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Diversity of Arbuscular Mycorrhizal Fungi Associated in a Mixed Natural Forest of Jeypore, Assam

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ABSTRACT

A study was conducted to investigate the diversity of arbuscular mycorrhizal fungi (AMF), spore population in rhizosphere soils and root colonization with trees in different seasons at Jeypore Reserve Forest, Assam, India. All 10 selected tree species had an AMF association with a varied range of root colonization and spore count in rhizosphere soil. Maximum percent root colonization was recorded in the rainy season (23-61%) which gradually declined in winter (13-58%) and was minimum in winter (11-40%), irrespective of the host species. Correspondingly, the rainy season recorded highest AMF spore count in rhizosphere soils (15-42) followed by winter (11-32) and least in summer (8-21). Based on morphological characteristics, 11 AMF species were recorded, representing four genera, viz. *Glomus* (5 spp.), *Acaulospora* (4 spp.), *Gigaspora* (1 sp.) and *Sclerocystis* (1 sp.), in which *Glomus* and *Acaulosporai* were found to be the dominant genera.

Keywords: AMF, percent root colonization, spore count
Abbreviations: AM, arbuscular mycorrhizae; **AMF**, arbuscular mycorrhizal fungi; **VAM**, vesicular arbuscular mycorrhiza

INTRODUCTION

Plant roots are colonized by many types of fungi (Parbery 1996), a sub-group being the arbuscular mycorrhizal fungi (AMF) which are associated with numerous plants (Harley and Harley, 1987). AMF belongs to the group Glomeromycota (Schubler *et al.* 2001) and play an important role in improving soil fertility. They are also plant growth enhancers and impart protection to plants against diseases (Bagyaraj and Varma 1995; Abas-Ali *et al*. 2007; Bagyaraj 2007; Smith and Read 2008; Mahmood and Rizvi 2010). These fungi form a symbiotic relationship with plant roots and supply nutrients and moisture to the host (Ferrol *et al*. 2004; Muthukumar *et al*. 2004; Mathur and Vyas 2007). AMF colonize root tissues bio-trophically and form an extensive network of extra-radical mycelia, providing a direct physical link between soil and plant roots (Smith and Read 1997). Dark septate endophytes (DSE) are a miscellaneous group of ascomycetous fungi that colonize roots of many plants, both intra- and intercellularly (Christie and Nicolson 1983; O'Dell *et al*. 1993; Ahlich and Sieber 1996; Jumpponen 2001). These fungi were first described as Mycelium radicis atrovirens (Smith and Read 1997) and were found to melanize hyphae and colonized the roots. Different species of AMF are an essential component of the ecosystem and have been reported to induce different growth patterns and biomass production in plants (Heijden *et al.* 1998; Scheublin *et al*. 2004). Although they prefer certain hosts, they are not usually host specific and have a wide host range (Abbott and Robson 1982; Ahmad 2004; Gracy and Bagyaraj 2005). Mycorrhizal diversity is greatly affected by changes in the rainfall, temperature and altitude of the locality (Adriano-Anaya *et al.* 2006; Gaur and Kaushik 2011).

Generally, chemical fungicides are used to control the disease but they are not economical; moreover, they constitute an eco-hazard. AM fungi have been reported to reduce disease infection in plants (Bharadwaj and Sharma 2006). Fungi like *Glomus mosseae*, *Glomus fasciculatum* and *Rhizobium leguminosarum* biovar *phaseoli* are considered important bio control agents (Wafaa *et al.* 2001; Aysan and Demir 2009; Askar Rashad 2010; Aysel and Semra 2011). The aim of the present work was to assess the species diversity of AM fungal species in an evergreen natural forest of Jeypore, Assam, India.

