

*Full Length Research Paper*

# Diversity of arbuscular mycorrhizal fungi in *Camellia sinensis* in Uttarakhand State, India

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Many microorganisms form symbioses with plants that range on a continuous scale, from parasitic to mutualistic. Among these, the most widespread mutualistic symbionts is the arbuscular mycorrhiza, formed between arbuscular mycorrhizal (AM) fungi and vascular flowering plants and other plants. A study of diversity of arbuscular mycorrhizal fungi in *Camellia sinensis* was conducted in four plantation territories of Uttarakhand. Microscopic analysis of the mycorrhizal status of roots has revealed that samples from all four locations belonged only to AM fungi. The mycorrhizal colonization level was found high thus reflecting the mycotrophic nature of *C. sinensis* (L.). Results of isolation and identification of spores from all field-collected soil samples has revealed relatively higher spore count. All recovered spores were found to belong to the *Glomales* Order, represented by *Glomaceae* family. Isolation has also brought into notice that three groups were dominant: the first one included light yellow colored spores, second, corresponded dark yellow-brown colored spores and third, magenta colored spores. The morphological characters indicated that the spore populations consisted of 3 – 6 morphotypes. The *Glomus* genus was represented by three species; that is, *Glomus mossae*, *Glomus fasciculatum*, *Glomus* sp. 1 (an unidentified species).

**Key words:** *Glomus mossae*, *Glomus fasciculatum*, Mycorrhiza, *Camellia sinensis*.

## INTRODUCTION

Tea is the agricultural product of the leaves, leaf buds and internodes of the *Camellia sinensis* plant, prepared and cured by various methods. "Tea" is also known as the aromatic beverage prepared from the cured leaves by combination with hot or boiling water, and is the common name for the *C. sinensis* plant itself. *C. sinensis* (L.) belongs to the Theaceae family. India and China or perhaps both of these countries are native home of tea. Total cultivated area for *C. sinensis* is estimated to be 394,866 hectares in India. *C. sinensis* is grown over a wide range of tropical and subtropical region, suitable for its cultivation. An annual rainfall of 100 - 150 cm and temperature of 24 - 30°C is required for cultivation, growth and maximum yield, it requires some shade for faster growth. In India, the principal growing *C. sinensis* is located in the North-East and South-region. Tea (*C. sinensis*) is highly appreciated as non-alcoholic beverage drink and possesses some

important health benefits (Mondal et al., 2004). Commercial tea is obtained from simple, elliptic to lanceolate, coriaceous and serrated leaves. Young leaves and unopened leaf bud of plant are turned into commercial tea. The quality of tea depends on the type of leaves.

Mycorrhiza acts as an important factor for plant growth, mineral nutrient status and resistance to transplanting stress (Mondal, 2002). In the colonies of arbuscular mycorrhizal (AM) fungi, several species of Arbuscular mycorrhizae fungi occur which help in plant growth and assist them in their mineral uptake. Arbuscular mycorrhizae create the most widespread plant fungi symbiosis that occurs in nature and so mycorrhizal fungi are key components of natural ecosystems. They are considered essential for ecosystem functioning (Koide and Mosse, 2004; Van der Heijden, 2002) because they play a fundamental role in soil fertility and in the maintenance of stability and biodiversity within plant communities (Giovannetti and Avio, 2002).

The main objective of this study is to find out diversity of *Glomus* according to colour and shape and also to

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Figure 1. Sketch of Uttarakhand showing the sampling area.

determine richness of *Glomus sp.* in Uttarakhand.

## MATERIALS AND METHODS

### Study sites

Four sites of *C. sinensis* cultivated areas were selected in Dehradun District (Figure 1) at which the study was conducted. The physical and chemical soil characteristics of each site were estimated.

