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Effects of inoculation of phosphate-solubilizing microorganisms and an arbuscular mycorrhizal fungus on mungbean grown under natural soil conditions

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Abstract The effect of inoculation of the phosphate-solubilizing microorganisms (PSM) *Bacillus circulans* and *Cladosporium herbarum* and the arbuscular mycorrhizal (AM) fungus *Glomus fasciculatum* with or without Mussoorie rockphosphate (MRP) was studied in a P-deficient natural non-disinfected sandy soil on mungbean (*Vigna radiata*). The AM levels increased following the addition of MRP or inoculation with PSM or *G. fasciculatum.* Both grain and straw yield of mungbean increased following inoculation with PSM or the AM fungus. In general, the increase in yield was higher in the presence of MRP and inoculation with a combination of PSM and AM fungus. Highest N and P uptake by mungbean was recorded after treatment with a combination of *B. circulans, C. herbarum* and *G. fasciculatum* in the presence of MRP. Generally the PSM population increased after AM fungus inoculation.

Key words Rockphosphate · Phosphate-solubilizing microorganisms · Arbuscular mycorrhizae · Mungbean

Introduction

Much of the inorganic P applied to soil as fertilizer is rapidly converted to unavailable forms with low solubility (Sanyal and De Datta 1991). Soluble P is released from insoluble phosphates by a variety of solubilization reactions involving rhizosphere microorganisms (Kapoor et al. 1989; Kucey et al. 1989). Mycorrhizal plants can take up more P than non-mycorrhizal plants, mainly from the same soluble P pool (Barea 1991). Inoculation with phosphate-solubilizing microorganisms (PSM) may help to solubilize native soil P as well as P from rockphosphates. The soluble P released by the activity of PSM is actively taken up by mycorrhizal roots (Barea et al. 1983; Kucey et al. 1989). Coinoculation of PSM and arbuscular mycorrhizas (AM) may enhance plant acquisition of P from insoluble P sources (Azcon et al. 1976; Azcon-Aguilar et al. 1986; Piccini and Azcon 1987). Most studies involving inoculation of PSM and AM fungi have been conducted in sterile soils where competition from native microflora is eliminated (Kucey 1987; Piccini and Azcon 1987; Toro et al. 1996). However, a few studies carried out with non-disinfected soils under pot conditions and involving coinoculation of PSM and AM fungi demonstrated an increase in dry matter production and P uptake (Singh and Singh 1993; Tilak et al. 1995). During the present investigation, we evaluated the effect of PSM and AM with and without Mussoorie rockphosphate (MRP) on the mycorrhization, dry matter production, and N and P uptake of mungbean under pot conditions in a natural unsterile sandy P-deficient soil. This was done to assess the potential of PSM and AM fungi for increasing availability of P from rockphosphate in alkaline soils.

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Material and methods

To study the interaction of P-solubilizing microorganisms *Bacillus circulans* and *Cladosporium herbarum,* and the AM fungus *Glomus fasciculatum* (Thaxter sensu Gerd) (Gerd & Trappe) a greenhouse experiment was carried out in a randomized block design. There were eight treatments with five replications each.

Soil

The soil used in the study was taken from Haryana Agricultural University dryland farm, Hisar and had clay 5.6%, silt 4.0%, sand 90.4%, 0.10% organic C, 2.5 ppm $NaHCO_3$-extractable P, 0.001% total P, 4.9 c mole (p^+) kg^{-1} cation exchange capacity and pH 7.6.

The AM inoculum

The AM endophyte *G. fasciculatum* (Thaxter sensu Gerd) (Gerd & Trappe) was obtained from Dr. D. J. Bagyaraj, University of

Agricultural Sciences, Bangalore, India was found to perform well with mungbean (*Vigna radiata* L. Wilczek cv. Asha). The inoculum consisted of chopped root segments and soil from a 10-week-old pot culture of *G. fasciculatum* grown on pearl millet (*Pennisetum typhoides*) in a sterile sandy soil.

Phosphate-solubilizing microorganisms

The P-solubilizing bacterium *B. circulans* and fungus *C. herbarum* were earlier isolated from soil and tested for phosphate solubilization under in vitro conditions using tricalcium phosphate and MRP as P sources. Inoculum was prepared in Pikovskaya medium (Pikovskaya 1948) at 30 °C for 5 days using tricalcium phosphate as a P source. Inoculation was carried out by dipping the mungbean seeds in cells/spore suspension for 10 min containing about 10^8 colony-forming units per ml.

