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Efficacy of native phosphate-solubilizing rhizobia on symbiotic parameters and grain yield in fieldpea (*Pisum sativum*)

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Fieldpea (*Pisum sativum* L.) is a pulse crop of *rabi* season, being a legume it can fix atmospheric nitrogen and helps in restoring soil fertility. It requires more phosphorus (P) as compared to cereals, but most of the soils are deficient in available form of P due to its precipitation with ions such as calcium and iron (Marra *et al.* 2011). To circumvent P-availability phosphate solubilizing bacteria (PSB) could play an important role in supplying P to plants in more eco-friendly and sustainable manner. The availability of soil P to plants is largely influenced by activity of soil microorganisms through their ability to solubilize and mineralize inorganic and organic soil P. The solubilization of phosphates by naturally abundant PSB is common under *in vitro* conditions, however, their performance *in situ* has to be ascertained. Among the PSB, *Rhizobium* is of particular interest because of its dual function, i e its ability to fix N_2 and to solubilize P (Kundu *et al*. 2009). Keeping this in view the present study was undertaken to isolate efficient P-solubilizing (PS) rhizobia and to assess their efficacy under field conditions.

Rhizobia were isolated from nodules of pea plants from ten locations in Punjab and screened for (PS) potential on Pikovskaya's medium. Rhizobia showing clear halo on medium were screened in Pikovskaya's broth and soluble P was assessed every third day up to 15 days of incubation (Jackson 1973). The final *p*H of the filtrate was also recorded.

Field experiments were conducted during *rabi* season (2007–09) using fieldpea variety FP 48 at research farms of Punjab Agricultural University, Ludhiana, (30° 56'N, 72° 52'E, and altitude 247 m) having sandy loam soil (*p*H 8.2). The soil had organic carbon (0.03%), available N (105 kg/ha), available P (16.8 kg/ha) and K (291 kg/ha). Eight treatments consisted of P-solubilizing rhizobia (R_1 and R_2), reference culture R (non P-solubilizer) and P amendments (0 and 40 kg/ha). The treatments were laid out in randomized block in triplicate, plot size 6.75 m^2 (row to row 30 cm). The seed (25 kg/acre) was treated with inoculants (10^8 cells/g carrier) and line sown. A basal dose of urea @ 26 kg/acre and P (single superphosphate) as per treatments was given and crop raised following the recommended agronomic practices. Observations on symbiotic and plant growth parameters, viz. nodule number and dry weight (NDW) 60 and 90 days after sowing (DAS), root (RDW) and shoot (SDW) dry weight, chlorophyll content (90 DAS) (Witham *et al.* 1971), N by micro-Kjeldahl method and P content (Tandon 1998) and grain yield at harvest was recorded, data subjected to analysis of variance using CPCS1.

P-solubilizing rhizobial (20) isolates varied in their PS efficiency as per the size of the halo on Pikovskaya's medium. Five of these evaluated for their relative PS ability using tricalcium phosphate (TCP) in liquid medium, solubilized higher amounts of TCP over control, highest being recorded with isolate R_2 (21.8 mg/100 ml) followed by R_1 (15.1 mg/100 ml) at 15 days incubation, thereafter it became stable. Turan *et al.* (2006) have also reported similar decline in PS activity and attributed this to depletion of nutrients in the medium. All the five rhizobial isolates showed a drop in *p*H of medium indicating production of certain acids, the *p*H drift was more in R_2 (4.9), followed by R_1 (5.1) as compared to control (6.7). Thus an inverse correlation between the amount of soluble-P and drop in *p*H due to PSB was observed in this study (Table 1). Marra *et al.* (2011) also reported a decline in *p*H of the medium.

Inoculation with *Rhizobium* increased symbiotic parameters (Table 2). Isolate R_2 outperformed amongst the three rhizobial cultures. *Rhizobium* R_1 and R_2 showed higher number of nodules as compared to R, however significantly higher nodulation was observed in R_1 and R_2 amended with P_2O_5 @ 40 kg/ha as compared to R_1 and R_2 alone. Similar results have also been reported by Zaeri *et al.* (2006). Similar

* Short note

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Table 1 Bacterial solubilization of tricalcium phosphate as a function of time

