

# **RESEARCH PAPER**

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# Diversity of vesicular and arbuscular mycorrhizal fungi in different land use systems

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# Abstract

Total numbers of 24 species of AM fungus were isolated and of which 20 species belong to *Golmus*, one species of *Gigaspora* and two species of *Acaulospora* respectively. The highest number of species were recorded in the Forest-I area. A small number of species were recorded in the Tea Garden soil. Spore density in soil samples for different areas ranging from 46 - 324 grains / 100gm soil. The bulk of the grain (218.8 spores / 100gm soil) was recorded in the Forest-I area and the minimum (66.4 spores / 100gm soil) in the Tea garden. Indication of diversity was highest in the Forest-I (2.30) area, followed by the Plantation Forest (2.03), the Home Garden (1.99) and the agricultural land (1.98). The index value of the variety was small in the tea garden (1.58). The dominant index of AM fungus species was highest in the Tea garden (0.21) due to the single dominance of the genus *Glomus occultum* (IVI 79.48). However, it was much lower in the Forest-I (0.115) region due to the co-dominance of *Glomus occultum, Glomus albidum* occupy several study sites followed by *Glomus aggregatum, Glomus mosseae* and *Glomus hoi*. In addition the formation of plant species may have a direct impact on the spread of plant species.

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## Introduction

Vesicular arbuscular mycorrhizal fungi are responsible for the symbiotic nature and the hyphae of the fungus can explore a large area around the root zone (Sharif and Moawad, 2006). It is well documented that mycorrhizal fungal hyphae can provide stable or stable mineral nutrients (P, Zn, and Cu) in common soil plants (Hayman, 1982; Allen *et al.*, 1995; Graw, 1979 and Mosse, 1973). In addition, Mycorrhizal interactions with plant roots can help plants overcome water stress (Kevin and Peterson, 1996; Robert, 2001) and disease resistance (Zeng, 2006).

The development of mycorrhiza in plants may vary from season to season (Staffeldt and Vogt, 1975) and soil nutrition status (Allen et al., 1981), physicochemical structures (Furlan and Fortin, 1977). Mycorrhizal fungi are not specific but the relative brightness of different species depends on the area with biotic and abiotic characteristics (Stahl and Christensen, 1982). Although it is present in a variety of plants but most of the plant species have not been tested by mycorrhizal organization (Muthukumar et al., 2003). Numerous studies have shown that arbuscular mycorrhizal fungi play an important role in plant structure and energy in different ecosystems (Koide and Dickie 2002; Ferrol et al., 2004). Mycorrhizal associations with plants are recorded in almost all natural systems but are commonly found in the tropical ecosystem (Johnson et al., 1997) with very high arbuscular mycorrhizal variability.

There are 102 species of Mycorrhizal occurring in the various land use systems in India (Manoharachary *et al.*, 2005) as the local natural forest is found in the Saraswati Range of Haryana (Thapar and Uniyal, 1996), forest soil and coastal regions of Andhra Pradesh (Manoharachary and Rao, 1991), the Nilgiris jungle plains (Raja *et al.*, 1991), the tropical coastal forest of Tamil Nadu (Ganesan *et al.*, 1991; Raghupathy and Mahadevan, 1991), and the forest soil planted with black pepper in Kerala (Lekha *et al.*, 1995). Several other works have also been done on the diversity of AM fungi in mangrove plants in West Bengal (Sengupta and Choudhuri, 1989, 1990), in the

dry areas of Rajasthan (Mohan and Verma, 1995) and in the grasslands of Maruthamalai Hills (Muthukumar and Udaiyan, 1995).

Arunachal Pradesh located in the eastern part of the Himalayas has been given a wide range of climates ranging from tropical climates to tropical and subtropical climates that can affect a variety of AM fungi. A literature review reveals that one research work has been conducted by Singh *et al.* (2003). There is not much information available on the diversity of mycorrhizal fungi from this area. Current research on AM fungal diversity has been carried out in various land use systems in Arunachal Pradesh.

#### Materials and methods

### Sampling areas

The investigation was carried out in different land use pattern in Papum Pare District (100-600m asl) of Arunachal Pradesh (26° 55' N - 28° 40' N and 92° 40' E - 94° 21' E). Annual precipitation ranges from 1100mm – 1600mm and day temperature vary from 12°C to 37°C experiencing a humid tropical climate.