Rockphosphate

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

Plant growth conditions

Four kg sieved (2 mm) soil was placed into earthenware pots. MRP was added to each pot in a uniform layer at a depth of 5 cm so as to supply 40 kg P_2O_5 ha^{-1}. A basal dose of nitrogen was applied as ammonium sulphate at 20 kg N ha^{-1} in the upper 5 cm prior to sowing. The mycorrhizal inoculum (5 g pot^{-1}) was added at a depth of 5 cm. Uniform inoculation with *Rhizobium* sp. *Vigna* S32 strain was obtained by putting 1 ml of culture (about 10^8 cells) over the seed hole. Ten seeds were sown at a 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 and the crop grown during June to August 1995. The mean ambient temperature during this period was 31.8 °C with a mean minimum of 26.3 and mean maximum of 37.3 °C. The mean bright sunshine was 6.2 hours and relative humidity 72%. Plants were watered daily to maintain moisture at field capacity and harvested at maturity after a growth period of 11 weeks for grain and straw yield.

Measurements

At harvest, the dry matter yield was determined after drying the grain and straw for 20 h at 70 °C. The N and P uptake were recorded. The microkjeldahl method was used for N determination (Bremner 1960) and total P was determined by the method of John (1970). The root clearing and staining method of Phillips and Hayman (1970) was used for studying root colonization by AM. 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 scored.

The pour-plate technique was applied for estimation of PSM populations in the rhizosphere soil (Pikovskaya 1948). The rhizosphere soil was collected by uprooting the plants and soil adhering to roots was serially diluted; 0.1 ml aliquots of appropriate dilution were spread on Pikovskaya medium plates. The plates were incubated at 30 °C for 1 week and colonies showing a clear zone of tricalcium phosphate dissolution were counted.

Table 1 Effects of the P-solubilizing microorganisms *Bacillus circulans* and *Cladosporium herbarum* alone or in combination with *Glomus fasciculatum* on root colonization of mungbean with and without Mussoorie rockphosphate (*MRP*)

Microbial treatment	Root colonization (%)	
	Without MRP	With MRP
Control	6.0	21.6
Bacillus circulans	17.0	29.3
Cladosporium herbarum	22.0	27.5
Bacillus circulans + *Cladosporium herbarum*	27.0	34.7
Glomus fasciculatum	66.6	68.2
Bacillus circulans + *Glomus fasciculatum*	73.3	79.8
Cladosporium herbarum + *Glomus fasciculatum*	72.3	82.0
Bacillus circulans + *Cladosporium herbarum* + *Glomus fasciculatum*	73.2	88.2
LSD (5%)	4.2	4.0

Results and discussion

AM root colonization

The AM root colonization at harvest ranged from 6.0 to 88.2% (Table 1) and it was poor without AM inoculation. Improvement of AM colonization was observed with *B. circulans* or *C. herbarum* either singly or in combination. Inoculation with *G. fasciculatum* alone gave 66.6% root colonization and this was further improved in the presence of PSM. In general, root colonization by the AM fungus in the presence of MRP was higher. The significant increase in the degree of mycorrhizal colonization by PSM inoculation alone may be due to phytohormone production by these microorganisms, which in turn may stimulate mycorrhizal colonization. These results confirm the findings of Azcon et al. (1978), who reported enhanced mycorrhizal development with *Lavandula, Lycopersicum* and *Medicago* plants due to phytohormones in the culture filtrate of P-solubilizing bacterium *Pseudomonas*. The enhancement of mycorrhizal root colonization by addition of MRP and inoculation with PSM may be attributed to effects on root morphology or on the physiology of the fungal symbionts, or could be due to the creation of more acidic conditions by an increasing population of native as well as the introduced PSM. Similar increases in root colonization by AM fungi in presence of rockphosphate have been observed previously (Barea and Azcon-Aguilar 1982; Piccini and Azcon 1987; Singh and Singh 1993).

Dry matter production

Both grain and straw yield of mungbean increased significantly after inoculation with PSM, particularly after

Table 2 Effects of the P-solubilizing microorganisms *Bacillus circulans* and *Cladosporium herbarum* alone or in combination with *Glomus fasciculatum* on dry matter yield (g pot^{-1}) in mungbean with and without Mussoorie rockphosphate (*MRP*)

Microbial treatment	Without MRP			With MRP		
	Grain	Straw	Total	Grain	Straw	Total
Control	0.630	1.702	2.332	0.642	1.768	2.410
Bacillus circulans	0.742	2.446	3.188	0.786	2.703	3.489
Cladosporium herbarum	0.765	2.904	3.694	0.795	3.218	4.013
Bacillus circulans + *Cladosporium herbarum*	0.809	3.520	4.329	0.824	3.405	4.229
Glomus fasciculatum	0.648	2.161	2.809	0.780	2.605	3.385
Bacillus circulans + *Glomus fasciculatum*	0.792	2.607	3.393	0.937	3.507	4.434
Cladosporium herbarum + *Glomus fasciculatum*	0.809	2.950	3.739	0.955	3.631	4.586
Bacillus circulans + *Cladosporium herbarum* + *Glomus fasciculatum*	0.868	3.800	4.668	0.966	4.282	6.664
LSD (5%)	0.048	0.260	0.291	0.122	0.028	0.029

