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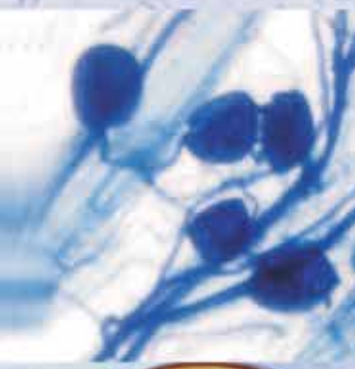


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Research finding papers

Effect of Arbuscular Mycorrhizal Fungus, *Bacillus polymeyxa*, PSB, and Rock Phosphate Application on Growth Biomass Improvement of *Terminalia bellerica* Roxb.

H C Lakshman and Jyoti Puttaradder*

Introduction

The problems of degradation of natural resources have received increasing attention in recent days. A total of 177 million hectares of land in India is subject to various types of degradation. This has many direct and indirect ill effects. It is important to re-vegetate the vast areas with high efficiency and valuable forest species to cater to the bona fide needs of the growing population. The role of arbuscular mycorrhizal fungi (AMF) in improving the quality and survival of forest nursery seedlings and their growth after planting has been well recognized (Trappe 1977; Lakshman 1996; Lakshmipathy *et al*. 2000).

Plant roots increase the efficiency of absorption of nutrients and thus enhance the growth of plants and trees (Tilak 1993; Rilling and Mumnery 2006). The existence of host preference has been suggested (Singh 1994; Bagyaraj 2006). This preferential association between certain plants and fungal species can be evaluated with respect to combined inoculation with two organisms that provide the greatest plant growth stimulations and the higher root colonization or maximum sporulation (Jasper 1994; Mukherjee *et al*. 2000). Combined inoculation of phosphate solubilizer (PSB) species with additional rock phosphate was found to be superior than uninoculated control plant (Mosse *et al.* 1976; Mamatha and Bagyaraj 2001; Gour 1990, 2002). *Terminalia bellerica* is one of the promising tree species, which could be taken as an alternative to achieve the goal of sustainability. *Terminalia bellerica* is a rare good timber tree, and its fruits and seeds are used medicinally in Ayurveda. In natural conditions, these plants do not form healthy symbiosis due to lack of specificity of the host. Therefore, there is exigency either to inoculate the species with specific PSB strains or to fertilize with phosphorus to get better nursery stock. Keeping in view the importance of PSB, AMF, and rock phosphate, the present studies were carried out to evaluate their influence on growth and nutrient dynamics of *T. bellerica* seedlings.

Materials and Methods

Greenhouse experiments were carried out in randomized block design with three replications at the greenhouse of Department of Botany, Karnatak University, Dharwad, during 2012–13. Earthen pots measuring (20 × 25 cm) length and breadth were filled with 4 kg of garden soil, which was sterilized in 5 per cent methyl bromide for one hour and stored at room temperature for one week. *Glomus macrocarpum* (AM fungus) was maintained on host plant *Panicum maximum Jacq*. in pot culture. Dried mixed 15 g inoculum of a *G. macrocanpun* was used. Lignite-based *Bacillus polymyxa* with 14×10^8 cells/g was inoculated at 0.4 g/pot in the form of slurry to the seeds of final product having 30 per cent moisture content before sowing. Four levels of rock phosphate

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$Ca_3(Po_4)_2CaF_2(P_2O_5)$; 10, 15, 30, and 40 mg/kg soil mixed in distilled water are used. For control treatment, the sieved soil suspension was added to maintain the rhizosphere of soil without inoculum. Pots were watered on alternate day, 15 ml Hoagland (–p) solution was added once a week. Plants were harvested after every 60 days up to 180 days.

Dry weight of shoots and roots was recorded after maintaining at 70°C in autoclave for 12 hrs. Total phosphorous (P) content of the plants was determined following the method of (Jackson 1973). Nitrogen (N) content of plants and root nodules were analysed following the procedure of (Bermner 1960). The percentage of mycorrhizal colonization was calculated after clearing the roots with 10 per cent KOH and stained with 0.05 per cent trypan blue in lactophenol (Phillips and Hayman 1970). Spore count in 50 g soil was determined by the following procedure of wet sieving and decanting technique of Gerdemann and Nicolson (1963).

Results and Discussion

Pot experiments were conducted in sandy loam with low P, 31.87/kg (Table 1). The data regarding the growth parameters of *T. bellerica* seedling is represented in Tables 2 and 3. The rock phosphate application with PSB had significant positive effect on seedling height, dry weight of shoot and roots, percentage of root colonization, and spore number when compared to non-inoculated (control) plants. These results revealed that inoculation of single bio-inoculant may influence the increase of mycorrhizal colonization in the roots and spore number in the rhizosphere soils. The lower level of rock phosphate, PSB with mycorrhizal inoculation does not influence *T. bellerica* biomass production. Varying levels of phosphate application with PSB plants showed difference in growth and yield parameters (Gour 2002). The increased biomass yield was recorded with rock phosphate at 30 mg Rk/kg soil with PSB and AM fungus. Similar results were reported by Cruz *et al.* (1988), Koide and Schreiner (1992), and Lakshman (1996, 2002). The number of leaves increased with an increased P level up to 30 mg Rk/kg soil with PSB and AM fungus inoculation. However, further increase in the levels of P had suppressing effect even in the presence of PSB. This may be due to the reason that when P is freely available in the soil, leaves may or may not take place, and even if the leaves are formed, they are in sterile state. At the same time, higher concentration may act as inhibitor for the symbiotic association (Allen 1992; Sandeepkumar and Lakshman 2009). Lower levels of phosphate were observed to be at par or sometimes better than the higher levels in producing vigorous growth and yield of inoculated plants (Michealson and Resendahl 1990).

Nitrogen uptake and phosphorus uptake in shoots increased with increase in rock phosphate along with PSB. This may be because of the slowly dissolved available P in the soil and P being the essential element for establishment of healthy symbiosis between PSB and *G. macrocarpum* (Lakshman 2002, 2009). The nutrient content increased significantly in rock phosphate amended experiment along with mycorrhizal PSB inoculated plants as compared to control plants (Table 3). Similarly, nitrogen and phosphate uptake increased with the inoculation of AMF + PSB + rock phosphate treatments. Therefore, these studies indicated that the mycorrhizal inoculation helps in the effective utilization of rock phosphate by changing into available form and PSB is taken up by the plants for better growth and development. With regard to the development of root system, it resulted in increase in the biomass and yield of aerial parts. However, the root/shoot ratio decreased with inoculation AM fungus PSB or rock phosphate treatment. Mycorrhizal inoculation with PSB and rock phosphate leads to change in distribution of dry matter between root and shoot. Thus, bio-inoculated plants showed lower root/shoot ratio than non-inoculated plants. Similar observation was made by early workers (Figure 1) (Smith 1980; Lakshman 1996).

Table 1: Physico-chemical characteristics of garden soil used for pot experiments

Parameters	Test value	Soil characteristics	Method employed
Texture	Sandy loam	Silt dominated solid	International pipette method (Jackson 1973)
pH	6.8	Acidic	1:2:5 water suspension
Organic carbon (percentage)	0.49	Low/medium	Walkley and Black Method 1934
Available N (kg/ha)	242.61	Low/Medium	Microkjeldhal prodecure (Brener 1960)
Available P (kg/ha)	31.87	Low	Olsen (1954)

Note: Each value is the mean of 12 samples.

Table 2: Effect of AMF (*G. macrocarpum*) PSB and phosphorus level and growth character of T. bellerica Roxb. seedlings for 180 days

Treatment	Plant height (cm)	No. of leaves/plants	Dry wt. of shoot (g)	Dry weight of root (g)	Root/shoot ratio (g/plant)
NM/NPSB	11.9	5.3	2.11	0.42	0.69
AMF	18.4	11.2	4.6	0.49	0.51
PSB	15.2	11.1	4.1	0.47	0.42
AMF + PSB + RPO	19.2	18.5	4.8	0.53	0.44
AMF + PSB + 10 mg/RP/kg	23.7	24.6	5.6	1.19	0.42
AMF + PSB + 15 mg/RP/kg	36.2	31.6	8.7	1.76	0.39
AMF + PSB + 30 mg/RP/kg	54.1	37.2	13.2	1.93	0.34
AMF + PSB + 40 mg/RP/kg	47.2	26.2	12.2	1.71	0.31
L.S.D. (P = 0.05)	0.11		0.03	0.01	0.06

Table 3: Effect of the *B. polymyxa* and phosphorus levels as nutrient content in shoots *T. bellerica* Roxb. seedlings for 180 days (replications)

Treatment	Spore number/50 g soil	Percentage of VMF colonization	P mg/Dry weight of shoot	N mg/Dry weight of shoot
NM/NPSB	23.1	–	–	2.16
AMF	34.3	189	47.5	2.23
PSB	33.1	157	34.2	4.11
AMF + PSB + RPO	34.5	151	47.3	5.14
AMF + PSB + 10 mg/RP/kg	35.7	202	52.4	6.13
AMF + PSB + 15 mg/RP/kg	35.6	207	55.3	9.15
AMF + PSB + 30 mg/RP/kg	49.2	213	68.5	11.30
AMF + PSB + 40 mg/RP/kg	33.1	109	51.2	8.22
L.S.D. (P = 0.05)	0.12	11	1.22	1.09

