

The interaction of rockphosphate, *Bradyrhizobium*, vesicular-arbuscular mycorrhizae and phosphate-solubilizing microbes on soybean grown in a sub-Himalayan mollisol

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Abstract. A factorial design $2^3 \times 4$ with two levels of Mussorie rockphosphate (RP) with or without vesicular-arbuscular mycorrhizal (VAM) fungi and *Bradyrhizobium japonicum*, and four treatments of phosphate-solubilizing microbes (PSM) *Pseudomonas striata*, *Bacillus polymyxa*, *Aspergillus awamori* was employed using Patharchatta sandy loam soil (Typic Hapludoll). The observations included mycorrhization, nodulation, grain and straw yield, N and P uptake, available soil P and the PSM population in the soil after crop harvest. Inoculation with endophytes alone caused about 70% root colonization. Addition of rockphosphate or inoculation with PSM, except *B. polymyxa*, stimulated root infection of native as well as introduced VAM endophytes. Application of RP or inoculation with *Bradyrhizobium japonicum*, mycorrhizal fungi or phosphate-solubilizing microorganisms significantly increased nodulation, N uptake, available soil P and the PSM population in the soil after the crop harvest. The grain and straw yields did not increase following RP addition or mycorrhizal inoculation but increased significantly after inoculation with *Bradyrhizobium* or PSM. In general, the application of RP, *Bradyrhizobium*, VAM and PSM in combinations of any two or three resulted in significant increases in nodulation, plant growth, grain yield and uptake of N and P. Among the four factor interactions, rockphosphate, *Bradyrhizobium* and *P. striata* in the absence of VAM resulted in maximal nodulation, grain and straw yields and N uptake by soybean. The highest P uptake by soybean grain was recorded with *Bradyrhizobium* and *A. awamori* in the absence of rockphosphate and VAM. Generally, available soil P and PSM population after crop harvest were not significantly increased by the treatment combinations giving the maximal uptake of nutrients. However, they increased significantly in response to PSM, which produced no significant increase in total uptake of nutrients.

Key words: Rockphosphate – *Bradyrhizobium* – Vesicular-arbuscular mycorrhizae – Phosphate-solubilizing microbes – Soybean

Introduction

Indian soils are generally low in available phosphorus. Sufficient deposits of low-grade rockphosphate are located in different states of India (Gaur 1985). Research has shown that vesicular-arbuscular mycorrhiza (VAM) in soils of low to moderate fertility (Rathore 1991) and phosphate-solubilizing microorganisms (PSM) (Gaur 1985) separately, VAM and PSM together (Raj et al. 1981), and VAM and *Rhizobium* together (Azcon and Barea 1981) increase the mycorrhization, growth, nodulation and nutrition of legumes and/or nonlegumes under greenhouse and field conditions. We have studied the interacting effects of RP, *Bradyrhizobium* VAM and PSM on soybean (*Glycine max*, L. Merr) cv. 'Bragg' in a mollisol in greenhouse experiments.

Materials and methods

An asymmetric ($2^3 \times 4$) completely randomised design with two levels of Mussorie rockphosphate (O—R₀ and 86 ppm P—R), VAM (uninoculated-M₀ and inoculated-M), *Bradyrhizobium japonicum* (uninoculated-I₀ and inoculated-I) and four treatments with phosphate-solubilizing microbes (uninoculated-S₀, *Pseudomonas striata*-S₁, 2.8×10^6 cells ml⁻¹, *Bacillus polymyxa*-S₂, 1.0×10^5 cells ml⁻¹ and *Aspergillus awamori*-S₃, 3.9×10^6 propagules ml⁻¹ was used in the study. All the 32 treatments were carried out in triplicate. The Patharchatta sandy loam (Typic Hapludoll) surface soil (0–15 cm) was transferred to plastic pots (23 cm × 23 cm) each receiving 4.14 kg soil (weighed oven dry). The soil had 19.7% water holding capacity, 1.12% organic C, 27 ppm available P, 143 ppm organic P, 538 ppm inorganic P, 6.8 C·Mole(p+)kg⁻¹ cation exchange capacity and pH 6.2.

