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Influence of Inoculation of Different Vesicular Arbuscular Mycorrhizal Fungi on Growth and Nutrient of Mungbean and Wheat

SATPAL SINGH and K.K. KAPOOR*

Department of Microbiology, CCS Haryana Agricultural University, Hisar-125 004, India

An experiment was conducted under greenhouse conditions to evaluate the effects of different vesicular-arbuscular mycorrhizal (VAM) fungi with and without Mussoorie rock phosphate (MRP) in P-deficient natural non-disinfected soil with mungbean (*Vigna radiata* L. Wilczek) and wheat (*Triticum aestivum* L. emend Thell) as test crops. Root colonization by native VAM fungi was poor in uninoculated soils which was improved with inoculation. The MRP amendment stimulated root colonization. The plant biomass and nutrient uptake were greater in the presence of MRP. Highest plant biomass, N and P uptake was observed on mungbean inoculated with *Glomus fasciculatum* in presence of MRP. In wheat *Glomus* sp. 88 promoted better plant growth and nutrient uptake than the other VAM fungi. There was increase in dry matter production, N and P uptake by 20.2, 110.5 and 160.4% respectively.

Key words: Vesicular-arbuscular mycorrhizae; Mussoorie rock- phosphate; *Vigna radiata, Triticum aestivum;* Nutrient deficient soils

Vesicular-arbuscular mycorrhizal (VAM) fungi are known to play an important role in plant growth and nutrient uptake especially the supply of P (Kucey *et al.* 1989; Barea 1991; Bolan 1991). The uptake of other nutrients such as zinc and copper are also reported to be increased by the inoculation with VAM fungi (Harley & Smith 1983; Faber *et al.* 1990). The successful mycorrhizal association depends on the presence of host, fungus and appropriate environment.

VAM fungi have little specificity and any of them infect virtually any potential host plant (Smith & Gianinazzi-Pearson 1988; Gianinazzi 1991). However, VAM endophytes differ in their effectiveness which appears to be more dependent on the specific host-endophyte combination. Therefore, the selection of efficient VAM fungi is essential for inoculation of field crops. Most of the experiments regarding the selection of efficient VAM fungi in different crops have been conducted in sterile soils where competition from native VAM fungi is eliminated (Powell 1979; Weber et al. 1993). Limited information is available under unsterile natural conditions (Smith & Gianinazzi-Pearson 1988; Barea et al. 1988). The objectives of the present investigation were: (a) to find a suitable host-endophyte combination under natural nutrient limited soil conditions for mungbean and wheat and (b) to find out the effectiveness in promoting growth and N and P uptake by the different VAM endophytes in the presence or absence of Mussoorie rock phosphate (an insoluble P source).

*Corresponding author: faculty@hau.hry.nic.in

Materials and Methods

Experimental design

To study the effect of inoculation of different VAM fungi such as *Glomus fasciculatum* (Thaxter sensu Gerd) (Gerd and Trappe), *Glomus mosseae* (Nicol and Gerd) (Gerdemann and Trappe) *Glomus* sp. 85, *Glomus* sp. 88, *Glomus* sp. 92 and Glomus sp.97, a green house experiment was conducted in a completely randomized factorial design (seven main treatments including inoculated control and two rock-phosphate levels) with three replicates each. The treatments means were compared at 5% level of significance.

Soil

The soil used in the study was taken from Haryana Agricultural University dryland farm, Hisar and it had the following characteristics: clay 5.6%, silt 4.0%, sand 90.4%, organic C, 0.10%; $NaHCO_3$-extractable P, 2.5 ppm; total P, 0.001%; 4.9 c mole (p+) kg^{-1} cation exhange capacity and pH 7.6. Four kg air dried sieved (2 mm) soil was placed in earthen pots with a hole in the bottom to drawn out excess water.

Greenhouse conditions

Ten seeds were sown at the depth of 2.5 cm and after germination thinned to 5 plants per pot. The pots were distributed at random in a greenhouse under natural conditions. The mean ambient temperature during growth of mungbean crop was 26.1°C. The mean bright sunshine was 8.2 h and relative humidity was 65%. The mean ambient temperature during wheat growth was 19.5°C with a mean minimum of 10.4°C and mean maximum of 28.5°C. The mean bright sunshine was 6.7 h and relative humidity was 84.3%. Plants were watered daily to maintain moisture at field capacity.

