



Diversity of Arbuscular mycorrhizal Fungal Association with *Quercus oblongata* D. Don

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Abstract

Arbuscular mycorrhizal fungi (AMF) form a pervasive, obligate, mutualistic association with plant roots to help and play an important role in their life. In the current study, the species diversity of AMF in the rhizosphere of *Quercus oblongata* D. Don was studied. Rhizospheric soil samples and roots were collected from three different locations (Ropadi Gad, Sakaryar, and Saron). The occurrence of AMF, species composition and the diversity of AM fungi were observed with *Q. oblongata* plants. At all the 3 locations, selected plants were found to be colonized with AMF, however, the extent of root colonization significantly varied at different locations. Morphologically different 12 AMF species were isolated and identified from the rhizosphere of *Q. oblongata*. Of those 12 AMF species, six were found from the Ropadi Gad location, seven from the Sakaryar, and the remaining five from the Saron location. The highest root colonization was observed in *Q. oblongata* at Sakaryar (53.32%), followed by Ropadi Gad (46.67%), while the least was observed at Saron (40.40%). The number of spores ranged from 185±2.54 to 312±3.60, with an average of 250.33±3.32 per 100 g of air-dried soil. The number of spores exhibited a significant positive correlation with root colonization. The number of arbuscules and vesicles also followed the same trend *viz.* the maximum number of arbuscules and vesicles at Sakaryar, followed by Ropadi Gad, and with a minimum at Saron.

Keywords – Arbuscular mycorrhizal Fungi – *Quercus oblongata* – root colonization – structural diversity

Introduction

Arbuscular mycorrhizal (AM) fungi are key members of any plant's microbiome. They are found in intimate symbiotic associations with the roots of higher plants (Kehri et al. 2018). About 40,000-50,000 species of fungi form a mycorrhizal association with approximately 250,000 species of plants (Smith & Read 2018). They play important roles in plant nutrition by often modifying soil biology and its biochemistry. AM fungi are beneficial to plants in many aspects as they improve plant nutrition, increase growth, boost crop production, increase plant antioxidants, vitamins, and essential trace elements, and promote stress tolerance and disease resistance (Pandey et al. 2019). Plant community diversity and their productivity is affected by AM fungal diversity (Öpik et al. 2008). The diversity of AM fungi is affected by environmental factors, e.g., soil type, soil depth, and climate (Wang et al. 2018; Pena-Venegas et al. 2019).

Diversity of AM fungi was reported in members of the family Fagaceae, e.g. *Castanea*, *Fagus*, *Castanopsis*, *Lithocarpus*, *Quercus*, etc. (Kremer et al 2012). Among the above, the genus *Quercus* is an important member of the family Fagaceae. Genus *Quercus* possesses nearly 450 species and is distributed worldwide (Joshi & Juyal 2017). Many species of *Quercus* are potential sources of fuel, timber, fodder, colors and secondary metabolites (Prasad et al 2015), and most of them are reported to be effective for groundwater conservation (Singh et al. 2015, Singh & Rawat 2012). They grow well on fertile clayey or loamy soils but not too well on dry sites (Joshi & Juyal 2017). Previously, AM fungi have been reported from *Q. myrsinifolia* (Tam & Griffiths 1993), *Q. agrifolia* (Warburton & Allen 2001), *Q. rubra* (Dickie et al., 2001), and *Q. garryana* (Moser et al. 2005).

