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Population dynamics of mycorrhizal fungi in rhizosphere of pigeon pea [*Cajanus cajan* (L.) Millsp.]

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Abstract
Soil samples were collected from the rhizosphere of pigeon pea [*Cajanus cajan* (L.) Millsp.] to evaluate the population dynamics of vesicular arbuscular mycorrhizae (VAM). Root colonization by native VAM fungi was recorded minimum in early stages of plant growth which significantly increased with the age of plants and became highest at maturity. Maximum VAM colonization in different blocks of the district ranged between 71-86% during both the years. Spore population was recorded maximum in soil samples collected at early stages of plant growth which significantly decreased of VAM fungi belonging 4 genera (*Glomus, Sclerocystis, Acaulospora, Endogon*) were identified. Among these *Glomus mosseae* and *Sclerocystis rubiformis* were found most dominating and widely distributed in all the blocks followed by *Acaulospora longula* and *Sclerocystis clavispora* which were distributed only in 12 blocks of the district. *Acaulospora* spinosa and *Glomus diaphanum* were recorded only from two blocks and their prevalence were least. Colonization per cent have negative correlation with spore density at different crop growth stages.

Keywords: Mycorrhizal fungi, pigeon pea, growth stage, population dynamics.

INTRODUCTION

Increased environmental awareness is progressively leading to a shift from conventional intensive agriculture to low input sustainable agricultural cropping system relying on biological processes rather than agro-chemical to maintain crop health and productivity. This shift has resulted in greater interest in naturally occurring soil organism that facilitate improvement of the soil fertility and/or stimulate plant nutrient and health either alone or via specific interactions. Vesicular arbuscular mycorrhizal (VAM) fungi are widely distributed and form mutualistic symbiosis with most vascular plants in grass land ecosystem (Smith and Read,2008). VAM fungi comprise the largest component (mycelia and spores) of the microbial mass in soil (Eriksen *et al.*, 2002; Muthukumar and Udaiyan, 2002) and can increase plant nutrient and water uptake in nutrient poor soil (Koide, 1991; Cui and Nobel, 1992; Khalvati *et al.*, 2010). VAM fungi therefore, play an important ecological role in determining the plant diversity, productivity and species composition in terrestrial ecosystem (Vander Heijden *et al.*, 1998; Umbanhowar and McCann, 2005; Vogelsand *et al.*, 2006 and Leigh *et al.*, 2009). The association and importance of fungi in agriculture and horticulture is well documented (Gerdemann, 1968; Mosse, 1973, Smith and Read, 2008). Most of the plants are found associated with VAM fungi in natural ecosystem in this region also but the extent of infection may vary from plant type, soil type and climatic conditions (Singh and Prasad, 2006; Singh, 2007 and Parmar *et al.*, 2010). Our knowledge about this symbiosis in economically important crop like pigeon pea [*Cajanus cajan* (L.) Millsp.] is meager. Hence, in present investigation population dynamic and morphology of mycorrhizal fungi in rhizosphere of pigeon pea have been studied at different location in Bahraich district of Uttar Pradesh.

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MATERIALS AND METHODS

Survey of 5 villages each from 14 blocks of Baharaich district was conducted time to time to evaluate the natural status and existing population of VAM fungi in the rhizosphere of pigeon pea. Soil samples containing soil and fine roots from the rhizosphere of pigeon pea plants were collected at three stages of plant growth (stage 1- One month after germination, stage-2, 4 month after germination, stage-3, At maturity of crop). For the quantitative studies and variability of VAM population in soil, few samples were collected randomly from each site. The rhizospheric soil of the plants were dug out with the help of trowel to a depth of 20 to 25 cm after scraping away the top soil upto 1-2 cm. Samples of entire root system were collected and mixed together to get single samples for each plants. The samples were kept in polythene bags and stored at 2^{o}C till their processing.

To asses the colonization of VAM fungi, clearing and staining of root segments were done as per procedures of Phillips and Hayman (1970). The per cent colonization of VAM-fungi was determined under microscope (100 root segments) as suggested by Giovanneti and Mosse (1980). Mycorrhizal spores were isolated by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). These spores were mounted in lactophenol and examined under stereo/research microscope for their counting and morphological feature for identification. Size of spores were measured with the help of ocular and stage micro-meter. The identification of spores were done by the basis of description given by Gerdemann and Trappe (1974) and Trappe (1982).

