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Native Diversity of Endotrophic Mycorrhizal Fungi of Forage Grass Species Occurring in Asan River Basin, Mussoorie Hills, Uttarakhand

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Abstract

Arbuscular Mycorrhizal (AM) fungi colonize more than 80% of plants on land in which grasses are known to have higher endomycorrhizal colonization. AM fungi are a common group of symbiotic fungi in the order, Glomale of Division, Zygomycota. These fungi are known to benefit growth through increased nutrient uptake especially phosphorus. In this study, a total of 21 grass species collected from the Asan river basin, Mussoorie hills, Dehradun, Uttarakhand were screened for AM fungal root colonization and their mycorrhizal diversity. Traditional method of sieving and decanting was used for isolating mycorrhizal spores whereas for studying colonization rapid staining and clearing method was used. The highest root colonization (95±2.9) and AM spore count (234±3.56) were observed in *Phalaris minor* whereas *Saccharum spontaneum* exhibited least colonization (30±0.53) and AM spore count (46.7±14.5), respectively. The Andropogoneae (Sorghum tribe) was observed to be the most diverse tribe in association with endomycorrhizal fungi among the studied grasses. This study confirms that the grass species are highly colonized and dependent on endomycorrhizal association. The diversity and colonization patterns of endotrophic mycorrhizal fungi are described in details in this research paper. The AM fungal association with grass species provides new vistas and insight on the functioning of any grass ecosystem and also helps in harnessing the benefits of AM fungi through their usage in waste and abundant land reclamation programmes.

Keywords: Abiotic stress, Arbuscular mycorrhiza, Mycorrhizal symbiosis, Plant microbe interaction, Root colonization, Spore count

Introduction

A multitude of microorganisms thrives in close proximity to the feeder roots of plants, where they perform essential functions in a wide array of physiological processes (Marx, 1975). Arbuscular mycorrhizal (AM) fungi are minuscule fungi that colonize the roots and their rhizosphere at the same time (Daei *et al.*, 2009) and spread out over numerous centimeters (Bonfante and Genre, 2010). AM fungi predominantly participate in the transfer of nutrients from the soil, with a particular emphasis on phosphorus and other nutrients that have limited mobility (O'Keefe and Sylvia, 1991; Rhodes and Gerdemann, 1975; Sanders and Tinker, 1973). AM fungal hyphae contribute extensively in terms of improving soil structure and its water holding capacity (Candido *et al.*, 2015; Daei *et al.*, 2009). AM fungi are renowned for enhancing plant growth and productivity, particularly in adverse abiotic stress conditions, *e.g.*, salinity, drought, cold, heat, mineral deficiency and metals (Daei *et al.*, 2009; Chen *et al.*, 2015). These fungi act as bio fertilizers, bio protectors and biocontrol agents and significantly have benefits to the plants (Hoeksema *et al.*, 2010; Jeffries and Barea, 2012; Sreenivasa and Bagyaraj, 1989). The term 'endomycorrhiza' is employed to characterize mutualistic

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symbiotic relationships between specific fungi and plant roots, wherein the fungal partner primarily develops within the root cortex and infiltrates the cells of the host plant root (Gianinazzi-Pearson and Diem, 1982). The symbiotic interactions between arbuscular mycorrhizal fungi and plants hold significant importance, considering that approximately 80% of all terrestrial plant species naturally engage in this type of symbiosis (Hart and Reader, 2002; van der Heijden *et al*., 1998). Knowledge and understanding of endotrophic mycorrhizal status on grass species is necessary for perceptive relation of eco-system functioning. Study conducted by Read (1991), proposed that in regions characterized by low mean annual temperatures, the rates of nutrient mineralization are sluggish; and consequently, this leads to a deficiency in the availability of crucial nutrients such as nitrogen and phosphorus. The endotrophic mycorrhizal associations have many benefits to plant communities in an ecosystem. Mycorrhizal associations exhibit clear connections with key climatic factors, including water and temperature, *etc*. which play a role in governing the distribution of plants, and are influenced by localized edaphic conditions (Brundrett, 1991; Daei *et al.*, 2009; Smith and Read, 2008). There is a very limited number of research studies concerned with correlation of mycorrhizal association with different grass species. The majority of endotrophic mycorrhizae are characterized by 'balanced' mutualistic relationships in which the fungus and plant engage in exchanges of essential resources necessary for their respective growth and survival (Brundrett, 2004). The primary objective of this study is to examine whether coexisting grass species exhibit divergent responses to arbuscular mycorrhizal fungi (AMF) associations. Additionally, the study aims to explore the variations in AMF communities based on the plant species within a particular group or family.