#### Isolation and collection of mycorrhizal fungi

For the isolation of VAM fungal spores, soil samples were taken from 7 different landuse systems of the district from a depth of 0-15cm. Mycorrhizal fungal spores were isolated from soil by the method as suggested by Gerdmann and Nicholson (1963), Daniel and Skipper (1982) and Tommercup (1992).

#### Root staining

To stain the roots, plant roots were cut into 1cm fragments and then cleared in 2.5% KOH for 30min at 90°C (Koske and Gemma 1989). After clearing, the root samples were then acidified with 5N HCl and stained with Trypan blue (Phillips and Hayman, 1970). The stained roots were examined under compound microscope for AM fungal structure. The proportion of root length colonized was estimated by the root slide technique (Brundreett *et al.*, 1996).

#### Culture establishment

Trap cultures were established from autoclaved sand with fresh soil samples mixed in a ratio of 3:1. Maize (*Zea maize* L.) was used as host plant to establish the mycorrhizal trap culture. Cultures were grown in a greenhouse at Department of Botany, Rajiv Gandhi University for 3 months with a temperature regime of  $28^{\circ}$ C (day) and  $15^{\circ}$ C (night) and a 14-hour photoperiod at a light intensity of  $250\mu$ mol m-2s-2 provided by supplementary illumination

# Identification of AM fungal spores

To identify AM fungi, spores were mounted on glass slides in polyvinyl-lacto-glycerol (PVLG) or PVLG + Malzers reagent (1:1, V/V). The spores were examined microscopically and identified according to current taxonomic criteria (Schenck and Perez 1990) and also using the internet information from the INVAM website (http://www.invam.caf.wvu.edu).

## Data analysis

Diversity of AM fungi in seven study sites was evaluated by observing the spores in 5 replicates each of per 100 gm of soil. Frequency was calculated as number of samples from a particular site in which the spores of a particular species occurred. Spore abundance was calculated as the number the ratio between the total number of spores of a particular species and the number of soil samples in which that species occurred. Importance value index (IVI) was calculated as the total sum of Relative density, Relative frequency and Relative abundance.

Species Diversity index, (Shannon-Weiner 1963) was measured as –

$$\begin{split} H &= -\sum\{(n_i/N) \ln{(n_i/N)}\}\\ \end{split}$$
 Where, H = Shannon-Weiner diversity index n\_i = IVI of individual species N = Total IVI of the community Dominance index (Simpson 1949)  $C &= \sum\{(n_i/N)^2\}\\ \end{aligned}$  Where, n\_i = IVI of individual species

N = Total IVI of the community

Similarity index (Sorensen 1948) was calculated to compare the similarity of species among different sites as –

$$S = \frac{2C}{(A+B)} \times 100$$

Where, C = Number of species common to site A and B

A = Number of species in site A

B = Number of species in site B

# Results

Physio-chemical properties of the soil

Soil texture from sandy loam to sandy loam and soil pH found to be slightly acidic (5.5-6.7). Organic soil carbon and nitrogen content were very high in the Forest-I area and very low in the vegetation forest. Although, the phosphorus found in the soil varied between 2.72-4.50µg mg-1 and was significantly higher in the Tea garden than in other sites (Table 1).

**Table 1.** Soil physico-chemical properties of differentland use types selected for arbuscular mycorrhizalfungal study.

Land use type	Soil texture	pН	Organio C (%)	c Ρ (μg mg <sup>-1</sup> )	N (%)
Forest site– I	Sandy loam			3.67	0.3
Jhum Fallow	Sandy loam	5.5	1.53	3.98	0.27
Plantation Forest	Sandy loam	5.76	0.84	3.8	0.21
Forest site- II	Sandy loam	5.93	1.61	3.27	0.27
Tea Garden	Loamy	5.63	1.4	4.5	0.24
Agricultural Field	Loamy sand	5.9	1.03	2.72	0.22
Home Garden	Loamy sand	6.07	1.52	3.2	0.28