Isolates	P-solubilized (mg/100 ml medium)					Final *p*H of medium
	Incubation period (days)					
	3	6	9	12	15	
Control	0.3	0.48	0.7	0.85	0.85	6.7
FP-R_1	6.5	7.8	8.95	11.25	15.1	4.9
FP-R_2	7.05	8.78	12.05	16.93	21.85	5.1
FP-R_3	4.3	6.89	9.01	10.85	11.0	5.5
FP-R_4	0.86	0.95	3.9	5.68	7.06	5.8
FP-R_5	0.77	0.86	2.85	4.6	6.08	6.2

Table 2 Effect of *Rhizobium* and P application on symbiotic, plant growth parameters and grain yield in field pea

Treatments	Number of nodules		Dry weight of nodules mg/plant		Dry weight of shoot g/plant 90 DAS	Dry weight of root mg/plant 90 DAS	Chlorophyll content (mg/g fresh wt of leaves) 90 DAS	N grains (%)	P grains (%)	Grain yield (kg/ha)
	60 DAS	90 DAS	60 DAS	90 DAS						
Control	14.5	19.8	26.9	36.7	2.735	291	2.06	3.90	0.085	1514
Rhizobium (R)	18.7	26.6	34.2	45.4	2.870	324	2.35	3.95	0.098	1663
R_1	20.9	28.3	38.1	47.9	2.965	318	2.64	3.98	0.111	1696
R_2	21.8	29.7	43.1	50.6	2.970	323	2.85	4.06	0.132	1708
40 kg P_2O_5/ha	18.5	27.8	35.4	47.3	3.350	321	2.25	4.19	0.106	1756
R + 40 kg P_2O_5/ha	20.9	31.0	41.0	56.2	3.500	358	2.64	4.44	0.088	1754
R_1 + 40 kg P_2O_5/ha	25.3	34.7	46.4	61.7	3.730	372	2.96	4.43	0.173	1789
R_2 +40 kg P_2O_5/ha	26.5	36.4	53.0	66.0	3.785	370	2.98	4.52	0.186	1810
CD (*P*=0.05)	3.6	4.3	6.3	5.9	NS	NS	0.058	0.10	0.009	61

trend was recorded with respect to NDW, although all the treatments significantly enhanced NDW, however, R_1 and R_2 amended with P_2O_5 significantly enhanced NDW (23% and 30%) over R_1 and R_2 alone.

Biomass yield is an important measure of plant vigour and health. Increase in SDW and RDW was observed with all the three rhizobial inoculants, maximum being with R_2 + P_2O_5 (3.78 g/plant and 370 mg/plant, respectively). Rhizobial inoculants have been reported to induce increased number of root hair and root laterals thereby favouring higher nutrient uptake. The PS ability of local isolates R_1 and R_2 may have increased the P-availability which could explain the increase in plant biomass which in legumes is reported to be enhanced with *Rhizobium* inoculation (Singh *et al.* 2005). It is known that chlorophyll content is significantly affected by adequate nutrient supply and biofertilizers and is reported to be positively associated with yield of legumes. All the rhizobial isolates enhanced chlorophyll content (Table 2), however, R_1 (2.64 mg/g fresh wt of leaves) and R_2 (2.85) showed significant increase as compared to R (2.35). Chlorophyll content was further enhanced with R_2 + P_2O_5 (2.98 mg/g fresh wt of leaves). Similarly P and N content increased significantly with R_2 (0.132 and 4.06, respectively) as compared to reference R (0.098 and 3.95, respectively). Highest P and N content were recorded in R_2 + P_2O_5 (0.186 and 4.52%, respectively). This could be attributed to the ability of R_2 to solubilize P thus facilitating better uptake by the plant. The grain yield also showed significant increase on inoculation, highest being with R_2 (1 708 kg/ha) as compared to control (1 514 kg/ha). The grain yield was enhanced with P fertilization @ 40 kg/ha however, maximum was recorded with R_2 + P_2O_5 (1 810 kg/ha). The availability of sufficient nutrients in soil for plant uptake greatly determines the yield of legume crops (Marra *et al*. 2011). Thus the present findings suggest the use of native P- solubilizing rhizobia for enhancing P-use efficiency and thus yield potential of field pea.

SUMMARY

The native P-solubilizing rhizobial isolates R_1 and R_2 enhanced nodule number and dry weight, root and shoot dry weight, chlorophyll, N and P content and grain yield as compared to non P- solubilizing rhizobia. Native rhizobia R_1 and R_2 along with P @ 40 kg P_2O_5/ha were found to augment the efficiency of applied P which enhanced symbiotic parameters and significantly improved yields of fieldpea.

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