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Arbuscular Mycorrhizal Fungal Diversity and Root Colonization in *Pisum sativum*

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ABSTRACT: Arbuscular mycorrhizal fungi (AMF) are soil fungi which form a mutualistic symbiosis with the roots of plants and enhanced uptake of immobile nutrients from the soil. The present study was carried out to study the association of arbuscular mycorrhizal fungi (AMF) with roots and rhizosphere of pea (*Pisum sativum*). A total of 17 AMF fungi belonging to 5 genera 17 species were isolated and identified from the rhizosphere soil. The dominant genus was *Glomus* (6 species), followed by *Acaulospora* (5 species); Boletus, *Gigaspora* (3 species), *Scutellospora* (2 species) and *Sclerocystis* represented by single species. Microscopic analyses of root samples revealed a variable degree of colonization by AM fungi. The different microscopic characters like size, colour, details of the wall layers and the nature of their subtending hyphae were also investigated to during this study.

Keywords: AM fungi, pea, root colonization, Mid-hill conditions, Himachal Pradesh.

INTRODUCTION

Pea (*Pisum sativum* L.) is a common leguminous crop belonging to the family "Fabaceae. "Faba" comes from *Latin* word which simply means "beans". It is third most important pulse crop commonly grown worldwide over six-million-hectare area. The major pea producing countries of the world are Germany, Italy, China and Canada followed by India, Australia, and the United States. France, Canada and Australia are major exporters of pea as they utilize over two million hectares of land area for pea cultivation. As per FAO Stat. (2014), pea occupies 4th position (10.53%) in area under cultivation and 5th position in total production (6.96%). Like other countries of the world, India also occupies a key position in pea production. Uttar Pradesh is the major pea growing state of India, produces about 49 % of total pea produced. In addition, Madhya Pradesh, Bihar and Maharashtra are also the major pea growing states of the country. Himachal Pradesh, a hilly state of India occupies 5th position in pea production with total production of 294.96 thousand metric tons per year.

Mycorrhiza is a non-disease-producing association in which the fungus invades the root to absorb nutrients. These fungi are found in a wide range of habitats usually inside the roots ramify into the surrounding bulk soil extending the root depletion zone around the root system. They transport water and mineral nutrients from the soil to the plant while the fungus is benefiting from the carbon compounds provided by the host plant (Warburton, 2005). This plant root association with these fungi play a vital role in supporting plant's health by improving plant nutrition (Jacoby *et al.,* 2017), suppress pathogen outbreaks (Pieterse *et al.,* 2014), nutrient exchange and modulation of abiotic stress tolerance (Cheng *et al.* 2019; Baum *et al.*, 2015). They mainly facilitate nutrient uptake, mainly phosphorus, nitrogen (Campo *et al.,* 2020) potassium, sulphur, copper, zinc, calcium etc. (Avio *et al.* 2006; Fanaei *et al.,* 2015; Prasad *et al.,* 2017; Wang *et al.,* 2018; Liu *et al.,* 2018) and enhance the availability of nutrients as well as their translocation (Rouphael *et al.*, 2015).

Keeping in view the key benefits of association of mycorrhiza fungi with plants roots, the present study was carried out to investigate the diversity of AM fungi

associated with pea in different location of mid hill conditions of Himachal Pradesh.

MATERIALS AND METHODS

A. Study area

The study areas selected for sample collections were lies in the mid hill regions of district Mandi, Himachal Pradesh. The areas selected for surveys and sample collection were Chachyot, Naugroun, Ganai and Gohar in Chail-Chowk areaand from Balh valley: Kummi, Ratti, Surandi, Dadour, Gagal and Sakroha. The areas are situated in mid hill conditions of District Mandi and fall in second zone, Mid-hill zone of Himachal Pradesh. Total five pea plants were randomly selected from each study site for the collection of plants and soil samples (Plate I). Soils up to the depth of 0-30cm were collected in sterile polythene bags and carried to the laboratory for further analyses.