VAM inoculum and inoculation

VAM endophtes *G. fasciculatum* (Thaxter sensu Gerd) (Gerd and Trappe) and *Glomus mosseae* (Nicol and Gerd) (Gerdemann and Trappe), were obtained from D.J. Bagyaraj, University of Agricultural Sciences, Bangalore, India while *Glomus* sp. 85, *Glomus* sp. 92 and *Glomus* sp. 97 were supplied by Dr. S.S. Dudeja, Department of Microbiology, Haryana Agricultural University, Hisar. The inoculum consisted of chopped root segments and soil from a 10-week old pot culture of different VAM fungi grown on pearlmillet *Pennisetum glaucum* (L. RBr) in a sterile sandy soil. Five grams of inoculum was band placed at a depth of 5 cm in the pots.

Rhizobium inoculation

Seeds of mungbean were inoculated with 72h old culture of Rhizobium sp. Vigna S32 strain by putting one ml of culture (about 10^8 cells) over the each seed hole.

Rock phosphate

Mussoorie rock phosphate (MRP) was obtained from M/S Pyrites, *Phosphates and Chemicals Ltd.*, Noida, India as a 100-mesh size powder. It contained 8.1% total P, 0.056 mg g^{-1} water-soluble P, 0.18 mg/g 0.5 M $NaHCO_3$-soluble P, 2.3 mg/g citric acid soluble P and a pH (1:2 MRP:Water) 8.7. Phosphorus in MRP is present as carbonate fluorapatite which consists of about 67% total rock phosphate and is of sedimentary origin.

Basal nutrients

MRP was mixed well in each pot so as to supply 40 and 60 kg P_2O_5/ha in mungbean and wheat respectively. A basal dose of nitrogen was applied to mungbean as ammonium sulphate at 20 kg N/ha while in case of wheat, N was added through urea to supply 120 kg N/ha in the upper 5 cm. The fertilizer doses were as per the recommendations based on earlier field experiments for each crop.

Plant sampling

Plant sampling for assessment of nodulation and nitrogen fixation in mungbean was done 35 days after sowing. Three pots was removed and analyzed for nodulation and nitrogenase activities (Hardy et al. 1968). The plants with other three pots for each treatments were harvested after 55 days to determine the root colonization, shoot and root biomass, and N and P uptake. Total N and P content of shoot and root were determined and uptake of N and P was calculated by multiplying with weight of root and shoot. Similarly, wheat plants from three pots were also harvested after 55 days of growth and analyzed in the same manner as for the mungbean crop.

Parameters measured

At harvest, the dry matter yield was determined after drying the shoot and root to constant weight. The N and P content of root and shoot were determined.

Assessment of mycorrhizal infection

The root clearing and staining method of Phillips & Hayman (1970) was used for studying root colonization by VAM. After removal of shoot, the roots were thoroughly washed under tap water to remove the soil. Mycorrhizal colonization in cortical root tissue was estimated by examining microscopically stained root segments (1 cm long). Fifty root segments per pot were examined and the percentage of root segments colonized were scored.

Nutrient analysis

The microkjeldhahl method was used for N determination (Bremner 1960) and total P was determined by the method of John (1970).

Results and Discussion

VAM root colonization

The VAM root colonization ranged from 6.0 to 88.1% (table 1) and it was poor without VAM inoculation both in mungbean and in wheat. Inoculation with different VAM fungi led to the significant increase in the degree of root colonization. Addition of MRP stimulated root colonization but it was non-significant. Maximum root colonization (88.1%) was osberved with Glomus fasciculatum in mungbean and Glomus sp. 88 inoculation in wheat (80.3%) in presnece of MRP. The interaction between rock phosphate levels and VAM inoculation was found to be nonsignificant.

Dry matter yield

The results of inoculation with VAM fungi on dry matter production (shoot and root) by mungbean in absence and presence of MRP are shown in table 2. Both shoot and root dry matter production were improved due to inoculation with VAM fungi over inoculated control, the increase was significant even within treatments. The improvement in total plant biomass was more when MRP was added to the soil. In case of mungbean the effectivity of Glomus fasciculatum in dry matter production was higher in presence of MRP (151%) as compared with that of without MRP (125%) over the inoculated control. The effect of two factor interaction of rock- phosphate with VAM fungi on total dry matter was nonsignificant. Similarly, in the case of wheat crop (table 3) the effectivity of Glomus sp. 88 in dry matter production was highest in the presence of MRP (153%) than in without MRP (116%) over the uninoculated control. Increased dry matter yield with different VAM inoculants over uninoculated control was significant.

