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Distribution of Vesicular-Arbuscular Mycorrhizal Fungi in the Natural Ecosystem

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ABSTRACT. Natural occurrence of vesicular-arbuscular mycorrhizal (VAM) fungi in Haryana soils showed that VAM sporulation was more intensive in the rhizosphere of nonlegumes than of legumes. Maximum number of spores (342 spores per 50 g of soil) was observed in the rhizosphere of mustard, followed by chickpea, wheat, pearl millet and pigeonpea. Four VAM genera *viz. Glomus*, *Gigaspora*, *Sclerocystis* and *Acaulospora*, were present there. Soil pH, total soil P, available P, type of soil, soil moisture and cropping season all variables influenced the VA mycorrhizal population in the natural ecosystem. Numbers of VAM spores highly correlated with the presence of total soil P and soil pH indirectly affected the VAM population through the total soil P. The spore population was abundant in sandy soils as compared to loamy sands. Drier soils had higher number of VAM spores. In summer, the VAM population in soil was less as compared to winter season.

Vesicular arbuscular (VA) mycorrhizal fungi are world wide in distribution and their occurrence varies with environmental conditions. In spite of immense importance, much less is known about the natural ecology of these fungal plant associations in field soils. A better understanding is needed because of their involvement in systems of sustainable agriculture (Deepika *et al.* 1996). VA mycorrhizal fungi form symbiotic association with the roots of plants and improve plant growth mainly through phosphorus nutrition. Mycorrhizal plants frequently show resistance to drought, salinity, environmental stresses and root pathogens. They also increase the activity of nitrogen-fixing organisms in the root zone.

The natural occurrence of VA mycorrhizal fungi in the soils is affected by soil ecological and environmental factors of physical, chemical and biological nature (Bagyaraj 1991). Soil physical factors include humidity, temperature and light; soil chemical factors include pH, soil N, soil P and organic matter. Biological factors include host and other soil microorganisms.

In Haryana State, wide variations in ecological conditions exists. The high soil temperature (shoots up to 52 °C in the upper 0–50 mm soil layer) and erratic rainfall which leads to rapid drying and wetting of the soil (Dudeja and Khurana 1989). In addition, variation in other soil abiotic factors, such as soil pH, available P and N in Haryana soils has been reported (Singh *et al.* 1992). The present investigation was therefore undertaken to find out the distribution of VA mycorrhizal fungi in the natural ecosystem of Haryana.

MATERIALS AND METHODS

Soil samples from the rhizosphere of pigeonpea, chickpea, mungbean, clusterbean, sesbania, mustard, wheat and pearl millet were collected from 91 different locations of Haryana. Soil samples were pooled from five plants from different locations in the same field and were mixed thoroughly. Composite soil samples of about 1 kg were brought to the laboratory for further studies.

Mycorrhizal spores were obtained by wet sieving and decanting technique (Abott and Robson 1991). For quantification of spores, 10 g of soil was added to 100 mL of water and stirred thoroughly. Heavier particles were allowed to settle down for 10 min and the supernatant was decanted through a set of sieves of different mesh sizes (22, 60, 100, 150, 200 and 500 μm mesh) arranged one over the other. The mycorrhizal spores were retained on the finest sieve. The material on the finest sieve was washed with water to free it from organic matter and other soil particles. The spore suspension was then taken on a slide of 1 cm^2 area and examined under the microscope and the spores were counted per microscopic field. Minimum of 20 microscopic fields were counted for each soil sample. The efficiency of spore retrieval from soil by using this combined decanting and sieving technique was

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60–65 %. This was determined by using spore-free soil and inoculating counted number of spores of *Glomus mosseae*. Using morphology and coloration of the spores, different VAM genera in the present study were identified up to genus level (Trappe and Schenk 1982).

Soil was suspended in a 1 : 1 ratio and thoroughly mixed. The suspension was allowed to settle for 30 min and the pH was measured. Total soil P was determined according to John (1970) and available P in the soil was estimated by the method of Olsen *et al.* (1954). Soils were categorized into three groups, depending upon the water content in soil during its collection from the agricultural fields. Soils which were collected from water-logged fields wer considered as water-logged soils; others were moist soils collected from the wet fields; very dry and loose soils collected either from sand dunes or the agricultural fields which were not irrigated for a long time. On visual basis, soils were classified into loamy sand (compact soils), sandy loam and sandy soils (loose soils).

