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Effect of Phosphate Solubilizing Bacteria on Nodulation and Growth Parameters of Greengram (*Vigna radiata* L. Wilczek)

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Abstract: Phosphorus is one of important macronutrients and plays an important role in metabolism of crop plants. In vertisols the availability of P is limited due to the problem of P fixation. Phosphate solubilizing microorganisms have the capability to solubilize P and make it available for plant uptake. In the present study ability of 16 isolates of Phosphate Solubilizing Bacteria (PSB) to promote growth parameters in greengram crop was tested under greenhouse conditions. The study consisted of 18 treatments which were replicated three times. Inoculation of greengram seeds with PSBV-14 recorded the highest nodule number, nodule dry weight, shoot dry matter and total dry matter in greengram plants 45 days after sowing. Similarly, treatment receiving the inoculation of PSBV-13 recorded the highest root length, root dry matter, P content and P uptake in root and shoot in greengram plants. Majority of PSB isolates tested in the present study were able to improve the growth parameters of greengram significantly compared to rock phosphate control and single super phosphate control. Among the various PSB isolates tested, PSBV-4, PSBV-9, PSBV-12, PSBV-13, PSBV-14 and PSBV-15 fared considerably better than the remaining ones. The highly efficient PSBs from the pot trial could be tested for their efficacy in field conditions before recommending them for commercial exploitation.

Keywords: Phosphate solubilizing bacteria, phosphorus uptake, plant growth promoting substances, vertisols, P fixation

INTRODUCTION

Phosphorus is one of the major plant nutrients required in optimum amounts for proper plant growth. About 98% of Indian soils have inadequate supply of available phosphorus (Hasan, 1996; Thiyageshwari and Selvi, 2006). In many soils although phosphate is available in aplenty, application of phosphatic fertilizers is a must to make up for the phosphorus lost due to fixation of soluble phosphate by soil constituents and phosphate runoff in P-loaded soil (Goldstein, 1986; Del Campillo *et al*., 1999). Phosphate fertilizers with available P_2O_5 when added to the soil get fixed in the soil and are unavailable for plant growth. The role of microorganisms in solubilizing inorganic phosphates in soil and making them available to plants is well known (Pal, 1998; Hilda and Fraga, 1999; Bhattacharya and Jain, 2000). They are called phosphate solubilizers and they convert the insoluble phosphates into soluble forms by acidification, chelation, exchange reactions and production of gluconic acid (Rashid *et al*., 2004; Rodriguez *et al*., 2004; Chung *et al*., 2005). Potential use of phosphate solubilizing microorganisms as inoculants with rock phosphates to increase phosphorus availability to plants have been studied intensively (Laheurte and Berthelin, 1988; Subba Rao, 1993).

The use of Phosphate Solubilizing Bacteria (PSB) as inoculants for crop plants has received considerable attention over the years (Gaind and Gaur, 1991; Defreitas *et al*., 1997; Dey *et al*., 2004;

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Chakraborty *et al*., 2006; Pandey *et al*., 2006). Inoculation of PSB has resulted in improving growth, yield and phosphorus uptake in several crops (Tomar, 1998; Zaida *et al*., 2003; Khalid *et al*., 2004; Hameeda *et al*., 2006a). It is believed that production of plant growth promoting substances by PSB may contribute to their stimulatory effect on plant growth (Sattar and Gaur, 1987; Lal, 2002; Hameeda *et al*., 2006b).

Although vertisols contain appreciable amounts of phosphorus it is not released for the crops in adequate amounts mainly due to its insoluble nature. Majority of the mineral phosphorus present in vertisols are in the form of poorly soluble calcium mineral phosphates (Ae *et al*., 1991). There is no proper response to applied phosphatic fertilizers even at higher doses in vertisols due to the problem of P fixation. Phosphate solubilizers developed elsewhere have not been very consistent in their performance everywhere owing to their poor adaptability to the changing soil and agroclimatic conditions (Alagawadi *et al*., 1992). Due to these problems there is an urgent need for development of locality specific strains that can be used in vertisols. The objective of the present study was to evaluate the effect of various PSB strains on nodulation, growth and phosphorus uptake of greengram plants under greenhouse conditions.