Materials and Methods

Mussoorie, known as the '*Queen of Hills*' is situated just 35 km away from the state capital of Dehradun with the GPS coordinates of 30°27'35.6724'' N and 78°3'59.0364'' E.

The mountain stands at an average elevation of 2,005 m (6,578 ft). To the hills' northeast lie the Himalayan snow ranges, while to the south, there are the Doon Valley and the Shiwalik ranges, including the Asan river basin. The Doon Valley is positioned within the Shiwalik ranges of the Himalayas, spanning from 78°00' E to 78°10' E longitude and 30°15' N to 30°25' N latitude (Jain, 2007). This valley is spread over 454.95 sq. km. It is enveloped by the Song River to the east, the Tons River to the west, the Himalayan ranges to the north, and lush Sal forests to the south (Gianinazzi-Pearson and Diem, 1982; Singh *et al*., 2013). Figure 1 illustrates the study area and the locations where data or samples were collected.

This study was undertaken with the objective of studying AMF association and diversity among different grass species. The survey and collection of different grass species samples/ rhizospheric root samples was done from different study areas in the Asan river basin, Mussoorie hills, Dehradun. Laboratory experiments on endotrophic mycorrhizal

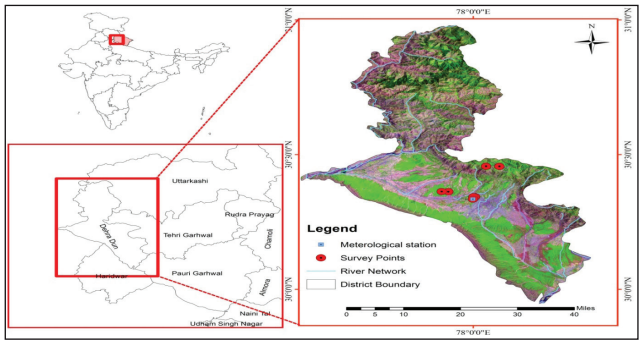

Figure 1: Map showing the Study area

association were carried out in the Forest Pathology Division, Forest Research Institute, Dehradun, Uttarakhand. The collected samples were studied for root colonization, AM spore count in its rhizosphere and their mycorrhizal diversity. Further biochemical analysis and forage quality analysis experiments are still under investigation.

A total of 21 samples of grass species were collected from different sites and their specimens were preserved in herbarium sheets for identification at Botany Division, Forest Research Institute, Dehradun. Rhizospheric soil specimens were collected by excavating a small quantity of soil (200 g) in close proximity to plant roots at depths ranging from 5 to 30 cm. These samples were carefully placed inside a polythene bag for subsequent analysis. To study the root colonization of arbuscular mycorrhizae, the rapid clearing and staining method was employed (Phillips and Hayman, 1970). The calculation of mycorrhizal root colonization percentage was performed utilizing the following formula,

$$\text{Percentage mycorrhizal root colonization} = \frac{\text{Total no. of infected root segments}}{\text{Total no. of root segments examined}} \times 100$$

