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Improving Phosphorus Use through Co-inoculation of Vesicular Arbuscular Mycorrhizal Fungi and Phosphate-Solubilizing Bacteria in Maize in an Acidic Alfisol

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Performance of three vesicular arbuscular mycorrhizal (VAM) fungi cultures and a phosphate-solubilizing bacteria (PSB) culture alone or in combination with or without 75% of the recommended P_2O_5 *dose based on soil-test crop response model was examined in maize in a phosphorus (P)-deficient acidic Alfisol in a glasshouse pot experiment. Sole application of VAM besides co-inoculation with PSB (*Pseudomonas striata*) and inorganic P stimulated mycorrhizal root colonization. Sole application of PSB,* VAM_T *(*Glomus intraradices*), and* VAM_I *(*Glomus mosseae*) as well as co-inoculation of VAM with PSB significantly improved crop productivity besides grain protein content, thus indicating a synergistic interaction between VAM and PSB. Application of* VAM_T *or* $VAM_I + PSB + 75\%$ P_2O_5 *remained at par with sole application of 100%* P_2O_5 *dose with regard to productivity, nutrient uptake, and soil fertility status (particularly P), thus indicating economization of fertilizer P to the tune of about 25% without compromising crop productivity and soil fertility in an acidic Alfisol.*

Keywords *Glomus mosseae*, maize, phosphate-solubilizing bacteria, productivity, soil fertility, vesicular arbuscular mycorrhizae

Introduction

Most mountain soils of the northwestern Himalayas are deficient in available phosphorus (P) because of their acidic nature (Anonymous 1997) and have high P-fixing power because of excess iron (Fe) and aluminium (Al) ions (Narsian and Patel 2009), resulting into low P availability for crop production (Sharma, Verma, and Bhumbla 1980). Again, phosphatic fertilizers constitute one of the most expensive inputs in agriculture, which warrants their efficient use. In view of environmental concerns and current developments in sustainability, research efforts are concentrated on development of agrotechniques that use less expensive

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sources of plant nutrients such as biofertilizers or inorganic P coupled with biofertilizers (Narsian and Patel 2009). In this context, use of vesicular arbuscular mycorrhizae (VAM) and phosphate-solubilizing bacteria (PSB) might be useful in reversing the process of P fixation in an acidic Alfisol of the northwestern Himalayas, thereby enhancing P supply to maize. The VAM will largely mobilize available P by increasing the surface area of roots and greater exploration of soil volume by its hyphal network, leading to higher overall nutrient use efficiency (George et al. 1992). The PSB will render insoluble phosphates soluble to some extent. The present study was undertaken with the objective of finding the extent to which the combined application of VAM and PSB might reduce P fertilizer dose in maize.

Materials and Methods

Experimental Details

Glasshouse studies were conducted in maize (Kharif 2003) as a test crop in pots filled with 10 kg of 2-mm sieved air-dried soil. Silty clay loam soil (Typic Hapludalf) used in the study had an initial organic carbon (C) status of 3.5 g kg^{-1} soil. The available nitrogen (N), P, and potassium (K) contents of experimental soil were 49.1, 2.2, and 124.1 mg kg^{-1} soil, respectively. The experimental design employed was completely randomized design (CRD) with three replications. An additional replication was included especially for making root VAM colonization studies at flowering/silking. Treatments were absolute control, three VAM fungi cultures [VAM_L (local VAM culture, *Glomus mosseae*, developed by CSK Himachal Pradesh Agricultural University, Palampur), VAM_T (VAM culture, *Glomus intraradices*, developed by Centre for Mycorrhizal Research, the Energy and Resources Institute, New Delhi), and VAM_I (VAM culture, *Glomus mosseae*, developed by Indian Agricultural Research Institute, New Delhi)], and a local PSB culture (*Pseudomonas striata*) alone or in combination with or without 75% of recommended P_2O_5 dose based on targeted yield concept following the soil-test crop response (STCR) precision model as well as one treatment with sole application of 100% of recommended P_2O_5 dose based on the STCR model (Ramamoorthy, Narasimham, and Dinesh 1967). The recommended N and K fertilizers (100%) were added in 75% and 100% doses of recommended P_2O_5 treatments based on the STCR model. Fertilizer N, P, and K were calculated as per targeted yield of the STCR concept (Ramamoorthy, Narasimham, and Dinesh 1967) using following fertilizer adjustment equations:

$$FN = 5.67\ T - 0.17\ SN$$

$$FP_2O_5 = 4.38\ T - 5.26\ SP$$

$$FK_2O = 2.29\ T - 0.10\ SK$$

where FN, FP_2O_5, and FK_2O stand for fertilizer N, P_2O_5, and K_2O in kg ha^{-1} respectively while T is the targeted yield (40 q ha^{-1}). The SN, SP, and SK are the available N, P, and K statuses of soil respectively in their elemental forms in kg ha^{-1}. Fertilizer calculations were made considering the average weight of 1 ha furrow slice (0–0.15 m) soil to be 2.24×10^6 kg (Suri et al. 2006a). The entire P and K doses were applied basally while N was applied in three equal splits (i.e., at basal, knee–high, and tasseling stages). Nitrogen, P, and K were supplied through urea (46% N), single superphosphate (16% P_2O_5), and muriate of potash (60% K_2O), respectively. Maize seeds were sown in pots on 10 June 2003. One maize seedling was raised and maintained until maturity. The crop was harvested on 15 September 2003.

VAM Inoculation

Soil mixed VAM cultures with VAM spores and fungal hyphae were used in the study. In all the three VAM cultures [VAM_L (*Glomus mosseae*), VAM_T (*Glomus intraradices*), and VAM_I (*Glomus mosseae*) cultures], the spore count was 100, 500, and 400 per 250 g air-dried soil, respectively. The VAM cultures were used at 12 kg ha^{-1} (54 mg pot^{-1}). These VAM cultures were used on spore equivalent basis taking VAM_T (*Glomus intraradices*) culture into consideration while using the VAM cultures. Local VAM (*Glomus mosseae*) culture was prepared by the investigating scientists (authors) themselves by raising the target crop (maize) until maturity in pots containing 7 kg sterilized soil + 2 kg FYM and 1 kg mother culture. After harvest, rhizosphere soil of pot as well as root biomass constituted the local VAM (*Glomus mosseae*) culture. Actual inoculation of maize seeds with above VAM cultures was performed by preparing soil slurry of cultures and dipping the seeds into it for half hour followed by shade drying for making seed pallets and then sowing in the pots.

Root Infectivity Studies

After 8–10 weeks of growth at flowering, the plants grown in pots were detached and the rhizosphere soil was carefully removed. The roots were washed thoroughly under running tap water. The cleaned roots were then chopped into small pieces (1 cm) and then subjected to fixation, cleaning, rinsing, and bleaching in potassium hydroxide (KOH) following standard techniques for microscopic observations (Rajapakse and Miller 1992). In case VAM propagules established infection, the infected portion in rhizosphere showed the presence of VAM fructifications. On the basis of root length(s) that were infected and uninfected, the percentage of infection was worked out.

Soil and Plant Chemical Analysis

Soil reaction (pH) was measured in a 1:2.5 soil/water suspension using a glass electrode pH meter (Jackson 1967). Organic C was determined by rapid titration method (Walkley and Black 1934), available N was measured by alkaline permanganate method (Subbiah and Asija 1956), available P was found by Olsen's method (Olsen et al. 1954), and available K was determined by neutral normal ammonium acetate extraction method (AOAC 1970). Plant analysis (grain and stover) was done by the standard procedures of Jackson (1967).