Table 1. Effect of rockphosphate (RP), *Bradyrhizobium*, vesicular-arbuscular mycorrhizal (VAM) fungi and phosphate solubilizing microbes on percent root infection by VAM fungi in soybean

Phosphate-solubilizing microbes	Without Bradyrhizobium (I ₀)		With Bradyrhizobium (I)	
	No VAM (M ₀)	With VAM (M)	No VAM (M ₀)	With VAM (M)
	Without rockphosphate (R ₀)			
Uninoculated (S ₀)	1.6	70.6	2.7	81.3
<i>Pseudomonas striata</i> (S ₁)	2.1	93.5	3.3	97.7
<i>Bacillus polymyxa</i> (S ₂)	0.7	71.4	1.7	70.5
<i>Aspergillus awamori</i> (S ₃)	5.5	83.5	6.5	90.0
	With rockphosphate (R)			
S ₀	5.3	90.5	6.0	90.0
S ₁	25.6	80.4	30.8	92.5
S ₂	18.5	75.2	21.4	79.4
S ₃	29.7	91.8	35.5	94.6

The Mussorie rockphosphate was applied in a uniform layer at a depth of 5 cm in the soil. Potassium (25 ppm) as chemical grade KCl was added in solution. No N was applied. Six seeds of soybean cv. Bragg were sown in each pot. About 0.3 g VAM-infected root segments (\approx 1 cm long with 21% infection) were placed in three depressions in the soil per pot and covered with a small amount of soil to avoid direct contact with seeds. A similar treatment as a mycorrhizal control was set up with sterilized root segments to overcome any effect arising from the decomposition of the plant roots. Broth cultures (1 ml each) of *Bradyrhizobium japonicum* and PSM were placed below seeds as inocula and the seeds covered with a small amount of soil. On germination, the plants were thinned to three per pot. The pots were watered at intervals to maintain moisture almost at field capacity. A 0.2% Endocol 35 EC solution was sprayed to control insects.

At maturity, the number and dry weight of nodules, VAM root colonization, grain and straw yield, N and P uptake, available soil P and PSM population in the soil after soybean were recorded. The micro-Kjeldal method was used for N determination (Jackson 1958), the 0.5 M NaHCO₃ extraction method for available P in soil (Jackson 1958), root clearing and staining method of Phillips and Hayman (1970) for VAM root infection, and the pour-plate dilution method for the PSM population (Pikovskaya 1948).

Results and discussion

VAM root colonization

VAM root infection ranged from 0.7 to 97.7% (Table 1). Without mycorrhizal inoculation, the addition of rockphosphate appreciably increased the percent root infection by native endophytes. In contrast to the *Bacillus polymyxa* (S₂) culture, inoculation with *Pseudomonas striata* (S₁) and *Aspergillus awamori* (S₃) phosphate-solubilizing cultures also stimulated VAM root colonization by native endophytes in the absence of introduced VAM and *Bradyrhizobium*. Inoculation with VAM alone caused 70% root infection and this was further increased in the presence of phosphate solubilizers. The enhancement of mycorrhizal root colonization by the addition of RP and inoculation with phosphate solubilizers may be attributed to the acidic conditions produced by increasing the population of native (due to addition of RP) and introduced phosphate so-

lubilizers. These results confirm the findings of Barea et al. (1974) who reported enhanced mycorrhizal infection in maize by the E₃ spore type of *Endogone* due to a decrease in pH caused by the bacteria.

Application of RP or inoculation with either *Bradyrhizobium japonicum*, mycorrhizal fungi or phosphate-solubilizing microorganisms significantly increased nodulation (Tables 2, 3). The increase in nodulation may be attributed to relatively high amounts of available phosphorus, as suggested by Mosse et al. (1976), who reported increased nodulation and nitrogenase activity with increasing P content in the nodules. It is also evident from the results of this experiment that the maximal nodule size formed by native *Bradyrhizobium* was observed with only the most efficient S₁ culture of PSM. Increasing nodulation due to *Bradyrhizobium* inoculation is well documented (Kumar et al. 1976; Goswami and Pareek 1978; Varela and Munevar 1978).