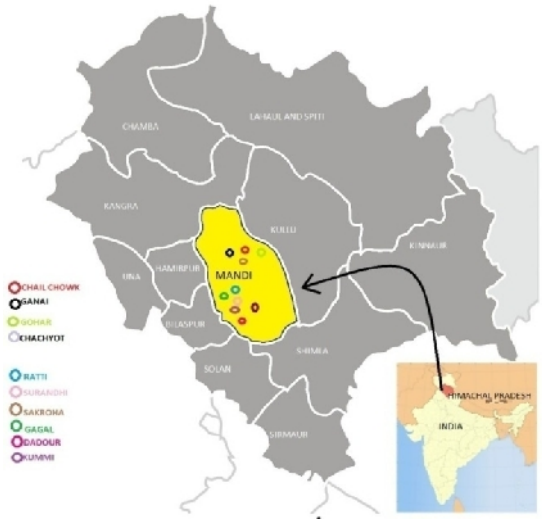

Plate I: Map of study area.

B. Assessment of root colonization by Arbuscular mycorrhizal fungi (AMF)

For staining of root to assess root colonization of AMF, a method described by Phillips and Hayman (1970) for roots and modified by Kormanik *et al.* (1980) was employed. Freshly collected roots of pea plants were washed thoroughly with tap water, cut it into 1 cm length and cleared in 10% (w/v) KOH for 1 hour at 90°C, acidified with 1 % HCl and stained with 0.05% trypan blue overnight and then finally de-stained with lactic acid- glycerin (1:1 by volume) at room temperature. Slides were prepared and observed under a compound microscope for any of structures associated with mycorrhizal fungi viz., hyphae, vesicles or arbuscules. Root colonization was assessed by using following formula:

$$\% \text{ colonisation} = \frac{\text{Total number of colonized root/tissues pieces}}{\text{Total number of root / tissue pieces examined}} \times 100$$

C. Isolation and Identification of AM Fungi

The isolation of AM fungal spores was carried out by wet-sieving and decanting method (Gerdeman and Nicolson 1963). The soil samples were carried to the laboratory in polythene bags and stored in a refrigerator at 4°C for isolation of AMF spores. Total 25g of soil was mixed in 100 ml of water in a glass beaker and stirred constantly with a glass rod to make a uniform suspension. The suspension was left for five minutes so that mycorrhizal debris floated on the top. The suspension was passed through a set of sieves of different sizes (240µm, 120µm, 100µm, 63µm, 30µm). The final decanted suspension of sieving was passed thorough what man filter paper. This process was repeated 8-10 times to trap all spores of AM fungi. The sieved material collected from sieves was observed under stereomicroscope and the spores were isolated using hypodermal needle. Spore population was expressed in terms of number of spores per 25 gm of dry soil.

To aid in the identification of AM fungi, the resting spores were mounted in polyvinyl lactic acid and the size, colour, details of the wall layers and the nature of their subtending hyphae were recorded as per the method suggested by (Phillips and Hayman 1970). The AMF isolates were identified at least to species level. The rhizosphere soil samples were expressed in term of percentage occurrence as per the given formula:

$$\%\text{age of occurance} = \frac{\text{Total no. of Spores of Individual AM fungi}}{\text{Total no. of Spores of AM Fungi}} \times 100$$

RESULTS

A. Root colonization by AM Fungi

The analyses of root samples confirmed association of Arbuscular mycorrhizal fungi (AMF) with Pea roots. The root samples collected from all the locations showed variable percentage of root colonization with various mycorrhizal fungi. The highest root colonization was observed with root samples plants collected from Kummi (56.6%) followed by Surandhi (53%) and Chachyot (42%) whereas, the range of root colonization form Ratti, Sakroha, Chail-Chowk, Ganai, Gagal, Dadour, Gohar was observed in the range of 34-40.5%. The detailed results of root colonization of pea plants with Arbuscular mycorrhizal (AM) fungi are presented in Table 1.

Table 1: Arbuscular mycorrhizal AM fungi colonization (percentage) of root or tissue of pea plants.