MATERIALS AND METHODS

Study site

The Jeypore Reserve Forest, with an area of 108 km^2, is located in Dibrugarh District of Assam, and which lies between 27°6′-27°16' N and 95°21′–95°29′ E longitude at an elevation of 1100-2600 m, was selected as the study site. The climate of the study site is humid tropical characterized by high rainfall and high humidity (up to 90%). The annual mean precipitation in the last three years ranged from 3600 to 5500 mm of which 82% is received during the monsoon season from May to August and 17% during dry periods from September to March. Mean ambient temperature is 27°C. The root and rhizosphere soil samples of 10 plants viz., *Shorea assamica* Dyer., *Dipterocarpus retusus* Blume., *Litsea salicifolia* Roxb., *Terminalia myriocarpa* Heurck and Muell., *Syzygium cumini* L., *Gmelina arborea* Roxb., *Begonia roxburghii* A.DC., *Mesua ferrea* L., *Vatica lanceaefolia* Bl. and *Baccaurea remiflora* Lour., belonging to eight families (Dipterocarpaceae, Lauraceae, Combretaceae, Myrtaceae, Vervenaceae, Begoniaceae, Guttiferrae and Phyllantsaceae, respectively) were collected.

AMF spore isolation, enumeration and identification

A total of 150 soil samples were collected from the rhizosphere of selected tree species from a depth of 5-30 cm during mid-May, late July, and early September in 2009-10. The samples (about 500 g for each) were air-dried for 2 weeks and stored in sealed plastic bags at 4°C. AMF were isolated by a wet sieving and decanting technique (An *et al*. 1990; Gerdemann and Nicholson 1963; Singh

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Research Note

Table 1 Seasonal variation of arbuscular mycorrhizal association in mixed natural forest of Joypur Assam in 2009-10.

Plant	Family	% Root colonization			No. of spores/50 g of soil		
		Rainy	Winter	Summer	Rainy	Winter	Summer
Begonia roxburghii	Begoniaceae	51	32	29	32	21	14
Dipterocarpus retusus	Dipterocarpaceae	49	31	24	39	27	19
Litsea salicifolia	Lauraceae	23	26	21	34	29	13
Terminalia myriocarpa	Combretaceae	22	30	26	42	22	18
Syzygium cumini	Myrtaceae	58	34	40	31	21	13
Gmelina arborea	Vervenaceae	32	58	32	15	32	21
Shorea assamica	Dipterocarpaceae	61	44	24	15	22	16
Mesua ferrea	Guttiferae	47	23	11	16	11	14
Vatica lanceaefolia	Dipterocarpaceae	58	37	24	38	27	13
Baccaurea remiflora	Phyllanthaceae	44	13	19	24	11	8

Table 2 Identified arbuscular mycorrhizal fungi and their occurrence frequencies.

Arbuscular mycorrhizal fungi	Absolute occurrence	Relative occurrence /frequency (%)
	143	**41.45**
Acaulospora bireticulata Rothw. & Trappe	13	3.77
Acaulospora denticulata Sieverding & Toro	82	23.77
Acaulospora foveata Trappe & Janos	33	9.57
Acaulospora scrobiculata Trappe	15	4.35
	193	**55.94**
Glomus claroideum Schenck & Smith	36	10.43
Glomus clarum Nicol. & Schenck	20	5.80
Glomus constrictum Trappe	27	7.83
Glomus fasciculatum (Thaxter) Gerd. & Trappe	13	3.77
Glomus monosporum Gerd. & Trappe	97	28.12
	3	**0.87**
Gigaspora gigantea (Nicol. & Gerd.) Gerd. & Trappe	3	0.87
	6	**1.74**
Sclerocystis coremioides Berk. & Broome	6	1.74
Total: AMF = 11 species	**345**	**100**

and Tiwari 2001). Fifty grams of soil was suspended in 250 ml of water, stirred with a magnetic stirrer for 10 min and then sieved. Spores and debris were collected on 40, 70, 100 and 150 µm sieves with tap water, filtered through filter paper and placed in a 9 cm Petri-dish for examination under a binocular stereomicroscope (Olympus BX 50F4, Japan) Each type of AMF spore was sequentially mounted in water, lactophenol, polyvinyl alcohol and Melzer's reagent (Morton 1988; Morton and Benny 1990) for identification. The spores were identified up to the species level with the help of a VAM fungi identification manual (Schenck and Perez 1990). The identification was based on spore colour, size, surface ornamentation and wall structure with reference to the descriptions and pictures provided by the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu) and originally published species descriptions.