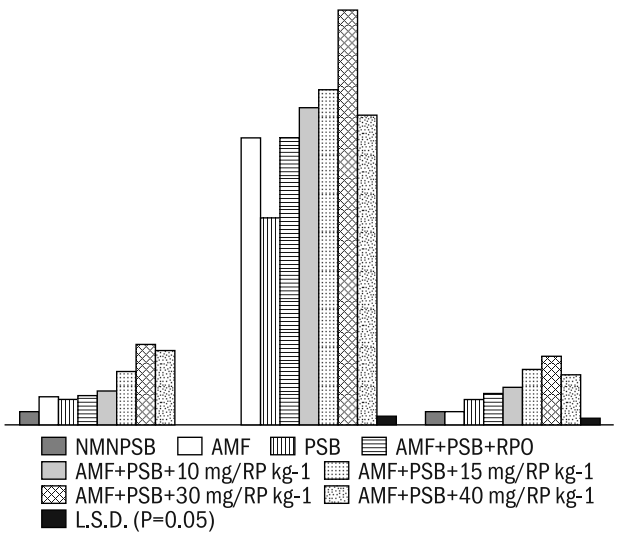

Figure 1: Dry weight of shoot, P and N content of *T. bellerica* Roxb. seedlings for 180 days

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Association of Arbuscular Mycorrhizal Fungi (AMF) with Common Leguminous Crops Cultivated in Aligarh District (Uttar Pradesh): A Survey

Rose Rizvi and Irshad Mahmood*

Introduction

Aligarh is located at 27.88°N latitude and 78.08°E longitude at an average elevation of 178 m (587 feet) above sea level in the mid of *Doab*, the land between the Ganges and Yamuna rivers. Aligarh experiences semi-arid and sub-tropical climate with hot dry summers and intense cold winters. The total area of Aligarh district is 3,696.94 sq. km, and it has 82 per cent of the geographical area under cultivation. The core sector of economy is agriculture, and crops raised in this district include wheat, paddy, pulses, oil seeds, potato, *ber*, and guava.

Arbuscular mycorrhizal fungi (AMF) are ubiquitous and form symbiotic association with roots of about 90 per cent plants in natural and agricultural ecosystems (Harley and Smith 1983; Powell and Bagyaraj 1982; Gabor 1991; Brundrett 2002; Muthukumar and Prakash 2009; Mahmood and Rizvi 2010). The significance of AMF in augmenting food production is increasingly appreciated as these organisms can increase plant growth (Smith and Read 1997), plant reproductive capacity (Lu and Koide 1994), plant water stress tolerance (Gupta and Kumar 2000), and plant health through antagonistic and competitive effects on pests and pathogens (Gange and West 1994). AMF are responsible for enhanced uptake of immobile mineral nutrients especially phosphorus from soil to the host plants and thereby enhancing vigour (Gerdemann 1964; Nicolsan 1967; Mosse 1972, 1973; Barea *et al.* 1988; Wani *et al.* 1991; Jakobsen 1999; Akhtar and Siddiqui 2008; Jalaluddin *et al.* 2008; Giasson *et al.* 2008; Sawers *et al.* 2008). Hence, they are referred as 'biofertilizers' and can be substantiated for substantial amounts of chemical fertilizers. Despite the importance of mycorrhizae in agriculture and forestry, little work has been done regarding their distribution, diversity, and association with the host plants in India. Hence in current research on AMF, there is a need for the selection of environmental and ecological specific plant–fungus association.

Legumes are important pulse cultivated throughout the Indian subcontinent, and cost effective cultivation of this agricultural crop depends on effective symbiotic nitrogen fixation in their nodules that relies on the rhizobial population of the soil (Postgate 1982). Mycorrhizal infection has particular value for legumes because nodulation and symbiotic nitrogen fixation by rhizobia require an adequate phosphorus supply and restrict root system leads to poor competition for soil phosphorus (Carling *et al.* 1978). AM association with different leguminous crops is currently of great interest because of the important role played by these crops in restoration of degraded soils and enriching soil fertility (Peoples and Crasswell 1992; Thomas *et al.* 1997). Thus, cultivation of leguminous crops is increasing steadily to maintain a steady supply and to support their increasing demand. But corresponding research works on the occurrence of AMF and their associations in legumes have received little attention as compared to the studies on forest species.

This has prompted evaluation of mycorrhizal status of leguminous crops in Aligarh district. Hence, the present study was undertaken to make a general survey on mycorrhizal colonization with different leguminous crops and spore population in rhizosphere soils at 12 blocks of Aligarh district.

Materials and Methods

Sites selection, Soil, and Crop Sampling

A qualitative and quantitative survey (2012–15) of species of distribution of AMF was carried out at different blocks viz., Atrauli (B_1), Akrabad (B_2), Bijauli (B_3), Chandaus (B_4), Dhanipur (B_5), Gangiri (B_6), Gonda (B_7), Iglas (B_8), Jawan (B_9), Khair (B_{10}), Lodha (B_{11}), and Tappal (B_{12}) of Aligarh district of Uttar Pradesh, India, during the year 2008–09.
Six commonly cultivated leguminous crops viz., pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), pigeon pea (*Cajanus cajan* L.) Mill sp., mung bean (*Vigna radiata* L.) Wilczek, cowpea (*V. unguiculata* L.) Walp., black gram (*V. mungo* L.) Hepper, and lentil (*Lens culinaris* Medikus) were selected for study at each site.

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Sampling was done for each crop separately from the fields of leguminous crops at different sites. Soil samples (soil cones of 5 cm diameter) were collected at random from each site with the help of soil auger up to a depth of 15 cm near the rhizosphere. Fifty such samples were collected from each plant species and were thoroughly mixed to make a composite sample. Six samples of 100 g soil were used for recovery of spores.

Six root samples for each plant species were collected at random in order to assess root colonization by AMF. A representative sample of the entire root system was obtained from five different portions of the root system after washing with tap water.

Soil Characteristics

During survey, soil characteristics of rhizosphere soil in terms of texture; pH; electrical conductivity (EC) (mmho/cm); particle size (percentage); organic carbon content; and nutrient levels such as nitrogen (N), phosphorus (P), and potassium (K) content of the soil were taken into consideration to determine the effect of these soil parameters on root colonization by AMF.

The soil samples were brought to laboratory, marked, and packed in polythene bags and their EC and pH were measured with EC meter (Rhoades 1996) and pH meter (McLean 1982), respectively, in 1:1 soil/water suspension (w/v). The texture of soil in relation to particle size was determined by hydrometer method (Allen *et al.* 1974); total organic carbon by Walkey (1947); total nitrogen by micro-Kjeldahl method (Nelson & Sommers 1972); total phosphorus by molybdenum blue method (Allen *et al.* 1974); and potassium by using flame photometer (Jackson 1973).

Isolation of Spores from the Soil

Mycorrhizal spore count of each soil sample was isolated by a modified wet sieving and decanting technique (Gerdeman and Nicolsan 1963). Samples of 100 g dry soil were taken in 1,000 ml water, thoroughly shaken, and left for a minute to settle down the heavier particles. The supernatant was poured first through a coarse soil sieve to remove large piece of organic matter and then decanted on to a series of varied size sieves, that is, 80, 100, 150, 250, and 400 meshes. Spores obtained on sieves were collected with water in separate beakers. The spore suspensions were repeatedly washed by Ringer's solution (NaCl: 6 g/l, KCl: 0.1 g/l, and $CaCl_2$: 0.1 g/l in distilled water of pH 7.4) in order to remove the adhered soil particles from the spores. The spores were counted in 1 ml of the suspension in nematode counting dish under the stereoscopic microscope. Spore numbers from the three replicates per samples were averaged and the result was expressed as spore number per 100 g of dry soil basis. The final number of AM spores of each species per 100 g of soil was calculated accordingly for each crop.

Identification of AMF Spores

The isolated spores of AMF were identified under a dissecting microscope with the help of synoptic keys suggested by various workers (Hall and Fish 1979; Trappe 1982; Schenck and Perez 1987; Rani and Mukerji 1988; Srinivas *et al.* 1988; Raman and Mohankumar 1988). For species identification, Perez and Schenck (1990) manual was followed. Different genera were identified on the basis of sporocarpic and spore characteristics such as spore size, shape, colour, surface structure, general nature of the contents, hyphal attachments, spore wall, and other morphological structures (Perez and Schenck 1990). The spores of different AM fungal species were separated with a microspatula and picked up by a pasteur pipette fitted with a rubber bulb and then spores were used for pot culture.

Clearing and Staining

Roots were washed with tap water and cut into 1-cm long segments and then boiled in 10 per cent KOH solution at 90°C for 45 minutes. KOH solution was then poured off and roots were rinsed well in a beaker until no brown colour appeared in the rinsed water. Roots were then treated with 0.05 per cent trypan blue (in lactophenol) and were kept for an hour. The specimens were then removed from trypan blue and kept overnight in a destaining solution, prepared with 875 ml acetic acid (laboratory grade), 63 ml glycerine, and 63 ml distilled water. The cellular contents were removed by this method and the AM fungal structures stained dark blue (Phillips and Hayman 1970). The percentage/proportion of root colonization by AMF was determined by the grid line intersecting method (Giovanetti and Mosse 1980). The presence or absence of colonization in each root segment was recorded and percentage of root colonization (mycorrhizal infection in the roots) was calculated as follows:

$$\text{Percentage of root colonization} = \frac{\text{(Number of AM positive segments)}}{\text{(Total number of segments screened)}} \times 100$$

Statistical Analysis

The data collected during this study was statistically analysed in simple randomized design by the method of Panse and Sukhatme (1985). All data were subjected to Analysis of Variance to assess the significance of variations in AM fungal variables among leguminous crop species. Minimum difference

required for significance (Critical Difference) at $P = 0.01$ and $P = 0.05$ was calculated.