Statistical Analysis

The experimental design was a completely randomized design (CRD), and the statistical analysis was done by the standard procedures suggested by Gomez and Gomez (1984).

Results and Discussion

Root Infectivity and Root Biomass

Maize root system inoculated with various VAM cultures gave greater VAM root infectivity at flowering than their noninoculated counterparts (Table 1). Data further revealed the

Table 1
Effects of co-inoculation of VAM fungi, PSB, and applied P on VAM colonization, root weight, crop productivity, and protein content

Treatments	Root colonization (%)	Root weight (g)	Yield (g/pot) Grain	Yield (g/pot) Stover	Grain protein content (%)
Control	8	3.24	28.07	77.23	8.18
VAM_L alone	12	3.42	31.03	79.87	8.25
VAM_T alone	16	3.56	33.97	83.27	8.37
VAM_I alone	16	3.54	33.23	82.17	8.25
PSB	10	3.52	34.07	84.97	8.31
VAM_L + PSB	16	3.57	33.00	83.07	8.37
VAM_T + PSB	20	3.69	35.10	90.10	8.43
VAM_I + PSB	18	3.60	33.97	88.07	8.37
VAM_L + PSB + 75% P_2O_5 based on STCR model	26	6.79	110.17	269.50	9.25
VAM_T + PSB + 75% P_2O_5 based on STCR model	30	6.98	117.03	292.23	9.37
VAM_I + PSB + 75% P_2O_5 based on STCR model	30	6.88	114.93	285.50	9.31
100% P_2O_5 based on STCR Model	16	7.02	118.00	294.77	9.31
CD ($P = 0.05$)	—	—	3.19	4.68	0.11

superiority of VAM_T (*Glomus intraradices*) and VAM_I (*Glomus mosseae*) cultures over the local VAM (*Glomus mosseae*) culture with respect to root infectivity. With dual inoculation of VAM cultures and PSB, VAM root colonization was further improved, indicating that there exists a synergistic interaction between VAM and PSB. Gryndler et al. (1990) and Singh and Singh (1993) have also reported similar results. Following the root infectivity pattern, root weight at flowering also increased with individual application of biofertilizers (i.e., VAM and PSB) over the control. Co-inoculation of PSB and VAM culture(s) further affected this parameter favorably. Maximum root weight, however, was recorded with sole application of 100% P_2O_5, which was closely followed by the combined application of VAM cultures and PSB along with 75% of the recommended P_2O_5 dose (Table 1). Thus, greater root biomass coupled with extensive VAM hyphal network in the soil–plant continuum can have great bearing on exploration of soil volume by the crop, leading to higher overall nutrient-use efficiency (George et al. 1992).

Crop Productivity

Like root infectivity, different VAM cultures enhanced the maize grain and stover yield over the control with significantly greater yields only in the cases of VAM_T (*Glomus intraradices*) and VAM_I (*Glomus mosseae*) cultures over the control (Table 1). The individual application of PSB also increased grain and stover yield significantly compared to the control. Following the co-inoculation of VAM_T (*Glomus intraradices*) + PSB and VAM_I (*Glomus mosseae*) + PSB; yield increases were 21.0%, 18.4%, and 21.4% with respect to grain and 7.8%, 6.4%, and 10.0% with respect to maize stover yield, respectively. Rathore and Singh (1995) have reported similar results. Beneficial effect of VAM inoculation on maize yield is attributed to greater exploration of soil volume by its hyphal network, leading to greater overall nutrient- and water-use efficiencies (George et al. 1992). The VAM are particularly known to induce many favorable changes in the crop rhizosphere by way of exudation/secretion of organic acids / chelating agents (Rovira 1969). These phenomena might have influenced maize yields favorably. The PSB helped in the solubilization of insoluble soil phosphates, thereby encouraging better acquisition of nutrients and strengthening the root system. Application of any of the 3 VAM fungi along with PSB and 75% of recommended P_2O_5 dose resulted in consistent and significant improvement in grain and straw yield of maize with superiority of VAM_T (*Glomus intraradices*) culture, which remained at par with sole application of 100% P_2O_5 dose with regard to grain and stover yield. This indicates that with the dual inoculation of efficient VAM strain and PSB along with 75% P_2O_5 dose, 25% of the fertilizer P dose can be saved without impairing the crop yield targets in maize in an acidic Alfisol of the northwestern Himalayas (Harrier and Watson 2003).