Sampling Sites	Samples	Number of roots colonized	Number of root segments examined	Rate of colonization (%age)	Average (%age)
Ratti	Sample 1	3	6	50	44
	Sample2	4	10	40	
	Sample 3	6	10	60	
	Sample 4	4	8	50	
	Sample 5	2	10	20	
Surandhi	Sample 1	4	8	50	53
	Sample2	4	8	50	
	Sample 3	6	10	60	
	Sample 4	2	8	25	
	Sample 5	8	10	80	
Sakroha	Sample 1	4	8	50	39
	Sample2	2	8	25	
	Sample 3	6	10	60	
	Sample 4	2	10	20	
	Sample 5	4	10	40	
Gagal	Sample 1	2	8	25	34
	Sample2	4	10	40	
	Sample 3	1	4	25	
	Sample 4	3	10	30	
	Sample 5	4	8	50	
Dadour	Sample 1	2	10	20	41
	Sample2	4	8	50	
	Sample 3	6	10	60	
	Sample 4	2	4	50	
	Sample 5	2	8	25	
Chail-Chowk	Sample 1	4	8	50	39
	Sample2	2	8	25	
	Sample 3	6	10	60	
	Sample 4	2	10	20	
	Sample 5	4	10	40	
Chachyot	Sample 1	3	10	30	42
	Sample2	5	10	50	
	Sample 3	7	10	70	
	Sample 4	2	5	40	
	Sample 5	1	5	20	
Ganai	Sample 1	4	10	40	34.5
	Sample2	2	10	20	
	Sample 3	3	12	25	
	Sample 4	5	8	62.5	
	Sample 5	2	8	25	
Gohar	Sample 1	5	10	50	40.5
	Sample2	6	12	50	
	Sample 3	3	8	37.5	
	Sample 4	2	8	25	
	Sample 5	4	10	40	
Kummi	Sample 1	4	10	40	56.5
	Sample2	6	10	60	
	Sample 3	8	10	80	
	Sample 4	5	8	62.5	
	Sample 5	4	10	40	

B. Association of AM Fungi

The soil samples collected from different study sites were analysed for occurrence of Arbuscular mycorrhizal (AM) fungal spores. The occurrence and abundance of AM fungi from different soil samples was calculated on 30th, 60th and 90th days after sowing of pea plants. A variation in diversity of Am fungi was observed with respect to collection sites however, no significant difference was observed age of the plants (30th, 60th and 90th days after sowing). The percentage of occurrence of Arbuscular mycorrhizal (AM) fungi on 30th day was observed highest in Dadour (20.04%) followed by Gohar (20%), Kummi (19.94) and Surandhi (19.73%). Similarly, this percentage of occurrence was observed maximum in Chail-chowk (20%) followed by Chachyot (20%) and Kummi (19.96%) and Gohar (18.86%)on 60th days after sowing whereas, on 90th day it was observed highest in Ratti (19.98%) and Chail-Chowk (19.98%) and then Sakroha (19.96%) and (15.23%). The detailed results of occurrence of Arbuscular Mycorrhizal fungi with pea plants on 30th, 60th, 90th days after sowing are presented in Table 2.

Table 2: Percentage occurrence of Arbuscular Mycorrhizal fungi on 30th, 60th, 90th days after sowing.