The number and dry weights of nodules per plant were significantly higher than the control in treatments with RP \times *Bradyrhizobium*, RP \times VAM, *Bradyrhizobium* \times PSM, VAM \times PSM, *Bradyrhizobium* \times VAM and RP \times PSM interactions (Tables 2, 3). Mosse et al. (1976) reported a concentration of phosphorus in the nodules twice that of parental roots and observed that in *Centrosema* legumes the nodules were very large and weighed as much as the roots. This may explain why relatively large amounts of phosphorus are needed for better nodulation. In the present experiment, the significantly higher nodulation is probably also due to increased content of phosphorus in the soil resulting from these interaction. In general, among two and three factors interactions, the maximal number and dry weight per plant were observed with the I \times S₁ and R \times I \times S₁ interactions, respectively. This may be attributed to the higher P uptake (Table 7) by plants due to these interactions, and also suggests that rockphosphate contributed to available phosphorus due to solubilization by an efficient culture of PSM (S₁) culture in the present study. Among four-factor interactions, the R \times I \times M₀ \times S₁ interaction resulted in maximal nodulation. Without *Bradyrhizobium* inoculation,

Table 2. Nodule number per plant in soybean as affected by the application of RP, *Bradyrhizobium*, VAM fungi and PSM. NS, Not significant

Phosphate-solubilizing microbes	I ₀			I			Overall mean (R × S)
	M ₀	M	Mean (R × I × S)	M ₀	M	Mean (R × I × S)	
	R ₀						
S ₀	1.8	7.8	4.8	8.5	22.8	16.6	10.2
S ₁	8.5	9.0	8.8	10.1	17.3	13.7	11.3
S ₂	25.1	4.1	14.6	19.8	35.0	27.4	21.0
S ₃	3.6	3.5	3.6	15.5	13.8	14.7	9.1
Mean (R × I × M)	9.8	6.1	—	13.5	22.2	—	—
	R						
S ₀	4.2	5.2	4.7	21.6	38.5	30.1	17.4
S ₁	3.0	1.9	2.4	40.7	30.3	35.5	19.0
S ₂	2.7	5.5	4.1	19.8	19.6	19.7	11.9
S ₃	6.9	3.5	5.2	11.3	14.3	12.8	9.0
Mean (R × I × M)	4.2	4.0	—	23.4	25.7	—	—
Overall mean (I × M)	7.0	5.1	—	18.5	24.0	—	—
Interaction	R × S	I × M	R × I × S	R × I × M	R × I × M × S		
LSD (5%)	22.5	15.9	31.6	NS	44.6		

Table 3. Dry weight of nodules (mg plant⁻¹) in soybean as affected by the application of RP, *Bradyrhizobium*, VAM fungi and PSM

Phosphate-solubilizing microbes	I ₀			I			Overall mean (R × S)
	M ₀	M	Mean (R × I × S)	M ₀	M	Mean (R × I × S)	
	R ₀						
S ₀	7.5	26.5	17.0	134.0	219.5	176.7	96.9
S ₁	35.0	26.0	30.5	150.0	161.0	155.5	93.0
S ₂	107.0	14.0	60.5	187.0	298.5	242.7	151.6
S ₃	15.0	28.5	21.8	173.5	157.5	166.5	93.6
Mean (R × I × M)	41.1	23.8	—	161.1	209.1	—	—
	R						
S ₀	18.0	26.5	22.3	170.5	258.5	214.5	118.4
S ₁	22.5	20.0	21.3	374.5	368.5	371.5	196.4
S ₂	13.5	20.0	16.8	238.0	283.0	260.5	138.6
S ₃	37.5	12.0	24.8	103.0	226.5	164.7	94.7
Mean (R × I × M)	22.9	19.6	—	221.5	284.1	—	—
Overall mean (I × M)	32.0	21.7	—	191.3	246.6	—	—
Interaction	R × S	I × M	R × I × S	R × I × M	R × I × M × S		
LSD (5%)	22.4	15.9	31.6	NS	44.6		

the R₀ × I₀ × M₀ × S₂ combination produced significantly higher amounts of nodule dry weight compared to the control. This is probably due to maximal phosphate-solubilizing and N-fixing capacity of the S₁ strain and *Bradyrhizobium*, respectively. *Bacillus polymyxa* (S₂) possesses both characteristics, as also reported by Seldin et al. (1983) who observed acetylene reduction by 13 out of 24 strains of *B. polymyxa* in their study on asymbiotic N₂ fixation.