RESULTS AND DISCUSSION

In different agricultural soils the number of VA mycorrhizal spores in the rhizosphere of pigeonpea, chickpea, mungbean, clusterbean and sesbania ranged from 10 to 668, 59 to 794, 167 to 235, 98 to 121 and 212 spores per 50 g of soil, respectively (*data not given in the tables*). On the average, in the soil samples collected for a particular legume, the maximum number of VAM spores present in the rhizosphere of chickpea was 288 spores, the minimum in the case of pigeonpea with 167 spores per 50 g soil (Table I). In Haryana soils, only four VAM genera, *viz. Glomus*, *Gigaspora*, *Sclerocystis* and *Acaulospora*, were present. *Glomus* was the predominant genus in the rhizosphere of all the legumes, contributing 87 % of the total spores, followed by *Gigaspora* (11 %), *Sclerocystis* and *Acaulospora*. *Sclerocystis* was observed only in the rhizosphere of pigeonpea.

Table I. Effect of host on the occurrence of VAM spores in Haryana soils

Host	Total VAM spores per 50 g soil	Spores of different VAM genera (per 50 g soil)			
		Glomus	*Gigaspora*	*Sclerocystis*	*Acaulospora*
Legumes					
Pigeonpea	167	144	16	5	2
Chickpea	288	242	41	–	5
Other legumes	181	170	9	–	2
Nonlegumes					
Pear millet	187	171	14	2	0
Wheat	220	220	0	0	0
Mustard	342	331	0	11	0
LSD (p = 0.05)	2.2		2.2		

In the rhizosphere of nonlegumes, pearl millet, wheat and mustard, the total number of VAM spores in 50 g soil ranged from 65 to 228, 163 to 277 and 130 to 717, respectively. On the average, 324 spores per 50 g soil were present in the case of soils where mustard crop was grown and minimum in the case of soil with pearl millet (187 spores per 50 g soil) as shown in Table I. In the rhizosphere of nonlegumes, *Glomus*, *Gigaspora* and *Sclerocystis* were observed. No spores of *Acaulospora* were detected. Again *Glomus* was the predominant genus in the rhizosphere of nonlegumes contributing to 96 % of the total VAM population. The effect of legume or nonlegume hosts on the distribution of total VA mycorrhizal spores or on spores of different genera was statistically significant.

The host plays an important role in the distribution, predominance and VA mycorrhizal infectivity of the host plant. The number of VAM spores present in Haryana soils was higher as compared with that reported in Karanatka State in different legume and nonlegume crops (Hiremath *et al.* 1990). Maximum spore density was reported in maize, followed by pearl millet, pigeonpea, chickpea and wheat in Madhya Pradesh soils (Singh and Pandya 1995). In wheat and lentil fields, the number of VAM spores has been reported to range from 78 to 272 per 100 g soil (Talukdar and Germida 1993). In the members of *Chenopodiaceae* and *Brassicaceae* minimum VAM colonization has been reported (Ocampo *et al.* 1980); however, in the present study, maximum number of VAM spores was observed in the rhizosphere of mustard intercropped with chickpea. Maximum rhizosphere colonization of VAM

could be due to the presence of accompanying chickpea plants. Further colonization of VAM has been reported in rapeseed and mustard to control root rot disease (Gupta *et al.* 1990, 1991, 1993). Variations in the spore number in the rhizosphere of different host species has been reported by several workers (Hiremath *et al.* 1990; Gupta and Ali 1990).

The soil pH of the 91 soil samples collected from different locations varied from 6.3 to 9.0 (Table II). At 12 locations the soil pH was between 6.3 to 7.0 with an average of 201 VAM spores per 50 g soil. Two VAM genera (*Glomus* and *Gigaspora*) were present in these soils. The soil pH ranged from 7.1 to 8.0 at 39 locations and on the average 217 spores per 50 g soil were present in these soils. All the four VAM genera were distributed in there. The remaining 40 locations, in the pH range of 8.1 to 9.0, contained 209 spores of all the four genera per 50 g of the soil. The effect of soil pH on distribution of VAM spores was statistically significant.