## Diversity and Distribution of mycorrhizal fungi

In the present study a total of 24 species of AM fungi were isolated (Table 2) of which 20 species belong to the *Golmus* genus, 1 of the *Gigaspora* and 2 of the *Acaulospora* respectively. The highest number of species (12) is recorded in the Forest-I area. This was followed by a Plantation forest where nine species were recorded. A small (5) species of species were recorded in the soil of the Tea Garden. Spore density in soil samples for different areas ranging from 46 -324 spores/100gm repetitive soil with an average of 131/100gm grains. Maximum diversity (218.8 spores/100gm soil) were recorded in Forest-I area and minimum (66.4/100gm soil; SD  $\pm$  14.54) in the Tea garden (Table 3). Indication of diversity was highest in the Forest-I (2.30) area, followed by the Plantation Forest (2.03), the Home Garden (1.99) and the agricultural land (1.98). The index value of the variety was small in the tea garden (1.58). The dominant index of AM fungus species was highest in the Tea garden (0.21) due to the single dominance of the genus Glomus occultum (IVI 79.48). However, it was significantly lower in Forest-I (0.115) due to the co-dominance of Glomus occultum, Glomus albidum and Glomus mosseae (Fig. 1). However in the present study Glomus occultum and Glomus albidum occupy several study sites followed by Glomus aggregatum, Glomus mosseae and Glomus hoi.

The results of the Sorensen similarity index showed that the highest similarity was between Forest-I Area and Home Garden (57.14%). This was followed by Forest-I area and Forest-II area and between Forest-I area and the Agricultural field (52.63%). However small similarities were found between Forest-I Area and Tea Garden (11.76%) (Table 4).

**Table 2.** AM fungal species isolated from different land use systems.

AM fungal species	F-I	JF	PF	F-II	TG	AF	HG
Acaulospora delicata	-	+	+	-	-	-	-
Acaulospora rugosa	+	-	-	+	-	+	-
Gigaspora candida	-	+	-	-	-	+	+
Glomus aggregatum	-	-	+	-	+	-	-
Glomus albidum	+	-	+	+	-	+	+
Glomus aurantium	+	+	-	+	-	-	-
Glomus claroidium	-	-	-	-	-	+	+
Glomus clarum	+	-	-	-	-	-	-
Glomus constrictum	+	-	-	-	-	-	-
Glomus coronatum	-	-	+	+	-	-	-
Glomus etunicatum	-	-	-	-	-	-	+
Glomus fasciculatum	-	-	-	-	+	-	-
Glomus geosporum	-	-	+	+	-	+	-
Glomus glomerulatum.	-	-	-	-	-	+	-
Glomus hoi	-	-	+	-	+	-	-
Glomus intraradices	+	+	-	+	-	-	+
Glomus macrocarpum	-	-	-	-	+	-	-
Glomus microcarpus.	+	-	-	-	-	-	+
Glomus mosseae	+	+	-	-	-	+	-
Glomus occultum	+	+	+	+	+	+	+
Glomus rubiforme.	+	-	-	-	-	-	-
Glomus xanthium	-	-	-	-	-	-	+
Glomus sp.4	+	-	+	-	-	-	-
Glomus sp.5	+	-	+	-	-	-	+

F-I – Forest Site I; JF – Jhum Field; PF – Plantation forest; F-II – Forest site II; TG – Tea garden; AF – Agro-forestry; HG – Home-garden.

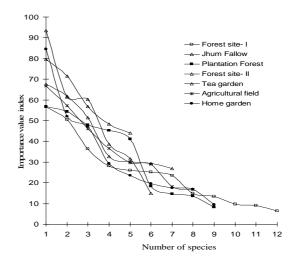
**Table 3.** Spore density (in 100gm soil), species richness, Frequency (%), Shannon-Weiner diversity index and percentage of AM colonization in different land use type.

Land use type	No. of	Density/	Diversity Dominance		
	Species	(100gm	index	index	
	species	of soil)	(H)	(c)	
Forest site-I	12	218.8±65.82	2.3	0.115	
Jhum Fallow	6	92.8±28.17	1.66	0.209	
Plantation Forest	9	132.8±17.6	2.03	0.144	
Forest site– II	7	118.8±30.18	1.88	0.162	
Tea Garden	5	66.4±14.54	1.58	0.21	
Agricultural Field	8	150.8±15.69	1.98	0.15	
Home Garden	9	134.6±42.1	1.99	0.162	
± SE					

**Table 4.** Sorensen Similarity index of AM fungi among different sites.

Sites	F-I	JF	PF	F-II	TG	AF	HG
F-I	100	44.44	38.1	52.63	11.76	52.63	57.14
JF		100	26.67	46.15	18.18	42.86	40
PF			100	50	28.57	35.29	33.33
F-II				100	16.67	40	37.5
TG					100	15.38	14.29
AF						100	47.06
HG							100
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<sup>•</sup>F-I' Forest site– I, 'JF' Jhum fallow, 'PF' Plantation forest, 'F-II' Forest site– II, 'TG' Tea Garden, 'AF' Agriculture field, 'HG' Home garden.