RESULTS AND DISCUSSION

Rhizospheric soil and root samples of pigeon pea were collected from different villages of various blocks of Baharaich district (Uttar Pradesh) at different stages of plant growth during 2009-10 and 2010-11 crop seasons. The collected soil samples were analysed for study of spore population and root samples for colonization of native VAM fungi. Root colonization by native VAM fungi increased with the advancing stage of the crops during both the years. In general, per cent root colonization of native fungi was found minimum in initial stage i.e. one month after germination. It increased significantly and became maximum in samples collected at maturity stage of the crops. Overall in the district mean minimum VAM colonization per cent of 8.34 and 8.47% was recorded in initial stage and maximum of 78.84 and 78.83% in samples collected at maturity stage of the crop during 2009-10 and 2010-11, respectively. Among the blocks on mean basis of villages maximum root colonization in samples collected at maturity stage ranged between 71.00-86.00% and 71.00-85.00% respectively during both the years. Average maximum root colonization by VAM fungi at maturity stage of the crop was recorded in block Mihinpurwa (86%) followed by Balha (84.00%) and Huzurpur (83.00%) during 2009-10 and block Prayagpur (85.00%) followed by Huzurpur (84.00%) and Risiya (84.00%) during 2010-11. All these were statistically at par among themselves, but were significantly higher over others. On the basis of mean value of all the three stages maximum root colonization of VAM fungi was recorded in block Mihinpurwa (49.00%) followed by Balha (47.33%) and Huzurpur (46.60) during first year and in block Risiya (48.33%) followed by Mihinpurwa (46.33%) and Prayagpur (45.53) during second year of study, respectively but all were at par. Interaction effect of location and stages of plant growth on VAM colonization was also found significant (Table-1).

Table 1. Mean per cent root colonization of VAM fungi in pigeon pea during 2009-10 and 2010-11 in different blocks

Name of Blocks	Per cent root colonization							
	2009-10				2010-11			
	Stage-1	Stage-2	Stage-3	Mean	Stage-1	Stage-2	Stage-3	Mean
Chittora	9.60 (17.80)	46.60(42.70)	78.00(62.17)	44.53(40.89)	6.60(14.73)	41.00(39.80)	75.00(60.16)	40.87(38.23)
Tejwawapur	6.00(14.02)	48.00(43.85)	81.00(64.26)	45.00(40.71)	8.60(16.93)	42.00(40.37)	76.00(60.73)	42.20(39.35)
Fakkarpur	4.00(8.86)	47.00(43.27)	77.00(61.49)	42.67(37.87)	2.00(5.17)	32.00(34.31)	71.00(57.46)	35.00(32.31)
Kaiserganj	9.60(17.80)	44.00(41.53)	81.00(64.26)	44.87(41.20)	8.60(16.93)	44.00(41.54)	81.00(64.26)	44.53(40.91)
Nanpara	5.00(11.18)	33.00(35.03)	72.00(58.15)	36.67(34.79)	7.00(15.13)	38.00(38.02)	73.00(58.79)	39.33(37.31)
Risiya	11.00(19.27)	38.60(38.39)	76.00(60.73)	41.87(39.46)	12.00(20.18)	49.00(44.43)	84.00(66.57)	48.33(43.72)
Mihinpurawa	11.00(19.27)	50.00(45.00)	86.00(68.20)	49.00(44.16)	13.00(21.05)	46.00(42.69)	80.00(63.80)	46.33(42.51)
Jarwal	9.60(17.80)	43.00(40.95)	82.00(65.06)	44.87(41.27)	13.00(21.05)	39.00(38.63)	77.00(61.49)	43.00(40.39)
Huzurpur	9.00(17.10)	47.00(43.27)	83.00(66.45)	46.60(42.28)	6.40(12.45)	39.00(38.63)	84.00(66.57)	43.13(39.22)
Prayagpur	5.00(11.44)	37.00(37.42)	79.00(62.82)	40.33(37.23)	7.60(15.83)	44.00(41.52)	85.00(67.44)	45.53(41.60)
Visheshwarganj	6.00(12.54)	42.00(40.37)	79.00(62.82)	42.33(38.58)	9.20(17.40)	38.00(38.02)	82.60(65.52)	43.27(40.31)
Balha	12.00(20.14)	46.00(42.69)	84.00(66.57)	47.33(43.14)	9.60(17.80)	40.00(39.17)	77.00(61.49)	42.20(39.49)
Shivpur	12.40(20.54)	41.00(39.80)	74.00(59.40)	42.47(39.91)	10.00(18.40)	45.60(42.47)	79.00(62.82)	44.87(41.23)
Nawabganj	6.60(14.73)	32.00(34.38)	71.00(57.46)	36.53(35.52)	5.00(11.44)	38.00(38.02)	79.00(62.82)	40.67(37.42)
Mean	8.34(15.89)	42.47(40.62)	78.84(62.85)		8.47(16.03)	41.11(39.83)	78.83(62.85)	
	SEm±	CD at 5%			SEm±	CD at 5%		
Blocks	1.249	3.488			1.262	3.524		
Stages	0.883	2.466			0.892	2.492		
Block x Stage	2.163	6.041			2.186	6.103		