Addition of RP did not significantly increase straw and grain yields. Inoculation with mycorrhiza alone or in most combinations with RP, *Bradyrhizobium* and PSM produced no increases in straw (Table 4). The grain yield also decreased (Table 5). The soil used in

the present experiment contained medium phosphorus. This agrees with the findings of Cooper (1974), who reported that where available phosphorus was not sufficiently limiting for species to be mycotrophic, the mycorrhizal inoculum often depressed growth. This effect was usually associated with a wide range of infection levels (a mean of 0–50%). However, individual plants with 70 to 90% infection were often relatively small (Cooper 1974). In general, inoculation with *Bradyrhizobium* or PSM significantly increased straw and grain yields. Increase in shoot and grain yields due to inoculation with *Rhizobium* or PSM were also reported by Sable and Khuspe (1977) and Sundara Rao (1964), respectively.

Table 4. Effect of RP, *Bradyrhizobium*, VAM fungi and PSM on straw dry weight of soybean (g plant⁻¹)

Phosphate-solubilizing microbes	I ₀			I			Overall mean (R × S)
	M ₀	M	Mean (R × I × S)	M ₀	M	Mean (R × I × S)	
	R ₀						
S ₀	4.43	5.15	4.79	6.78	6.02	6.40	5.60
S ₁	6.25	5.78	6.02	6.58	5.84	6.21	6.12
S ₂	5.90	5.85	5.88	6.13	5.88	6.01	5.94
S ₃	5.93	5.80	5.87	6.02	6.07	6.04	5.96
Mean (R × I × M)	5.63	5.65	—	6.38	5.95	—	—
	R						
S ₀	5.28	5.67	6.45	6.45	6.43	6.44	5.96
S ₁	6.12	5.65	5.89	6.82	5.67	6.24	6.07
S ₂	5.97	5.85	5.91	5.88	5.75	5.82	5.86
S ₃	5.82	5.55	5.69	6.25	5.85	6.05	5.87
Mean (R × I × M)	5.80	5.68	—	6.35	5.93	—	—
Overall mean (I × M)	5.71	5.66	—	6.36	5.94	—	—
Interaction	R × S	I × M	R × I × S	R × I × M	R × I × M × S		
LSD (5%)	0.11	0.08	0.15	NS	0.31		

Table 5. Effect of RP, *Bradyrhizobium*, VAM fungi and PSM on grain yield (g plant⁻¹) of soybean cv. Bragg

Phosphate-solubilizing microbes	I ₀			I			Overall mean (R × S)
	M ₀	M	Mean (R × I × S)	M ₀	M	Mean (R × I × S)	
	R ₀						
S ₀	1.38	1.41	1.39	2.46	2.65	2.55	1.97
S ₁	1.72	2.15	1.93	3.03	2.75	2.89	2.41
S ₂	1.75	1.89	1.81	3.12	3.07	3.09	2.45
S ₃	1.99	2.02	2.00	3.04	3.01	3.02	2.52
Mean (R × I × M)	1.71	1.86	—	2.91	2.87	—	—
	R						
S ₀	1.50	1.54	1.52	3.02	3.16	3.09	2.30
S ₁	1.74	1.82	1.78	3.20	3.21	3.23	2.51
S ₂	1.94	1.88	1.91	2.89	2.93	2.91	2.41
S ₃	1.58	1.51	1.54	2.94	2.72	2.83	2.19
Mean (R × I × M)	1.69	1.69	—	3.02	3.00	—	—
Overall mean (I × M)	1.70	1.78	—	2.97	2.93	—	—
Interaction	R × S	I × M	R × I × S	R × I × M	R × I × M × S		
LSD (5%)	0.06	0.04	0.08	0.06	0.12		

The R × I × M₀ × S₁ interaction resulted in maximum straw (about 54% higher than the control) and grain (about 130% more than the control) yields of soybean (Table 5). This suggests that in addition to native soil phosphates, the S₁ strain of PSM also solubilized applied RP in the soil, resulting in maximal straw and grain yields due to increased P availability to the plant. Some bacteria that hydrolyse insoluble phosphate in vitro (Greaves and Webley 1965), including RP (Azcon et al. 1976), have been reported to improve phosphate uptake and growth of inoculated over uninoculated plants when RP was added to the soil.