MATERIALS AND METHODS

Soil Type, Seeds and Fertilizer

Medium black clay soil collected from 0-15 cm depth of E block, plot number 125 of Main Research Station (MRS), University of Agricultural Sciences, Dharwad was used in the experiment. The soil was then mixed thoroughly, sieved and filled in earthen pots of 30 cm diameter and 30 cm in height at the rate of 18 kg pot^{-1}. The required quantity of farm yard manure (90 g pot^{-1}) was weighed separately for each pot and incorporated into the soil. The soil used in the study had a pH of 7.5, organic carbon (0.40%), available nitrogen (170 kg ha^{-1}), available phosphorus (30 kg ha^{-1}) and available potassium (290 kg ha^{-1}). The population of bacteria, fungi, actinomycetes and phosphate solubilizers in the soil were 62×10^6, 17×10^3, 8×10^3 and 12×10^3 cfu g^{-1} soil. Greengram (*Vigna radiata* (L.) Wilczek) seeds of variety China mung obtained from Main Research Station, University of Agricultural Sciences, Dharwad were used in the pot culture experiment. The recommended dose of fertilizer for greengram (25:50 kg NP ha^{-1}) was applied. Nitrogen in the form of urea and phosphorus in the form of single superphosphate or Mussoorie rockphosphate were applied in calculated quantities as basal dose at the time of sowing as per the treatment schedule. The treatments of the experiment were Single Super Phosphate (SSP) control (no inoculation with SSP as P source), Mussoorie Rock Phosphate (MRP) control (no inoculation with MRP as P source) and PSB strains PSBV-1 to PSBV-16 with recommended dose of P in the form of MRP. The study was based on a completely randomized design having 18 treatments which were replicated three times.

Seed Inoculation and Sowing

Healthy and bold seeds of greengram were sown in pots at 10 seeds pot^{-1} to which one ml inoculum $seed^{-1}$ was added as per the treatment schedule. After germination two seedlings were maintained in each pot. The pots were watered regularly and the plants were allowed to grow up to 45 days. After 45 Days After Sowing (DAS), greengram plants were uprooted gently and growth parameters like height, root length, plant dry matter content, number of leaves, number and dry weight of nodules, phosphorus content (Jackson, 1973), phosphorus uptake and available phosphorus content in soil (Jackson, 1973) were recorded. The population of all P solubilizing bacterial cultures at the time of sowing was to the range of 10^8 cfu mL^{-1}. The PSB strains used in the study were PSBV-1 (*Xanthomonas* sp.), PSBV-2 (*Bacillus* sp.), PSBV-3 (*Pseudomonas* sp.), PSBV-4 (*Xanthomonas* sp.), PSBV-5 (*Pseudomonas* sp.), PSBV-6 (*Xanthomonas* sp.), PSBV-7 (*Xanthomonas* sp.), PSBV-8

(*Pseudomonas* sp.), PSBV-9 (*Pseudomonas* sp.), PSBV-10 (*Pseudomonas* sp.), PSBV-11 (*Bacillus* sp.), PSBV-12 (*Serratia* sp.), PSBV-13 (*Serratia* sp.), PSBV-14 (*Serratia* sp.), PSBV-15 (*Bacillus* sp.) and PSBV-16 (*Enterobacter* sp.). The PSB strains obtained from the culture collection of Department of Agricultural Microbiology, UAS, Dharwad were maintained on tricalcium phosphate agar medium. The pot culture experiment was conducted under greenhouse conditions in the Department of Agricultural Microbiology, UAS, Dharwad.

Number and Dry Weight of Nodules per Plant

The number of root nodules per plant at 45 DAS was recorded by carefully uprooting the plants, followed by dipping the roots in water to remove soil without losing the nodules. The number of root nodules on the two plant roots was counted and their average number was expressed as number of nodules per plant. The root nodules collected from the plants were dried in an oven (Scientific Engineering Corporation, New Delhi, India) at 70°C to constant weight and the average weights were expressed as mg plant^{-1}.

Dry Matter Content

The root and shoot portions of greengram plants were separated and air-dried. They were then oven dried at 70°C to constant weight in a hot air oven (Scientific Engineering Corporation, New Delhi, India). The shoot and root dry weights were recorded separately and the average weight of two plants was expressed in g plant^{-1}.

Phosphorus Content in Plant Samples

The oven-dried samples were ground to fine powder and then used for estimation of phosphorus content. The phosphorus content in shoot and root samples was estimated separately by following the standard Vanadomolybdate phosphoric yellow color method of Jackson (1973). Five hundred mg of root or shoot sample was taken in a 250 mL conical flask to which 2.5 mL of concentrated HNO_3 was added. The flask was swirled to moisten the entire sample. It was then placed on a hot sand bath for 30 min and later on an electric hot plate (Scientific Engineering Corporation, New Delhi, India) at 180 to 200°C. The suspension was boiled until it became completely dry.