Results

Soil Characteristics

It was found that all the sites (B_1 to B_{12}) surveyed have sandy loam soil and their compositions were almost the same with narrow pH range of 7.5 (B_2) to 8.9 (B_9). The EC was lowest at Akrabad (B_2) and highest at Jawan (B_9). Organic carbon content of the soil at block B_1 was highest, followed by B_{12}, B_8, B_4, B_5, B_2, B_7, B_{10}, B_9, B_6, B_{11}, and B_3 blocks. As far as the soil nutrients are concerned, nitrogen content was highest at Atrauli (184 kg/ha) and phosphorus and potassium content ranged between 7.54 (B_2) to 42.17 (B_1) and 102.30 (B_3) to 252.60 (B_1) kg/ha, respectively. Soil at Atrauli (B_1) has comparatively high N, P, and K content than the soil at other blocks and Chandaus (B_4) have the largest area under pulses production, that is, 2,840 hectares (Table 1).

Root Colonization and Spore Population

The data provided in Table 3 indicates that the root colonization (percentage) and total AMF spore number per 100 g of soil varied with the crop as well as with the 12 sites investigated.

At block B_1, AM colonization was observed in almost all grain legumes. The highest colonization (60.2 per cent) was observed in chickpea and the lowest (14.4 per cent) in lentil. Black gram and lentil showed very poor colonization compared to other legumes. Results clearly indicated that root colonization diversified within the members of the same family. Total spore numbers ranged from 155 in lentil to 227 in chickpea per 100 g dry soil. AMF spores per 100 g soil was more than 200 in chickpea, pigeon pea, and mung bean, and it was more than 100 in pea, cowpea, black gram, and lentil (Table 3). At block B_2, the level of mycorrhizal colonization varied from 53.0 to 88.0 per cent in different leguminous crops, highest being exhibited by chickpea. High percentage of root colonization was observed in pea, chickpea, pigeon pea, mung bean, and cowpea (Table 3). The spore numbers ranged from 395 (black gram) to 456 (chickpea) per 100 g dry soil. At block B_3, the maximum colonization (85.0 per cent) was observed in pigeon pea and minimum (50.0 per cent) in black gram. The spore numbers per 100 g dry soil in the rhizosphere ranged from 366 in black gram to 435 in pigeon pea. Chickpea and pigeon pea recorded more than 400 spores per 100 g dry soil while pea, mung bean, cowpea, lentil, and black gram recorded more than 300 spores per 100 g dry soil (Table 3). At block B_4, lowest colonization (23.1 per cent) was found in black gram and highest in chickpea (70.0 per cent). Pigeon pea, mung bean, cowpea, and pea also showed higher root colonization. Total spore numbers ranged from 197 (black gram) to 260 (chickpea) per 100 g dry soil. All the legumes recorded more than 200 spores per 100 g dry soil except black gram. At block B_5, different leguminous crops showed different percentages of root colonization by AMF. Percentage of root colonization varied from 35.2 to 77.1 per cent. Chickpea, pigeon pea, mung bean, and pea exhibited comparatively better performance than cowpea, lentil, and black gram, respectively. The rhizosphere soil contained AMF spore number ranging from 278 in

Table 1: Some physico-chemical characteristics of the rhizosphere soil surveyed at 12 blocks under Aligarh district

Different Blocks in Aligarh	Soil Texture	pH	Electrical Conductivity (Mmho/Cm)	Particle Size (Percentage)			Organic Carbon (Percentage)	Available Nutrients (Kg/Ha)			Area Under Pulses (Ha)
				Sand	Silt	Clay		N	P	K	
Atrauli (B1)	Sandy Loam	8.4	5.1	70.3	20.5	9.2	0.30	184	42.17	252.60	1,906
Akrabad (B2)	Sandy Loam	7.5	3.8	76.4	17.8	5.8	0.26	160	7.54	205.79	2,247
Bijauli (B3)	Sandy Loam	7.7	3.9	74.9	19.1	6.0	0.21	101	7.80	102.30	1,085
Chandaus (B4)	Sandy Loam	8.3	4.8	75.6	15.9	8.5	0.27	133	18.00	151.00	2,840
Dhanipur (B5)	Sandy Loam	7.9	4.0	77.1	16.4	6.5	0.27	154	12.45	194.26	2,329
Gangiri (B6)	Sandy Loam	7.8	3.9	70.7	23.0	6.3	0.23	108	8.11	118.94	2,170
Gonda (B7)	Sandy Loam	8.5	5.3	72.3	17.2	10.5	0.26	142	10.57	176.83	1,163
Iglas (B8)	Sandy Loam	8.0	4.2	75.2	17.4	7.4	0.28	165	14.55	220.32	1,355
Jawan (B9)	Sandy Loam	8.9	5.9	74.5	19.4	12.6	0.24	116	19.35	125.61	3,020
Khair (B10)	Sandy Loam	8.7	5.6	71.0	17.7	11.3	0.25	139	15.91	163.52	1,760
Lodha (B11)	Sandy Loam	8.0	4.3	71.8	20.2	8.0	0.23	171	17.30	235.10	2,744
Tappal (B12)	Sandy Loam	8.1	4.5	69.1	22.6	8.3	0.28	127	9.23	137.44	1,695

Table 2: Arsbuscular mycorrhizal structure (hyphae, arbuscules, and vesicles) in different leguminous crops surveyed at 12 blocks under Aligarh district

Crops	Atrauli (B1)				Akrabad (B2)				Bijauli (B3)				Chandaus (B4)				Dhanipur (B5)				Gangiri (B6)			
	H	A	V	VS	H	A	V	VS	H	A	V	VS	H	A	V	VS	H	A	V	VS	H	A	V	VS
Pea	+	+	–	–	+	+	–	–	+	+	–	–	+	+	–	–	+	–	–	–	+	+	–	–
Chickpea	+	+	+	O, R	+	+	+	O, S	+	+	–	–	+	+	–	–	+	+	+	O, R	+	+	+	O, S
Pigeonpea	+	+	+	O, R	+	+	+	O, S	–	+	+	O, R	+	+	–	–	+	+	+	O, S	+	+	–	–
Mung bean	+	+	+	O, S	+	+	+	R, S	+	+	+	O, S	+	+	+	O, S	+	+	+	O, S	+	+	–	–
Cowpea	+	–	–	–	+	+	–	–	+	+	+	O, R	+	+	+	O, S	+	+	–	–	+	+	+	O, S
Black gram	+	+	–	–	+	+	–	–	+	–	–	–	+	+	+	S	+	–	–	–	+	+	–	–
Lentil	+	+	–	–	–	+	+	O, S	+	+	–	–	–	–	+	O, S	+	–	–	–	+	+	–	–

Crops	Gonda (B7)				Iglas (B8)				Jawan (B9)				Khair (B10)				Lodha (B11)				Tappal (B12)			
	H	A	V	VS	H	A	V	VS	H	A	V	VS	H	A	V	VS	H	A	V	VS	H	A	V	VS
Pea	+	+	–	–	+	–	–	–	+	+	–	–	+	–	–	–	+	+	–	–	+	–	–	–
Chickpea	+	+	–	–	+	+	–	–	+	+	–	–	+	+	+	O, S	+	+	–	–	+	+	+	O, R
Pigeon pea	+	+	+	O, R	+	+	+	O, S	+	+	–	–	+	+	–	–	+	+	–	–	+	+	–	–
Mung bean	+	+	–	–	+	+	+	R, S	+	+	+	O, S	+	+	+	R, S	+	+	+	O, S	+	+	–	–
Cowpea	+	+	+	O, R	+	–	–	–	+	+	–	–	+	–	–	–	+	+	–	–	+	+	+	O, R
Black gram	+	–	–	–	+	+	+	S	+	+	–	–	+	–	–	–	+	+	–	–	+	+	+	S
Lentil	–	–	+	O, S	–	+	+	O, S	+	+	–	–	+	–	–	–	+	–	–	–	+	+	–	–

H = Hyphae; A = Arbuscules; V = Vesicle; VS = Vesicle Shape; S = Spherical; O = Oval; R = Round.

lentil to 327 in chickpea per 100 g dry soil. More than 200 spores per 100 g dry soil were divulged in all the legumes (Table 3). At block B_6, the mycorrhizal colonization was highest (82.1 per cent) in chickpea and lowest (46.1 per cent) in black gram. Total spore numbers ranged from 341 in black gram to 406 in chickpea per 100 g dry soil. AMF spores per 100 g soil was more than 300 in all the leguminous crops (Table 3). At block B_7, the mycorrhizal colonization varied from 39 to 79 per cent in different leguminous crops, highest being exhibited by pigeon pea. High percentage of root colonization was observed in pea, chickpea, pigeon pea, mung bean, and cowpea. The spore numbers ranged from 294 (black gram) to 352 (pigeon pea) per 100 g dry soil (Table 3). At block B_8, maximum colonization (75 per cent) was observed in chickpea and minimum (31 per cent) in black gram (Figure 1b). The spore numbers per 100 g dry soil in the rhizosphere ranged from 251 in black gram to 310 in chickpea. At block B_9, the lowest colonization (20 per cent) was found in lentil and the highest in chickpea (66.0 per cent). Pigeon pea, mung bean, cowpea, and pea also showed higher root colonization. Total spore numbers ranged from 178 (lentil) to 245 (chickpea) per 100 g dry soil (Table 3). At block B_{10}, percentage of root colonization varied from 28.5 to 73.2 per cent. Chickpea, pigeon pea, mung bean, and pea exhibited comparatively better performance than cowpea, lentil, and black gram. The rhizosphere soil contained AMF spore number ranging from 227 in black gram to 289 in chickpea per 100 g dry soil (Table 3). At block B_{11}, highest colonization (71 per cent) was observed in chickpea and lowest (25 per cent) in black gram. Total spore numbers ranged from 206 in black gram to 270 in chickpea per 100 g dry soil. AMF spores per 100 g soil was more than 200 in all the legumes. At block B_{12}, the level of mycorrhizal colonization varied from 42.3 to 80.4 per cent in different leguminous crops, highest being exhibited by pigeon pea. The spore numbers ranged from 318 (black gram) to 378 (pigeon pea) per 100 g dry soil (Table 3).