Nutrient uptake

The results of the inoculation of different VAM fungi on P uptake of mungbean and wheat are depicted in tables 4 and 5. There was more P uptake in shoot as compared to root in both crops. Inoculation significantly improved P uptake both in the presence or absence of MRP. However, the P uptake was greater when the soil was amended with MRP. Amongst the different VAM fungi the maximum P uptake was observed on plants inoculated with Glomus fasciculatum in mungbean and Glomus sp. 88 in wheat and this was statistically significant.

Tables 6 and 7 show the effects of inoculation of different VAM fungi on N uptake. VAM inoculation also significantly improved N uptake and maximum N uptake both in the absence and presence of MRP was observed on inoculation with Glomus fasciculatum in mungbean and with Glomus sp. 88 in wheat.

Nodulation and nitrogenase activity

There was no significant effect of VAM inoculation on nodule number, nodule dry weight and nitrogenase activity in mungbean (data not shown). Application of MRP did not improve nodulation and nitrogenase activity.

The results of the inoculation of different VAM fungi showed variation in their root colonization ability. The different levels of root colonization of a host by different endophytes gave an indication of a certain degree of compatibility between host and the VAM fungus (Gianinazzi 1991). The enhancement of mycorrhizal root colonization by addition of MRP may be attributed due to effect on root morphology or on the physiology of fungal symbiont. Similar increases in root colonization by VAM fungi in presence of rock phosphate have been observed previously (Singh & Singh 1993, Toro et al. 1996, Singh & Kapoor 1998).

Both shoot and root biomass increased significantly in presence of VAM fungi which may be attributed due to the increased nutrient supply resulting from root colonization by VAM endophyte. The increase in plant growth on VAM inoculation has been earlier reported in various field crops (Schubert & Hayman 1990; Pearson & Jakobsen 1993; Rathore & Singh 1995).

The significant increase in P uptake by root and shoot in presence of different VAM fungi may be attributed due to increased physical exploration of the soil by VAM hyphae. The enhancement of P uptake in presence of MRP and VAM fungi as compared to without MRP may be due to better utilization of P from native soil P as well as from rock phosphate (Bolan 1991, Rathore & Singh 1995, Singh & Kapoor 1998). Utilization of P from sparingly soluble P source such as rock phosphate by VAM fungi implies the ability of the latter to solubilize P from MRP (Singh & Kapoor 1998). In addition, the presence of VAM fungi in rhizosphere harbour soil microbial population which may include bacteria that have the ability to solubilize P and make it available to the plants (Fitter & Garbaye 1994).

Table 1. Effect of different VAM fungi on percent root colonization of mungbean and wheat with and without Mussoorie rock phosphate.

Treatments	Root Colonization (%)					
	Mungbean			Wheat		
	R_0	R	Mean	R_0	R	Mean
Control (uninoculated)	6.6	6.0	6.31	8.3	13.3	10.8^a
Glomus sp. 85	73.4	80.3	76.9^b	60.0	65.6	62.8^d
Glomus sp. 88	62.2	67.8	65.0^d	73.3	80.3	76.8^b
Glomus sp. 92	74.3	81.3	77.8^b	64.0	70.0	67.0^{cd}
Glomus sp. 97	66.6	75.5	71.1^c	66.6	71.2	68.9^c
Glomus mosseae	68.8	73.3	71.1^c	65.6	68.3	66.9^c
Glomus fasciculatum	80.2	88.1	84.2^e	62.6	69.3	66.0^c
Mean	61.7^a	64.5^a	-	57.2^a	62.6^a	-
LSD (P=0.005)						
Treatments (T)	4.6			4.8		
Rock Phosphate (R)	6.7			7.9		
T x R	NS			NS		

Treatment means followed by different letters are significantly different at 5% level of probability.

Table 2. Effect of different VAM fungi on dry matter yield of mungbean with and without Mussoorie rock phosphate.

Treatments	Root Colonization (%)								
	Mungbean			Wheat			Total		
	R_0	R	Mean	R_0	R	Mean	R_0	R	Mean
Uninoculated	6.6	6.0	6.31	8.3	13.3	10.8^a	1.042	1.074	1.058^a
Glomus sp. 85	73.4	80.3	76.9^b	60.0	65.6	62.8^d	1.205	1.302	1.253^b
Glomus sp. 88	62.2	67.8	65.0^d	73.3	80.3	76.8^b	1.190	1.318	1.254^b
Glomus sp. 92	74.3	81.3	77.8^b	64.0	70.0	67.0^{cd}	1.157	1.321	1.239^b
Glomus sp. 97	66.6	75.5	71.1^c	66.6	71.2	68.9^c	1.134	1.179	1.157^c
Glomus mosseae	68.8	73.3	71.1^c	65.6	68.3	66.9^c	1.215	1.146	1.181^c
Glomus fasciculatum	80.2	88.1	84.2^e	62.6	69.3	66.0^c	1.308	1.623	1.466^d
Mean	61.7^a	64.5^a	-	57.2^a	62.6^a	-	1.179^a	1.280^a	-
LSD (P=0.05)									
Treatments (T)	0.039			0.037			0.070		
Rock Phosphate (R)	0.042			0.050			0.096		
T x R	0.051			NS			NS		

Treatment means followed by different letters are significantly different at 5% level of probability.