Species *Quercus oblongata* D. Don (synonyms: *Q. dealbata* Wall, *Q. incana* Roxb., *Q. lanata* var. *incana* Wenz., *Q. leucotrichophora* A. Camus ex Bahadur, *Q. leucotrichophora* A. Camus), commonly known as 'Banj oak' or 'Ban oak', is an important evergreen tree in the tropical and temperate areas. In the Himalayan region, the 'Banj oaks' are distributed at altitudes between 1500 m to 2300 m above the mean sea level (Thadani & Ashton 1995). Ethno-medicinally, *Q. oblongata* is an important species. In Swat valley of Pakistan, the powder made from fruits of these species are used in the treatment of urinary infection; In Ladakh (Jammu and Kashmir) of India, barks of these species are used to cure toothache and piles, leaves in the treatment of diarrhoea and gum-resins in stomachache (Pandey et al. 2019). In absence of a report on the diversity of AM fungal association with such an important plant, i.e., *Q. oblongata* led us to choose this species for the present study.

Materials & Methods

Description of Study Site

Samples were collected from Mandi District in three areas; Ropadi Gad, Sakaryar, and Saron. Mandi District is located at the latitude of 31.5892° N and 76.9182° E of Himachal Pradesh and at an altitude of 760 meters from sea level. The plant *Quercus oblongata* was found in the above three areas of Mandi, Himachal Pradesh. *Quercus oblongata* grows up to elevation, 1500 to 2400 feet above sea level. Soil and root samples were collected in December. Laboratory studies were conducted at Eternal University, Baru Sahib, Himachal Pradesh, India.

Isolation, Estimation, and Identification of the AMF spores from the soil samples

Isolation of AMF spores from the soil samples (3 samples from each location) was done using the wet-sieving and decanting method of Gerdmann & Nicolson (1963). A procedure provided by Gour & Adholleya (1994) for the estimation of AM fungal spores was adopted. Identification of AMF fungi was done following the different identification keys (Schenck & Perez, 1990; www.amf.phylogeny.com).

Collection, Staining, and Management of root samples

Roots were carefully extracted with the aid of a hoe without damaging fine roots. Separated finer feeder roots were gently shaken to remove adhering soil particles. All the root samples were brought to the lab in labelled polythene bags. The root samples soaked in FAA (Formalin-Acetic Acid-Alcohol) can be kept up to 2.5 years before assay with no adverse effect on the samples (Brundrett et al. 1984). To view internal features of plant tissues, clearing procedures that used chemical agents to extract cell contents and cell wall pigments were commonly used. The root clearing and staining method of Phillips & Hayman (1970) was followed.

Assessment of Colonization

The slide system of Giovannetti & Mosse (1980) was used to determine colonization by AMF fungi. One centimeter (1 cm) long root segment was chosen randomly from stained samples and placed on microscopic slides in a group of ten bits. In each of the ten parts, the presence or absence

of root colonization were reported with a minimum of 100 root segments for each site. The percentage of roots colonized was calculated by the following formula:

$$\text{Fungal Colonization (\%)} = \frac{\text{Number of intersections with fungal structures}}{\text{Total number of intersections studied}} \times 100$$

Frequency, Density and Relative density of AM fungi

The frequency of different AM fungal species was calculated at different sites based on their occurrence with the dominant plant species selected.

$$\text{Frequency (F) \%} = \frac{\text{No. of host plants having a AM species}}{\text{Total No. of host plants examined}} \times 100$$

Density and relative density of each site and each AM fungi species were calculated using the following formula:

$$\text{Density} = \frac{\text{Total no. of individuals of AMF species in all fields}}{\text{Total no. of fields studied}}$$

$$\text{Relative density} = \frac{\text{Density}}{\text{Total Density}} \times 100$$