### Sample collection

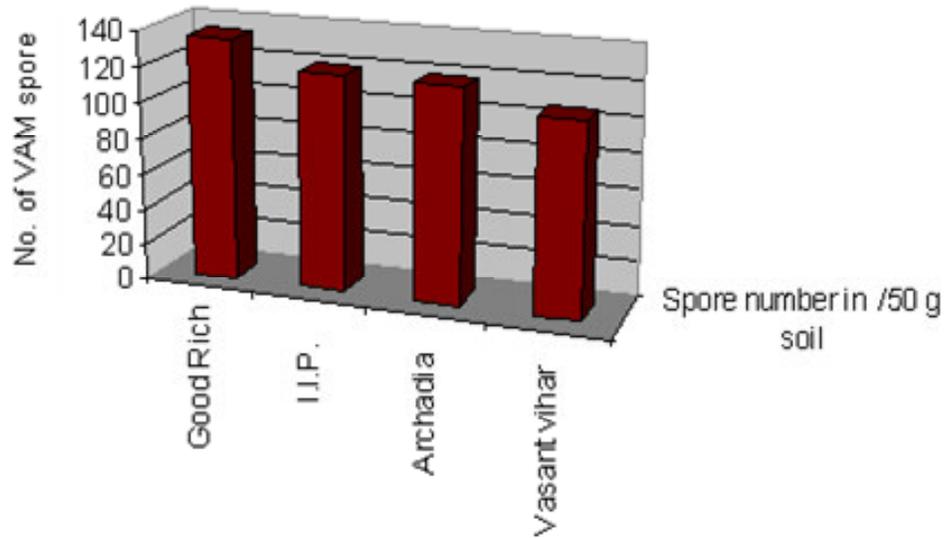
From every site, approximately 3 to 5 kg of soil was collected around and from *C. sinensis* roots. Rhizospheric soil sample were collected from the depth of 10 to 70 cm and homogenized to obtain a representative sample for the entire site. The process was repeated for all locations selected. A 3 kg sub sample of homogenized soil was taken to the laboratory for physicochemical analyses and arbuscular mycorrhizal spore extraction. *C. sinensis* fine roots were collected at the same time.

### Root clearing and staining

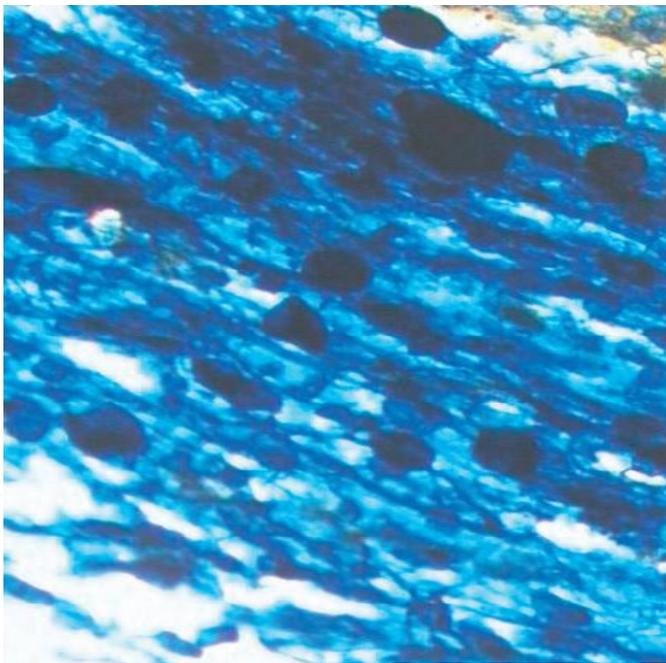
One to five grams (1 - 5 g) of *C. sinensis* fine roots were collected and maintained in a glycerol/ethanol/distilled water (GEE) solution (Ducouso, 1991). Roots were then cleared in 10% KOH and stained with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970) to reveal fungal structures. Stained roots were cut into 1 cm fragments and crushed on slides in a drop of polyvinyl alcohol-lacto-glycerol (Koske and Tessier, 1983). 5 to 10 fragments were mounted on each slide with 10 replications. Each fragment was observed under a microscope (10× and 40× magnification) to estimate the extent of arbuscular mycorrhizal infection as described by Trouvelot et al., (1986).

### Extraction and counting of AM fungus spores

The Gerdemann and Nicolson (1963) method was used to extract Glomalean spores from the soil. 100 g of soil was wet sieved on 500 to 50 µm mesh sieves and centrifuged in a water sucrose solution (50% w/v) for 10 min at 1500 rpm. Spores were counted under a stereomicroscope and grouped according to their morphological characteristics.