Diversification of AM Structure

In the root system, AM fungal structures of the selected leguminous crops diversified irrespective of legume species (Table 2). At block B_1, 100 per cent legumes had hyphae and 90 per cent had arbuscules. A total of 30 per cent plants produced oval- and round-shaped vesicles and 10 per cent plants showed oval and spherical type of vesicles. At block B_2, all the leguminous crops contained hyphae except lentil and all crops had arbuscules. Chickpea, pigeon pea, mung bean, and lentil had vesicles. The shapes of vesicles were both oval and spherical in chickpea, pigeon pea, and lentil while mung bean contained round- and spherical-shaped vesicles.

Table 3: Root colonization and spore population of AM fungi in some leguminous crops surveyed at 12 blocks under Aligarh district

Crops	Atrauli (B1)		Akrabad (B2)		Bijauli (B3)		Chandaus (B4)		Dhanipur (B5)		Gangiri (B6)	
	Root Colonization (percentage)	Number of AMF Spore per 100 G Soil	Root Colonization (percentage)	Number of AMF Spore per 100 G Soil	Root Colonization (percentage)	Number of AMF Spore per 100 G Soil	Root Colonization (percentage)	Number of AMF Spore per 100 G Soil	Root Colonization (percentage)	Number of AMF Spore per 100 G Soil	Root Colonization (percentage)	Number pf AMF Spore per 100 G Soil
Pea	46.0 ± 2.3	200.0 ± 10.0	78.0 ± 3.9	420.0 ± 21.0	73.0 ± 3.7	389.0 ± 19.4	52.5 ± 2.6	236.0 ± 11.8	63.2 ± 3.2	298.0 ± 14.9	71.0 ± 3.5	363.0 ± 18.1
Chickpea	60.2 ± 3.0	227.0 ± 11.4	88.0 ± 4.4	456.0 ± 22.8	85.0 ± 4.3	435.0 ± 21.8	70.0 ± 3.5	260.0 ± 13.0	77.1 ± 3.9	327.0 ± 16.4	82.1 ± 4.1	406.0 ± 20.3
Pigeonpea	51.0 ± 2.5	214.0 ± 10.7	84.0 ± 4.2	440.0 ± 22.0	82.3 ± 4.1	411.0 ± 20.5	58.0 ± 2.9	253.0 ± 12.6	71.4 ± 3.6	314.0 ± 15.7	79.0 ± 3.9	384.0 ± 19.2
Mungbean	53.1 ± 2.7	221.0 ± 11.1	80.0 ± 4.0	428.0 ± 21.4	78.1 ± 3.9	397.0 ± 19.8	55.0 ± 2.8	248.0 ± 12.4	66.2 ± 3.3	303.0 ± 15.1	75.0 ± 3.8	370.0 ± 18.5
Cowpea	32.0 ± 1.6	186.0 ± 9.3	71.1 ± 3.6	414.0 ± 20.7	67.0 ± 3.3	386.0 ± 19.3	40.0 ± 2.0	221.0 ± 11.1	51.3 ± 2.6	286.0 ± 14.3	64.1 ± 3.2	355.0 ± 17.8
Blackgram	14.4 ± 0.7	155.0 ± 7.8	53.0 ± 2.6	395.0 ± 19.8	50.0 ± 2.5	366.0 ± 18.3	23.1 ± 1.2	197.0 ± 9.8	35.2 ± 1.8	278.0 ± 13.9	46.1 ± 2.3	341.0 ± 17.0
Lentil	19.0 ± 1.0	170.0 ± 8.5	62.0 ± 3.1	404.0 ± 20.2	59.2 ± 3.0	374.0 ± 18.7	29.1 ± 1.5	209.0 ± 10.5	43.0 ± 2.1	284.0 ± 14.2	55.1 ± 2.8	348.0 ± 17.4
CD (P = 0.05)	3.96	17.82	6.75	NS	6.49	35.46	4.57	20.97	5.49	26.86	6.22	33.03
CD (P = 0.01)	5.55	24.99	9.46	NS	9.10	49.72	6.40	29.40	7.69	37.66	8.71	46.31

Crops	Gonda (B7)		Iglas (B8)		Jawan (B9)		Khair (B10)		Lodha (B11)		Tappal (B12)	
	Root Colonization (percentage)	Number of AMF Spore per 100 G Soil	Root Colonization (percentage)	Number of AMF Spore per 100 G Soil	Root Colonization (percentage)	Number of AMF Spore Per 100 G Soil	Root Colonization (percentage)	Number of Amf Spore Per 100 G Soil	Root Colonization (percentage)	Number of AMF Spore per 100 G Soil	Root Colonization (percentage)	Number of AMF Spore per 100 G Soil
Pea	65.0 ± 3.3	316.0 ± 15.8	60.0 ± 3.0	281.0 ± 14.0	48.0 ± 2.4	222.0 ± 11.1	57.1 ± 2.9	265.0 ± 13.3	54.0 ± 2.7	247.0 ± 12.4	68.4 ± 3.4	334.0 ± 16.7
Chickpea	79.0 ± 3.9	352.0 ± 17.6	75.0 ± 3.8	310.0 ± 15.5	66.0 ± 3.3	245.0 ± 12.3	73.2 ± 3.7	289.0 ± 14.4	71.0 ± 3.5	270.0 ± 13.5	80.4 ± 4.0	378.0 ± 18.9
Pigeonpea	73.0 ± 3.7	333.0 ± 16.7	69.0 ± 3.5	298.0 ± 14.9	54.0 ± 2.7	232.0 ± 11.6	65.0 ± 3.3	277.0 ± 13.9	63.0 ± 3.2	262.0 ± 13.1	76.1 ± 3.8	362.0 ± 18.1
Mungbean	74.0 ± 3.7	339.0 ± 16.9	62.0 ± 3.1	284.0 ± 14.2	50.0 ± 2.5	230.0 ± 11.5	60.0 ± 3.0	272.0 ± 13.6	64.4 ± 3.2	265.0 ± 13.3	72.1 ± 3.6	345.0 ± 17.3
Cowpea	56.0 ± 2.8	312.0 ± 15.6	49.0 ± 2.5	260.0 ± 13.0	36.0 ± 1.8	205.0 ± 10.3	45.0 ± 2.3	247.0 ± 12.4	42.2 ± 2.1	231.0 ± 11.5	60.0 ± 3.0	330.0 ± 16.5
Blackgram	39.0 ± 1.9	294.0 ± 14.7	31.0 ± 1.5	251.0 ± 12.6	20.0 ± 1.0	178.0 ± 8.9	28.5 ± 1.4	227.0 ± 11.3	25.0 ± 1.3	206.0 ± 10.3	42.3 ± 2.1	318.0 ± 15.9
Lentil	48.3 ± 2.4	301.0 ± 15.1	39.0 ± 1.9	256.0 ± 12.8	23.0 ± 1.2	193.0 ± 9.6	34.1 ± 1.7	235.0 ± 11.8	30.0 ± 1.5	220.0 ± 11.0	52.0 ± 2.6	323.0 ± 16.2
CD (P = 0.05)	5.78	28.86	5.23	25.03	4.21	19.46	4.98	23.36	4.85	21.93	5.97	30.77
CD (P = 0.01)	8.11	40.47	7.34	35.10	5.90	27.28	6.99	32.75	6.80	30.74	8.37	43.14

Data mean ± standard deviation of five replicates. CD = Critical difference.

At block B_3, hyphae were found in all the leguminous crops except pigeon pea and all crops had arbuscules except black gram. Both arbuscules and vesicles were found in pigeon pea, mung bean, and cowpea. Oval- and round-shaped vesicles were displayed in pigeon pea and cowpea while oval- and spherical-shaped vesicles were found in mung bean (Table 2). At block B_4, all the leguminous crops contained hyphae and arbuscules except lentil. Mung bean, cowpea, and black gram contained both arbuscules and vesicles. Mung bean, cowpea, and lentil contained both oval- and spherical-shaped vesicles. Only black gram had spherical-shaped vesicles. At block B_5, hyphae were found in all the leguminous crops. Arbuscules were present in chickpea, pigeon pea, mung bean, and cowpea; vesicles were found in chickpea, pigeon pea, and mung bean. The shape of vesicle was oval and spherical in pigeon pea and mung bean; chickpea had oval- and round-shaped vesicles. At block B_6, all the leguminous crops contained hyphae and arbuscules. Both oval- and spherical-shaped vesicles were present in chickpea and cowpea. At block B_7, all the leguminous crops contained hyphae except lentil. Both arbuscules and vesicles were found in pigeon pea and cowpea (Table 2). Oval- and round-shaped vesicles were displayed in pigeon pea and cowpea while oval- and spherical-shaped vesicles were found in lentil. At block B_8, hyphae were present in all the leguminous crops except lentil. Pigeonpea, mung bean, black gram, and lentil contained both arbuscules and vesicles. Black gram had only spherical-shaped vesicle; pigeon pea and lentil contained both oval- and spherical-shaped vesicles; mung bean had round- and spherical-shaped vesicles. At block B_9, all the leguminous crops contained hyphae and arbuscules. Only mung bean contained oval- and spherical-shaped vesicles. At block B_{10}, hyphae were found in all the leguminous crops. Arbuscules were present in chickpea, pigeon pea, and mung bean. The shape of vesicles was oval and spherical and round and spherical in chickpea and mung bean, respectively. At block B_{11}, hyphae were found in all the leguminous crops and all crops had arbuscules except lentil. Oval- and spherical-shaped vesicles were displayed in mung bean only. At block B_{12}, hyphae were found in all the leguminous crops and all crops had arbuscules except pea. Chickpea and cowpea contained both oval- and round-shaped vesicles. Only black gram had spherical-shaped vesicle (Table 2).