Results

Arbuscular Mycorrhizal Fungal Species

In the present study, a total of 12 AMF species belonging to five genera were identified, associated with *Q. oblongata* rhizosphere at selected locations, viz. Ropadi Gad, Sakaryar, and Saron of the Mandi District of Himachal Pradesh, India. The *Acaulospora*, *Diversispora*, *Gigaspora*, *Glomus*, and *Scutellospora* spores are present in the rhizosphere soils of *Q. oblongata* (Table 1; Fig. 1). Among them, the *Acaulospora* accounts for 41.67% of all the identified AMF species, followed by *Glomus* at 33.33%. Only one species was found from each of the *Diversispora*, *Gigaspora* and *Scutellospora* genera, accounting for 8.33% of the total number of AMF species (Table 1; Fig. 1). Out of the 12 AMF species, six (6), i.e., *Acaulospora denticulate*, *A. longula*, *Diversispora pustulata*, *Gigaspora albida*, *Glomus etunicatum*, and *G. hoi*, were found in Ropadi Gad, while seven, i.e., *A. denticulate*, *A. lacunose*, *A. mellea*, *A. myriocarpa*, *G. etunicatum*, *G. hoi*, *Secutellospora calospora*, were found in Sakaryar. Of them, four (4) AMF species were different from those of Ropadi Gad and three (3) were similar. At the Saron, five (5) AMF species, i.e., *A. denticulate*, *A. mellea*, *G. caledonium*, *G. hoi* and *G. pansihalos*, were found. Among which, two (2) species were similar to the isolates from Ropadi Gad, and three (3) with those from Sakaryar (Table 1; Fig. 1).

Table 1 Species composition of Arbuscular mycorrhizal fungi.

Location	Number of Species	AMF species observed	Simpson index (D)	Dominance index approximation
Saron	5	<i>A. denticulate</i> , <i>A. mellea</i> , <i>G. caledonium</i> , <i>G. hoi</i> , <i>G. pansihalos</i>	0.15	0.63
Ropadi Gad	6	<i>A. denticulate</i> , <i>A. longula</i> , <i>D. pustulata</i> , <i>G. albida</i> , <i>G. etunicatum</i> , <i>G. hoi</i>	0.23	0.78
Sakaryar	7	<i>A. denticulate</i> , <i>A. lacunose</i> , <i>A. mellea</i> , <i>A. myriocarpa</i> , <i>G. etunicatum</i> , <i>G. hoi</i> , <i>S. calospora</i>	0.32	0.86