Table II. Effect of soil pH on the occurrence of VAM spores in Haryana soils

pH range[a]	Total VAM spores per 50 g soil	Spores of different VAM genera (per 50 g soil)			
		Glomus	*Gigaspora*	*Sclerocystis*	*Acaulospora*
6.0–7.0 (12)	201	177	24	0	0
7.1–8.0 (39)	217	192	18	4	3
8.1–9.0 (40)	209	181	22	4	2
LSD (p = 0.05)	2.1		2.8		

[a]Numbers in parentheses indicate the number of soil samples in the given range.

Distribution of VAM spores in soils of different pH has no significant correlation. This could be attributed to the fact that effects of pH are difficult to evaluate since many chemical properties of soil changed with pH as was observed in the present case; soil pH correlated with total P, available P and total N. These results were supported by other workers (Kendrick and Berch 1985; Kianmehr 1990). *Glomus* was found to be predominant in all the soils which indicated that the dominance of a particular genus is not dependent on the soil pH. However, Abbot and Robson (1991) observed that at a particular pH, certain genera of VAM predominated. In acidic alfisols of Konkan region the spore density ranged from 164 to 932 spores per 50 g soil; in Solapur region, at pH 5.6 to 8.7, the spore count ranged from 25 to 48 per 50 g soil (Dalal and Hippalgoankar 1995).

Table III. Effect of soil phosphorus on the occurrence of VAM spores in Haryana soils

Soil P ppm[a]	Total VAM spores per 50 g soil	Spores of different VAM genera (per 50 g soil)			
		Glomus	*Gigaspora*	*Sclerocystis*	*Acaulospora*
Total soil P					
≤1600 (54)	183	156	22	3	2
1601–3200 (35)	242	221	14	5	2
≥3201 (2)	725	578	122	0	25
LSD (p = 0.05)	2.0		2.5		
Available soil P					
≤20 (49)	220	192	24	2	2
21–40 (32)	209	178	23	5	3
≥41 (10)	170	148	17	2	3
LSD (p = 0.05)	2.5		3.2		

[a]Numbers in parentheses indicate the number of soil samples in the given range.

Out of 91 soil samples, in 54 the total P contents were less than 1600 ppm and the spore number in these soils was 183 spores per 50 g soil (Table III). In other 35 soil samples the total P ranged between 1601 and 3200 ppm. In these soils on an average 242 spores per 50 g soil were present. Only two locations were found to have soil P levels above 3201 ppm. In these high total P soils, the average number of spores were 725 spores per 50 g of soil. The effects of total P contents on the distribution of

VAM spores was statistically significant. Again *Glomus* was the dominant genus and with increase in total P levels the number of *Glomus* spores decreased.

Available P in different soils ranged from 6.1 to 57 ppm. At 49 locations, the available P was less than 20 ppm and on the average 220 spores were present per 50 g of soil (Table III). The available P in other 32 soil samples lay between 21 to 40 ppm and the spore number was 209 spores per 50 g soil. The available P was more than 41 ppm at 10 locations and these soils contained 170 spores per 50 g soil on the average. With increasing levels of available P, a decreasing trend in the VAM population in different soils was observed and these were statistically significant. The availability of P in the Haryana soils did not affect the distribution of different genera of VAM present in the Haryana soils. *Glomus* contributed 85–87 % of the total VAM population followed by *Gigaspora*.

The population of VA mycorrhizal fungi was significantly correlated with the total P contents (Table IV). However, the amount of available P adversely affected the distribution of VA mycorrhizal fungi in the soils. This relationship between spore number and available soil P was not significant as also reported by other researchers (Dalal and Hippalgoankar 1990; Krishna and Bagyaraj 1982). Contrary to this, a high spore count has been reported with high soil P (Khalil and Loyanachan 1994).

Table IV. Correlation coefficient (γ) of distribution of VA mycorrhizal fungi with different soil factors

Soil factors	pH	Total P[a]	Available P
Number of VAM spores per 50 g soil	0.0303	0.3997**	-0.1116
pH	—	0.2362*	-0.1510
Total P	—	—	-0.0076

[a]Correlation coefficient at 5 % level (*) = 0.21, at 0.1 % level (**) = 0.34.