Nutrient uptake, available soil phosphorus and the PSM population in the soil

The significant increases in total N uptake by the crop due to RP application and mycorrhizal inoculation (Table 6) may be attributed to increase in the number and dry weight of nodules. The significant decrease in P content in soybean grain is not understood (Table 7). Inoculation with *Bradyrhizobium japonicum* alone resulted in a significant increase in total N uptake (98.88% more than uninoculated controls) and P uptake by grain (about 39% higher than the controls), but significantly reduced the available phosphorus in soil after harvesting of the crop (Table 8). However, the decrease in the PSM population was not statistical-

Table 6. Nitrogen uptake by soybean crop as affected by RP, *Bradyrhizobium*, VAM fungi and PSM (mg pot⁻¹)

Phosphate-solubilizing microbes	I ₀			I			Overall mean (R × S)
	M ₀	M	Mean (R × I × S)	M ₀	M	Mean (R × I × S)	
	R ₀						
S ₀	305	365	335	644	628	636	485
S ₁	421	481	451	849	724	787	619
S ₂	428	455	442	753	815	784	613
S ₃	428	463	445	780	822	801	623
Mean (R × I × M)	395	441	—	756	747	—	—
	R						
S ₀	356	379	367	762	854	808	588
S ₁	385	429	407	1017	947	982	694
S ₂	390	409	399	740	836	788	594
S ₃	396	350	373	811	818	815	594
Mean (R × I × M)	382	391	—	833	864	—	—
Overall mean (I × M)	388	416	—	794	804	—	—
Interaction LSD (5%)	R × S 9.4	I × M 6.7	R × I × S 13.3	R × I × M 9.4	R × I × M × S 18.8		

Table 7. Phosphorus uptake by soybean grain as affected by RP, *Bradyrhizobium*, VAM fungi and PSM (mg/pot)

Phosphate-solubilizing microbes	I ₀			I			Overall mean (R × S)
	M ₀	M	Mean (R × I × S)	M ₀	M	Mean (R × I × S)	
	R ₀						
S ₀	33	38	35	62	59	60	48
S ₁	50	58	54	80	57	69	61
S ₂	46	49	47	78	62	70	59
S ₃	52	55	53	84	63	73	63
Mean (R × I × M)	45	50	—	76	60	—	—
	R						
S ₀	39	44	41	63	62	62	52
S ₁	47	51	49	68	65	67	58
S ₂	52	51	52	67	61	64	58
S ₃	44	41	43	55	55	55	49
Mean (R × I × M)	46	47	—	64	61	—	—
Overall mean (I × M)	45	48	—	70	60	—	—
Interaction LSD (5%)	R × S 1.8	I × M 1.2	R × I × S 2.5	R × I × M 1.8	R × I × M × S 3.5		

ly significant (Table 9). Increase in dinitrogen fixation (Sundara Rao 1971; Vincent 1974) and phosphorus uptake by the grain (Jones et al. 1977; Singh and Saxena 1977) with effective rhizobial strains have been reported. The decrease in available phosphorus in the soil after soybean cropping is obviously the result of greater uptake by a more vigorously growing crop.

Each of the cultures of PSM significantly increased uptake of total N, grain P, available soil P and the PSM population after the crop harvest. Among phosphate solubilizers, maximal uptake of N and P, and the PSM population in the soil after harvesting of soybean crop were observed after *Pseudomonas striata* (S₁) inoculation. However, the maximal increase in available soil P was observed with the *Aspergillus awamori* (S₃) culture

followed by the S₁ culture. Because of the maximal enhancement in root infection by native VAM, the greatest benefit would be obtained by inoculation with the S₁ culture.