AM (Arbuscular Mycorrhizal) spores were separated and isolated through the wet sieving and decanting technique (Gerdemann and Nicolson, 1963). The quantitative assessment of AM spores was conducted using a modified version of the method described by Gaur and Adholeya (1994). The AM fungal isolates, along with their corresponding accession numbers, have been conserved by trapping them on host plants and are currently stored at the Biofertilizers Research Laboratory, Forest Research Institute, Dehradun, Uttarakhand, India. The identification of the AM fungi was carried out using taxonomic keys from the various sources (Morton and Benny, 1990; Morton and Redecker, 2001; Pérez and Schenck, 1990; Sharma *et al*., 2008; Sharma *et al*., 2009; Srivastava *et al*., 1996; Taber and Trappe, 1982; Walker, 1992).

Results and Discussion

Twenty one different grass species were collected during the survey and a herbarium was made for their confirmed identification at Botany Division, Forest Research Institute, Dehradun. The detailed list of the collected grass species along with common name and their uses are tabulated in table 1.

Table 1: Target grass species naturally occurring in Asan river basin, Mussoorie hills, Dehradun

Sl. No.	Botanical names	English/ Common name	Usage (if any)
1	*Arundinella nepalensis* Stapf Trin.	River grass	Used as forage. Long canes are used for thatching and fencing.
2	*Arundo donax* L.	Elephant grass	Used as forage. Also used as a fuel source.
3	*Avena fatua* L.	Common wild oat	Used for cattle forage, food and medicine.
4	*Axonopus compressus* (Sw.) P. Beauv.	Carpet grass	Horses forage, permanent pasture, fodder conservation.
5	*Cynodon dactylon* (L.) Pers.	Doob, Dharuva	Sacred grass which is used to feed the cows, folk remedy of cough, diarrhea and also eaten by dogs for stomach ailments.
6	*Dichanthium annulatum* (Forssk.) Stapf	Marvel grass	Forage for local livestock.
7	*Eragrostis tenella* (L.) P. Beauv.	Love grass, Bharbhusi	Grazed by cattle and wild herbivores.
8	*Eulaliopsis binata* (Retz.) C.E. Hubb.	Sabai grass, Bhabhar	Used as fodder, culms are used for making ropes, mats *etc*., used for large scale for the production of paper pulp and also pulp is used for making high-quality printing and writing papers.
9	*Imperata cylindrica* (L.) P. Beauv.	Cogon grass	Used as forage. Thatching the roofs of traditional homes, paper-making, traditional Chinese medicines.
10	*Lopatherum gracile* Brongn.	Bambu-buluh	Used as forage. Used in treating diseases including sunstroke, fever, chicken pox and measles by local people/ villagers.
11	*Oplismenus burmannii* (Retz.) P. Beauv.	Burmann's basket grass	As forage for cattle. An ointment made up from the leaves.
12	*Paspalum scrobiculatum* L.	Kodo millet	Used as forage for horses. Seed- eaten cooked.
13	*Phalaris minor* Retz.	Gulli danda	Fodder or forage for livestock, birdseed, *etc*.
14	*Phragmites karka* (Retz.)	Tall reed, Narkul	Used as dry fodder. The panicles can be arranged in a fan-like manner form a broom, stems can be used for fuel, for weaving coarse hats, mats, hurdles, *etc*.
15	*Poa annua* L.	Annual Blue grass	Cattle fodder. Used by grazing horses as meadow grass species.
16	*Pogonatherum paniceum* P. Beauv.	Baby panda grass	Livestock forage, cultivated as an ornament plant species also.
17	*Polypogon monspeliensis* (L.) Desf.	Annual -beard grass	Forage for livestock milk yield. Seeds are eaten, plant ashes have been used in the treatment of heart related problems.
18	*Saccharum spontaneum* L.	Kans grass	Used as cut fodder. Grass is harvested to thatch roofs. Also used in Ayurvedic preparations.
19	*Sporobolus diander* (Retz.) Beauv.	Indian drop seed	Pasture grass. Seeds are eaten raw or cooked. It can be ground into flour. The plant is used to enrich the blood, reduce swellings and correct gonorrhea.
20	*Thysanolaena maxima* (Roxb.) Kuntze	Broom grass	Green forage for livestock, the dried up stems can be used as stakes to support growing vegetables and also making of brooms.
21	*Aegilops speltoides* Tausch	Goat grass	Awns are eaten. As forage for cattle growing in riverside forest areas.