Wet Oxidation

Five ml of tri acid mixture (concentrated HNO_3, concentrated H_2SO_4 and $HClO_4$ in the ratio of 10:1:4) was added to the pre digested sample and further digestion was carried out at 180 to 200°C on a digestion mantle until the residue in the flask became clear white. The contents of the flask were cooled, added with 10-15 mL of 6 N HCl and stirred well. The acid digest was transferred to 50 mL volumetric flask and the volume was made up to 50 mL with distilled water. From this wet oxidized digested sample, P was estimated by Vanadomolybdate phosphoric yellow color method (Jackson, 1973). Ten milliliter of wet oxidized digested sample was taken in a 50 mL volumetric flask, to which 10 mL of vanadomolybdate reagent was added. The volume was made up to 50 mL with distilled water and allowed to react for 30 min. The yellow color developed was read at 490 nm using spectrophotometer (UV-VIS Spectrophotometer SL 150, Elico Ltd, Hyderabad, India). The P content in the samples was obtained by the standard curve. To obtain a standard curve, 0.439 g of KH_2PO_4 was dissolved in distilled water and the volume was made up to 1000 mL in a volumetric flask. Aliquots of 1 to 10 mL were transferred to a series of 50 mL volumetric flasks and the 10 mL vanadomolybdate reagent was added to each flask including blank. The volume was made up to 50 mL with distilled water. The yellow color developed was read after 10 min in a spectrophotometer at 490 nm. The standard curve was obtained by plotting a graph with concentration along X axis and corresponding absorbance along Y axis.

Statistical Analysis

Statistical analysis of the data obtained in the present study was carried out by assigning the treatments according to a completely randomized design with three replicates. Sampling and measurements of various response variables were carried out at 45 days after sowing (DAS). The means of different treatments were compared using LSD (least significant difference) at $p = 0.05$. The Hierarchical Cluster Analysis (HCA) of all response variables was performed using cluster procedure of SAS (1999) to calculate the Euclidean distance between group centers, as a measure of similarity of groups. This distance was used to construct a similarity measure matrix and a dendrogram to better visualize the PSB isolate grouping leading to the identification and classification of efficient isolates of PSB with similar pattern of effects on response variables.

RESULTS

Hierarchical Cluster Analysis (HCA)

A hierarchical cluster analysis of PSB isolates and the controls based on 14 different response variables representing as measures of growth, nodulation, phosphorous content and uptake of greengram plants and available P content in soil classified them into three distinctive main clusters including all PSB isolates, RP (Rock phosphate) and SSP (Single super phosphate) control treatments (Fig. 1). RP and SSP controls were distinctively separated from all the other treatments. Therefore, the RP and SSP controls formed one cluster namely "the control group". All the other PSB isolates were clustered as follows: G1= PSBV-1+RP and PSBV-3+RP; G2=PSBV-2+RP and PSBV-10+RP; G3=PSBV-4+RP, PSBV-6+RP, PSBV-8+RP and PSBV-11+RP; G4=PSBV-5+RP, PSBV-7+RP, PSBV-9+RP and PSBV-15+RP; G5=PSBV-12+RP and PSBV-13+RP; G6=PSBV-14+RP and PSBV-16+RP (Fig. 1).