Table 3. Effect of different VAM fungi on dry matter yield of wheat with and without Mussoorie rock phosphate.

Treatments	Dry matter yield (g pot^{-1})								
	Shoot			Shoot			Total		
	R_0	R	Mean	R_0	R	Mean	R_0	R	Mean
Uninoculated	0.627	0.637	0.632^a	0.455	0.455	0.460^a	1.082	1.102	1.092^a
Glomus sp. 85	0.695	0.758	0.727^b	0.550	0.620	0.585^b	1.245	1.378	1.312^b
Glomus sp. 88	0.708	0.928	0.818^d	0.712	0.762	0.737^c	1.420	1.690	1.555^c
Glomus sp. 92	0.678	0.786	0.732^b	0.578	0.604	0.591^b	1.256	1.390	1.323^b
Glomus sp. 97	0.686	0.717	0.702^c	0.574	0.679	0.627^b	1.260	1.396	1.328^b
Glomus mosseae	0.702	0.816	0.759^b	0.578	0.624	0.601^b	1.280	1.440	1.360^b
Glomus fasciculatum	0.668	0.724	0.696^c	0.596	0.638	0.617^b	1.264	1.362	1.313^b
Mean	0.681^d	0.767^b	-	0.578^a	0.627^a	-	1.258^a	1.394^a	-
LSD (P=0.005)									
Treatments (T)	0.031			0.081			0.130		
Rock Phosphate (R)	0.027			0.071			0.132		
T x R	NS			0.092			NS		

Treatment means followed by different letters are significantly different at 5% level of probability.

Table 4. Effect of different VAM fungi on P uptake by mungbean with and without Mussoorie rock phosphate.

Treatments	P uptake ($\mu g\ pot^{-1}$)								
	Shoot			Root			Total		
	R_0	R	Mean	R_0	R	Mean	R_0	R	Mean
Uninoculated	42	46	44^{a}	28	32	30^{a}	70	78	74^{a}
Glomus sp. 85	58	69	64^{b}	38	48	43^{b}	96	117	107^{b}
Glomus sp. 88	52	61	57^{d}	37	58	48^{b}	89	119	104^{b}
Glomus sp. 92	56	81	69^{c}	30	52	41^{b}	86	133	110^{b}
Glomus sp. 97	49	59	54^{ab}	36	54	45^{b}	85	103	94^{b}
Glomus mosseae	52	78	65^{c}	48	56	52^{bc}	100	134	117^{b}
Glomus fasciculatum	73	88	81^{c}	55	66	61^{d}	128	154	141^{c}
Mean	55^{a}	69^{a}	-	39^{a}	52^{b}	-	93^{a}	131^{b}	-
LSD (P=0.005)									
Treatments (T)	16			8			30		
Rock Phosphate (R)	14			10			27		
T x R	NS			15			NS		

Treatment means followed by different letters are significantly different at 5% level of probability.

Table 5. Effect of different VAM fungi on P uptake by wheat with and without Mussoorie rock phosphate.

Treatments	P uptake ($\mu g\ pot^{-1}$)								
	Shoot			Root			Total		
	R_0	R	Mean	R_0	R	Mean	R_0	R	Mean
Uninoculated	32	37	35^{a}	17	20	19^{a}	49	57	53^{a}
Glomus sp. 85	69	89	79^{b}	49	67	58^{b}	118	158	138^{b}
Glomus sp. 88	82	122	102^{c}	66	89	78^{c}	148	211	180^{c}
Glomus sp. 92	51	74	63^{b}	40	52	46^{b}	91	126	109^{d}
Glomus sp. 97	46	72	59^{b}	36	56	46^{b}	82	138	110^{d}
Glomus mosseae	61	118	90^{c}	47	84	66^{bc}	108	202	155^{b}
Glomus fasciculatum	56	90	73^{b}	36	78	57^{b}	92	168	138^{b}
Mean	57^{a}	86^{b}	-	42^{a}	64^{b}	-	98^{a}	151^{b}	-
LSD (P=0.05)									
Treatments (T)	18			14			25		
Rock Phosphate (R)	15			12			28		
T x R	22			18			NS		

Treatment means followed by different letters are significantly different at 5% level of probability.