Note: Figures given in parentheses are sine arc transformed values.

Spore counts per 25 g soil at different stages of plant growth were also found variable among the blocks and villages during both the years. Spore population was noted little higher during 2010-11 in comparison to 2009-10 in soil samples collected at different crop growth stage. Spore population on mean basis was found maximum (80.11 and 81.67/25 g soil) in samples collected at early stage of plant growth which gradually decreased and became significantly lower in samples collected at maturity stage (55.34 and 56.14/25 g soil). Among the blocks spore count/25 g soil collected at different stages of plant growth on mean basis was recorded maximum in Risiya (77.87) followed by Jarwal (77.27) and Balha (75.53) during first year and Tejawapur (75.80) followed by Nanpara (75.20) and Chittora (74.13), respectively during second year of testing, but all were at par among themselves. Significance differences were noted only in some blocks. Interaction effects of location and stage on plant growth were also recorded significant in some blocks (Table-2).

Giovannetti (1985) and Diaz and Hanrubia (1994) studied the effect of seasonal difference on mycorrhizal population and found highest spore population in month of July and October and root colonization during flowering period of each plants. In present findings highest spore population at early stage of plant growth and maximum root colonization at maturity stage of the crop may also be due to seasonal variations, which supports this views. Lowering of spore population in later stage with increasing root colonization may be the utilization of spores in VAM colonization. Thus in present study spore population in soil showed negative correlation with root colonization by VAM fungi at different stages of plant growth. Variation in spore population and root colonization in soil of different blocks may be due to changes in physico-chemical properties of soil of different places as earlier workers had studied and reported soil

pH, available nitrogen, phosphorus, potassium and calcium negatively or positively influence the spore density and VAM colonization in different plants (Mosse, 1972; Hayman, 1982, Land *et al.*, 1990; Sasai, 1990; Singh and Prasad, 2006; Singh, 2007).

Table 2. Spore population of VAM fungi in samples pigeon pea [*Cajanus cajan* (L.) Millsp.]

Name of Blocks	Spore count (25 g soil)							
	2009-10				2010-11			
	Stage-1	Stage-2	Stage-3	Mean	Stage-1	Stage-2	Stage-3	Mean
1. Chittora	80.00	77.20	57.80	70.67	84.00	80.20	58.20	74.13
2. Tejwawapur	83.80	79.60	58.00	73.80	84.60	82.20	60.60	75.80
3. Fakkarpur	79.60	76.00	56.80	70.80	83.40	78.60	59.20	73.73
4. Kaiserganj	77.60	74.80	58.60	70.33	82.00	79.60	56.00	72.53
5. Nanpara	80.20	75.60	55.00	70.27	85.40	81.40	58.80	75.20
6. Risiya	87.80	83.80	62.00	77.87	84.00	80.40	57.60	74.00
7. Mihinpurawa	75.00	72.00	51.80	66.27	80.40	76.80	55.60	70.93
8. Jarwal	87.60	83.20	61.00	77.27	82.60	78.80	58.40	73.27
9. Huzurpur	74.40	71.00	52.00	65.80	80.40	76.80	55.00	70.73
10. Prayagpur	73.80	69.40	48.80	64.00	77.60	73.20	51.20	67.13
11. Visheshwarganj	76.60	72.20	50.20	66.33	79.80	77.20	56.40	71.27
12. Balha	84.40	79.80	58.40	75.53	81.60	77.00	55.20	72.13
13. Shivpur	79.20	74.40	53.20	69.93	82.60	78.80	55.00	65.07
14. Nawabganj	77.60	73.20	51.20	67.33	75.00	71.40	48.80	71.95
Mean	80.11	75.87	55.34		81.67	78.03	56.14	
	SEm±	CD at 5%			SEm±	CD at 5%		
Blocks	2.149	6.002			1.709	4.773		
Stages	1.520	4.244			1.209	3.375		
Block x Stage	3.723	10.396			2.960	8.267		