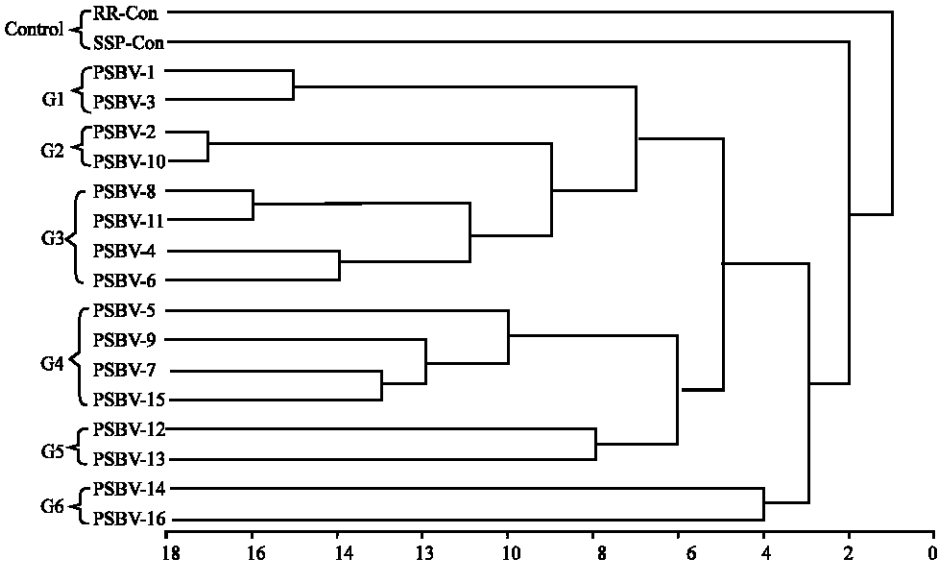

Fig. 1: Classification of efficient PSB isolates based on their impact on Nodulation (measured as Number of nodules plant^{-1} and Nodule dry weight (mg plant^{-1}), growth (Number of leaves plant^{-1}), Shoot and Root length (cm), Shoot, Root and Total dry matter (g plant^{-1}) of greengram plants at 45 days after sowing and phosphorous content in shoot and root (in percentage), P uptake in root, shoot and total (mg plant^{-1}) and available P content of soil (mg kg^{-1}). SSP-Con=Single super phosphate control, RP-Con = Rock phosphate control, PSBV-1 to PSBV-16 = PSB isolates + Rock phosphate

Effect of PSB Isolates on Nodulation

All the PSB isolates showed significant increase in nodule number over RP control (Table 1). Isolates of PSB belonging to PSBV-12, PSBV-13, PSBV-14, PSBV-15 and PSBV-16 showed significant increase in nodule number over SSP control while rest of them were on par with SSP control. Maximum number of nodules (18 plant^{-1}) was recorded in plants inoculated with PSBV-14 closely followed by PSBV-16 (17 plant^{-1}) both of which were significantly superior over all other treatments (Table 1). A similar trend was observed with respect to nodule dry weight per plant. All the treatments differed in a significant manner over RP control with respect to dry weight of nodule per plant. PSBV-1, PSBV-5, PSBV-7, PSBV-9, PSBV-12, PSBV-13, PSBV-14, PSBV-15 and PSBV-16 recorded nodule dry weights that were higher in a significant manner than SSP control. Highest nodule dry weight was recorded from PSBV-14 (54.2 g) which was significantly better than rest of the treatments (Table 1).

The maximum number of leaves per plant at 45 DAS was observed in greengram plants inoculated with PSBV-5, PSBV-12, PSBV-13, PSBV-14 and PSBV-15 all of which gave 22 leaves (Table 1). Although none of the PSB strains differed significantly from RP and SSP control, most of them recorded more number of leaves than those controls. Also, no significant differences existed between the strains with respect to the number of leaves.

Shoot and Root Growth

The maximum shoot length of greengram at 45 DAS was recorded in the treatment receiving inoculation of PSBV-12 (61.3 cm) followed by PSBV-14 (61.2 cm), PSBV-15 and PSBV-7 (both with 61.0 cm), PSBV-5, PSBV-8 and PSBV-13 (all 60.3 cm), PSBV-4 (60.0 cm), PSBV-9 (59.3 cm) and PSBV-11 (58.6 cm) which differed significantly compared to SSP control and RP control (Table 1).

The greengram plants inoculated with PSBV-13 recorded the maximum root length (34.8 cm) followed by PSBV-5 (34.3 cm), PSBV-9 (34.0 cm) and PSBV-15 (33.5 cm) which were significantly superior over all other strains and SSP control. Most of the PSB strains were able to improve the root and shoot growth of greengram plants compared to SSP control and RP control.

Dry Matter Accumulation

PSB strains belonging to PSBV-5, PSBV-7, PSBV-8, PSBV-9, PSBV-10, PSBV-12, PSBV-13, PSBV-14 and PSBV-15 significantly increased the root, shoot and total dry matter compared to both SSP control and RP control (Table 2). The highest root dry matter was recorded by PSBV-5, PSBV-9

Table 1: Effect of different isolates of PSB on nodulation and growth parameters of greengram plants at 45 DAS