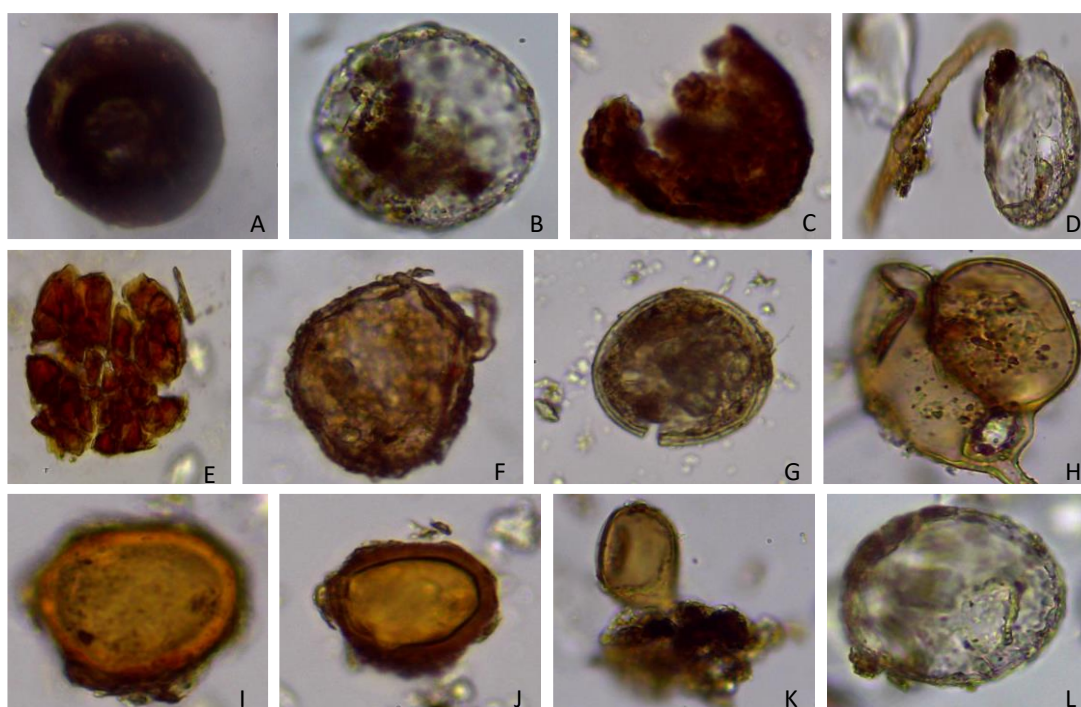


Fig. 1 – Different AMF species associated with *Q. oblongata* at all the sites. A *Acaulospora denticulate*, B *A. lacunose*. C *A. longula*. D *A. mellea*. E *A. myriocarpa*. F *Diversispora pustulata*. G *Gigaspora albida*. H *Glomus caledonium*. I *G. etunicatum*. J *G. hoi*. K *G. pansihalos*. L *Scutellospora calospora*.

Root colonization, spore number and Diversity of AMF

Table 2 displays the pH, root colonization, spore numbers, number of arbuscules, number of vesicles, and diversity of AMF in the rhizosphere soils of *Q. oblongata*. The highest pH (7.60) was recorded at Sakaryar, while the other two locations did not show a significant difference from each other. The highest root colonization was observed in *Q. oblongata* at Sakaryar (53.32%), followed by Ropadi Gad (46.67%) and Saron (40.40%). The number of spores ranged from 185 ± 2.54 to 312 ± 3.60 , with an average of 250.33 ± 3.32 per 100 g of air-dried soil (Table 2; Fig. 2). The number of spores exhibited a significant positive correlation with root colonization. The number of arbuscules and vesicles also followed a similar trend, viz., the maximum number of arbuscules and vesicles were observed at Sakaryar, followed by Ropadi Gad and Saron. A total of 12 AMF species within five genera were identified associated with *Q. oblongata* rhizosphere. *Acaulospora*, *Diversispora*, *Gigaspora*, *Glomus* and *Scutellospora* spores were present in the rhizosphere soils of *Q. oblongata* (Table 2). At the Sakaryar location, a maximum of seven (7) AMF species were observed, while six (6) AMF species were found at Ropadi Gad. At the Saron, only five (5) AMF species were recorded. There is a positive correlation observed between the number of AMF species and spore numbers, arbuscules and vesicle numbers (Table 2).

Table 2 pH, root colonization, number of spores, number of arbuscules, vesicles and diversity of AMF with *Q. oblongata* roots.

S. No.	Location	pH value	Root Colonization (%)	spore number / 100 g soil	No. of arbuscules / cm root	No. vesicles/ cm root	No. of species
1.	Ropadi Gad	7.32 ± 0.15	46.67 ± 2.45	312 ± 3.60	1.16 ± 0.11	2.02 ± 0.12	6
2.	Sakaryar	7.60 ± 0.25	53.32 ± 2.71	254 ± 2.94	1.30 ± 0.11	3.65 ± 0.13	7
3.	Saron	7.28 ± 0.25	40.40 ± 1.70	185 ± 2.54	0.93 ± 0.03	1.63 ± 0.11	5