Table 3. Spore characters of VAM fungi found in rhizosphere of Pigeon pea in different blocks

Name of blocks	Spore size (lem)	Shape of spores	Colour of spores	Identification of VAM fungi
Chittora	48.80-145.80	Globose	Black, Brown	*Acaulospora longula, Glomus microcarpum, G. monocarpum, G. mosseae, G. tenue, Sclerocystis clavispora, S. coccogena, , S. dussii, S. rubiformis*
Tejwawapur	86.60-126.60	Sub-globose, globose	Black	*Acaulospora longula, A. trappei, Endogon,pisiformis, Glomus fasciculatum, G. monosporum, G. mosseae, G. tenue, Sclerocystis clavispora, S. dussii, S. rubiformis , S. sinuosa,*
Fakkarpur	32.40-129.60	Globose	Yellow-brown	*Acaulospora longula, A. spinosa, Glomus gerdemanii, G. fasciculatum, G. microcarpum, G. mosseae, Sclerocystis clavispora, S. dussii, S. rubiformis*
Kaiserganj	34.40-142.80	Ellipsoid, Globose	Brown, Black	*Acaulospora longula, A. trappei, Glomus diaphanum, G. mosseae, G. occulatum, Sclerocystis clavispora, S. coccogena, S. rubiformis, S. sinuosa*
Nanpara	48.60-162.00	Globose, Ellipsoid, Irregular	Brown, Black	*Acaulospora longula, Endogon pisiformis, Glomus diaphanum, G. fasciculatum, G. mosseae, Sclerocystis clavispora, S. dussii, S. rubiformis, S. sinuosa*
Risiya	66.80-192.40	Globose, Irregular	Brown, Black, Yellow brown	*Acaulospora longula, A. trappei, Endogon pisiformis,Glomus,fasciculatum, G. microcarpum, G. mosseae, G. occulatum, Sclerocystis dussii, S. rubiformis, S. sinuosa*
Mihinpurawa	16.40-176.20	Globose, Ellipsoid	Yellow -brown, Black	*Acaulospora longula, A. trappei, Endogon pisiformis,Glomus,fasciculatum,G. monosporum, G. mosseae, G. tenue, Sclerocystis clavispora, S. dussii, S. sinuosa*
Jarwal	18.40-114.80	Globose	Black, Brown	*Acaulospora longula, A. trappei, Glomus fasciculatum, G. microcarpum, G. monosporum, G. mosseae, Sclerocystis clavispora, S. dussii, S. rubiformis, S. sinuosa*
Huzurpur	34.60-144.80	Globose	Yellow-brown	*Glomus gerdemanii, G. fasciculatum, G. microcarpum, G. mosseae, G. tenue, Sclerocystis coccogena, S. rubiformis, S. sinuosa*
Prayagpur	50.00-130.60	Globose	Black	*Acaulospora longula, Glomus microcarpum, G. monosporum, , G. mosseae, G. tenue, Sclerocystis clavispora, S. coccogena, S. dussii, S. rubiformis*
Visheshwarganj	45.20-144.80	Globose	Yellow-brown	*Acaulospora longula, Endogon pisiformis, Glomus,fasciculatum, Glomus mossae, G. tenue, Sclerocystis clavispora, S. dussii, S. rubiformis*
Balha	34.80-130.40	Globose, Sub-globose	Black, Brown	*Acaulospora longula, Glomus microcarpum, G. monosporum, , G. mosseae, G. tenue, Sclerocystis clavispora, S. dussii, S. rubiformis, S. coccogena*
Shivpur	48.60-145.80	Globose, Ellipsoid	Yellow-brown, Brown	*Acaulospora longula, Endogon pisiformis, Glomus,fasciculatum, Glomus mossae,G. occulatum, G. tenue, S. clavispora, S. dussii, S. rubiformis, S. sinuosa*
Nawabganj	32.40-178.20	Globose	Yellow-brown	*Acaulospora trappei, A. spinosa, Glomus gerdemanii, G. fasciculatum, G. microcarpum, G. mosseae, Sclerocystis clavispora, S. dussii, S. rubiformis*