Treatments[1]	Nodule No. plant^{-1}	Nodule dry weight (mg plant^{-1})	No. of leaves plant^{-1}	Root length (cm)	Shoot length (cm)
SSP Control	10.00^{de2}	30.10^{h}	21^{a}	28.50^{i}	52.00^{f}
RP Control	8.00^{e}	23.80^{i}	19^{a}	27.00^{i}	49.00^{g}
PSBV-1	11.00^{cd}	33.00^{g}	21^{a}	31.00^{de}	56.00^{e}
PSBV-2	10.00^{de}	30.10^{h}	20^{a}	29.00^{hi}	52.00^{f}
PSBV-3	10.00^{de}	30.30^{h}	20^{a}	30.30^{f}	56.50^{e}
PSBV-4	10.00^{de}	30.40^{h}	21^{a}	30.60^{ef}	60.00^{ab}
PSBV-5	11.00^{cd}	33.00^{g}	22^{a}	34.30^{ab}	60.30^{ab}
PSBV-6	10.00^{de}	30.30^{h}	21^{a}	29.60^{gh}	57.30^{de}
PSBV-7	12.00^{bcd}	36.10^{f}	21^{a}	31.00^{de}	61.00^{a}
PSBV-8	10.00^{de}	30.00^{h}	21^{a}	30.00^{fg}	60.30^{ab}
PSBV-9	12.00^{bcd}	36.30^{f}	20^{a}	34.00^{bc}	59.30^{bc}
PSBV-10	10.00^{de}	30.20^{h}	20^{a}	29.00^{hi}	53.00^{f}
PSBV-11	10.00^{de}	29.80^{h}	20^{a}	29.40^{gh}	58.60^{cd}
PSBV-12	14.00^{b}	42.40^{d}	22^{a}	29.60^{gh}	61.30^{a}
PSBV-13	14.00^{b}	43.30^{c}	22^{a}	34.80^{a}	60.30^{ab}
PSBV-14	18.00^{a}	54.20^{a}	22^{a}	29.50^{gh}	61.20^{a}
PSBV-15	13.00^{bc}	39.10^{e}	22^{a}	33.50^{c}	61.00^{a}
PSBV-16	17.00^{a}	51.30^{b}	20^{a}	31.30^{d}	53.00^{f}
LSD at 0.05	2.45	0.79	3	0.68	1.32

[1]Each treatment was replicated three times; [2]Within each column, means followed by same letter(s) are not significantly different from each other at p = 0.05; DAS: days after sowing; PSB: Phosphate Solubilizing Bacteria

Table 2: Effect of different isolates of PSB on dry matter accumulation and phosphorus content of greengram plants at 45 DAS

Treatments[1]	Root dry matter (g plant^{-1})	Shoot dry matter (g plant^{-1})	Total dry matter (g plant^{-1})	P content in root (%)	P content in shoot (%)
SSP Control	0.41^{ef2}	5.48^{g}	5.89^{i}	0.592^{f}	0.739^{ij}
RP Control	0.37^{f}	5.09^{h}	5.46^{j}	0.437^{h}	0.634^{k}
PSBV-1	0.41^{ef}	5.60^{g}	6.01^{h}	0.605^{e}	0.746^{hi}
PSBV-2	0.41^{ef}	5.87^{f}	6.28^{g}	0.586^{f}	0.789^{e}
PSBV-3	0.42^{e}	5.50^{g}	5.92^{hi}	0.634^{e}	0.755^{g}
PSBV-4	0.42^{e}	6.02^{def}	6.44^{ef}	0.585^{fg}	0.761^{fg}
PSBV-5	0.59^{a}	6.18^{bcd}	6.76^{b}	0.619^{d}	0.768^{ef}
PSBV-6	0.42^{e}	5.96^{ef}	6.37^{fg}	0.606^{e}	0.746^{hi}
PSBV-7	0.49^{cd}	6.10^{de}	6.50^{de}	0.620^{d}	0.761^{fg}
PSBV-8	0.51^{c}	6.02^{def}	6.53^{de}	0.648^{ab}	0.773^{de}
PSBV-9	0.59^{a}	6.36^{ab}	6.95^{a}	0.620^{d}	0.732^{j}
PSBV-10	0.53^{bc}	6.14^{cde}	6.67^{bc}	0.642^{bc}	0.754^{gh}
PSBV-11	0.45^{de}	6.06^{def}	6.51^{de}	0.606^{e}	0.793^{bc}
PSBV-12	0.53^{bc}	6.17^{bcde}	6.70^{bc}	0.642^{bc}	0.779^{d}
PSBV-13	0.59^{a}	6.35^{abc}	6.94^{a}	0.656^{a}	0.821^{a}
PSBV-14	0.57^{ab}	6.41^{a}	6.97^{a}	0.648^{ab}	0.798^{b}
PSBV-15	0.58^{a}	6.02^{def}	6.60^{cd}	0.634^{e}	0.776^{de}
PSBV-16	0.43^{e}	5.99^{def}	6.42^{ef}	0.577^{g}	0.741^{i}
LSD at 0.05	0.04	0.22	0.11	0.008	0.008

