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Observations on arbuscular mycorrhiza associated with important edible tuberous plants grown in wet evergreen forest in Assam, India

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ABSTRACT

Kumar R, Tapwal A, Pandey S, Rishi R, Borah D. 2013. Observations on arbuscular mycorrhiza associated with important edible tuberous plants grown in wet evergreen forest in Assam, India. Biodiversitas 14: 67-72. Non-timber forest products constitute an important source of livelihood for rural households from forest fringe communities across the world. Utilization of wild edible tuber plants is an integral component of their culture. Mycorrhizal associations influence the establishment and production of tuber plants under field conditions. The aim of present study is to explore the diversity and arbuscular mycorrhizal (AMF) colonization of wild edible tuber plants grown in wet evergreen forest of Assam, India. A survey was conducted in 2009-10 in Sunaikuchi, Khulahat, and Bura Mayong reserved forest of Morigaon district of Assam to determine the AMF spore population in rhizosphere soils and root colonization of 14 tuberous edible plants belonging to five families. The results revealed AMF colonization of all selected species in all seasons. The percent colonization and spore count was less in summer, moderate in winter and highest in rainy season. Seventeen species of arbuscular mycorrhizal fungi were recorded in four genera viz. *Acaulospora* (7 species), *Glomus* (5 species), *Sclerocystis* (3 species) and *Gigaspora* (2 species).

Key words: AMF, root colonization, wild edible tuber

INTRODUCTION

Wild edible plants refer to species that are neither cultivated nor domesticated, but available from their natural habitat and used as source of food (Beluhan and Ranogajec 2010). They are collected by forest fringe communities for their requirement of food and livelihoods. Earlier works have reported the wild edible plants as a potential source of nutrition and many of them have higher nutrition than conventionally eaten crops (Grivetti and Ogle 2000). Arbuscular mycorrhizal fungi (AMF) colonize the roots of higher plant as obligate symbionts, where the host generally benefited through increased nutrient uptake, improved growth and better survival (Linderman 1994; Akhtar and Siddiqui 2007; Smith and Read, 2008). Soil is characterized by the presence of a diverse population of microorganisms of which mycorrhizal fungi constitutes one of important component. Arbuscular mycorrhizal (AM) fungi are the most common types among all mycorrhizae and represent a major group of soil microbial community (Linderman 1992). Arbuscular Mycorrhiza is a widespread mutualistic symbiosis between land plants and fungi belonging to the phylum Glomeromycota. Their occurrence as root symbionts has been reported from exceptionally wide range of plants (Sharma et al. 2007). The AMF association may also increase the tolerance of host plant against biotic (Hol and Cook 2005; Akhtar and Siddiqui 2007) and abiotic stresses, including salinity and drought (Cartmill et al. 2007). In modern years, AM fungi gained considerable importance in horticulture, agriculture, afforestation and land reclamation (Javot et al. 2007) because of their potentially to improve growth and yield of the plants by increasing the nutrient uptake (Jensen 1984). AM fungal association found in all organs of plants which are concerned with the absorption of substances from the soil (Srivastava et al. 1996). The occurrence of AM fungi association with the portions other than roots was reviewed by Nazim (1990). Presence of AM association has been reported in tubers of *Pueraria tuberosa* (Willd.) DC (Rodrigues 1996), *Colocasia esculenta* (L.) Scott (Bhat and Kaveriappa 1997), garlic bulbs (Kunwar et al. 1999) and tubers of *Gloriosa superba* L. (Khade and Rodrigues 2003). AMF colonization varies with season and its effects also influence the establishment of plants under field condition (Giovannetti and Nicolson 1983). Information on AM association with tuberous plants is scanty. Therefore, the present study is aimed to determine the AMF spore population in rhizosphere soils and its colonization for wild edible tuberous plants during different seasons in Sunaikuchi, Khulahat and Bura Mayong reserve forest of Morigaon district in Assam, India.

MATERIALS AND METHODS

Study area

The Sunaikuchi, Khulahat, and Bura Mayong Reserved Forests are situated in Morigaon district of Assam, India

between 26.15° to 26.5° Northern latitude and 92° to 95.5° Eastern longitude (Figure 1). These three Reserved Forest, (RF) of Morigaon district formed under Assam Forest Regulation Act, 1891. The area receives annual rainfall is about 1530.9 mm and the annual average maximum temperature is 30.4°C and the minimum is 19.8°C. Fringe area of the RF is inhabited by a few ethnic groups such as Karbis, Bodos, Kukis, Dimasas, Hmars, Garos, Rengma Nagas and Tiwas. These communities are dependent on forest for habitat and other needs for well-being; the forest contributes livelihoods to many households as well.