Some of the species like *Poa annua*, *Phalaris minor*, *Eragrostis tenella* are utilized as good sources of food and fodder for cattle. Some of the species are used by locals as their food like *Aegilops speltoides*, *Avena fatua*, *Paspalum scrobiculatum*. The grass species *i.e.*, *Cynodon dactylon* is a sacred grass, local name: *Dhoob*, which is used to feed the

cows as well as dogs for their stomach ailment (Table 1). The collected rhizospheric samples were analyzed to determine the quantity of AM spores within their rhizospheric soil. The results indicated great variation among these rhizospheric samples. The highest density of AM spore was observed in the case of *Phalaris minor* (234±3.56), followed by *Avena fatua* L. (183±4.08); whereas, *Saccharum spontaneum* exhibited lowest endomycorrhizal density (30±0.53), followed by *Thysanolaena maxima* (Roxb.) Kuntze (11±2.44) (Table 2).

Table 2: Endomycorrhizal quantification of the screened grass species occurring in Asan river basin, Mussoorie hills, Dehradun

Sample Nos.	Botanical Names	AM Spore (per 50 g soil)
Sample 1	*Poa annua* L.	39 ± 4.9
Sample 2	*Dichanthium annulatum* (Forssk)	24.3 ± 4.08
Sample 3	*Paspalum scrobiculatum* L.	20.6 ± 4.06
Sample 4	*Imperata cylindrica* (L.) P. Beauv.	79.3 ± 3.26
Sample 5	*Axonopus compressus* (Sw.) P. Beauv.	62.3 ± 1.63
Sample 6	*Cynodon dactylon* (L.) Pers.	80 ± 4.08
Sample 7	*Phalaris minor* Retz.	234 ± 3.56
Sample 8	*Aegilops speltoides* L.	22 ± 4.08
Sample 9	*Avena fatua* L.	183 ± 4.08
Sample 10	*Sporobolus diander* (Retz.) Beauv.	78 ± 3.76
Sample 11	*Arundinella nepalensis* Stapf Trin.	45 ±1.63
Sample 12	*Polypogon monspeliensis* (L.) Desf.	166 ± 3.26
Sample 13	*Pogonatherum paniceum* P.Beauv.	89 ± 2.86
Sample 14	*Saccharum spontaneum* L.	30 ± 0.53
Sample 15	*Thysanolaena maxima* (Roxb.) Kuntze	11 ± 2.44
Sample 16	*Lopatherum gracile* Brongn.	33 ± 1.63
Sample 17	*Phragmites karka* (Retz.)	66 ± 0.82
Sample 18	Arundo donax L.	19 ± 3.26
Sample 19	*Eulaliopsis binata* (Retz.) C.E. Hubb.	67 ± 0.98
Sample 20	*Eragrostis tenella* (L.) P. Beauv.	30 ± 2.44
Sample 21	*Oplismenus burmannii* (Retz.) P. Beauv.	45 ± 3.26

