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Response of cowpea [*Vigna unguiculata* (L.) Walp.] to phosphate solubilizing bacteria isolated from rhizosphere

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A pot culture experiment was carried out to assess the impact of novel phosphate solubilizing bacteria on yield parameters, nodulation and nutrient uptake of cowpea [*Vigna unguiculata* (L.) Walp.]. The experiment consists of nine treatments. Among them plants receiving phosphate solubilizing bacteria identified as *Pseudomonas* sp. and *Acetobacter* sp. amended with or without rock phosphate was superior over other treatments in all respects and were comparable with the treatment receiving *Pseudomonas striata*.

Key words : Phosphate solubilizing Bacteria, *Vigna unguiculata*,Rhizosphere.

INTRODUCTION

Phosphorus is one of the most important plant nutrients, which is required in optimum amount for proper growth of plants and soil microorganisms. About 98% of Indian soils have inadequate supply of available phosphorus (Ghosh and Hassan, 1979). Various number of soil bacteria possess mineral phosphate solubilizing activity (Mikanova and Kubat, 1994). In addition, microorganism involved in P solubilization as well as better scavenging of soluble P can enhance plant growth by increasing the efficiency of biological nitrogen fixation, enhancing the availability of other trace elements such as Fe, Zn etc. and by production of plant growth promoting substances (Kucey *et al.*, 1989). Many plants have shown to benefit from the association with microorganisms under phosphorus deficient conditions. Taking these points into account an investigation was carried out to evaluate the effects of local phosphate solubilizing bacteria on the yield parameters, nodulation and nutrient uptake of cowpea.

MATERIALS AND METHODS

Twenty seven phosphate solubilizing bacterial isolates were obtained from rhizosphere soil of different crops *viz.* coconut, vanilla, rubber, banana, paddy, ladies finger, pea and pumpkin. All the bacterial isolates were subjected to *in vitro* tricalcium phosphate solubilization. Zone of clearance produced in Pikovskaya's agar was also taken as criteria for selection. Based on these two isolates (*Acetobacter* sp. and *Pseudomonas* sp.) and a standard culture (*Pseudomonas striata*) obtained from IARI, New Delhi was used for further study.

Experiments with cowpea as test crop was conducted in loamy soil obtained from Kottayam district to evaluate the effect of inoculation of selected strains of phosphate solubilizing bacteria on yield and uptake of nutrients by cowpea. The soil collected from the top 15 cm layer was air dried, passed through 2 mm sieve and filled in polythene bags of 20´15 cm size. The soil is loamy with pH 6.1, electrical conductivity $0.1x10^{-3}$ mhos/cm, organic carbon level 1.3%, available phosphorus 8.8 kg/ha and available potassium content 11 kg/ha. Fertilizers at the rate of Urea 32.5 kg/ha, Muriate of potash 21.375 kg/ha, Farm yard manure 20 t/ha and Lime 250 kg/ha were weighed separately for each pot and added to the soil as basal dose before sowing.

The seeds were mixed with *Rhizobium japonicum* culture and rice water for two hours and then dried in shade and used for sowing. 2 ml of the 3 days old cultures of the phosphate solubilizing bacteria (OD 1) were poured over the seeds. Super phosphate and rock phosphate were added to the respective pots after one week to allow the bacterial cultures to establish themselves in the soil. When the plants were established the total number of plants in each pot was thinned to three. The plants were watered regularly to maintain the optimum soil moisture regime. Plant protection measures were taken up whenever necessary. A booster inoculation of cultures was given (2 ml suspension of 3 days old culture) in the rhizosphere of each plant after 30 days. Observations were taken at

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regular intervals. The crops were harvested at 90 days after sowing. The pods from each plant were cut to their base and sun dried. The seeds were separated by hand threshing.

Dry matter yield, number of seeds, dry weight of seeds, number of pods and dry weight of pods were checked. The plants were uprooted from the pots and were washed with water jet to remove the soil adhering to roots. The roots were washed in a tray taking care not to loose a single nodule during washing. The nodules were separated from the roots with the help of forceps. Total number of nodules and its weight was recorded treatment wise. Dry weight of nodules was recorded after drying in an oven to constant weight.

The available P level of soil was estimated by Olsen's method (Jackson, 1973). The population of PSB was enumerated by serial dilution method (10^{-5}) by plating in Pikovskaya's medium.

Sample of straw and seeds of all the treatment were dried and powdered separately for the purpose of chemical analysis. For the estimation of nitrogen the samples were digested by Kjeldahl digestion method and the total nitrogen present in the samples were determined colorimetrically using Technicon Nitrogen Autoanalyser (Tecator Kjeltec 1030 Auto Analyser Sweden). In case of total phosphorous estimation, 1g of the powdered plant was digested with 15 ml of triacid mixture (con. HNO_3: con. H_2SO_4: 60% $HClO_4$ in 10:1:4 ratio) on a hot plate and the volume of the digested material was made upto 100 ml with distilled water. Phosphorus was estimated by taking a suitable aliquot filtrate by the Vanadomolybdate phosphoric yellow colour method (Jackson, 1973).

Statistical Analysis was performed by Analysis of Variance (Two way and One way ANOVA). As post test the means were compared by Tukey multiple comparison test.