Distribution of AM Genera

It was evident from the Figure 1 that distribution of different AM genera in the rhizosphere of selected legume crops diversified considerably. The rhizosphere soils of various crops represent six AM genera. The most commonly distributed genera are *Glomus*, *Gigaspora*, and *Acaulospora* followed by *Scutellospora*, *Entrophosphora*, and *Sclerocystis*. *Glomus*, *Gigaspora*, and *Acaulospora* were observed abundantly in the rhizosphere soil of almost all leguminous crops surveyed in the 12 blocks. *Scutellospora*, *Entrophosphora*, and *Sclerocystis* were found rarely with a small number of legume crops under all the blocks. This investigation discloses that AMF are common in all the legumes assessed from various blocks. *Glomus* was the most predominat genus among all the six genera recorded in this study (Figure 1).

Distribution of AM Species

Figure 2 shows the spore number of different species of the most frequent AM genera surveyed viz., *Glomus mosseae*, *Gl. constrictum*, *Gl. fasciculatum*, *Gl. intraradices*, *Gl. aggregatum*, *Gigaspora gigantea*, and *Acaulospora scrobiculata* at different blocks in relation to different leguminous crops. At block B_1, *Gl. fasciculatum* has the highest spore numbers followed by *Gl. mosseae*, *Gl. intraradices Gl. constrictum*, *Gl. aggregatum*, *A. scrobiculata*, and *Gi. gigantea*, highest being exhibited by chickpea (180, 174, 168, 165, 159, 30, and 28 spores). At block B_2, the highest spore number was observed by *Gl. fasciculatum* in all the legumes. *Gigaspora gigantea* has the lowest spore number. At block B_3, *Gl. mosseae* has highest spore number than *Gl. fasciculatum* in all the crops. The lowest number of spores was found in *Gi. gigantea*. At block B_4, *Gl. fasciculatum* has the highest spore numbers as compared to other AM species. Lowest spore numbers were recorded for *A. scrobiculata*. At block B_5, the highest spore numbers were recorded for *Gl. fasciculatum* followed by *Gl. mosseae*, *Gl. constrictum*, *Gl. intraradices*, *Gl. aggregatum*, *Gi. gigantea*, and *A. scrobiculata*. At this site, pigeon pea showed greater number of spores than chickpea. At block B_6, *Gl. fasciculatum* showed the highest spore numbers in all the legumes as compared to other AM species; lowest being recorded in *Gi. gigantea*. Chickpea and pigeon pea recorded almost similar spore numbers. At block B_7, *Gl. mosseae* recorded highest spore numbers than *Gl. fasciculatum*. Almost same number of spores was exhibited by pigeon pea and chickpea. At block B_8, the highest spore number was observed by *Gl. fasciculatum* in all the legumes. *Acaulospora scrobiculata* has the lowest spore number. Chickpea showed highest spore number. At block B_9, *Gl. fasciculatum* has the highest spore numbers followed by *Gl. mosseae*, *Gl. intraradices Gl. constrictum*, *Gl. aggregatum*, *Gi. gigantea*, and *A. scrobiculata*, highest being exhibited by chickpea (210, 207, 196, 192, 184, 20, and 12 spores). This block recorded highest number of spores as compared to other blocks surveyed. At block B_{10}, the highest spore numbers were recorded for *Gl. fasciculatum* followed

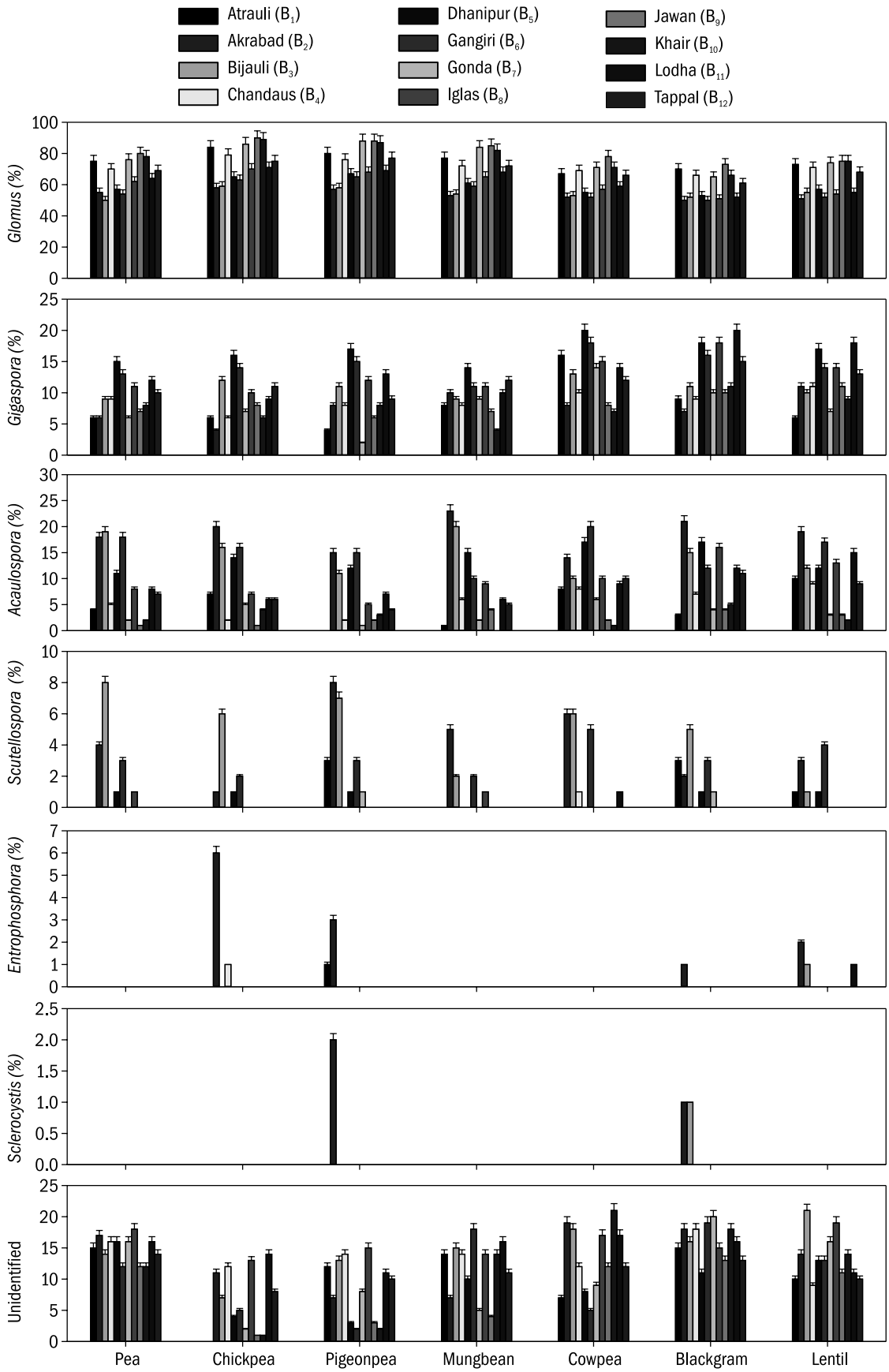

Figure 1: Distribution of AM genera in the rhizosphere soils of different leguminous crops surveyed at 12 blocks under Aligarh district.