**Figure 2.** Occurrence of spores in the soil samples of different sampling sites.



**Figure 3.** *C. sinensis* root colonization by AM fungi (10x magnification).

#### Spore identification

##### Morphological characters

Spore size and colour were assessed in water under a stereomicroscope (Olympus SZ H10 research stereomicroscope). Spore wall structures and other specific attributes were observed under a microscope on permanent slides prepared according to Azcon-Aguilar et al., 2003 (Azecon-Aguilar et al., 2003). Spore identification was mainly based on morphological features, e.g. colour, size and wall structure (Morton and Benny, 1990).

## RESULTS

### Natural mycorrhizae of *C. sinensis*

The cytological organisation of mycorrhizae was the same in all samples. Microscopic observations of stained roots showed that *C. sinensis* formed abundant endomycorrhizae (Figure 3). In some cases, different endomycorrhizal structures were observed, including number of *Glomus* sp. spores outside the *C. sinensis* root cortex in the roots (Figure 4).

### Diversity of arbuscular mycorrhizal fungi (AMF) spores

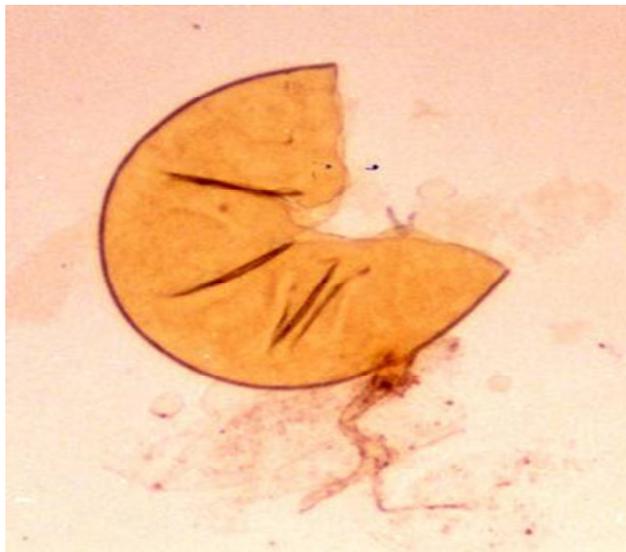
The number of spore morphotypes detected at each site, according to shape, colour and size are shown in Figure 5. Most of the morphotypes were common to all sites, and few were specific. All spores belonged to the Theaceae order represented by *C. sinensis* root colonization by AM fungi (10 x magnification) (Figure 3). The most representative spore morphotypes of this family were divided into three groups: The 1st one included light yellow spores (Figure 5), the 2nd corresponded dark yellow-brown spores (Figure 6) and 3rd, magenta (Figure 4). A detailed analysis of the morphological characteristics of this spore community revealed the presence of the genera *Glomus*.

### *Glomus*

It is characterised by a generally multilayered wall that blended with the wall of subtending hyphae. Species in this genus were the most abundant, sometimes accounting



**Figure 4.** Number of *Glomus* Sp. magenta spore outside the *C. sinensis* root cortex.



**Figure 5.** Light yellow *Glomus* Sp. showing in *C. sinensis*.

for up to 72% of all spores counted. Three distinct species were observed: *Glomus mossae*, *Glomus fasciculatum* and *Glomus* sp. 1. Soil texture has been shown in Table 1. Likewise, pH of soil and organic matter have been presented in Table 2. The data of mycorrhizal spore present as number per 50 g soil in *C. sinensis* have been given in Table 3, and Table 4 showed diversity of AM fungal spores in *C. sinensis* plantation lands. Distribution of AMF species at the 4 studied sites were given in Table 5. The cluster analysis was done and Euclidian distance was calculated for determining the similarities between the four different sampling sites. The result showed that



**Figure 6.** Dark yellow-brown spore of *Glomus* sp. in *C. sinensis*.

**Table 1.** Soil texture in the soil samples collected from different sampling sites of Utrankhand.

| S/N | Sampling site | Sand (%) | Silt (%) | Clay (%) |
|-----|---------------|----------|----------|----------|
| 1   | A             | 51.3     | 33.0     | 15.7     |
| 2   | B             | 50.3     | 43.3     | 6.40     |
| 3   | C             | 53.1     | 32.6     | 14.3     |
| 4   | D             | 55.0     | 23.5     | 21.5     |

A = Good rich; B = IIP; C = Archadia; D = Vasant Vihar.