Analysis of AMF and DSE colonization

Roots were washed thoroughly in tap water and cut into approximately 1-cm long segments. The roots were cleared in 10% (w/v) KOH by heating at 90°C for 1 to 2 h, depending on the degree of lignifications of the roots, then washed and stained with stamp pad ink (Das and Kayang 2008). The stained root samples were mounted on slides and examined for AM colonization under a light microscope. The colonization of root length with arbuscules, vesicles, hyphae and dark septate endophytes per sample were quantified by the magnified intersections method (McGonigle *et al.* 1990). Percent root colonization was determined using the following formula:

$$\% \text{ Root Colonization} = \frac{\text{Number of positive segments}}{\text{Number of segments observed}} \times 100$$

RESULTS AND DISCUSSION

All of the selected tree species exhibited an AM association and the colonization in roots and spore population in the rhizosphere soil revealed a wide range of variation in different seasons (**Table 1**). Percent root colonization and mycorrhizal spore counts steadily increased during the rainy season, which recorded the highest infection (61%) with *Shorea assamica* and the least count (22%) with *Terminalia myriocarpa*, while in summer the count was highest (40%) with *Syzygium cumini* and lowest (11%) with *Measua ferrea.* In winter, maximum root colonization (58%) was observed with *Gmelina arborea* and minimum (13%) with *Baccaurea remiflora.*

The spore count in 50 g of rhizosphere soil also recorded the highest values in the rainy season followed by winter and summer, respectively. In the rainy season, the highest spore count was 42 for *Terminalia myriocarpa* and least was 15 for *Gmelina arborea* and *Shorea assamica.* In winter, however, the lowest count (32 spores) was for *Gmelina arborea* and the minimum spore count (11) was for *Measua ferrea* and *Baccaurea remiflora.* During summer, the highest spore population of 21 spores was recorded for *Gmelina arborea* and the least (8 spores) was recorded for *Baccaurea remiflora.* The variable spore levels are likely due to their differential capacity of each AMF species to sporulate (Bever *et al.* 1996). Several factors can affect spore density and species richness in the host rhizosphere. Previous researchers also reported variable spore counts with different plant species (Chaurasia *et al*. 2005; Shi 2007; Susana *et al.* 2008). AMF diversity depends on season, climatic conditions, host, age of host and soil type (Zhao1999; Zhao *et al*. 2001; Yang *et al*. 2010).

The 11 AMF species identified belong to four genera: *Acaulospora bireticulata*, *A. denticulata*, *A. foveata* and *A. scrobiculata*; *Gigaspora gigantean*; *Glomus claroideum*, *G. clarum*, *G. constrictum*, *G. fasciculatum* and *G. monosporum*; and *Sclerocystis coremioides*. The frequency of occurrence of the four genera was 41.45, 0.87, 55.94 and 1.74%, respectively (**Table 2**). *Acaulospora* and *Glomus* were the dominant genera while *A. denticulata*, *A. foveata*, *G. claroideum*, *G. clarum*, *G. constrictum* and *G. monosporum* were the dominant AMF associated with selected tree species of Joypur Reserve Forest, Assam (**Table 2**). The occurrence of dominant AMF with more than one tree species indicates a non-host specific relationship. Little AMF biodiversity was also found in a tropical rainforest of

Xishuangbanna, southwest China, possibly because of the non-host-specific nature of AMF (Zhao *et al.* 2003).

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