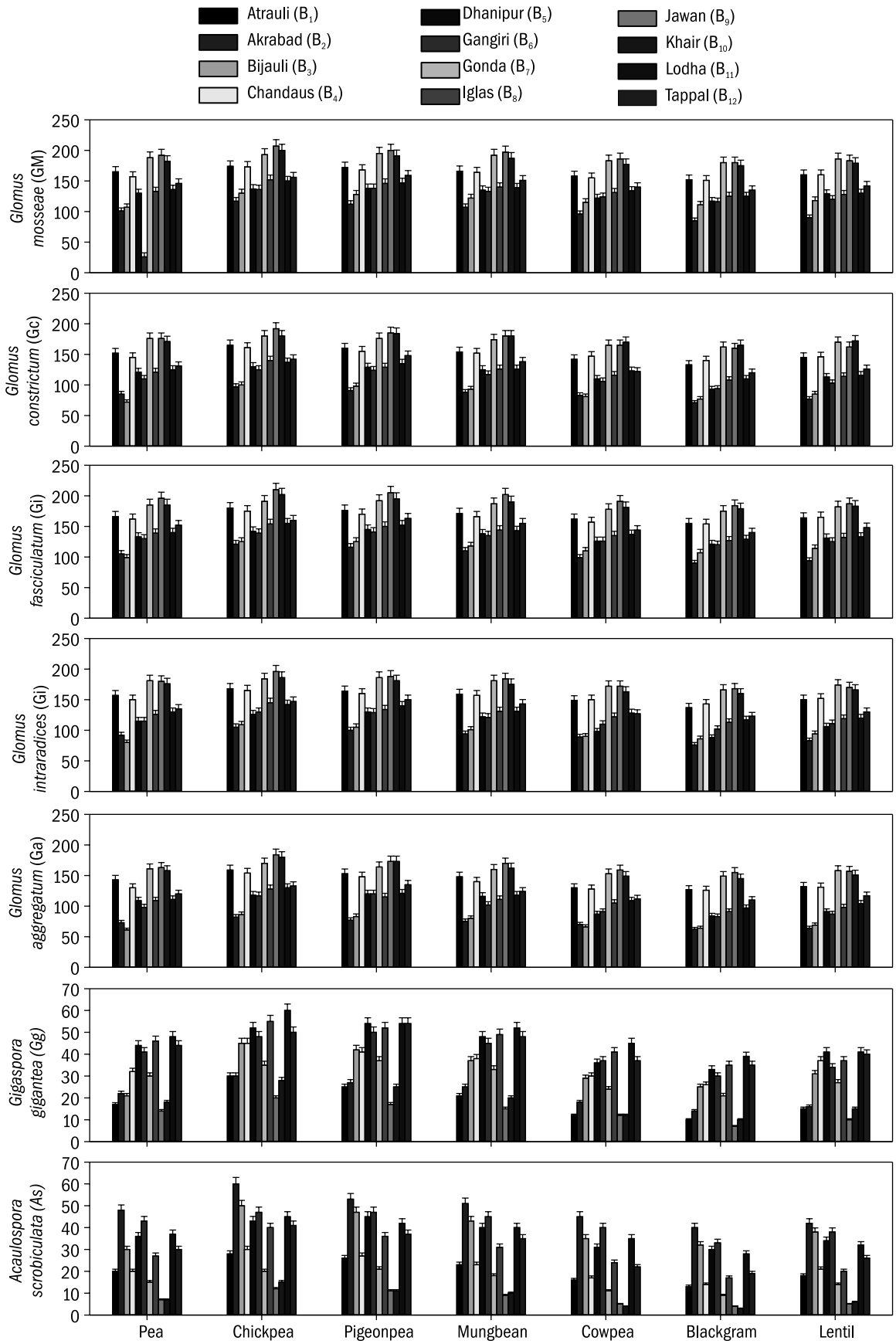

Figure 2: Distribution of AM species in the rhizosphere soils of different leguminous crops surveyed at 12 blocks under Aligarh district.

by *Gl. mosseae*, *Gl. constrictum*, *Gl. intraradices*, *Gl. aggregatum*, *Gi. gigantea*, and *A. scrobiculata*. At block B_{11}, the highest spore numbers recovered were *Gl. fasciculatum* in all the legumes as compared to other AM species and the lowest were *A. scrobiculata*. At block B_{12}, *Gl. fasciculatum* showed the highest spore numbers and *A. scrobiculata* the lowest. At this site, pigeon pea showed greater number of spores than chickpea (Figure 2).

Discussion

The occurrence of AMF in relation to soil characteristics has been studied by a number of workers (Rovira *et al.* 1983; Abbott and Robson 1985; Meyer and Linderman 1986; Muthukumar *et al.* 1996; Bhardwaj *et al.* 1997; Siddiqui and Mahmood 1998; Ananthakrishnan *et al.* 2004; Molla and Solaiman 2009; Nasrullah *et al.* 2010; Chatterjee *et al.* 2010; Muthukumar and Tamilselvi 2010). The result obtained in the present study show that all the crops investigated are mycorrhizal in nature, as reported by Mosse (1977) and all the crops are colonized by AMF to a great extent (Tables 1–3 and Figures 1 and 2).

In the present study, it has been observed that all the crops investigated are colonized by the different species of AMF extensively showing direct relationship with spore number prevailing at the site. The data further indicates that the AM fungal species prefer chickpea and pigeon pea to a greater extent compared to other leguminous crops investigated.

Results clearly indicate a high percentage of root colonization in chickpea, pigeon pea, mung bean, and pea. Root colonization was detected at all the blocks and ranged from 14.4 per cent (B_1) to 88 per cent (B_2). High root colonization could be attributed to low soil phosphorus and optimum moisture condition. Due to different structure of root system, the colonization rate of AMF might be changed among different plant species in the same site (Hetrick *et al.* 1992). Chickpea exhibited highest AM root colonization. Solaiman *et al.* (2005) and Khanam *et al.* (2006) reported 70 and 68 per cent colonization in chickpea. The diversities of root colonization in grain legumes were observed in respect of differences in the structure of the root system and the amounts of sugars and amino acids in the root exudates (St. John *et al.* 1981; Hetrick *et al.* 1992; Isobe and Tsuboki 1998; Khanam *et al.* 2004).

Leguminous crops exhibited high degree of root colonization by AMF. Low P content of plants could correlate with a decrease in phospholipid levels under low phosphorus nutrition and an increase in root membrane permeability would favour mycorrhizal colonization (Graham *et al.* 1981). Nodulating legumes generally require a large amount of phosphorus for optimum growth, nodulation, and nitrogen fixation (Hayman 1986). In spite of less extensive root systems, legumes are able to fulfil their nutritional requirements depending on colonization by native AMF (Bethlenfalvay and Newton 1991). Solaiman *et al.* (2005) and Khanam *et al.* (2005) reported that AM colonization and spore population were increased in leguminous crops.

Soil nitrogen is significantly correlated with soil K. However, soil phosphorus is negatively correlated with root colonization. The role of K in root colonization and spore numbers of AMF is little known as compared to P and N. Potassium has previously been reported to have no effect on AMF (Daniels and Trappe 1980).

Legume crops occupied higher number of spores. Similar findings were observed (Khalil *et al.* 1992; Khanam *et al.* 2004; Lakshman *et al.* 2010) in Jawa, Bangladesh, and Karnataka soils, respectively. Highest spore population per 100 g dry soil was recovered in the rhizosphere soil at B_1 (155) and lowest at B_2 (456). The variation in spore population at different sites might be due to biotic and abiotic components of the soil ecosystem and the associating plant species (Bethlenfalvay and Linderman 1992). The agricultural significance of the above root colonization percentage and spore number indicate more nutrient uptake by the legume crops specially orthophosphates, zinc, and copper.

The presence of hyphae, vesicles, and arbuscules characterize endomycorrhizal colonization. The formation of arbuscules is influenced by the stage of plant development and short-lived structure (Neeraj *et al.* 1991). During active vegetative growth, arbuscules are normally formed because of the availability of new cortical cells and high nutrient requirement by the host. Arbuscules formation decrease and vesicles formation increase at flowering and fruiting stages (Mosse *et al.* 1981). Formation of AM structure was inconsistent and fluctuating from site to site in the present study. Round, oval, and spherical vesicles were observed in the present study. Our present findings are in agreement with the earlier report (Khanam *et al.* 2003, 2004, 2006; Khanam 2007; Molla and Solaiman 2009).

A multiple infection has been observed at all the 12 blocks in the present study. Diversity of spore types has been found to be very high, and AM fungal chlamydospores are quite common in all the samples. Results of similar kind have been reported by Mosse (1973), Hayman and Stovold (1979), and Khalil *et al.* (1992). Five fungal genera viz., *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora*, and *Entrophosphora* were isolated from the study sites. Of these, *Glomus*, *Gigaspora*, and *Acaulospora* were found in all the soils sample of all the blocks of Aligarh district. *Glomus*

species (*Gl. fasciculatum*, *Gl. mosseae*, *Gl. constrictum*, *Gl. intraradices*, and *Gl. aggregatum*) were most abundant in the rhizosphere indicatives of their broad host range. In the present study, the spores of *Glomus* sporulated abundantly regardless of legume species and sites. Padmavathi *et al.* (1991) have also come across spores of different AMF occurring often in agricultural soils and found that *Glomus* was the predominant genus among the AMF, in addition to *Acaulospora* and *Gigaspora*. It has been further found that the colonization varies to different levels at different sites. A similar situation has been reported by Sulochana and Manoharachary (1990), Peng and Shen (1990), and Blaszkowski (1993).

Slightly alkaline soils favoured occurrence of *Glomus* followed by *Gigaspora*, *Acaulospora*, and *Scutellospora*. These reports are in agreement with Mosse *et al.* (1981), who exhibited that a neutral to slightly alkaline soil favoured the predominance of *Glomus* and acidic soil allowed *Acaulospora*. *Glomus* was predominant in all the soils, which indicated that the dominance of particular genus is not dependent on the soil pH. These results were supported by Bhardwaj *et al.* (1997).

AMF are wide spread in occurrence and due to their potential for crop improvement they have been investigated extensively (Powell and Bagyaraj 1982; Mukerji and Dixon 1992; Mukerji 1995). The natural occurrence of AMF in the soils was affected by soil ecological and environmental factors of physical, chemical, and biological nature (Bagyaraj 1991). Natural occurrence and predominance of *Glomus*, *Gigaspora*, and *Acaulospora* in the cultivated soils at all the 12 blocks indicate that there is a need of maintaining them in cultivated soils through proper management, which may be further utilized as biofertilizer and biocontrol agents.

Conclusion

Among the seven leguminous crops surveyed at 12 blocks of Aligarh district, chickpea and pigeon pea were found highly mycotrophic. Lentil and blackgram showed poor mycorrhizal colonization. *Glomus* was the most common genus of AMF with five common species (*Gl. mosseae*, *Gl. constrictum*, *Gl. fasciculatum*, *Gl. intraradices*, *Gl. aggregatum*) followed by *Gi. gigantea* and *A. scrobiculata* while *Sclerocystis* was the least prevalent genus in the rhizosphere soil.