**Table 2.** pH and Organic matter of soil collected from the sampling sites.

| S/N | Sampling site | pH  | Organic matter (%) |
|-----|---------------|-----|--------------------|
| 1   | A             | 5.7 | 1.55               |
| 2   | B             | 5.1 | 1.35               |
| 3   | C             | 4.8 | 1.2                |
| 4   | D             | 4.7 | 2.2                |

A = Good rich; B = IIP; C = Archadia; D = Vasant Vihar.

the sites 1 and 3 are more similar and the sites 2 and 4 are similar to each other with reference to the presence to mycorrhizal spores (Figure 7).

## DISCUSSION

Microscopic analysis of *C. sinensis* roots revealed a general presence of higher AM fungi and mycorrhizal colonization levels in all root samples, reflecting the amyotrophic nature of the three species. It is known that

**Table 3.** Number of mycorrhizal spores of *C. sinensis* in the soil sample of the studied sites.

| S/N | Sampling sites | Spore number in 50 g soil |
|-----|----------------|---------------------------|
| 1   | Good rich      | 135±2                     |
| 2   | IIP            | 120±1                     |
| 3   | Archadia       | 119±3                     |
| 4   | Vasant Vihar   | 107±4                     |

**Table 4.** Diversity of AM fungal spores in *Camellia sinensis* plantation sites.

| S/N | Sites name   | Colour                                     |
|-----|--------------|--|
| 1   | Good rich    | Dark brown, yellow and Light yellow        |
| 2   | IIP          | Light yellow and Magenta Dark brown yellow |
| 3   | Archadia     | Dark brown, yellow and Light yellow        |
| 4   | Vasant Vihar | Magenta, Dark brown, yellow                |

**Table 5.** Distribution of AMF spores at the 4 studied sites.

| S/N | Species                    | Good Rich | I.I.P. | Archadia | Vasant Vihar |
|-----|----------------------------|-----------|--------|----------|--------------|
| 1   | <i>Glomus mossea</i>       | P         | N      | P        | P            |
| 2   | <i>Glomus fasciculatum</i> | P         | P      | P        | N            |
| 3   | <i>Glomus</i> sp. 1        | P         | N      | N        | N            |

N = Nil; P = Presence.

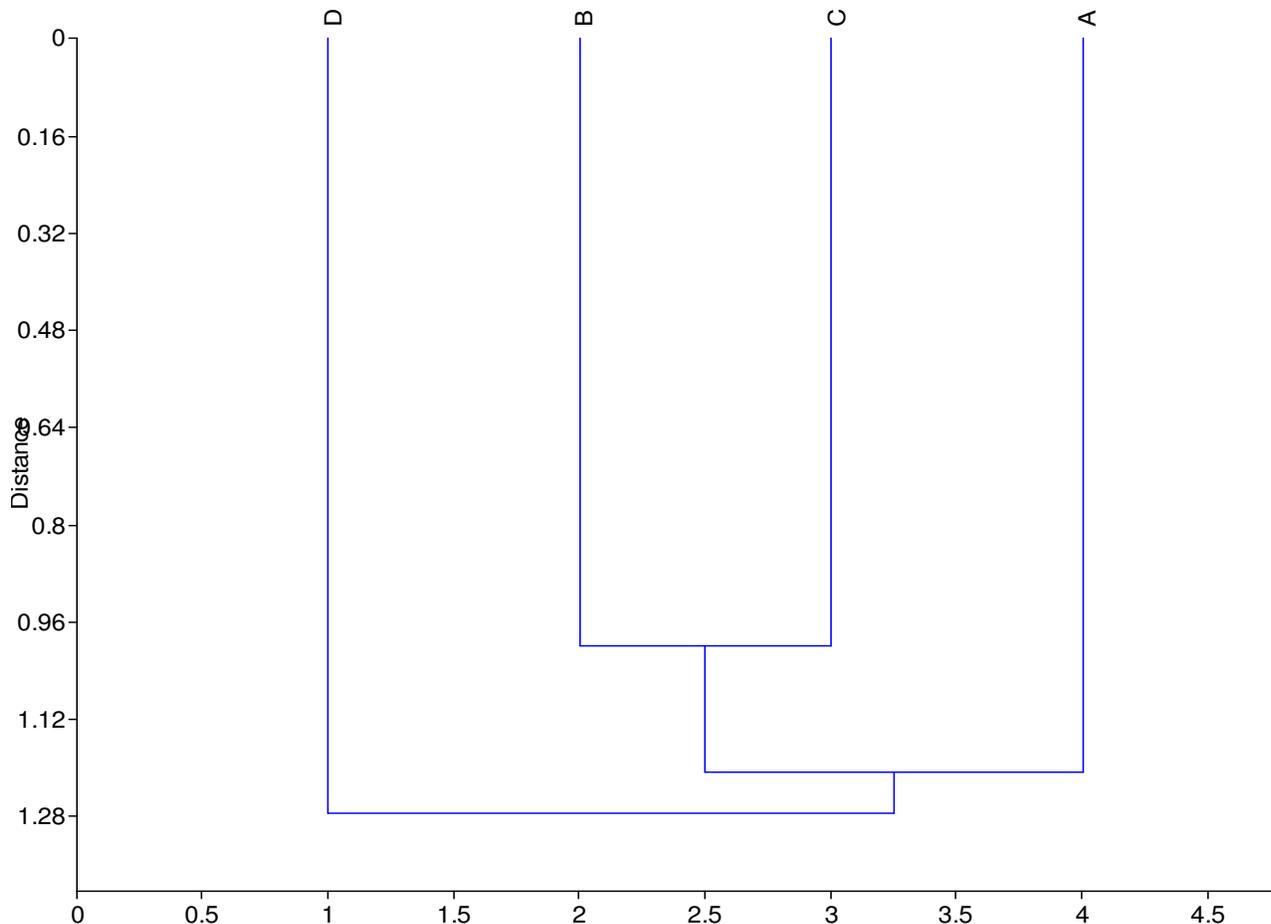
*C. sinensis* is naturally infected by arbuscular mycorrhizal fungi (Diaz and Honrubia, 1993a). Diaz and Honrubia (1993b) experimentally found that mycorrhizal infection was clearly visible in 2 month old *C. sinensis* seedlings. Between the 2nd and 7th month, the percentage of infection increased from 20 to 70%.