Grain Protein Content

Overall, grain protein content followed a trend similar to that of grain yield (Table 1). There was a significant increase in grain protein content over the control in the case of sole inoculation of VAM_T (*Glomus intraradices*) culture and PSB. It was notable that the combined application of various VAM cultures and PSB was additionally effective in enhancing maize grain protein content, indicating a synergistic interaction between VAM and PSB biofertilizers (Sreenivasa and Krishnaraj 1992). Results, on the whole, suggested that co-inoculation of VAM and PSB coupled with the 75% P_2O_5 dose generated savings of 25% of the fertilizer P in maize.

Nutrient Content and Uptake

Data presented in Table 2 reveal that nutrient (NPK) contents and their uptake in maize grain and stover were enhanced with the sole application of either VAM cultures or PSB inoculation though respective magnitude remained at par with that of the control. However, combined application of VAM cultures and PSB improved the NPK contents and their uptake in maize grain/stover with significant improvement in the case of VAM_T (*Glomus intraradices*) and VAM_I (*Glomus mosseae*) cultures alongwith PSB. Dual inoculation of either VAM_T (*Glomus intraradices*) or VAM_I (*Glomus mosseae*) cultures along with PSB and 75% of the recommended P_2O_5 did not excede sole application of 100% P_2O_5 in these parameters, but the values tended to be close to each other, which indicates that these biofertilizers have some role in nutrient dynamics in the soil–plant system (Narsian and Patel 2009).

Soil Fertility Status

Co-inoculation of VAM_T (*Glomus intraradices*) or VAM_I (*Glomus mosseae*) cultures along with PSB resulted in significant reductions in available N, P, and K levels in soil after crop harvest in pot experiment (Table 3), mainly because of higher utilization of these

Table 2
Effect of co-inoculation of VAM fungi, PSB, and applied P on nutrient content (NPK) and uptake in maize

	Nutrient content (%)						Nutrient uptake (mg/pot)					
	N		P		K		N		P		K	
Treatments	Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover	Grain	Stover
Control	1.31	0.40	0.13	0.046	0.21	0.40	367.3	308.6	36.3	37.1	58.0	308.5
VAM_L alone	1.32	0.41	0.13	0.050	0.21	0.41	409.6	327.5	41.7	40.0	67.3	327.6
VAM_T alone	1.34	0.42	0.14	0.054	0.23	0.43	455.1	350.1	47.5	45.1	76.7	358.1
VAM_I alone	1.32	0.42	0.14	0.051	0.22	0.42	438.8	345.3	46.7	41.9	73.0	345.1
PSB	1.33	0.42	0.15	0.055	0.23	0.42	452.8	356.9	49.8	47.3	78.3	356.9
VAM_L + PSB	1.34	0.42	0.14	0.056	0.23	0.43	442.2	348.9	46.2	46.5	75.9	357.4
VAM_T + PSB	1.35	0.43	0.16	0.060	0.25	0.44	470.8	390.1	56.5	51.4	89.1	396.2
VAM_I + PSB	1.34	0.43	0.15	0.058	0.24	0.44	455.2	378.7	50.9	51.0	81.5	387.7
VAM_L + PSB + 75% P_2O_5 based on STCR model	1.48	0.51	0.25	0.091	0.33	0.55	1630.6	1373.3	275.4	245.3	363.6	1482.1
VAM_T + PSB + 75% P_2O_5 based on STCR model	1.50	0.53	0.27	0.110	0.35	0.57	1755.6	1548.5	316.1	321.9	409.7	1665.8
VAM_I + PSB + 75% P_2O_5 based on STCR model	1.49	0.53	0.26	0.104	0.34	0.56	1712.5	1513.2	295.0	296.7	390.8	1598.8
100% P_2O_5 based on STCR model	1.49	0.52	0.27	0.118	0.35	0.56	1758.2	1533.0	314.8	347.9	413.2	1641.0
CD ($P = 0.05$)	0.02	0.02	0.02	0.011	0.03	0.03	49.1	52.7	13.6	13.6	22.7	56.6