RESULTS AND DISCUSSION

Different yield parameters of all the treatments were recorded (Table 1). Maximum dry matter yield was shown by the treatments inoculated with *Pseudomonas* sp.+RP. Number of seeds, dry weight of seeds and dry weight of pods were also highest to *Pseudomonas* sp.+RP immediately followed by the *Pseudomonas* striata+RP. Number of pods was same for *Pseudomonas* sp.+RP and *Pseudomonas striata* +RP. Increased dry matter yield of leguminous crops was reported Khalafallah *et al.* (1982) who reported significant increase in dry matter of cowpea, gram and *Vicia faba*, respectively due to PSB inoculation. The findings are in conformity with findings of Gaur (1990).

At the time of harvest number and weight of nodules of different treatments were noted (Table 2). Maximum nodule number and weight were shown by the treatments inoculated with *Acetobacter* sp. Nodule number was very low in case of the uninoculated control. Number and weight of nodules of *Acetobacter* sp. treated soil was 97.66 and 2.03 g, respectively whereas in the case of uninoculated control it was 11.66 and 0.22 g. Phosphate solubilizing bacteria stimulates the multiplication of rhizobia and was found conducive for the development of mobile forms which are essentially required to migrate through the soil towards the root system (Madhok, 1961). The supply of phosphorus to host plants also influences

Table 1 :Effect of PSB inoculation on different yield parameters of cow pea.

No.	Treatments	Dry matter Yield (g.)	No. of seeds	Dry weight of seeds (g.)	No. of pods	Dry weight of pods (g.)
T_1	Control	3.60	5.00	0.032	2.00	0.116
T_2	RP	4.66	5.00	0.034	2.33	0.121
T_3	SP	4.66	5.00	0.034	2.33	0.093
T_4	Acetobacter sp.	21.16	6.66	0.057	4.66	0.173
T_5	Pseudomonas sp.	30.66	8.33	0.061	6.33	0.200
T_6	Pseudomonas striata	31.66	7.66	0.600	6.00	0.186
T_7	Acetobacter sp. + RP	21.68	6.66	0.058	6.33	0.220
T_8	Pseudomonas sp. + RP	34.50	8.66	0.069	8.33	0.230
T_9	Pseudomonas striata + RP	32.60	8.56	0.068	8.33	0.266
	Mean	20.24	6.83	0.048	5.18	0.178
	CD (5%)	1.55	1.028	0.045	1.191	0.196

Table 2 : Effect of PSB inoculation on nodulationand available P level of cowpea growing soil (90 days).

No.	Treatments	Nodule number	Nodule weight (g)	Available P (Kg/ha)	PSB count ($x10^5$)
T_1	Control	11.66	0.22	10.23	5.66
T_2	RP	25.66	0.53	10.60	11.66
T_3	SP	13.33	0.33	18.32	11.00
T_4	Acetobacter sp.	97.66	2.03	14.06	16.66
T_5	Pseudomonas sp.	88.33	1.83	16.04	12.00
T_6	Pseudomonas striata	49.33	1.56	17.62	10.66
T_7	Acetobacter sp. + RP	161.66	2.43	15.67	18.00
T_8	Pseudomonas sp. + RP	106.00	2.10	19.79	17.66
T_9	Pseudomonas striata + RP	89.33	1.80	19.58	12.00
	Mean	71.44	1.42	15.76	12.81
	CD (5%)	3.43	0.445	1.08	1.80

Table 3 : Nutrient uptake by cowpea as influenced by PSB inoculation.

No.	Treatments	Straw		Seeds	
		P_2O_5 uptake (mg/pot)	N uptake (mg/pot)	P_2O_5 uptake (mg/pot)	N uptake (mg/pot)
T_1	Control	4.07	36.16	0.02	0.802
T_2	RP	5.13	49.80	0.06	1.267
T_3	SP	8.29	56.00	0.06	1.630
T_4	Acetobacter sp.	10.22	97.63	0.31	8.032
T_5	Pseudomonas sp.	20.87	338.95	0.50	8.634
T_6	Pseudomonas striata	21.63	313.92	0.58	9.125
T_7	Acetobacter sp. + RP	15.76	105.58	0.50	5.859
T_8	Pseudomonas sp. + RP	31.20	521.32	0.80	12.306
T_9	Pseudomonas striata + RP	27.03	345.17	0.62	11.645
	Mean	16.02	207.17	0.38	6.58
	CD (5%)	0.490	1.295	0.133	0.060

nodulation (Schreven, 1958). Available P level and PSB count were high for treatments receiving PSB+RP (Table 2). Maximum available P level was shown by the soil treated with *Pseudomonas* sp. and RP (19.79 Kg/ha) where as maximum PSB count was shown by soil that received *Acetobacter* sp. and RP ($18x10^5$). Peterson (1958) and Rovira (1965) reported that the host plant and its growth stages are important in determining the rhizosphere microflora. Jones and Sreenivasa (1993) also reported significantly higher count of P-solubilizers in the rhizosphere of sunflower, inoculated with *P. striata*. The results on increased available P content of soil due to inoculation were reported by Salih *et al.* (1989).

The data on nutrient uptake by straw and seeds at harvest is presented in Table 3. All the inoculation treatments were found to be superior over control. In the case of straw samples highest phosphorus and nitrogen uptake was shown by *Pseudomonas* sp. +RP (31.20 and 521.32 mg/pot, respectively). Similarly in the case of seed samples also highest phosphorus and nitrogen uptake was shown by *Pseudomonas* sp.+RP (0.80 and 12.306 mg/ pot respectively). The phosphorus uptake of Bengal gram was more due to PSB inoculation (Gaur and Gaind, 1992). Yadav *et al.* (1986) reported increased nitrogen uptake with increased level of phosphatic fertilizers. Here the results indicate that phosphate solubilizing bacteria have a positive effect on the nutrient uptake of cowpea.

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