**Fig. 1.** Importance value index of AM fungal species collected from soils of different sites.

# Discussion

Higher numbers of AM fungi were recorded from the natural ecosystem (forest) than manmade one (tea garden).

AM fungus is an important component of the soil that forms interactions with many plant species (Smith and Read, 1997). They are said to improve plant nutrition and promote plant diversity (Van der Heijden *et al.*, 1998) and are generally abundant in soils deficient in phosphorus (Smith and Read, 1997). The sites selected for the present study show slightly acidic soils and are deficient in phosphorus that allow the growth and proliferation of AM fungi. However due to the use of inorganic fertilizers in the Tea garden, the phosphorus level was higher than other natural ecosystems. This may be one of the possible reasons for small number of AM fungal species in the tea garden soil.

The diversity of species (24 species) in the present study is compared to the diversity as reported by Zhao et al. (2003) in the tropical forests of China (27 species), Gai et al., 2006 in the grassy suburb of southern Tibet (25 species). Diversity of AM fungal species in the present study found higher than that reported by Guadarrama and Alvarez-sanchez (1999) in Mexico's Tropical Rainforest (16 species). However, the diversity of species is lower than reported by Muthukumar and Udaiyan (2000) from six different ecosystems of Western Ghat, India (35 species). The richness of the varieties in the present study is due to the high pH (5.5-6.7), high disturbances and high rainfall. The texture of sandy loam soils with loamy-sand of study areas may be another factor that may contribute to the growth of fungal mycelium and hence the diversity of species.

Out of all the 24 species in the present study, 21 were contributed by *Glomus*, one by *Gigaspora* and two by *Acaulospora*. This is because Glomus species often occur in a variety of natural ecosystem and the limited distribution of one generation reflects the greater variability of *Glomus* under different soil conditions (Manoharacharya *et al.*, 2005) or may be due to sporogenous factors (Bever *et al.*, 1996; Zhao *et al.*, 2003). However it may be due to the high efficiency of the *Glomus* species to flourish in slightly acidic soil to close to neutral soil pH (Graw 1979). Rapid mineralization of nutrients in tropical climates favors the

rapid production and adaptability of the *Glomus* species (Hepper, 1984). The current study of higher species of *Glomus* is also in line with Danesh *et al.* (2006).

The Shannon Wieners diversity index on Forest site-I (2.30) shows similarities with those found by Gai et al. (2006) in the natural grasslands of Southern Tibet. Values in the field of Agriculture and Home Garden were higher than those found by Gai et al. (2006) in Farmland. The low level of variability in Jhum fallow may be due to the high rate of disturbance and the burning process may reduce fungal variability. In the Tea Garden, however, the use of high quality of inorganic fertilizers and the frequent use of fungicides to control pathogens on tea leaves, which are likely to prevent mycorrhizal fungal growth. Thus the role of mycorrhizal fungi in the abandoned Jhum land of a different cycle may be the scientific interest in restoring damaged soil. Similarity in diversity of fungal species isolated during investigation was mostly with the studies carried out in the tropical ecosystems.

The high similarity of species formation between the Forest-I and Home Garden site may be due to the diversity of vegetation present on both sites. Arbuscular mycorrhizal fungi are soil microorganisms that form symbiotic relationship with vascular plant species as well as with agricultural plants. The arbuscular mycorrhizal symbiosis is probably the most widespread beneficial interaction between plant and microorganisms. The richness in fungal species in the forest land and plantation site may be due to the interaction and stability of AM fungal species. Presence of plant community and land use system has direct or indirect impact on the diversity of AM fungal species. AM fungi are vulnerable to land use changes and any disturbance often lead to decrease in AM fungal diversity. High plant density and diverse plant species provide more niches hosting AM fungi. It may be ascribed that land use systems have impact on the distribution and diversity of AM fungi.

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