Table 4. Prevalence of VAM fungi in soil samples of pigeon pea (Pooled data of 2009-10 and 2010-11 in different blocks

Name of blocks	Identified species of VAM fungi																
	Al	At	Aspi	Ep	Gd	Gg	Gf	Gmi	Gmon	Gmos	Go	Gt	Scl	Sco	Sd	Sr	Ss
Chittora	+							+	+	+		+	+	+	+	+	
Tejwawapur	+	+		+			+		+	+		+	+		+	+	+
Fakkarpur	+		+			+	+	+		+			+		+	+	
Kaiserganj	+	+			+					+	+		+	+		+	+
Nanpara	+			+	+		+			+			+		+	+	+
Risiya	+	+		+			+	+		+	+				+	+	+
Mihinpurawa	+	+		+			+		+	+		+	+			+	+
Jarwal	+	+					+	+	+	+			+		+	+	+
Huzurpur						+	+	+		+		+		+		+	+
Prayagpur	+							+	+	+		+	+	+	+	+	
Visheshwarganj	+			+			+			+		+	+		+	+	
Balha	+							+	+	+		+	+	+	+	+	
Shivpur	+			+			+			+	+	+	+		+	+	+
Nawabganj		+	+			+	+	+	+	+			+		+	+	

Al = *A. longula,*
At = *A. trappei*
Aspi = *A. spinosa*
Ep = *Endogone pisiformis*
Gd = *Glomus diaphanum*
Gg = *G. gerdemanii*
Gf = *Glomus fasciculatum*
Gmi = *Glomus microcarpum*
Gmon = *Glomus monosporum*
Gmos = *Glomus mosseae*
Go = *G. occulatum*
Gt = *G. tenue*
Scl = *Sclerocystis clavispora*
Sco = *S. coccogena*
Sd = *S. dussii*
Sr = *Sclerocystis rubiformis*
Ss = *Sclerocystis sinuosa*

Vesicular arbuscular fungi were identified on the basis of morphology of their resting spores. The detail information about characteristics of spores and their morphological features are given in table-3. The identification of these spores were done on the basis of presence of vesicles and arbuscles which are the most important diagnostic criteria for identifying the VAM fungi in root. Shape of spores are generally globose, sub-globose or ellipsoid. In some cases they may be irregular also. The colour of spores were variable and they may be black, brown, and yellow-brown. Total 17 species of VAM fungi belonging to 4 genera were isolated and identified from samples collected from different locations at different stages of plant growth of pigeon pea fields. Among these species *Glomus mosseae* and *Sclerocyctis rubisformis* were found widely distributed in different villages of all the blocks under study. It was followed by VAM fungi *Acaulospora longula* and *Sclerocyctis clavispora* which were prevalent in 12 blocks, while *S. dussii* was isolated in the samples collected from 11 blocks only. VAM fungi *Acaulospora spinosa* and *Glomus diaphanum* were obtained only from two blocks. Prevalence of the rest of the species were given in table-4. Concurrent with present findings several scientist have also identified different species of VAM fungi belonging to 4 genera and reported domination of *Glomus* species in different leguminous plant from different places Kim and Kim, 1992; Ahn *et al.*, 1992; Damjanova and Dabolyi, 1993; Trimurtulu and Johri, 1998; Nagabhusan *et al.,* 1999). Domination of VAM fungi *Sclerocyctis rubisformis* in case of pigeon pea in this region is new report and may be due to environmental effect and/or specialty of physico-chemical properties of the soil which needs further investigations.

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