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Morphotaxonomy of *Paraglomus majewskii* (Accession CMCC/AM 1701)

*Kiran Sunar, Priya Ahuja, Aditi Pandit, and Alok Adholeya**

Morphological characterization of any given isolate is considered as one of the preliminary characterizations that are mostly based on visible characteristics. In case of describing an arbuscular mycorrhizal fungus (AMF) species, characteristics such as spore structure, shape, size, wall layers, surface ornamentations, hyphal attachments, type of spore formations, and reaction of wall layers towards different reagents play an important role. To this end, we have considered all the possible visible characteristics of one of our unique AMF available in Centre for Mycorrhizal Culture Collection Bank with the accession number CMCC/AM 1701.

This culture has been isolated and raised from the soils of Jaisalmer district of Rajasthan, India. Initially the soil samples were suspended in water and were passed through a series of sieves of 60, 100, and 300 BSS (British Standard Size) and were analysed for AMF spore density and diversity. In the next step, all healthy spores were categorized according to their size, structure, and colour. Similar types of spores were grouped and were used to obtain pure single species culture of AMF. Voucher specimen of this potential monosporal was prepared and morphotaxonomic analysis of the spore and its wall layers, hyphal attachment, etc., was done under compound microscope (10×, 40×, and 100×) after mounting in Polyvinyl Lacto glycerol (PVLG) and PVLG: Melzer's reagent (1:1). Selected healthy single AMF spores were used to raise monospecific cultures that were inoculated to pre-germinated seed of a suitable host. After a successful growth period of three to six months, the host roots were evaluated for colonization and sporulation. Cultures showing colonized roots and spores were considered as successful cultures for raising monosporals and were considered to be pure when the spores isolated from them were found to be morophotaxonomically similar to the voucher specimen prepared from the mother cultures that were used during the initiation of the monosporals.

Spore Morphology and Shape

The spores of this accession were formed singly in the soil without any sporocarp. The spores are formed blastically at the tip of sporogenous hyphae continuous with mycorrhizal extraradical hyphae. Spores are mostly hyaline; however, some of the mature spores appear light brown to pale yellow in colour. Spores are globose to sub-globose in shape and sometime slightly oval with a smooth surface (Figure 1). A thin subtending hypha is also seen in young spores, which are difficult to locate in mature spores. Scanning electron micrograph (SEM) of the spores revealed a slightly rough outer surface. The outer wall of the spores is mucilaginous and appears rough with organic debris adhered to it (Figure 2). The average diameter of the spores were found to be in the range of 140.15 (210.76) to 320.18 μm (Figure 3).

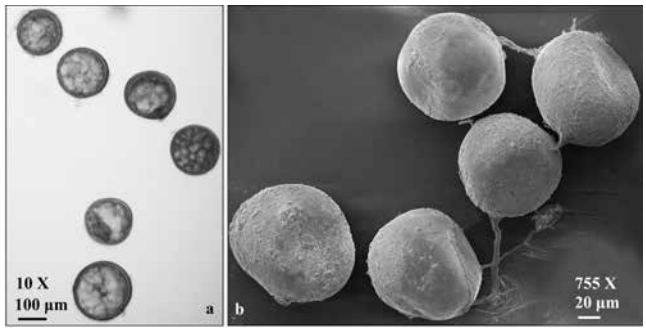

Figure 1: Compound microscopic images (10×) and SEM (755×) of the spores of accession CMCC/AM-1701 showing singly formed spores with a small subtending hypha.

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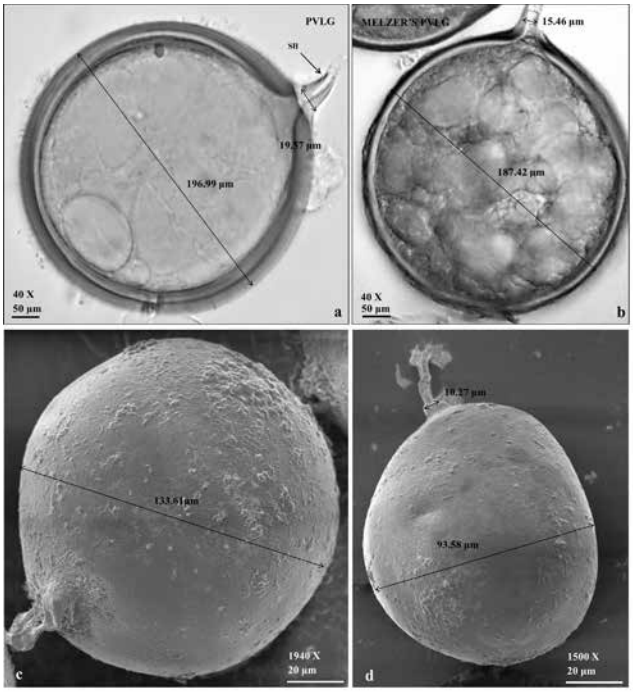

Figure 2: Compound microscopic images (10×) and SEM (755×) of mature spores of accession CMCC/AM-1701 showing globose to slightly oval shaped spore with a single subtending hyphae. SEM images reveal a smooth outer layer of the spore with a remnant of mucilaginous layer appearing rough with organic debris adhered to it.

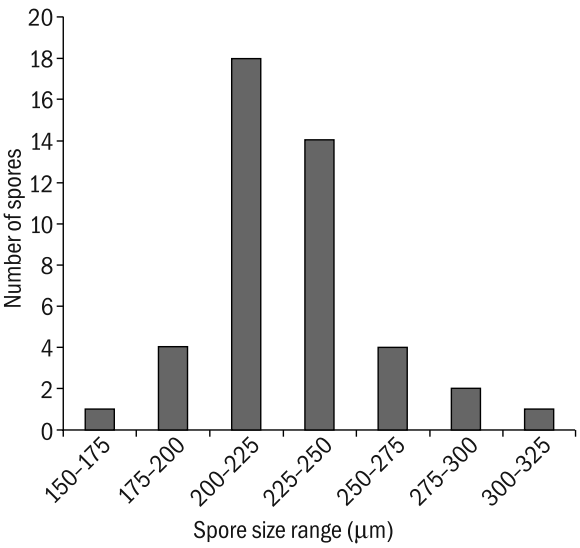

Figure 3: Analysis of spore diameters of 50 healthy spores obtained from one-year old monosporal culture of CMCC/AM-1701.

Subcellular Structure of the Spore

Spores of this accession show very less or no reaction towards both polyvinyl alcohol-lactic acid glycerol (PVLG) and Melzer's: PVLG; however, mature spores are composed of the following sub-cellular structures and wall layers (Figure 4):

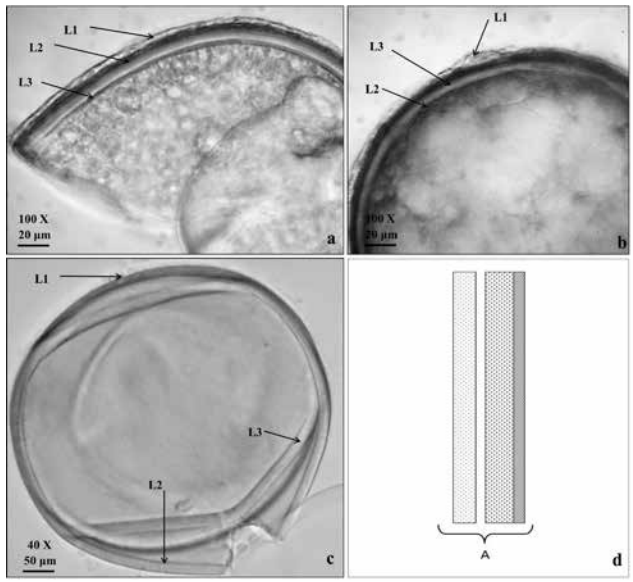

Figure 4: Compound microscopic images of young and mature spores of accession CMCC/AM-1701 showing distinct wall layers. L1 is mucilaginous layer appearing rough with organic debris adhered to it while L2 is laminated and L3 is flexible permanent layer. Young spore (a) and a mature spore (b and c). Murograph of composite spore wall.

Spore Wall Layer 1 (L1): This is the outermost mucilaginous hyaline layer that is formed in young juvenile spores. An evanescent layer that is slightly roughened on its upper surface due to organic debris adhered to it. This layer is almost deteriorated or completely sloughed in mature spores and does not show any reaction in Melzer's: PVLG. The average thickness of this layer lies between 0.5 and 1.75 µm.

Spore Wall Layer 2 (L2): This is a laminated layer next to the outermost layer. Seen as a single layer in juvenile spores, but subsequent adherent sub-layers are formed as the spore matures. The layer is smooth and appears to be the thickest wall layer of the spore. This layer is also slightly hyaline and shows no or very less reaction with Melzer's: PVLG. The average thickness of this layer lies between 1.5 and 4.5 µm.

Spore Wall Layer 3 (L3): This layer lies closely adhered to the innermost sub-layer of L2 but sometimes separate in vigorously crushed spores. L3 is flexible and smooth with an average thickness of 0.2 to 0.6 µm and does not show any strong reaction towards Melzer's: PVLG. Both the layers L2 and L3 are continuous with the subtending hyphae.