Target species

The root and rhizosphere soil samples of 14 wild edible tuberous plants belonging to five families were collected viz; *Ipomoea batatas* (L.) Lam., *Pueraria thomsonii* Benth., *Pueraria tuberosa* (Wild.) D.C, *Vigna vexillata* (L.) Rich., *Alocasia odora* (Roxb.) C. L. Koch, *Alocasia cucullata* Schott., *Colocasia esculenta* (L.) Schott., *Sagittaria sagittifolia* L., *Amorphophallus campanulatus* Roxb., *Dioscorea pentaphylla* L., *Dioscorea puber* (Bl.), *Dioscorea alata* L., *Dioscorea esculenta* Burk., *Dioscorea batatas* Decene (Figure 2), belonging to four families Fabaceae, Araceae, Araceae and Dioscoreaceae respectively) and studied.

AMF spore isolation, enumeration and identification

A total of 150 soil samples were collected from the rhizosphere of 14 plants species having tuber from a depth of 5-30 cm during mid-May, late July, and early September

Figure 1. Study sites at Sunaikuchi, Khulahat and Bura Mayong Reserved Forests (●) in Morigaon district of Assam, India

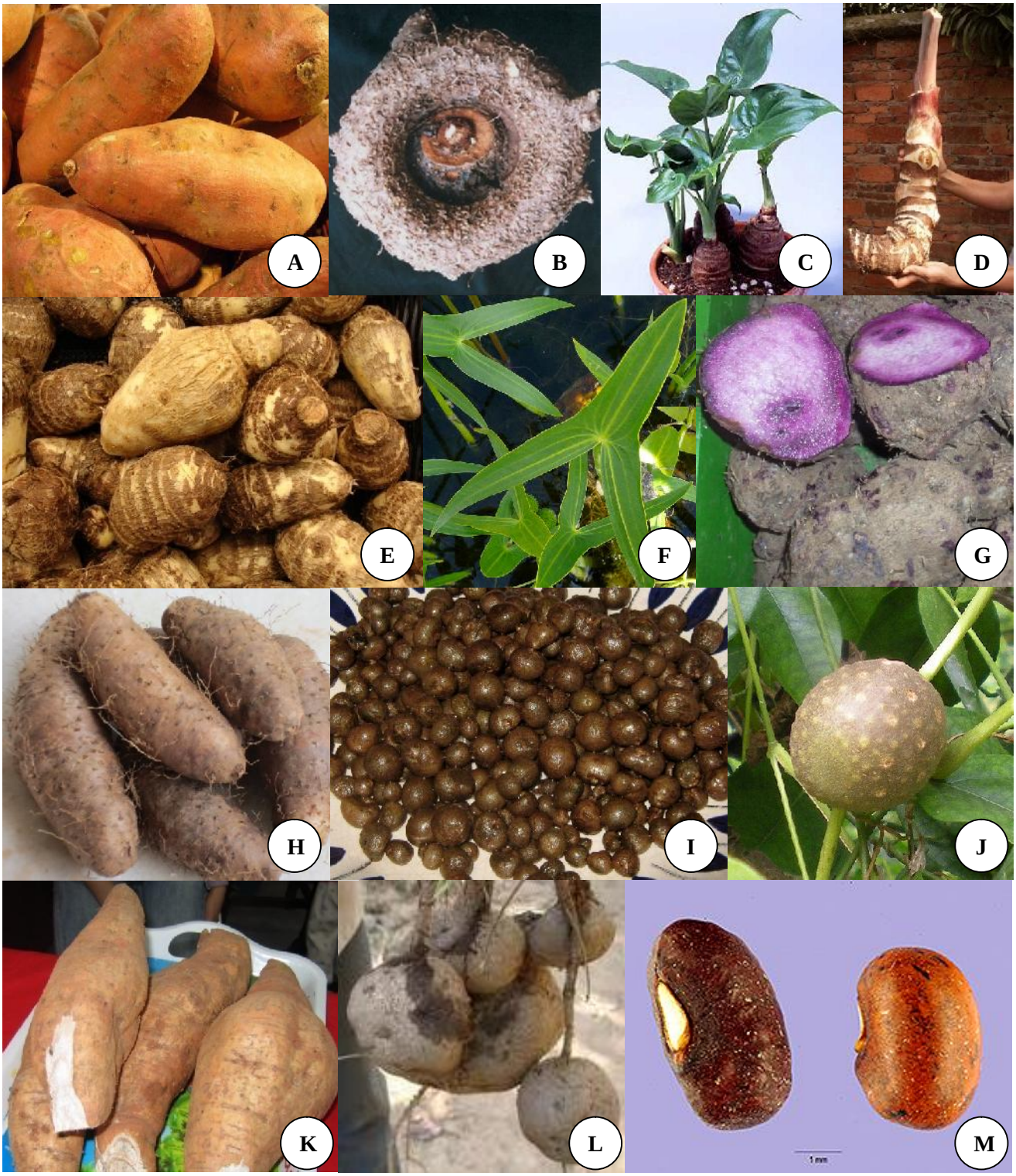

Figure 2. A. *Ipomoea batatas*, B. *Amorphophallus campanulatus*, C. *Alocasia cucullata*, D. *Alocasia odora*, E. *Colocasia esculenta*, F. *Sagittaria sagittifolia*, G. *Dioscorea alata*, H. *Dioscorea esculenta*, I. *Dioscorea batatas*, J. *Dioscorea pentaphylla*, K. *Pueraria thomsoni*, L. *Pueraria tuberosa*, M. *Vigna vexillata* (photos from many sources).

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 (Gerdemann and Nicholson 1963; An et al. 1990; Singh and Tiwari 2001). Fifty grams of soil was suspended in 250 ml of water, stirred with a magnetic stirrer for 10 min and sieved. Spores and debris were collected on 150, 100, 70 and 40 µm sieves under tap water, filtered through Whatman filter paper and placed in a 90 mm Petri-dish for examination under a binocular stereomicroscope (Olympus BX 50F4, Japan). Each type of AMF spore was sequentially mounted in water, lactophenol, Poly vinyl

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 color, 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 1cm 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:

$$\% \text{ Root colonization} = \frac{\text{No. of positive segments}}{\text{No. of segments observed}} \times 100$$

RESULTS AND DISCUSSION