Different AM spores were pricked up from the samples for qualitative analysis through above mentioned technique. After careful examination under the microscope and with the help of keys as described above in materials and methods, the various AM spores were identified. Various species of genera like *Glomus, Acaulospora,* and *Gigaspora* were identified whereas very limited numbers of AM spores were observed in case of genera like *Entrophospora, Scutellospora* and *Sclerocystis* (Table 3). Figure 2 shows some of the isolated AM spores from these grass species. The most diverse endomycorrhizal mycoflora was observed in case of *Poa annua* where AM spores of 4 different genera were observed and a total of 8 species were identified. If generic diversity of AM fungi is considered then *P. annua* and *Phalaris minor* were the most diverse grass species/ samples with 4 different genera. Whereas if species diversity is taken into consideration then after *P. annua, Axonopus compressus* were the most diverse grass species in terms of AM fungal diversity. Root colonization was investigated from root segments of target grasses (Figure 3) and was tested for the presence of characteristic mycorrhizal structures *viz*., hyphae, arbuscules and vesicles. Table 4 shows presence and absence of these structures in the screened grass species. It was found that vesicles are present in most of the grass species except *Avena fatua*, *Arundo donax* and *Oplismenus burmannii*; whereas, arbuscules are not present in many grass species *i.e.*, *Paspalum scrobiculatum*, *Imperata cylindrica*, *Cynodon dactylon*, *Polypogon monspeliensis*, *Pogonatherum paniceum*, *Saccharum spontaneum*, *Lopatherum gracile*, *Phragmites karka* and *Arundo donax*. Highest root colonization (%) was observed in *Phalaris minor* (95±2.9) followed by *Arundinella nepalensis* (91.7±4.4) whereas lowest in *Saccharum spontaneum* (46.7±14.5) followed by *Arundo donax* (53.3±9.7). It was revealed that the data of AM spore count and root colonization are in similar trends. The various AM spores which are isolated and identified are shown in figure 2.

Similar kind of work was also undertaken by Sathiyadash *et al*. (2010) where screening of a total of 50 grasses for fungal association from the south Indian region was analyzed. Among these grass species they had also studied *C. dactylon* and *S. spontaneum*. The results were not in accordance with the observations noted in this study. In this study, the observation in case of *C. dactylon* highlighted higher root colonization (65±17.5) and AM spore count (80±4.08) as compared to the lower colonization percentage (22.2±4.0) and spore count (10.7±1.8) detected by Sathiyadash *et al*. (2010) from the grass of south Indian region. One other striking fact was the presence/ absence of specialized endotrophic mycorrhizal structures in the colonized grass species. In this study, we observed the absence of vesicles and arbuscules both; whereas, Sathiyadash *et al*. (2010) reported absence of vesicles but presence of arbuscules. This difference in observations can be attributed to the variation in site/ origin where the observations made in one case were from a grass of north Indian region and the other being of south Indian region as it is a pre-eminent fact that the same organism has different features only because of

