.4	11.1	0	0	5	0	0
Gmelina arborea	55.9	21	6.5	23	3	0	1.8	0	0
Swietenia macrophylla	68.1	22.1	4.3	20	0.5	0	0	0	0

P, Poor; M, Moderate; A, Abundant.

Table 3. Intensity of arbuscular mycorrhizal structural colonization in different forest trees during monsoon season

Species	Intensity of colonization (%)								
	Mycelium			Vesicle			Arbuscule		
	P	M	A	P	M	A	P	M	A
Albizia saman	21.6	2.7	0	0	0	0	0	0	0
Acacia auriculiformis	18.2	8.8	0	10	0	0	1	0	0
Albizia lebbeck	22	8.2	0.5	10	0	0	1.5	0	0
Chickrassia tabularis	36.6	13.8	1.6	0	0	0	0	0	0
Eucalyptus camaldulensis	26.5	6.5	0	0	0	0	0	0	0
Gmelina arborea	34.9	12.6	0.5	5	0	0	5	0	0
Swietenia macrophylla	22.6	4.9	0	0	0	0	0	0	0

P, Poor; M, Moderate; A, Abundant.

Table 4. Intensity of arbuscular mycorrhizal structural colonization in different forest trees during post monsoon season

Species	Intensity of colonization (%)								
	Mycelium			Vesicle			Arbuscule		
	P	M	A	P	M	A	P	M	A
Albizia saman	25.9	4.8	0	15	0	0	0	0	0
Acacia auriculiformis	25.4	10.9	2.7	24	0.5	0	0	0	0
Albizia lebbeck	42.71	16.01	8.8	29.1	7.2	1	0	0	0
Chickrassia tabularis	34.9	14.9	8.1	47	14	0.5	0	0	0
Eucalyptus camaldulensis	41	13.8	1.5	30.6	8.6	0	0	0	0
Gmelina arborea	32.2	8.8	0	15	1	0	0	0	0
Swietenia macrophylla	42.1	16	6.5	17.3	0	0	0	0	0

P, Poor; M, Moderate; A, Abundant.

Table 5. VA mycorrhizal spore populations in different tree species and Mean spore density (spore 100-1 gm dry soils) in different seasons

Species	Total spore population/100 gm dry soils		
	(Pre-monsoon)	(During monsoon)	(Post monsoon)
Albizia saman	376	206	219
Acacia auriculiformis	242	33	206
Albizia lebbeck	325	175	241
Chickrassia tabularis	269	310	299
Eucalyptus camaldulensis	164	125	194
Gmelina arborea	308	271	203
Swietenia macrophylla	265	27	258
Mean spore density (spore 100^{-1} gm dry soils)			
Mean spore density	278	164	231

AM structural colonization was also different in different forest tree species during monsoon season (Table 3). Poor, moderate and abundant intensity were recorded 18.2-36.6%, 2.7-13.8% and 0.5-1.6% for mycelia colonization; in some species, poor colonization 5-10% for vesicles and 1-5% for arbuscules were also recorded during monsoon season.

AM structural colonization was also different in different forest tree species during post monsoon season (Table 4). Poor, moderate and abundant intensity were recorded 25.4-42.71%, 4.8-16.01% and 1.5-8.8% for mycelia colonization; 15-47%, 0.5-14% and 0.5%-1% for vesicular colonization and there were no arbuscular colonization during post monsoon season.

Arbuscular mycorrhizal spore population

Total population of AM fungal spores was counted and recorded 164-376/100 g dry soil in pre-monsoon season, 27-310 in monsoon season and 194-299 in post monsoon season (Table 5). The highest was recorded with *A. saman* (376) in pre-monsoon, *C. tabularis* (27) during monsoon and *C. tabularis* (299) in post monsoon season and the lowest was with *E. camaldulensis* (164) in pre-monsoon, *S. macrophylla* (27) during monsoon and *E. camaldulensis* (194) in post monsoon season. Spore population was less during monsoon season compared to pre-monsoon and post monsoon season. *Glomus* was dominant in all the species in all the seasons. Out of six recognized genera of AM fungi, *Glomus, Acaulospora, Sclerocystis, Entrophospora, Scutellospora* and other unidentified spores were observed. Sporocarp of some spores was also found in the rhizosphere soils of these species. The mean spore density was higher in the pre-monsoon season compared to monsoon and post monsoon seasons (Table 5).

Discussion

The present result indicated that the studied seven forest tree species are mycorrhizal, but their root infection and intensity of infection of AM fungi differed from species to species and season to season. In this study, maximum AM colonization and spore population were found in pre-monsoon season and minimum were in monsoon season which

is similar with the study conducted by D'Souza and B. F. Rodrigues (2012) on seasonal diversity of AMF in mangroves of Goa, India where maximum spore density and AM species richness were recorded in the pre-monsoon season, while minimum spore density and richness were observed during monsoon season. Gerdemann (1975) discussed the occurrence of VA mycorrhiza in different tropical plant species to a great length. A perusal of the biodiversity of the structural colonization reveals that all these tree species is more or less arbuscular mycorrhizal fungal infected plant. The pattern of arbuscular mycorrhizal colonization was different among the tree species studied. The results are in consistent with that of Saif (1977).

These differences might be due to the presence of diverse type of AMF in the rhizosphere soils of individual plant species and might be a manifestation of greater host susceptibility to AMF (Mehrota 1998), variation of water availability (Kiran et al. 1989; Mohan et al. 1995) and other unknown factors. The spore number and root colonization recorded by Mohan (1996) in different tree species grown in the nursery were almost similar with the record of the selected tree species of our study.

Spore density showed variation, maximum in pre-monsoon and minimum in the monsoon season. Similar observations have been reported in earlier studies (S.S. Dhillion and R.C. Anderson 1993), (D'Souza, James and Rodrigues, Bernard Felinov 2012) and (J.N. Gemma et al. 1989). Higher spore density in pre-monsoon season is thought to be an indication of root senescence and available nutrients, stimulating fungal sporulation as plant nutrient requirement is reduced (Hetrick 1988; Bentivenga and Hetrick 1992). Higher AM spore density in pre-monsoon may also be attributed to soil temperature, as previous studies by D.S. Hayman, 1970 and A. Saravanakumar et al. 2008 suggested that high soil temperature favours AM fungal sporulation. The decline in the spore population in monsoon season may be due to depletion of nutrient status, pH, soil moisture, low microbial activity and stress conditions (Hetrick 1988).

Of the AMF species recorded in this study, *Glomus* spp. was the most common AMF but *Acaulospora* spp, *Entrophospora. Sclerocystis, Scutellospora* and other unidentified spores were also observed. Nure Ferdousee et al. 2012 also observed *Glomus* spp., *Acaulospora* spp. and *Entrophospora* spp. as most common AMF at the forest tree species in Chittagong university campus. The present findings are in agreement with the numerous reports on the wide spread occurrence of *Glomus* spp. throughout the world (Gerdemann and Trappe 1974; Blaszkowski 1989; Talukdar and Germida 1993). The presence or absence of host plant, nutrient availability, soil aeration, soil moisture content, altitude are the most important factors responsible for the fluctuation among the locations and seasons (Janos 1987; Hetrick 1988; Barea 1991).

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