Table 6. Effect of different VAM fungi on N uptake by mungbean with and without Mussoorie rock phosphate.

Treatments	N uptake ($\mu g\ pot^{-1}$)								
	Shoot			Root			Total		
	R_0	R	Mean	R_0	R	Mean	R_0	R	Mean
Uninoculated	0.282	0.302	0.292^{a}	0.185	0.192	0.189^{a}	0.467	0.494	0.481^{a}
Glomus sp. 85	0.435	0.662	0.549^{b}	0.410	0.500	0.455^{b}	0.845	1.162	1.004^{b}
Glomus sp. 88	0.467	0.674	0.571^{b}	0.431	0.512	0.472^{b}	0.898	1.186	1.042^{b}
Glomus sp. 92	0.442	0.728	0.585^{b}	0.436	0.532	0.484^{b}	0.878	1.260	1.069^{b}
Glomus sp. 97	0.489	0.792	0.641^{bc}	0.463	0.564	0.514^{b}	1.952	1.356	1.154^{b}
Glomus mosseae	0.507	0.682	0.595^{b}	0.495	0.625	0.560^{bc}	1.002	1.310	1.156^{c}
Glomus fasciculatum	0.585	0.868	0.727^{d}	0.53	0.684	0.607^{d}	1.115^{a}	1.552	1.334^{c}
Mean	0.458^{a}	0.673^{b}	-	0.421^{a}	0.516^{b}	-	0.880^{a}	1.189^{b}	-
LSD (P=0.05)									
Treatments (T)	0.071			0.079			0.098		
Rock Phosphate (R)	0.074			0.052			0.075		
T x R	NS			NS			NS		

Treatment means followed by different letters are significantly different at 5% level of probability.

Table 7. Effect of different VAM fungi on N uptake by wheat with and without Mussoorie rock phosphate.

Treatments	N uptake (μg pot^{-1})								
	Shoot			Root			Total		
	R_0	R	Mean	R_0	R	Mean	R_0	R	Mean
Uninoculated	0.232	0.257	0.245^{a}	0.205	0.217	0.211^{a}	0.437	0.474	0.456^{a}
Glomus sp. 85	0.429	0.512	0.471^{b}	0.382	0.416	0.399^{b}	0.811	0.928	0.870^{b}
Glomus sp. 88	0.569	0.592	0.581^{c}	0.534	0.563	0.549^{c}	1.103	1.555	1.129^{c}
Glomus sp. 92	0.457	0.522	0.490^{b}	0.391	0.484	0.438^{b}	0.848	1.006	0.927^{b}
Glomus sp. 97	0.428	0.487	0.458^{b}	0.401	0.463	0.432^{b}	0.829	0.952	0.891^{b}
Glomus mosseae	0.506	0.568	0.537^{cd}	0.482	0.552	0.517^{c}	0.988	1.120	1.054^{c}
Glomus fasciculatum	0.463	0.536	0.500^{c}	0.402	0.518	0.460^{b}	0.865	1.054	0.960^{b}
Mean	0.441^{a}	0.496^{b}	-	0.400^{a}	0.459^{b}	-	0.840^{a}	0.956^{b}	-
LSD (P=0.05)									
Treatments (T)	0.038			0.050			0.104		
Rock Phosphate (R)	0.021			0.039			0.081		
T x R	NS			NS			NS		

Treatment means followed by different letters are significantly different at 5% level of probability.

Our results did not show significant effect of inoculation with Rhizobium, and different VAM fungi on nodule number, their dry weight and nitrogenase activity of mungbean. This is in contrast to other studies where VAM inoculation has been shown to enhance nodulation and nitrogen fixation by rhizobia (Barea et al. 1988, Fitter & Garbaye 1990). The significant increase in total N uptake by both the crops due to VAM inoculation and MRP application could have been due to greater translocation of soil N by VAM hyphae (Johansen et al. 1992; George et al. 1991).

The results of the present study show that plant biomass and nutrient uptake of mungbean and wheat crops improved by inoculation with VAM fungi and amendment of rock phosphate. However, different VAM fungi performed differentially with host plant. Glomus fasciculatum and Glomus sp. 88 were found to perform best with mungbean and wheat respectively in the presence of MRP amendment.

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