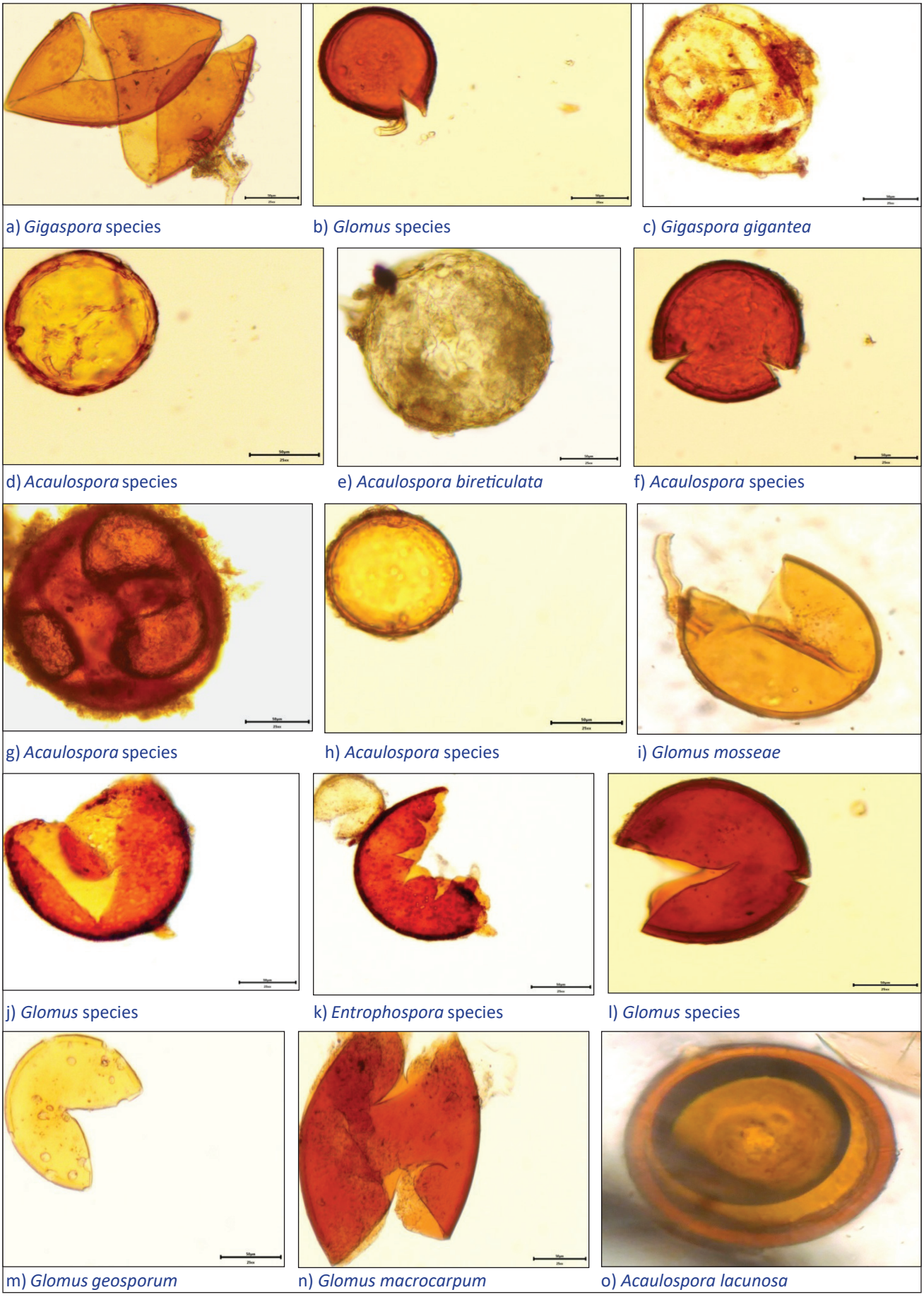

Figure 2: Some of the identified AM spores isolated from different grass species

Table 3: Natural occurrence and diversity of AM fungi in grass species occurring in Asan river basin, Mussoorie hills, Dehradun

Sl. No.	Grass species	Glomus								Acaulospora						Gi.	En.	Scu.	Scl.
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	*P. annua*							+	+		+		+	+	+	+	+		
2	*D. annulatum*	+		+				+	+										
3	*P. scrobiculatum*		+					+				+				+			
4	*I. cylindrica*		+			+										+			
5	*A. compressus*		+		+		+	+		+				+		+			
6	*C. dactylon*	+	+												+				
7	*P. minor*	+							+							+	+		
8	*A. speltoides*	+	+						+						+			+	
9	*A. fatua*							+											
10	*S. diander*							+							+	+			
11	*A. nepalensis*							+							+	+			+
12	*P. monspeliensis*							+							+	+	+		
13	*P. paniceum*							+							+				
14	*S. spontaneum*	+						+											
15	*T. maxima*							+											
16	*L. gracile*							+							+				
17	*P. karka*							+							+	+			
18	*A. donax*																		+
19	*E. binata*							+							+				
20	*E. tenella*							+											
21	*O. burmannii*							+							+	+			