[1]Each treatment was replicated three times; [2]Within each column, means followed by same letter(s) are not significantly different from each other at p = 0.05; DAS: Days After Sowing; PSB: Phosphate Solubilizing Bacteria

and PSBV-13 (all of which recorded 0.59 g) while highest shoot and total dry matter was recorded by PSBV-14 (6.41 and 6.97 g, respectively) (Table 2). Most strains of PSB were able to improve the dry matter accumulation in greengram plants in comparison with SSP control and RP control.

Phosphorus Content in Shoot and Root

Among different treatments, the treatment involving inoculation of PSBV-13 recorded the highest P content in shoot (0.821%) followed by PSBV-14 (0.798%), PSBV-11 (0.793%), PSBV-2 (0.789%), PSBV-12 (0.779%), PSBV-8 (0.773%), PSBV-5 (0.768%), PSBV-4 (0.761%), PSBV-7 (0.761%), PSBV-3 (0.755%) and PSBV-10 (0.754%) (Table 2). All these treatments recorded P contents in shoot which were significantly superior to SSP control and RP control. PSBV-13 increased shoot P content in a significant manner compared to other PSB strains. Highest P content in root was recorded by PSBV-13 (0.656%) followed by PSBV-8 (0.648%), PSBV-14 (0.648%), PSBV-10 (0.642%), PSBV-12 (0.642%), PSBV-3 (0.634%), PSBV-15 (0.634%), PSBV-7 (0.620%), PSBV-9 (0.620%), PSBV-5 (0.619%), PSBV-6 (0.606%), PSBV-11 (0.606%) and PSBV-1 (0.605%) (Table 2). All PSB strains were significantly better than SSP control and RP control with regard to root P content of greengram plants.

Phosphorus Uptake

Almost all PSB strains except PSBV-1 (2.48 mg), PSBV-2 (2.40 mg), PSBV-4 (2.47 mg) and PSBV-16 (2.48 mg) recorded significantly higher P uptake in root than SSP control (2.43 mg) while all the strains were superior to RP control (1.62 mg) with respect to root P uptake (Table 3). Among all the treatments tested, PSBV-13 recorded the highest root (3.91 mg), shoot (52.13 mg) and total P uptake (56.04 mg) in greengram plants at 45 DAS (Table 3). All strains of PSB tested in this study resulted in significantly higher shoot P uptake and total P uptake compared to RP control and SSP control.

Available P Content in Soil

The inoculation of PSB strains significantly increased the available P content in soil over RP control (6.13 mg kg^{-1}) (Table 3). Highest available P content in soil was recorded from PSBV-4

Table 3: Effect of different isolates of PSB on phosphorus uptake in greengram plants and available P content in soil at 45 DAS

Treatments[1]	Root P uptake (mg plant^{-1})	Shoot P uptake (mg plant^{-1})	Total P uptake (mg plant^{-1})	Available P (mg kg^{-1})
SSP Control	2.43^{hi}	40.49^{l}	33.89^{o}	8.78^{d}
RP Control	1.62^{j}	32.27^{m}	42.92^{n}	6.13^{h}
PSBV-1	2.48^{gh}	41.78^{j}	44.26^{m}	10.61^{b}
PSBV-2	2.40^{i}	46.31^{g}	48.71^{j}	9.62^{c}
PSBV-3	2.68^{f}	41.53^{k}	44.21^{m}	9.37^{c}
PSBV-4	2.47^{ghi}	45.81^{j}	48.28^{k}	11.59^{a}
PSBV-5	3.63^{b}	47.46^{d}	51.09^{d}	8.91^{d}
PSBV-6	2.52^{g}	44.43^{i}	46.98^{l}	10.41^{b}
PSBV-7	3.03^{e}	46.42^{fg}	49.45^{i}	8.82^{de}
PSBV-8	3.30^{d}	46.65^{e}	49.95^{g}	8.40^{g}
PSBV-9	3.66^{b}	46.55^{ef}	50.21^{f}	11.44^{a}
PSBV-10	3.40^{c}	46.29^{g}	49.69^{h}	8.76^{def}
PSBV-11	2.73^{f}	48.06^{c}	50.79^{e}	8.69^{defg}
PSBV-12	3.40^{c}	48.06^{c}	51.46^{c}	8.46^{fg}
PSBV-13	3.91^{a}	52.13^{a}	56.04^{a}	9.36^{c}
PSBV-14	3.62^{b}	51.09^{b}	54.79^{b}	8.67^{defg}
PSBV-15	3.66^{b}	46.66^{e}	50.35^{f}	8.52^{efg}
PSBV-16	2.48^{gh}	44.39^{i}	46.87^{l}	10.54^{b}
LSD at 0.05	0.08	0.16	0.16	0.30