Table 3 Effects of the P-solubilizing microorganisms *Bacillus circulans* and *Cladosporium herbarum* alone or in combination with *Glomus fasciculatum* on P uptake (mg pot^{-1}) and P content (% dry wt.) of mungbean with and without Mussoorie rockphosphate (*MRP*)

Microbial treatment	Without MRP P uptake			P content	With MRP P uptake			P content
	Grain	Straw	Total		Grain	Straw	Total	
Control	0.051	0.084	0.135	0.006	0.066	0.210	0.276	0.011
Bacillus circulans	0.066	0.147	0.213	0.007	0.074	0.302	0.375	0.011
Cladosporium herbarum	0.074	0.162	0.236	0.009	0.090	0.310	0.400	0.010
Bacillus circulans + *Cladosporium herbarum*	0.100	0.218	0.378	0.009	0.112	0.412	0.522	0.012
Glomus fasciculatum	0.059	0.186	0.245	0.009	0.110	0.350	0.460	0.014
Bacillus circulans + *Glomus fasciculatum*	0.069	0.276	0.345	0.010	0.136	0.382	0.517	0.012
Cladosporium herbarum + *Glomus fasciculatum*	0.079	0.286	0.365	0.010	0.133	0.462	0.595	0.013
Bacillus circulans + *Cladosporium herbarum* + *Glomus fasciculatum*	0.106	0.375	0.491	0.010	0.195	0.518	0.713	0.011
LSD (5%)	0.048	0.089	0.078	0.001	0.044	0.068	0.072	0.001

combined inoculation (Table 2). Total plant biomass increased in the presence of MRP except with the combined inoculation. The effectivity of PSM on grain production was similar both in the presence and absence of MRP but straw production was higher (207%) without MRP than with MRP (192%). Thus addition of MRP and microbial inoculation affected partioning of biomass between grain and straw (Azcon-Aguilar et al. 1986). There was a synergistic effect of triple inoculation with *B. circulans, C. herbarum* and *G. fasciculatum.* Both grain and straw yield were higher when *G. fasciculatum* and PSM were inoculated in combination than in treatments with only PSM or *G. fasciculatum.* The increase in dry matter may be attributed to better P utilization from soil as well as from rockphosphate in the presence of these organisms (Raj et al. 1981; Piccini and Azcon 1987).

Nutrient uptake and the population of PSM in the soil

The concentration of P in plant biomass was higher in the presence of MRP (Table 3). Inoculation with PSM or the AM fungus significantly improved the concentration of P in the plant biomass in absence of rockphosphate. However, in the presence of rockphosphate, P concentration increased only in treatments receiving the AM fungus or the AM fungus in combination with *C. herbarum.* This may be due to better utilization of P from the pool of native soil P as well as from the MRP by the action of PSM and *G. fasciculatum.*

Significant increases in total N and P uptake due to inoculation of PSM and *G. fasciculatum* were observed (Tables 3, 4) in the crop at maturity. Inoculation with *B. circulans* or *C. herbarum* alone or with *G. fasciculatum* enhanced P uptake into both grain and straw, as compared with uninoculated controls. P uptake was higher when the soil was amended with MRP. The higher P uptake in plants inoculated with PSM and the AM fungus in presence of MRP may be attributed to greater absorption of P by AM (Barea 1991). Many studies have shown the synergistic effect of coinoculation of PSM and AM fungi on growth and nutrition of plants. Azcon et al. (1976) using lavender as test crop in a steam-sterilized soil observed higher P uptake with mycorrhiza plus P-solubilizing bacteria than plants with either mycorrhiza or bacteria separately. In a greenhouse experiment on wheat and beans, better utilization of P from rockphosphate was observed in the presence of an AM fungus and a P-solubilizing *Penicillium*

Table 4 Effects of the P solubilizing microorganisms *Bacillus circulans* and *Cladosporium herbarum* alone or in combination with *Glomus fasciculatum* on N uptake (mg pot^{-1}) of mungbean with and without Mussoorie rockphosphate (*MRP*)