A. *Glomus mosseae* (Nicolson & Gerd.); B. *Glomus geosporum* (Nicolson & Gerd.); C. *Glomus sinuosum* (Gerd. & Bakshi); D. *Glomus pelvinatum* (Henn.) Trappe & Gerd.; E. *Glomus microcarpum* (Tul. & Tul.); F. *Glomus reticulatum* (Bhatt. & Mukerji); G. *Glomus* species; H. *Acaulospora trappei* (Ames & Linderman); I. *Acaulospora lacunose* (Morton); J. *Acaulospora foveata* (Trappe & Janos); K. *Acaulospora rehmii* (Sieverding & Toro); L. *Acaulospora bireticulata* (Rothwell & Trappe); M. *Acaulospora scrobiculata* (Trappe); N. *Acaulospora* species; O. *Gigaspora* species; P. *Entrophospora* species; Q. *Scutellospora* species; R. *Sclerocystis* species

Table 4: Different types of endomycorrhizal colonization in roots of the grass species occurring in Asan river basin, Mussoorie hills, Dehradun

Grass species	Hyphae	Arbuscules	Vesicles	Root colonization (%)
P. annua	+	+	+	81.3 ± 3.7
D. annulatum	+	+	+	71.7 ± 7.3
P. scrobiculatum	+	-	+	55 ± 21.8
I. cylindrica	+	-	+	63.3 ± 14.5
A. compressus	+	+	+	75 ± 13.2
C. dactylon	+	-	+	65 ± 17.5
P. minor	+	+	+	95 ± 2.9
A. speltoides	+	+	+	81.6 ± 9.2
A. fatua	+	+	-	68.3 ± 18.3
S. diander	+	+	+	81.7 ± 9.3
A. nepalensis	+	+	+	91.7 ± 4.4
P. monspeliensis	+	-	+	63.3 ± 23.3

Table 4: Continue...

Grass species	Hyphae	Arbuscules	Vesicles	Root colonization (%)
P. paniceum	+	-	+	56.7 ± 8.8
S. spontaneum	+	-	+	46.7 ± 14.5
T. maxima	+	+	+	68.3 ± 4.4
L. gracile	+	-	+	75 ± 2.9
P. karka	+	-	+	66.7 ± 10.9
A. donax	+	-	-	53.3 ± 9.7
E. binata	+	+	+	85 ± 2.9
E. tenella	+	+	+	75 ± 5.8
O. burmannii	+	+	-	68 ± 9.6

[NB: + present, - absent, ± SEm]

different place of origins/ habitats. Another possible reason for this variation can be the time of sample collection as at which stage of the life cycle the root samples were collected is important to the extent of endomycorrhizal colonization. Tahira *et al*. (2012) worked out the root colonization of *P. minor* and observed relatively lower root colonization (66.5%); whereas, highest root colonization was observed during this study. Javaid *et al.* (1995) studied the AM infection with respect to allelopathic and non-allelopathic grasses and concluded that AM infection was relatively lower in the grasses with allelochemicals presence and vice versa. They also screened *Dicanthium annulatum*, *Imperata cylindrica*, *Cynodon dactylon*, *Phalaris minor* and *Polypogon monspeliensis* for AM infection and the results exhibited a similar pattern of infection in comparison to this study. There was no any dark septate endophytes (DSE) association observed in these grass species. The variation in arbuscular mycorrhizal infection and vesicular mycorrhizal root infection in roots of studied grass species are shown in figure 3.