Sampling sites	Samples	%age of occurrence			Average (%age)		
		30th Day	60th Day	90th Day	30th Day	60th Day	90th Day
Ratti	Sample 1	7.14	9.67	11.11	19.99	19.96	19.98
	Sample 2	17.85	19.35	19.44			
	Sample 3	28.57	22.58	22.22			
	Sample 4	25.01	25.80	25			
	Sample 5	21.42	22.58	22.22			
Surandhi	Sample 1	18.75	19.04	19.23	19.73	19.94	19.96
	Sample 2	18.75	14.28	15.38			
	Sample 3	12.05	19.04	19.23			
	Sample 4	37.05	33.33	30.76			
	Sample 5	12.05	14.28	15.38			
Sakroha	Sample 1	22.22	19.04	19.23	19.99	19.94	19.96
	Sample 2	11.11	19.04	19.23			
	Sample 3	22.22	14.28	15.38			
	Sample 4	11.11	33.33	30.76			
	Sample 5	33.33	14.28	15.38			
Gagal	Sample 1	25.92	26.66	2.64	19.99	19.96	15.23
	Sample 2	40.74	40	38.23			
	Sample 3	14.81	16.66	20.58			
	Sample 4	11.11	6.66	8.82			
	Sample 5	7.40	10	5.88			
Dadour	Sample 1	30.01	25.80	28.12	20.04	19.96	19.96
	Sample 2	10.01	12.90	15.62			
	Sample 3	20.01	22.58	25			
	Sample 4	23.33	19.35	21.87			
	Sample 5	16.66	19.35	9.37			
Chail-Chowk	Sample 1	46.66	40	37.5	19.99	20	19.98
	Sample 2	6.66	10	12.5			
	Sample 3	6.66	10	16.66			
	Sample 4	13.33	15	8.33			
	Sample 5	26.66	25	25			
Chachyot	Sample 1	18.18	18.75	18.18	19.99	20	19.96
	Sample 2	18.18	18.75	22.72			
	Sample 3	18.18	18.75	18.18			
	Sample 4	36.36	31.25	27.27			
	Sample 5	9.09	12.5	13.63			
Ganai	Sample 1	21.73	23.07	22.58	19.99	19.94	19.96
	Sample 2	13.04	7.69	9.67			
	Sample 3	30.43	30.76	29.03			
	Sample 4	8.69	11.53	12.90			
	Sample 5	26.08	26.92	25.80			
Gohar	Sample 1	16.66	17.64	18.18	20	18.86	19.96
	Sample 2	25.01	23.52	27.27			
	Sample 3	16.66	17.64	13.63			
	Sample 4	25.01	17.64	9.09			
	Sample 5	16.66	17.77	31.81			
Kummi	Sample 1	15.90	17.77	17.30	19.99	19.96	19.94
	Sample 2	20.45	22.22	21.15			
	Sample 3	13.63	24.44	23.07			
	Sample 4	22.72	15.55	15.38			
	Sample 5	16.66	20	23.07			

C. Identification and Diversity assessment of Arbuscular Mycorrhizal Fungi

Total five genera and 17 species of mycorrhizal fungi were isolated from the rhizosphere of Pea (*Pisum sativum*) from all samples collected from various sampling sites of mid hill conditions of district Mandi, Himachal Pradesh. The genus *Glomus* was isolated with maximum 6 species followed by *Acaulospora* (05 sp.), *Gigaspora* (03 sp.), *Scutellospora* (02 sp.) whiles *Sclerocystis* with single species. The genus *Glomus* is classified as a mycorrhizal type with a wide distribution found in almost all ecosystems (Ibou *et al.,* 2021; Lara-Capistran *et al.,* 2021; Sukmawati *et al.,* 2021). While the genera *Scutellospora* and *Acaulospora* have limited distribution (Ibou *et al.,* 2021; Lara-Capistran *et al.,* 2021; Sukmawati *et al.,* 2021). The various microscopic characteristics of AM fungi observed in present study are summarized in Table 3, Plates II&III.

Table 3: Types and density of Arbuscular Mycorrhizal fungi (AMF) spores.

Sr. No.	AM fungi	Identification parameters			
		Diameter	Wall width	Colour	Hypha
1.	*Glomus fugianum*	264 × 231µm	16µm	Dark brown to yellow	Absent
2.	*Glomus macrocarpum*	132 × 165µm	4µm	Light yellow & transparent	Present
3.	*Glomus melanosporum*	231 × 231µm	3µm	Dark black and brown inside	Present
4.	*Glomus multicauli*	297 × 297 µm	15µm	Brown and black	Absent
5.	*Glomus spercum*	40.5 × 98.5µm	10 µm	Dark yellow to black inside	Absent
6.	*Glomus* sp.	297 × 198 µm	10 µm	Dark yellow to brown inside.	Absent
7.	*Acaulospora denticulata*	191 × 165 µm	8.5 µm	Yellow to brown	Absent
8.	*Acaulospora bireticulata*	181.5 × 214.5 µm	15 µm	Dark brown	Absent
9.	*Acaulospora rehmii*	264 × 231 µm	16 µm	Yellow to brown	Absent
10.	*Acaulospora dilatata*	171.6 × 188.1µm	16 µm	Brown	Present
11.	*Acaulospora undulata*	181.5 × 214.5µm	10 µm	Brown to dark brown	Present
12.	*Gigaspora albida*	264 × 231µm	8 µm	Dark brown	Present
13.	*Gigaspora margarita*	184.5 × 132µm	13 µm	Yellow	Absent
14.	*Gigaspora rosea*	214.5 × 198µm	6 µm	Black	Present
15.	*Scutellospora dipurpurscens*	297 × 297 µm	6 µm.	Brown	Present
16.	*Scutellospora minuta*	397 × 297 µm	4 µm	Transparent shade	Present
17.	*Sclerocystis* sp.	330 × 330 µm	4 µm	Dark brown	Absent