Microbial treatment	Without MRP			With MRP		
	Grain	Straw	Total	Grain	Straw	Total
Control	0.890	0.336	1.226	1.168	1.689	2.857
Bacillus circulans	1.142	0.859	2.001	1.305	2.102	3.317
Cladosporium herbarum	1.216	1.139	2.355	1.415	2.170	3.586
Bacillus circulans + *Cladosporium herbarum*	1.340	1.576	2.916	1.640	2.479	4.119
Glomus fasciculatum	1.135	1.525	2.660	1.484	1.955	3.439
Bacillus circulans + *Glomus fasciculatum*	1.293	1.929	3.222	1.560	2.345	3.905
Cladosporium herbarum + *Glomus fasciculatum*	1.370	2.026	3.396	1.695	2.485	4.180
Bacillus circulans + *Cladosporium herbarum* + *Glomus fasciculatum*	1.586	2.416	4.002	2.220	2.908	5.128
LSD (5%)	0.108	0.168	0.208	0.210	0.215	0.672

Table 5 Effect of the P-solubilizing microorganisms *Bacillus circulans* and *Cladosporium herbarum* alone or in combination with *Glomus fasciculatum* on the population of P-solubilizing microorganisms (g^{-1} soil) in the rhizosphere soil of mungbean with and without Mussoorie rocksphosphate (*MRP*)

Microbial treatment	Without MRP		With MRP	
	P-solubilizing bacteria	P-solubilizing fungi	P-solubilizing bacteria	P-solubilizing fungi
Control	3.3×10^4	2.0×10^2	3.8×10^4	2.3×10^2
Bacillus circulans	5.0×10^6	3.7×10^2	19.2×10^6	4.3×10^2
Cladosporium herbarum	2.7×10^4	5.0×10^3	5.1×10^4	16.2×10^3
Bacillus circulans + *Cladosporium herbarum*	8.8×10^6	6.3×10^3	21.2×10^6	19.0×10^3
Glomus fasciculatum	3.0×10^4	1.3×10^2	2.6×10^4	3.4×10^2
Bacillus circulans + *Glomus fasciculatum*	15.0×10^6	5.3×10^2	29.8×10^6	6.3×10^2
Cladosporium herbarum + *Glomus fasciculatum*	3.8×10^4	11.5×10^3	8.8×10^4	28.0×10^3
Bacillus circulans + *Cladosporium herbarum* + *Glomus fasciculatum*	17.8×10^6	15.3×10^3	42.3×10^6	36.3×10^3

bilaji isolate (Kucey 1987). Similar positive interactions of a calcium phosphate-solubilizing isolate of *Bacillus* sp. and *G. mossae* on the growth of *Pueraria phaseoloides* and nutrient uptake have also been observed (Toro et al. 1996). On the other hand, Azcon-Aguilar et al. (1986) reported no increase in the utilization of P fertilizer by soybean after inoculation with AM, *Rhizobium japonicum* and P-solubilizing bacteria in a gamma-irradiated soil amended with tricalcium phosphate. However, there was an increase in shoot N concentration.

There was no significant effect of PSM or AM alone or in combination on nodulation of mungbean, either in the presence or absence of MRP (the number of nodules varied from 21 to 30 per pot). This is in contrast to earlier studies where AM inoculation was shown to enhance nodulation and nitrogen fixation of legumes by rhizobia (Manjunath and Bagyaraj 1986; Barea et al. 1988). The higher N uptake by mungbean due to inoculation with PSM and *G. fasciculatum* may be due to better P nutrition of mungbean, which subsequently improved utilization of soil N. The significant increase in total N uptake by the crop due to MRP application and inoculation with PSM along with the AM fungus could also have been due to AM hyphae translocating soil N to the plant. Johansen et al. (1994) observed increased translocation of soil N to cucumber plants inoculated with *G. intraradices* when ^{15}N-labelled NH_4NO_3 was added to soil, indicating hyphal transport of inorganic N.

There was no change in the population of P-solubilizing bacteria in the rhizosphere of mungbean after inoculation of the fungi (Table 5). Addition of MRP stimulated the population of both PSM in the rhizosphere of mungbean. A larger population of PSM was maintained in the presence of *G. fasciculatum.* These effects may be attributed to higher metabolic activities of PSM for longer periods in the rhizosphere after MRP amendment and AM inoculation (Singh and Singh 1993).

The results of the present study show that yield of mungbean can be improved by a combination of an AM fungal symbiont and saprophytic PSM in a non-disinfected soll. This double inoculation helps the plants to obtain P from insoluble P sources such as rockphosphate and also leads to better N nutrition of the legume. The potential of using this dual inoculation to enhance utilization of P from rockphosphate in a neutral soil is important, since P availability from rockphosphate is low in such a soil.

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