The soils collected from different parts of Haryana were classified on the basis of loam and sand contents into three types. This classification was done visually and three categories of soils were loamy sand, sandy loam and sandy soils (Table V). In 40 loamy sand soils from different locations the spore count ranged from 10 to 407 with an average of 154 per 50 g soil. Twenty-four soils were of sandy loam type and the spore count in these soils varied from 114 to 717 with an average of 257 per 50 g soil. In sandy soils (27) the VAM population varied with different locations (59 to 794 spores) and on the average 267 spores were present per 50 g soil. The effects of soil type on VAM population was statistically significant. The VAM population contained mostly *Glomus* spores in sandy loam soil and comparatively fewer were present in loamy sand soil. The counts of *Glomus* were followed by *Gigaspora* and *Sclerocystis*.

Table V. Effect of other soil factors on the occurrence of VAM spores in Haryana soils

Soil factors[a]	Total VAM spores per 50 g soil	Spores of different VAM genera (per 50 g soil)			
		Glomus	*Gigaspora*	*Sclerocystis*	*Acaulospora*
Soil type					
Loamy sand (40)	154	134	15	4	1
Sandy loam (24)	257	239	12	4	2
Sandy (27)	267	225	38	1	3
LSD (p = 0.05)	2.1		2.7		
Soil moisture					
Water logged soils (12)	131	113	15	–	3
Moist soils (40)	194	173	15	5	1
Dry soils (39)	267	225	38	1	3
LSD (p = 0.05)	2.3		3.0		

[a]Numbers in parentheses indicate the number of soil samples.

Differences in the number of VAM spores with different types of soil were also observed by Hiremath *et al.* (1990) and Land *et al.* (1990). The native VA mycorrhizal population was reported to be less in black soil (vertisol) as compared to red soil (alfisol). This may be attributed to clay or loam contents of the soil which influenced the VA mycorrhizal population because with the increase in loam contents in Haryana soils the number of VAM spores decreased.

Soil samples were collected from water-logged fields, soils with optimum moisture contents and dry soils. The number of VAM spores in water-logged soils ranged from 49 to 407 spores per 50 g soil (*data not given in the table*) and the average number of spores in these 12 soils was 131 per 50 g soil (Table V). Moist and dry soil samples contained 194 and 267 spores per 50 g soil, respectively. The spore population was significantly different under different soil moisture conditions. Distribution of different VAM genera in these soils was similar.

Soil water stress has been known to have many direct and indirect effects on mycorrhizal infection and growth promotion. Unfavorable conditions, such as water stress, caused more sporulation of VA mycorrhizal fungi, thereby increasing the VAM population in the soil. Similar observations were made by other workers (Hiremath *et al.* 1990; Land *et al.* 1990; Khalil and Loyanchan 1994).

Table VI. Effect of cropping season on the occurrence of VAM spores in Haryana soils

Cropping seasons[a]	Total VAM spores per 50 g soil	Spores of different VAM genera (per 50 g soil)			
		Glomus	*Gigaspora*	*Sclerocystis*	*Acaulospora*
Summer crop (52)	152	144	4	3	1
Winter crop (39)	283	264	14	4	1
LSD (p = 0.05)	2.4		3.8		

[a]Numbers in parentheses indicate the number of soil samples collected in the given cropping season.

In summer when the soil temperature was very high, the number of VA mycorrhizal fungi in the rhizosphere of pigeonpea, mungbean, pearl millet and clusterbean ranged from 10 to 668 spores with an average of 152 spores per 50 g soil (Table VI). However, during winter in the rhizosphere of chickpea, wheat and mustard the number of VAM spores was greater and ranged from 59 to 794 spores with an average of 283 spores per 50 g soil. The distribution of VAM spores during different cropping seasons was significantly different. These reports were not in agreement with others (Lopez Sanchoz and Honrubia 1990, 1992; Methew *et al.* 1990; Sulochana and Manoharachray 1990; Vardavakis 1992) where higher counts of VAM spores were found in summer crops. This could be attributed to the prevalence of high temperature (52 °C) in Haryana during summer (Dudeja and Khurana 1989), which may be lethal for VAM spores.

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