Five-hundred and eighteen arbuscular mycorrhizal fungal spore samples were wet-sieved from the 150 soil samples. Seventeen species of arbuscular mycorrhizal fungi were identified. The morphological characters of some identified arbuscular mycorrhizal fungi are illustrated in (Table 1). All the fourteen plant species studied exhibited AM fungal association. AMF colonization in roots and the spore population in the rhizosphere soil samples of all fourteen plant species having tubers showed wide range of variation under different seasons (Table 1). The level of AM fungal association depends on root morphology, metabolism and rate of plant growth (Warmer et al. 1980). Percent root colonization and mycorrhizal spore counts steadily increased in rainy season. Earlier reports also revealed higher percent root colonization during rainy season (Raghupathy and Mahadevan 1993; Kumar et al. 2013). The maximum infection (73%) was recorded in *Sagittaria sagittifolia* whereas minimum infection (45%) in *Amorphophallus campanulatus* were observed during rainy season in 2009-10. However, the maximum percent colonization was (53%) in *Vigna vexillata* in winter and (51%) in summer only. In the present study, the percent root colonization recorded higher in rainy season than in winter and summer. Least activity of AM fungi in other seasons may be due to reduced translocation of carbohydrates towards the roots. The spore population was also least in summer and gradually increased in July. The spore population varied from 15-61 spores, 13-41 spores and 7-27 spores during rainy, winter and summer seasons respectively (Table 2). Khade and Rodrigues (2007) also observed maximum number of spore density while studying the occurrence of AM fungi in plants with underground storage organs. The identified species of arbuscular mycorrhizal fungi belonged to the genera of *Acaulospora* (7 species), *Glomus* (5 species), *Sclerocystis* (3 species) and *Gigaspora* (2 species).The occurrence frequency of the five genera was 42.62%, 36.67%, 12.92%, and 7.71%, respectively (Table 2). The results indicated that *Acaulospora* and *Glomus* were the dominant genera, and *A. denticulata*, *A. spinosa*, *A. tuberculata*, *G. clarum*, *G. constrictum* and *G. monosporum* and *S. clavispora* were the dominant species (Table-3). It is also observed that Acaulospora and *Glomus* species usually produce more spores than *Gigaspora* and *Sclerocystis* species in the same environment. This may be due to their smaller spore size and require a short time to produce spores (Hepper 1984; Bever et al. 1996).