Table 3
Effect of co-inoculation of VAM fungi, PSB, and applied P on available nutrient status ($mg\ kg^{-1}$ soil) of soil after maize harvest

	Available nutrient status ($mg\ kg^{-1}$ soil)		
Treatments	N	P_2O_5	K_2O
Control	34.8	1.7	114.7
VAM_L alone	33.0	1.7	113.4
VAM_T alone	29.9	1.6	111.2
VAM_I alone	31.2	1.6	112.5
PSB	29.9	1.7	111.2
VAM_L + PSB	29.5	1.6	112.5
VAM_T + PSB	27.7	1.6	109.8
VAM_I + PSB	28.6	1.6	110.3
VAM_L + PSB + 75% P_2O_5 based on STCR model	27.7	1.6	104.9
VAM_T + PSB + 75% P_2O_5 based on STCR model	25.9	1.5	102.2
VAM_I + PSB + 75% P_2O_5 based on STCR model	26.8	1.5	103.1
100% P_2O_5 based on STCR model	25.0	1.5	101.3
CD ($P = 0.05$)	2.2	0.1	1.8
Intial status	49.1	2.2	124.1

nutrients (Table 2) and consequently better crop growth (Table 1). The performance of local VAM culture (*Glomus mosseae*) remained at par with control particularly in terms of soil-available P status. Dual inoculation of biofertilizers further depleted available N, P, and K because of the enhanced exploration of soil reserve nutrients. VAM_T (*Glomus intraradices*) mobilized soil nutrients to the maximum extent followed by VAM_I (*Glomus mosseae*), thereby reducing soil nutrient status significantly over control. This may be ascribed to mobilization of soil N, P, and K from organic and inorganic complexes by the action of VAM fungi due to its mycelial growth or release of organic acids in rhizosphere (Pare, Gregorich, and Nelson 1999). The PSB inoculation also followed the same trend. However, available P in soil under PSB treatment remained at par with the control despite a greater uptake, unlike VAM_T (*Glomus intraradices*) and VAM_I (*Glomus mosseae*) cultures. These facts suggest that PSB inoculation is useful in replenishing the available P status of soil (Suri et al. 2006a). Co-inoculation of PSB with either VAM_T (*Glomus intraradices*) or VAM_I (*Glomus mosseae*) coupled with 75% recommended P_2O_5 dose remained statistically at par with the 100% P_2O_5 dose with respect to soil fertility status. Thus, this study indicates that dual inoculation of PSB and VAM coupled with 75% of the recommended P_2O_5 dose based on targeted yield STCR precision model can bring an economy of about 25% of applied P in maize in a P-deficient acidic Alfisol (Suri et al. 2006).

Conclusions

From this study, it can be inferred that application of VAM fungi alone or with PSB with or without inorganic P fertilization improved the crop productivity, protein content, and nutrient uptake while VAM fungi in combination with PSB as well as 75% of recommended

P_2O_5 dose helped in economizing soil-test and yield-target-oriented P dose using the STCR precision model by about 25% in maize in a P-deficient acidic Alfisol of the northwestern Himalayas. The *Glomus intraradices* culture also showed its superiority over the other two VAM cultures in terms of crop yield, grain quality, and nutrient uptake in maize.

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