[1]Each treatment was replicated three times; [2]Within each column, means followed by same letter(s) are not significantly different from each other at p = 0 05;00S: days after sowing; PSB: Phosphate Solubilizing Bacteria

(11.59 mg kg^{-1}) followed by PSBV-9 (11.44 mg kg^{-1}), PSBV-1 (10.61 mg kg^{-1}), PSBV-16 (10.54 mg kg^{-1}), PSBV-6 (10.41 mg kg^{-1}), PSBV-2 (9.62 mg kg^{-1}), PSBV-3 (9.37 mg kg^{-1}), PSBV-13 (9.36 mg kg^{-1}) and PSBV-5 (8.91 mg kg^{-1}) which differed significantly with SSP control (8.78 mg kg^{-1}) (Table 3).

DISCUSSION

Role of Phosphate Solubilizing Bacteria (PSB) in solubilizing fixed form of soil P and making it available to plants is very well known. These bacteria have been shown to enhance the growth, increase the yield and P uptake when applied to crop plants (Gaind and Gaur, 1991; Abd-Alla, 1994; Tomar *et al*., 1998; Khalid *et al*., 2004; Pandey *et al*., 2006; Hameeda *et al*., 2006a). In vertisols, the problem of P fixation is usually higher due to the fact that the available P is fixed in the form of poorly soluble calcium mineral phosphates and is unavailable for plant uptake (Ae *et al*., 1991). In the present investigation, an attempt was made to test the efficacy of PSB isolates on nodulation, growth and nutrient uptake of greengram plants under pot culture conditions.

The inoculation of PSB strains significantly increased the number and dry weight of nodules per plant over RP control at 45 DAS. PSBV-14, PSBV-16, PSBV-13, PSBV-12, PSBV-15, PSBV-9 and PSBV-7 showed an increase in the nodule dry weight by 123.59, 111.13, 78.35, 74.31, 61.48, 49.48 and 49.03%, respectively over RP control. Inoculation of peanut seeds with plant growth promoting and P solubilizing fluorescent pseudomonad isolates, PGPR1, PGPR2 and PGPR4, significantly enhanced the nodule number and dry weight over the control in a three-year study (Dey *et al*., 2004). Seed inoculation with microphos was shown to significantly increase the number and dry weight of nodules per plant in soybean, groundnut and mungbean plants over uninoculated control (Khamparia, 1995). Results of significant increases in nodulation and dry weights were obtained in soybean inoculated with P solubilizing *Bacillus* sp. (Pal, 1997). In the present investigation results of similar nature were also recorded with respect to nodule number and dry weights following PSB inoculation.

Improvement in nodulation due to inoculation of P solubilizers along with application of P fertilizers was also noticed in chickpea and other pulses (Alagawadi and Gaur, 1988; Tiwari *et al*., 1989; Nagaraju *et al*., 1995). In legumes it was shown that the number and density of root nodules was

significantly stimulated by P (Chhonkar and Subba Rao, 1967; Tang *et al*., 2001). The observed enhancement in nodulation following inoculation of PSB suggests an increase in available P to the plants through solubilization of insoluble P. Legumes require high amounts of P for nodule formation and for maintenance of high rate of activity of bacteria in nodules. Consequently P availability affects nodulation in leguminous plants (Leidi and Rodriguez-Navarro, 2000; Zaman-Allah *et al*., 2007).