The five genera and 17 species of mycorrhizal fungi were isolated during the present study are as *Glomus fugianum, G. macrocarpum, G. melanosporum, G. multicauli, G. spercum, Glomus* sp., *Acaulospora denticulate, A. bireticulata, A. rehmii, A. dilatata, A. undulate, Gigaspora albida, G. margarita, G. rosea, Scutellospora dipurpurscens, S. minuta* and *Sclerocystis* sp. The isolated AM fungi showed a great degree of variations in occurrence at different sampling sites. The diversity and distribution of AM fungi at different sampling sites is given in Table 4.

Table 4: The distribution of AM fungi in different sampling sites.

AM Fungi	Balh Valley						Chail –Chowk valley					
	Ku	Sa	Su	Da	Ra	Gag	Cc	Ch	Gan	Go	Ft 1	Ft 2
Glomus fugianum	+	+	+	-	-	-	+	+	-	+	-	-
Glomus macrocarpum	+	+	+	+	-	-	-	+	-	-	+	-
Glomus melanosporum	+	-	+	+	+	-	+	-	-	-	+	+
Glomus spercum	+	-	-	+	+	+	-	-	-	+	-	+
Glomus spp.	-	+	+	-	+	-	-	+	-	+	-	-
Acaulospora denticulate	+	-	+	-	-	-	+	-	+	-	+	-
Acaulospora bireticulata	+	+	-	-	-	-	-	+	-	-	-	-
Acaulospora rehmii	-	-	-	-	+	+	-	+	+	-	-	-
Acaulospora dilatata	-	-	-	-	+	-	-	-	-	-	+	-
Acaulospora undulate	+	-	-	+	-	-	+	+	+	+	-	+
Gigaspora albida	+	-	+	-	-	+	+	+	+	-	+	-
Gigaspora margarita	+	+	+	+	+	-	+	-	-	+	+	+
Gigaspora rosea	-	+	-	-	+	-	-	+	-	-	-	-
Scutellospora dipurpurscens	-	-	-	-	-	-	-	+	-	-	+	+
Scutellospora minuta	-	-	-	-	-	-	+	-	-	+	-	-
Sclerocystis spp.	-	-	-	-	-	-	-	+	-	+	-	-

Ku= Kummi, Sa= Sakroha, Su= Surandhi, Da= Dadour, Ra= Ratti, Gag= Gagal, Cc= Chail-Chowk, Ch= Chachyot, Gan= Ganai, Go= Gohar, Ft1=Field trial=1, Ft2=Field trial 2.

DISCUSSION

Root colonization was checked first to observe the AM fungi presence or absence in plant root samples. The variation in percentage root colonization with various AM fungi under natural conditions was observed in this study. Variability in humidity, temperature, moisture texture, pH of soil and available nutrients played an important role in root colonization by AM fungi (Herold *et al.,* 2014; Bhardwaj and Chandra 2018; Liu *et al.,* 2016). The root colonization by different AM fungi has already been studied on very wide scale throughout the world. The plant like *Asparagus* sp.,

Smilax sp., *Rhizophagus* sp., *Withania* sp., *Claroideoglomus* sp. has been investigated for association of AMF with plant roots (Thangavelu and Raji 2016; Yaseen *et al.*, 2016; Johny *et al.*, 2021).