In general, application of RP, *Bradyrhizobium*, VAM and PSM in combinations of any two or three resulted in greater uptake of N and grain P. Among two-and three-factor interactions, the maximal uptake of N and P were recorded with the I × S₁ and I × M₀ × S₁ interactions. Either in the absence or in the presence of *Bradyrhizobium* with S₁ or S₃ cultures, mycorrhizal inoculation decreased N uptake, but this increased with the S₂ culture. Without *Bradyrhizobium*, the R₀ × I₀ × M₀ × S₂ combination alone gave a significant increase in N uptake because of the dinitrogen-

Table 8. Available phosphorus (ppm) in soil after soybean cropping as affected by RP, *Bradyrhizobium*, VAM fungi and PSM

Phosphate-solubilizing microbes	I ₀			I			Overall mean (R × S)
	M ₀	M	Mean (R × I × S)	M ₀	M	Mean (R × I × S)	
	R ₀						
S ₀	11.0	16.4	13.7	14.2	15.9	15.1	14.4
S ₁	19.4	20.6	20.0	16.4	14.7	15.5	17.8
S ₂	18.4	16.2	17.3	13.2	15.0	14.1	15.7
S ₃	19.2	28.0	23.6	16.9	15.9	16.4	20.0
Mean (R × I × M)	17.0	20.3	—	15.2	15.4	—	—
	R						
S ₀	15.9	17.9	16.9	13.2	13.9	13.5	15.2
S ₁	16.4	14.2	15.3	18.9	14.4	16.7	16.0
S ₂	14.4	14.4	14.4	17.9	13.2	15.5	15.0
S ₃	16.9	13.3	15.1	19.4	13.4	16.4	15.8
Mean (R × I × M)	15.9	15.0	—	17.3	13.7	—	—
Overall mean (I × M)	16.5	17.7	—	16.3	14.6	—	—
Interaction	R × S	I × M	R × I × S	R × I × M	R × I × M × S		
LSD (5%)	1.1	0.8	1.5	NS	2.2		

Table 9. Population of PSM ($\times 10^5$ cells g⁻¹) in soil after soybean cropping as affected by RP, *Bradyrhizobium*, VAM fungi and PSM

Phosphate-solubilizing microbes	I ₀			I			Overall mean (R × S)
	M ₀	M	Mean (R × I × S)	M ₀	M	Mean (R × I × S)	
	R ₀						
S ₀	0.3	5.9	3.1	7.7	15.1	11.4	7.3
S ₁	21.7	14.8	18.2	11.7	10.6	11.1	14.7
S ₂	9.5	14.5	12.0	10.1	14.4	12.2	12.1
S ₃	18.0	15.6	16.8	15.0	9.8	12.4	14.6
Mean (R × I × M)	12.4	12.7	—	11.1	12.5	—	—
	R						
S ₀	3.1	15.9	9.5	7.5	12.2	9.8	9.7
S ₁	23.3	11.8	17.5	10.6	17.3	14.0	15.8
S ₂	16.7	11.3	14.0	3.4	14.4	8.9	11.4
S ₃	11.5	11.0	11.3	13.4	18.4	15.9	13.6
Mean (R × I × M)	13.6	12.5	—	8.7	15.6	—	—
Overall mean (I × M)	13.0	12.6	—	9.9	14.1	—	—
Interaction	R × S	I × M	R × I × S	R × I × M	R × I × M × S		
LSD (5%)	NS	1.6	3.2	2.3	4.6		

fixing characteristics of *Bacillus polymyxa* (S₂) (Seldin et al. 1983).

Among four-factor interactions, the maximal N uptake (233.44% more than the control) and grain yield (136.23% more than control) resulted from the R × I × M₀ × S₁ interaction, while that maximal P uptake by soybean grain was due to the R₀ × I × M₀ × S₃ interaction followed by the R₀ × I × M₀ × S₁ interaction. As would be expected, the available soil P and PSM population in general were not significantly increased by treatment that gave the maximal uptake of nutrients. However, they increased significantly with interactions of PSM that resulted no significant increases in total uptake of nutrients. The maximal increase in available soil P due to these interactions may be be-

cause of lower P uptake by the crop due to the limiting supply of N and higher P-solubilizing capacity of the PSM.

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