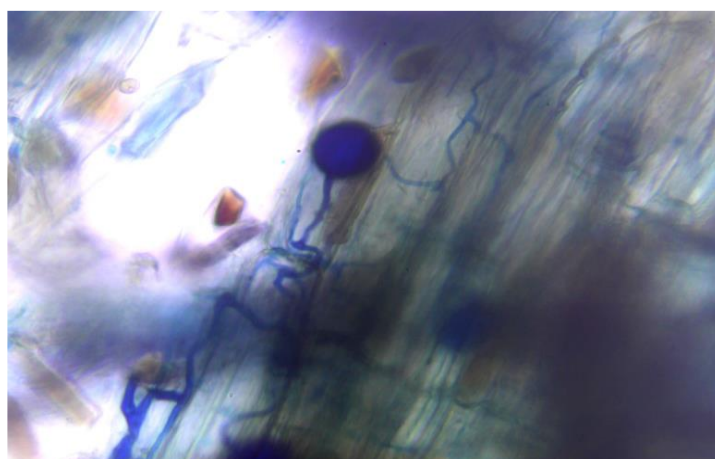


Fig. 2 – Root colonization with AMF species *Q. oblongata* roots

Occurrence, density, frequency and importance value of AM fungi

The occurrence, density, relative density, frequency and importance value of different AM fungi associated with *Q. oblongata* as observed in different locations are presented in Table 3. *Acaulospora denticulate* and *G. hoi* recorded the highest density (1.00), the highest relative density (16.72) at a frequency of 100% with *Q. oblongata*, followed by *A. mellea*. *Glomus etunicatum* showed a density of 0.67, a relative density of 11.24, and a frequency of 66.67%. *Acaulospora longula*, *A. lacunosa*, *A. myriocarpa*, *D. pustulata*, *G. albida*, *G. caledonium*, *G. pansihalos* and *S. calospora* supported the least density (0.33), least relative density (5.52) at a frequency of 33.33% (Table 3).

Table 3: Occurrence, density, frequency and importance value of AM fungi in *Q. oblongata* at different locations.

Sr. No.	AMF Species	Location			Density	Relative Density	% Frequency	Importance Value
		Ropadi Gad	Sakaryar	Saron				
1	<i>A. denticulate</i>	+	+	+	1.00	16.72	100.00	58.36
2	<i>A. lacunosa</i>	-	+	-	0.33	5.52	33.33	19.43
3	<i>A. longula</i>	+	-	-	0.33	5.52	33.33	19.43
4	<i>Acaulospora mellea</i>	-	+	+	0.67	11.24	66.67	38.96
5	<i>A. myriocarpa</i>	-	+	-	0.33	5.52	33.33	19.43
6	<i>D. pustulata</i>	+	-	-	0.33	5.52	33.33	19.43
7	<i>G. albida</i>	+	-	-	0.33	5.52	33.33	19.43
8	<i>Gl. caledonium</i>	-	-	+	0.33	5.52	33.33	19.43
9	<i>Gl. etunicatum</i>	+	+	-	0.67	11.24	66.67	38.96
10	<i>Gl. hoi</i>	+	+	+	1.00	16.72	100	58.36
11	<i>Gl. pansihalos</i>	-	-	+	0.33	5.52	33.33	19.43
12	<i>S. calospora</i>	-	+	-	0.33	5.52	33.33	19.43

Relative abundance (RA), frequency (F), and species number of the total AMF genera identified at three locations on *Q. oblongata* are shown in Table 3. The *Acaulospora* genus showed a maximum number of species (5), whereas four (4) species were observed at Sakaryar and two (2) each at Ropadi Gad and Saron locations. *Acaulospora* also showed the highest frequency (33.33%)

and the highest relative abundance (50.00%) at Sakaryar. *Glomus* was the second most dominant genus, recorded with four (4) species. Of them, three (3) were observed at Saron, followed by Sakaryar with two (2), and only one (1) at Ropadi Gad. *Diversispora* and *Gigaspora* genera were only observed at Ropadi Gad. *Scutellospora* genus was observed only at Sakaryar in association with *Q. oblongata* plants (Table 3).