The AM fungi in the rhizosphere of pea plant revealed the association of five genera and 17 species. However, a great variability in the diversity of these fungi was observed from soil samples collected from various study sites. This variation could be due to the reason of variation in physical and chemical properties of the soil (Urcoviche *et al.*, 2014; Liu *et al.*, 2016; Abedi and Esfandiari 2017) and seasonal periods and host plant (Guyonnet *et al.*, 2017). Being a commercial agricultural crop, tillage as well as land use intensity also affects the diversity and structure of arbuscular mycorrhizal fungal communities (Jansa *et al.*, 2002; Oehl *et al.*, 2004; Mathimaran *et al.*, 2005). A study of Krüger *et al.*, (2012) also reported the isolation of *Glomus* is the most diverse of the genus from rhizosphere of some medicinal plants. Similarly, Garampalli *et al.* (2012) also isolated *Glomus as* predominant genus in the rhizosphere 46 medicinal plants whereas, Bhat *et al.* (2014) also isolated it as predominant genus in the rhizosphere *Catharanthus roseus.* Several academics are working on identifying certain mycorrhizal fungus and their role in phytochemical production (Kumar *et al.*, 2021).

CONCLUSION

In conclusion, the present study was focused mainly on investigation of pea (*Pisum sativum* L.) roots and rhizosphere soil samples for the association of Arbuscular Mycorrhizal fungi. As pea is one of the major commercial crop of mid hill regions of Himachal Pradesh and the associations of AM Fungi in general may be useful to improve soil microbial status and overall performance of these plants. considering the importance of pea as commercial crop and usefulness of AM fungi, further studies should be focused on the evaluation of dominant mycorrhizal fungi association with agricultural crops and impact on plant growth and metabolite production.

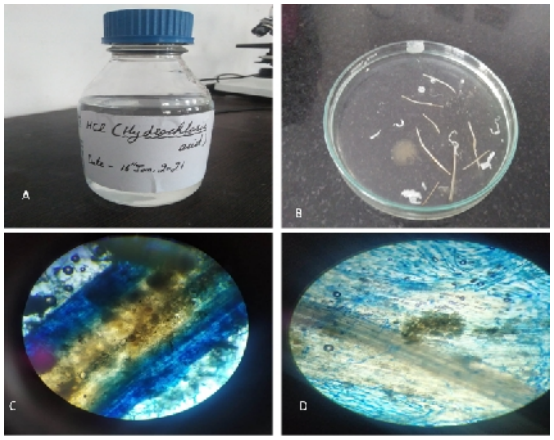

Plate II: Root colonization by AM fungi A) HCl solution; B) Fine roots acidified in HCl solution; C) & D) microscopic view of AMF colonized roots.

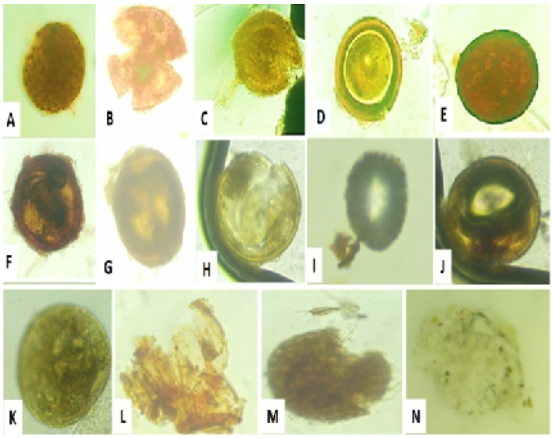

Plate III: Different species of the genus *Glomus* isolated from rhizosphere of pea: A) *Acaulospora denticulate*, B) *Acaulospora bireticulata*, C) *Acaulospora rehmii*, D) *Acaulospora dilatata*, E) *Acaulospora undulate*. F) *Glomus fugianum*, G) *Glomus macrocarpum*, H) *Glomus melanosporum*, I) *Glomus multicauli*, J) *Glomus spercum*, K) *Glomus* sp., L) *Scutellospora dipurpurscens*, M) *Scutellospora minuta*, N) *Sclerocystis* sp.

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