### Diversity of AMF in *C. Sinensis*

Our observations reported here are in accordance with that reported in the findings of Singh et al. (2008). The fungus develops simplistically and spreads directly from cell to cell within the root cortex while studying the presence of mycorrhizal symbionts in *C. sinensis*. This is an important step in assessing the diversity and richness of the AM fungal community in this area (Abbas and Abourouh, 2002). We thus focused on identifying AM fungi in soils and roots according to the morphological characteristics of the fungi. The number of spores recovered from the soil samples was relatively high (Figure 2). In some cases, this number is lower, probably due to soil degradation. Indeed, degraded areas often exhibit low densities of indigenous mycorrhizal propagates (Sieverding, 1991). In our investigations, the morphological characteristics of the spores indicated the presence of AM fungi belonging to the Glomineae order. These observations were confirmed by the frequent

presence of vesicles in all samples. The higher numbers of *Glomus* sp. were found in all sampling sites. *C. sinensis* seedlings are usually mycorrhized by *Glomus* (Diaz and Honrubia, 1993a), but the most effective species observed by these authors was *Glomus fasciculatum* and *Glomus mosseae*. This community composition pattern could be due to the type of *C. sinensis* plantation land. Differences in characters could be explained by the presence of fungal ecotypes in soil samples obtained from the areas of study (Diallo et al., 1999; Jeffries and Barea, 2001), because *C. sinensis* species can also differentially alter fertility and other physical and chemical characteristics of soils (Singh et al., 2008), which in turn can affect the AM community structure.

The diversity of AM fungi present in the rhizosphere of *C. sinensis* indicated degradation of this area and corroborated with surveys on AM fungus species richness in degraded weathered acidic soil in the humid and subhumid tropics environments (Azcon-Aguilar et al., 2003; Stutz and Morton, 1996). In conclusion, our results showed a high relative abundance of spores in some cases, which should be preserved and utilized in such ecosystems by including them in nursery plant production programs. Indeed, mycorrhizal inoculation technologies can partially overcome problems of dieback of *C. sinensis* seedlings after transplanting, as observed by Morte and Honrubia (1996). Further investigations are also required to identify *Glomus* sp. at the species level in order to



**Figure 7.** Cluster analysis showing the similarities among the different sampling sites. A = Good rich; B = IIP; C = Archadia; D = Vasant Vihar.

determine the symbiotic performance of AM fungi with *C. sinensis*.

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