Plant growth parameters like shoot and root length, number of leaves and dry matter content of greengram at 45 DAS were significantly increased due to inoculation of PSB strains along with RP application as compared to RP control. The inoculation of PSBV-12, PSBV-14, PSBV-15, PSBV-7, PSBV-13, PSBV-5 and PSBV-8 recorded an increase of 25.1, 24.89, 24.49, 24.49, 23.06, 23.06 and 23.06 percent respectively over RP control with regard to shoot length of greengram. Similar trend was also noticed with regard to root length of greengram plants. Increase in shoot and root length of several crop plants due to inoculation of P solubilizing microorganisms have been reported in a number of studies (Patil 1990; Tomar *et al*., 1994; Dubey, 1996; Defreitas *et al*., 1997; Dey *et al*., 2004). Increased cell elongation and multiplication due to enhanced nutrient uptake by plants following inoculation of P solubilizing microorganisms may have caused the increased plant height (Black, 1968). All the PSB strains used in the present study enhanced the P uptake in greengram plants significantly over RP and SSP control. The increase in shoot and root length may also be attributed to the production of plant growth promoting substances as many PSB are known to produce IAA, GA and cytokinin like substances (Sattar and Gaur, 1987; Lal, 2002; Ponmurugan and Gopi, 2006; Chakraborty *et al*., 2006; Hameeda *et al*., 2006b). The role of these plant growth promoting substances in shoot and root elongation as well as plant growth is well established (Brown, 1975). The strains used for inoculation in the present study exhibited the capacity to produce both IAA and GA (Vikram *et al*., 2007) and therefore might have contributed to the enhanced shoot and root growth.

The inoculation of PSBV-14, PSBV-9, PSBV-13 and PSBV-5 recorded an increase of 27.66, 27.29, 27.11 and 23.81% in dry matter content of greengram plants respectively over RP control. Significant increases in dry matter content of greengram, groundnut, soybean and gram due to inoculation of PSB were reported by Gaind and Gaur (1991), Dubey (1996) and Mudalagiriyappa *et al*. (1997). Significant increases in dry matter content of chickpea and barley plants was observed in *Mesorhizobium mediterraneum* PECA21 inoculated plants compared to uninoculated control (Peix *et al*., 2001). The increased dry matter accumulation following inoculation of PSB and RP application could be attributed to increased nodulation, increased shoot and root growth and enhanced number of leaves per plant, which are the main contributing factors for plant biomass. Increased number of leaves per plant in greengram due to inoculation of PSB along with RP application was observed by Jones (1992).

In the present study, significant increases in the phosphorus content and uptake in greengram plants was observed at 45 DAS after inoculation of PSB along with RP application when compared with RP control and SSP control. Due to the inoculation of PSBV-13 and PSBV-14, the phosphorus content in shoot was increased by 29.49 and 25.71%, respectively over RP control whereas in roots it was 50.11 and 48.28%, respectively over RP control. Similar results of increase in P content of mungbean and soybean due to PSB inoculation was observed by Gaind and Gaur (1991) and Dubey (1996). Phosphate nutrition in maize, fingermillet, amaranthus and buckwheat was improved due to seed inoculation with phosphate solubilizing *Bacillus* sp (Pal, 1998). In barley and chickpea growing in soils treated with insoluble phosphates and inoculated with *Mesorhizobium mediterraneum* PECA21, the phosphorous content was significantly increased by 100 and 125%, respectively compared to uninoculated control (Peix *et al*., 2001). The total P uptake in greengram plants was also increased due to inoculation of PSB strains over RP control. PSBV-13, PSBV-14, PSBV-12, PSBV-5 and PSBV-11 recorded an increase of 65.36, 61.67, 51.84, 50.75 and 49.87%, respectively in total P uptake of greengram plants over RP control. Ahmad and Jha (1977), Maurya and Sanoria (1982) and Mohod *et al*. (1989) also observed increased P uptake in gram and rice. Inoculation of PSB like *Serratia*

marcescens, *Pseudomonas fluorescens* and *Bacillus* spp. improved the P uptake of shoot and grain in maize and peanut plants (Dey *et al*., 2004; Sahin *et al*., 2004; Hameeda *et al*., 2006a). The inoculated organisms in the present study increased the available P content of soil, probably by well establishing in the rhizosphere, which ultimately resulted in enhanced P uptake by plants. Among the various PSB isolates tested, PSBV-4, PSBV-9, PSBV-12, PSBV-13, PSBV-14 and PSBV-15 improved the growth parameters and P uptake of greengram significantly compared to the remaining PSB strains. These efficient PSB strains from the pot trial can be tested for their efficacy under field conditions before they can be recommended for exploitation under commercial conditions.

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