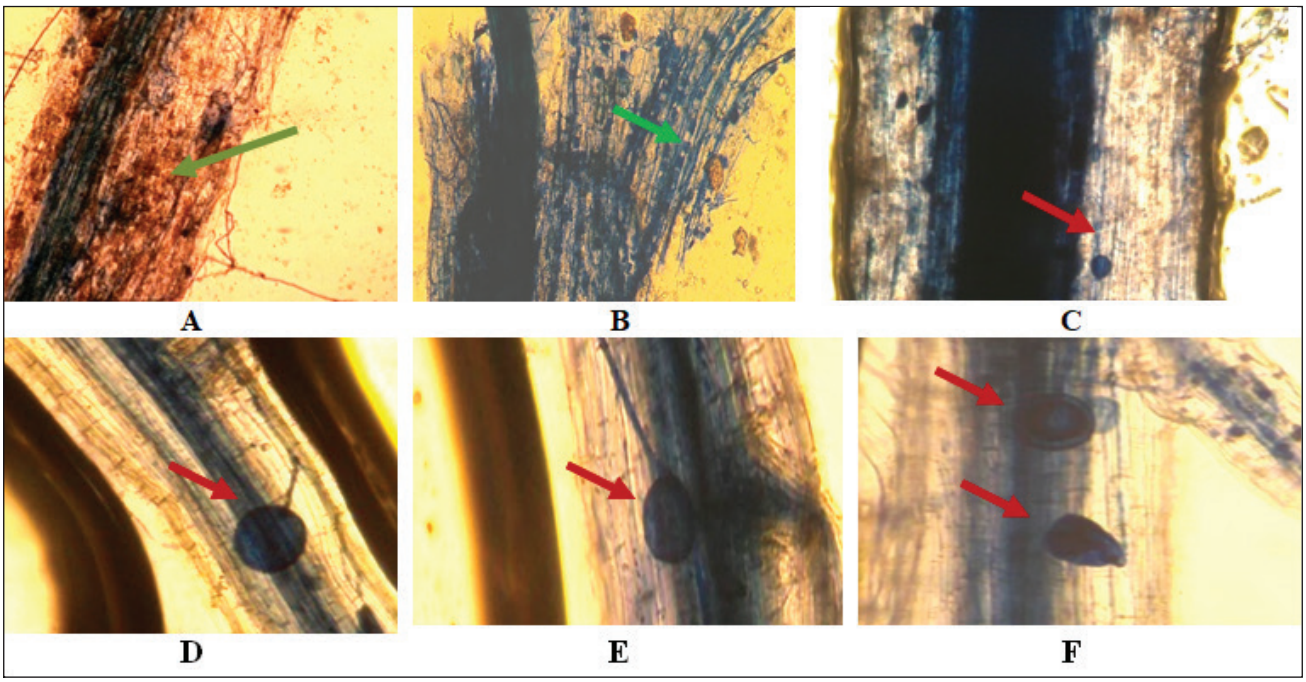

Figure 3: A-B, Extraradical and arbuscular mycorrhizal infection/s (green arrows) in root systems and C-F, vesicular mycorrhizal root infection/s (red arrows show round to elliptical vesicles) in cortical region of roots of studied grass species

Conclusion

This investigation was carried out to know about the endotrophic mycorrhizal fungi colonized with grass species of Asan river basin, Mussoorie hills, Dehradun, Uttarakhand in India. Total 21 species of grasses were selected and the rhizospheric soil sample was taken from each grass species. The study sites show the higher diversity of grass species with wide range and spread of habitats across the Asan river basin. It has also been demonstrated that the presence of arbuscular mycorrhizal (AM) fungi on plant communities varied from 30-234 in AM spore count. In this study, it is also found that the highest AM spore density was found in *Phalaris minor* and lowest was in *Saccharum spontaneum*. It represents that the *P. minor* grass species support the diverse community of AM fungi among the studied grass species. This study additionally affirms that grass species

exhibit a significant dependency on endotrophic mycorrhizal associations. There was no any dark septate endophytes (DSE) association found in these grass species. Measurement of the variation in the response of a plant species to different AMF species will be useful in comparative plant ecology and in defining functional groups of plant species within plant communities (van der Heijden *et al.*, 1998). The AM fungi association with plant species provides new insight for understanding of any ecosystems and also benefits and role of AM fungi in nature. The potential advantages of AM fungi can be utilized in waste and abundant land reclamation programmes.

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