Subtending Hyphae and Occlusion

Majority of spores at maturity shows cylindrical to slightly flared hyaline subtending hyphae. The width

of the subtending hypha at the spore bases ranges from 2.54 to 3.83 µm. The subtending hypha consists of two wall layers (hyphal wall layer 1 [HWL-1] and hyphal wall layer 2 [HWL- 2]) that are continuous to the inner wall layers L2 and L3 of the spore, respectively. The innermost layer L3 of the spore forms an occlusion just at the point of attachment that plugs the spore at the base. The occlusion is generally a thin-curved septum originating from the inner wall layer L3 of the spore wall (Figures 5 and 6).

Mycorrhizae

Root colonization assay of *Sorghum bicolor* roots after three months of inoculation showed profuse colonization with numerous arbuscules evenly distributed. No any vesicles were observed in the root fragment. Arbuscules consisted of a short trunk developed from a parent hypha and numerous branches with fine tips. Intraradical hypha was seen to grow parallel to the root axis and sometimes formed H- or Y-shaped branches and coils (Figure 7).

Conclusion and Classification Level

On the basis of above morphotaxonomic analysis of the accession CMCC/AM-1701, many distinguishing features regarding the family, genera, and the species

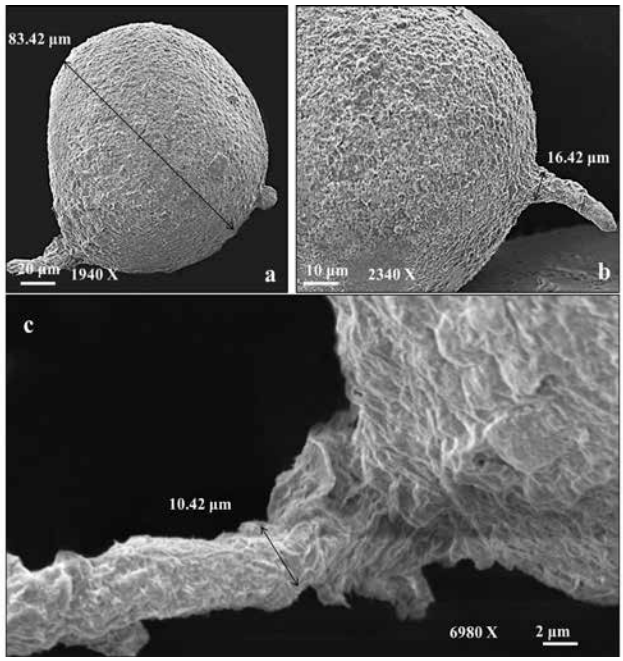

Figure 6: SEM of young and mature spores of accession CMCC/AM-1701 showing cylindrical and flared subtending hyphae attached to the spore. Higher magnification of the spore base reveals that the outer warty mucilaginous layer is continuous with the subtending hyphae (c), which broadens at the base of the spore.

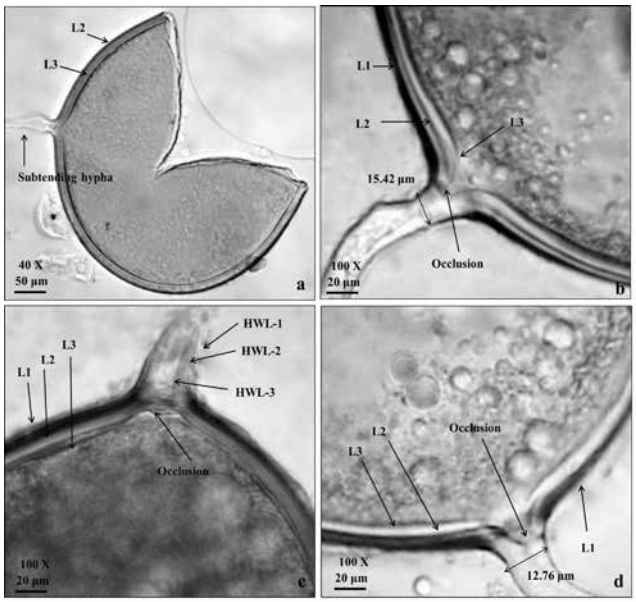

Figure 5: Compound microscopic images of young and mature spores of accession CMCC/AM-1701 showing the attachment of subtending hyphae with the spore. Subtending hyphae is slightly flared and cylindrical. L2 and L3 of the spore are continuous with the HWL-2 and HWL-3, respectively. A small occlusion that plugs the spore is seen at the base of the spore formed by L3 of the spore.

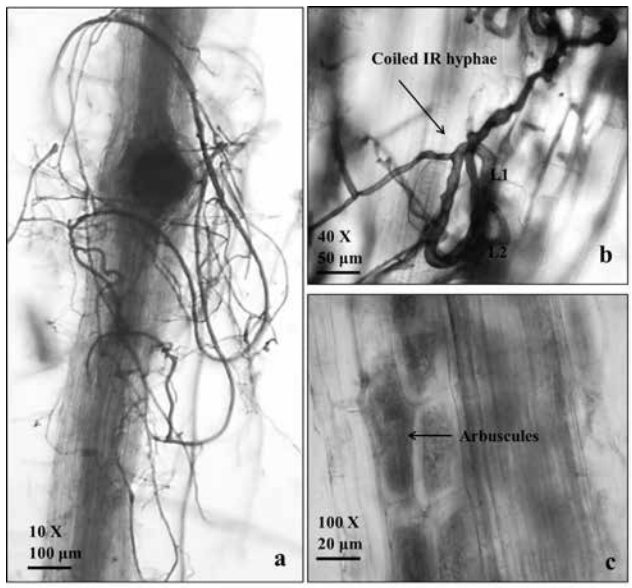

Figure 7: Compound microscopic images of colonized roots of *Sorghum* after three months of inoculation with CMCC-AM-1701 showing intensive colonization of the cortical cells with branched hyphae (a) and abundant arbuscules. Intraradical hyphae are coiled. Vesicles are absent (b and c).

could be derived. The following features were taken into consideration for characterization:

- Asexual spores produced singly with spore wall layers continuous with the subtending hypha.
- Wall layer containing outer hyaline mucilaginous layer, a laminated inner second layer, and a third flexible innermost layer. Both the layers—L2 and L3—are continuous with the subtending hyphal wall.
- Spores characteristically hyaline to pale yellow in colour with shapes ranging from globose to sub-globose as well as oval. Formation of highly coiled intraradical hyphae within the cortical cells observed. Extraradical hyphae and vesicles absent in the mycorrhiza.

All these features suggest that the culture CMCC-AM-1701 belongs to the family Paraglomeraceae (Morton and Redecker 2001) and the order Paraglomerales.

Some of the unique diagnostic morphotaxonomic characters of this accession are as follows:

- Frequent production of asexual spores produced singly with spore wall layers continuous with the subtending hyphae.
- Spore shape ranges from globose to sub-globose as well as oval with average spore size ranging from 140 to 300 µm in diameter.
- A majority of spores at maturity show cylindrical to slightly flared subtending hyphae. The subtending hypha also consists of two wall layers (L1 and L2) that are continuous to the wall layer of the spores.
- The spore wall layers are continuous with the subtending hypha. The innermost wall layer L3 of the forms a bridging structure and resembles a septum.
- Formation of intraradical hyphae within the cortical cells observed are highly coiled. Extraradical hyphae and vesicles absent in the mycorrhiza.

The culture resembles all the characters of previously characterized isolate of species of the genus *Paraglomus* by Morton and Redecker (2001), wherein they have proposed a separate order and family for those genera that do not form vesicles in their mycorrhiza, however the spores are formed blastically at the tip of each extraradical hypha. The only morphological distinction between *Paraglomus* spp. and *Glomus* spp. is in properties of mycorrhizae. Although the arbuscules of *Paraglomus* spp. are similar to those of *Glomus* spp., that is, have cylindrical or slightly flared trunks with branches progressively tapering in width toward tips (Morton 2002; Morton and Redecker 2001), the mycorrhizae of *Paraglomus* spp. do not contain vesicles and their intraradical hyphae are frequently coiled within and between cortical cells. In contrast, *Glomus* spp. frequently produces vesicles, and coils in mycorrhizae of these fungi rarely occur, except near entry points. The main visible evidence of mycorrhizae of *Paraglomus* spp. is their light staining or the lack of any staining reaction in trypan blue or other stains despite the presence of extraradical hyphae.

The distinctive morphological characters of the present culture CMCC/AM-1701 is its small spores produced singly in soil and remaining hyaline throughout their life cycle, their three-layered spore wall structure in which layer 3 is flexible and the unusually narrow subtending hypha. These characters match those described by Blaszkowski *et al.* 2012.

Systematic Classification

Glomeromycota
Glomeromycetes
Paraglomerales
Paraglomeracea
Paraglomus majewskii

The family Paraglomeraceae was erected recently to accommodate the ancestral AMF that forms small glomoid spores based on rDNA phylogenetic analysis (Morton and Redecker 2001; Schu¨ßler *et al.* 2001). The type species within Paraglomeraceae are *Paraglomus occultum*, *P. brasilianum*, and *P. laccatum*. This genus is renamed based on molecular evidence. The spores, although appearing to be *Glomus* spp, are phylogenetically very distant from other members in that group. Morphotaxonomic characterization for species identification has got its own limitations. With many unique characters having tendency to vary from host to host and different Geographic locations, the identities of similar species become quite tough if they are solely based on visible taxonomic scores. This limitation has been successfully overcome with the advent of molecular methods for identification of organisms. Sequence analysis of rDNA regions has often confirmed the morphologically defined species and the molecular data have characterized new genera and families in AM taxonomy. It is, therefore, advised to our distinguished readers to kindly correlate their morphotaxonomic studies with the molecular data.

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The latest additions to the network's database on mycorrhiza are published here for the members' information. The list consists of papers from the following journals:

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