Table 1. Important wild edible plants with tubers in Sunaikuchi, Khulahat, and Bura Mayong Reserved Forest, Assam in 2009-2010 and seasonal variation of arbuscular mycorrhizal association in wild edible tuberous plants

Name of plants	Family	Local name	% root colonization			No. of spores/ 50g of soil		
			Rainy	Winter	Summer	Rainy	Winter	Summer
Alocasia cucullata Schott.	Araceae	Panchamukhi Kachu	58	33	40	21	19	16
Alocasia odora (Roxb.) C.L. Koch	Araceae	Baibing	66	35	16	44	31	21
Amorphophallus campanulatus Roxb.	Araceae	Pani kachu	45	32	41	21	18	14
Colocasia esculenta (L) Schott.	Araceae	Kachu	66	42	34	26	19	18
Sagittaria sagittifolia L.	Araceae	Ole kachu	73	45	37	38	25	13
Ipomoea batatas (L.) Lam.	Convolvulaceae	Ranga alu, Mitha alu	53	41	22	44	31	14
Dioscorea alata L.	Dioscoreaceae	Kath alu	62	41	32	26	24	16
Dioscorea batatas Decene.	Dioscoreaceae	Gosh alu	66	38	31	39	25	19
Dioscorea esculenta Burk.	Dioscoreaceae	Mua alu	58	39	28	15	13	7
Dioscorea pentaphylla L.	Dioscoreaceae	Paspatia alu	51	40	32	22	18	13
Dioscorea puber BL	Dioscoreaceae	Jangali alu	67	38	35	24	23	9
Pueraria thomsonii Benth.	Fabaceae	Mayong (Mis), Pani alu	42	35	24	39	32	18
Pueraria tuberosa (Wild.) D.C	Fabaceae	Urahi alu	68	44	32	58	33	21
Vigna vexillata (L.) Rich	Fabaceae	Bonoria urahi	72	53	51	61	41	27

CONCLUSION

The study revealed that the plants with tubers growing in the tropical wet ever green forest of Sunaikuchi, Khulahat, and Bura Mayong Reserved Forest of Assam, India are colonized by arbuscular mycorrhizal fungi. It is also apparent that rainy season may considered as the best season for the propagation of plants by the application of AMF as bio-inoculants even for the plants of rare and threatened species. Our results also revealed that uneven spatial distribution (clumped distribution) of arbuscular mycorrhizal fungal spores and the complex below ground structure of tropical wet ever green forests are major factors that affect the spore density.

Table 2. Identified arbuscular mycorrhizal fungi and their occurrence frequencies

Arbuscular mycorrhizal fungi	Absolute occurrence	Relative occurrence/ frequency (%)
Acaulospora	221	42.62
Acaulospora bireticulata Rothw. & Trappe	16	3.08
Acaulospora denticulata Sieverding & Toro	67	12.93
Acaulospora foveata Trappe & Janos	18.4	3.55
Acaulospora mellea Spain & Schenck	19.4	3.74
Acaulospora scrobiculata Trappe	13.2	2.54
Acaulospora spinosa Walker & Trappe	53.3	10.28
Acaulospora tuberculata Janos & Trappe	33.7	6.50
Glomus	190	36.67
Glomus claroideum Schenck & Smith	20	3.86
Glomus clarum Nicol. & Schenck	83	16.02
Glomus constrictum Trappe	33	6.37
Glomus fasciculatum (Thaxter) Gerd. & Trappe	17	3.28
Glomus monosporum Gerd. & Trappe	37	7.14
Sclerocystis	67	12.92
Sclerocystis clavispora (Trappe) Almeida & Schenck	33	6.37
Sclerocystis coremioides Berk. & Broome 8 1.52	19	3.66
Sclerocystis sinuosa (Gerd. & Bakshi) Almeida	15	2.89
Gigaspora	40	7.71
Gigaspora gigantea (Nicol. & Gerd.) Gerd. &Trappe	19	3.66
Gigaspora margarita W.N. Becker & I.R. Hall	21	4.05
Total AMF = 17Species	518	100

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