Discussions

In the present study, we isolated and identified AMF in the rhizosphere of *Q. oblongata* at three different locations. A total of 12 AMF species belonging to five genera were identified (Table 1), indicating that *Q. oblongata* has fewer AMF species than what was reported on apples (40 AMF species) in apple orchards of Himachal Pradesh (Mehta and Bharat 2013) and from the different land-use system (24 AMF species) of Arunachal Pradesh (Bordoloi et al. 2015). The number of species we identified is similar to that reported in the study of Vyas et al. (2006) in the subtropical regions of Madhya Pradesh, associated with the wheat crop. The dominant AMF genera in all three locations associated with *Q. oblongata* were *Acaulospora* and *Glomus*, and the dominant species was *A. denticulate*. Similar to this study, *Glomus* and *Acaulospora* were widely distributed and dominant in the rhizosphere of plant species in Madhya Pradesh (Singh et al. 2011), and similar results were also observed in China (Bi et al. 2019) where *Acaulospora* and *Glomus* were dominant. These results confirm that the two (2) AMF genera are widely distributed, broad-spectrum ecotypes in ecosystems (Singh et al. 2010).

In the current study, some AMF species occurring in natural habitats were detected in association with *Q. oblongata* at three (3) locations, i.e., *Acaulospora*, *Glomus*, *Diversispora*, *Gigaspora*, and *Scutellospora*. AMF species, *A. denticulate*, *A. lacunose*, *A. longula*, *A. mellea*, *A. myriocarpa*, *D. pustulata*, *G. albida*, *G. caledonium*, *G. etunicatum*, *G. hoi*, *G. pansihalos*, and *S. calospora* were found in association with *Q. oblongata* plant. Our results are supported by Prasanthi et al. (2016), who isolated AMF from two medicinal plants belonging to four genera *Acaulospora*, *Entrophospora*, *Glomus*, and *Scutellospora*. They described *Glomus* as the most dominant genus in contrast to *Acaulospora* in this study (Table 2). Chanda et al. (2014) also found the dominance of *Glomus* genus in Assam (India) in the Eastern Himalayan region, with species such as *G. fasciculatum*, *G. macrocarpum*, and *G. mosseae* as the most dominant. Pramod et al. (2006) found *Glomus* as the most occurring species with the highest spore count, and the genera *Acaulospora*, *Entrophospora*, *Gigaspora*, *Sclerocystic*, and *Scutellospora* occurred regularly, with lower spore densities in apple orchards in Himachal Pradesh.

This was the first study investigating AMF species associated with *Q. oblongata*. Twelve (12) morphologically different species of AMF were recorded at all three locations. *Quercus oblongata* plantations had different AMF species composition, spore abundance and species richness. There is a high impact of *Q. oblongata* plant on the native AMF community. All the observed characteristics were compared with those of plants in the natural vegetation in an undisturbed environment. The presence of AMF populations in the root zone of the plants most likely owes a great deal to agricultural inputs, such as fertilizer.

In the present study, spore numbers were positively correlated with high root colonization of plant roots. High root colonization can directly affect the absorption of nutrients and translocation of the solutes from soil to the plant roots. Differences in root colonization rates of the plants at different locations were also observed. Similar results have been reported by other researchers (Shukla et al. 2015; Liu et al. 2016). The variation in spore number at different locations was considerable, and may best be explained by spore production capacity (Clapp et al. 2010) because spore density was not correlated with the plant root colonization percentage (Tian et al. 2009). AMF richness, diversity and community structure were investigated, with individual AMF species on morphological bases as *Acaulosporaceae* spores have key characters permitting identification at the species level. Many studies of AMF have been carried out using this approach, such as da Silva et al. (2017), who reported 37 AMF species representing 12 genera similar to this study.

The AMF diversity and distribution in different places and soils were studied. The maximum number of AMF species were found at Sakaryar, a total of seven (7) AMF species, at Saron five (5) AMF species, and Ropadi Gad recorded six (6) AMF species. The great density and diversity of AMF found in protected precious forests dominated by local plant species demonstrate (Singh et al. 2011) the value of mycorrhizal symbionts, the genetic reservoirs in their natural environments (Liu et al. 2016). The present study also serves as a revelation on the influence of plant communities on AMF diversity and spore abundance.

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