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Efficacy of Phosphate Solubilizing Bacteria on the Yield and Phosphorus Uptake of Green Gram (*Vigna radiata* (L.) Wilczek)

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ABSTRACT

The efficacy of phosphate solubilizing bacteria (PSB) on the yield and phosphorus uptake of green gram (*Vigna radiata* L.) was studied in a field experiment at the Main Research Station, University of Agricultural Sciences, Dharwad, India. All the strains of PSB used, except for strain PSBV-7, significantly increased the grain and straw yield of green gram as compared to rock phosphate control. The highest grain and straw yield was recorded by strain PSBV-13 which differed significantly over single super phosphate control and other strains of PSB. The phosphorus content in shoot, root and grain increased by 29-45, 17-30 and 12-17%, respectively, due to inoculation of PSB over the rock phosphate control. In most treatments there were significant increases in the rhizosphere population of the PSB suggesting their successful establishment. The results suggest the possible use of efficient strains of PSB as a biofertilizer to enhance crop production.

Keywords: phosphate use efficiency, phosphorus uptake, vertisol

INTRODUCTION

Phosphorus plays a key role in the balanced nutrition of plants. Indian soils are rich in P but more than two thirds of the native phosphates are in a chemical form that cannot be absorbed by plants (Hasan 1996; Thiyageshwari and Selvi 2006). Furthermore, applied P fertilizers are rendered unavailable due to its chemical fixation in the soil (Vassilev and Vassileva 2003). Phosphate solubilizing microorganisms are a group of organisms that convert the unavailable forms of phosphorus to available forms from soils, phosphatic fertilizers like rock phosphate (RP) and from crop residues and organic manures (Pal 1998; Hilda and Fraga 1999; Bhattacharya and Jain 2000). An important group of phosphate solubilizing microorganisms is the phosphate solubilizing bacteria (PSB) which includes several genera like *Pseudomonas*, *Bacillus*, *Serratia*, etc. PSB inoculants play an important role in making P available to crop plants and thereby increase the yield of crop plants (Gaur and Gaind 1992; Tomar 1998; Zaida *et al*. 2003; Khalid *et al*. 2004; Hameeda *et al*. 2008). The production of organic acids by phosphate solubilizing microorganisms has been shown to be the main cause for solubilization of insoluble inorganic compounds but other mechanisms such as carbonic acid, H_2S and alkalinity are also implicated (Gaur 1990). The performance of PSB varies with the soil type and although their performance in vertisols result in yield increases, in some cases it is associated with inconsistent results (Kucey *et al*. 1989; Gaur 1990). Phosphate solubilizing microorganisms developed in one geographical location do not perform well at a different location which might be attributed to their poor adaptability to the changing soil and agroclimatic conditions (Alagawadi *et al*. 1992). Thus there is a need to develop locality-specific strains for use in vertisols, as such efforts will enhance the phosphate use efficiency in these soils. In studies conducted earlier PSB strains belonging to the genus *Pseudomonas*, *Serratia* and *Xanthomonas* enhanced the nodulation and growth parameters of green gram under greenhouse conditions (Vikram and Hamzehzarghani 2008). In the present study the efficacy of these PSB strains to improve the yield and phosphorus uptake in green gram was tested under field conditions with a view of using these strains of PSB as biofertilizer.

MATERIALS AND METHODS

Bacterial cultures

The PSB strains used in the study were PSBV-1 (*Xanthomonas* sp.), PSBV-4 (*Xanthomonas* sp.), PSBV-5 (*Pseudomonas* sp.), PSBV-7 (*Xanthomonas* sp.), PSBV-9 (*Pseudomonas* sp.), PSBV-13 (*Serratia* sp.), and PSBV-14 (*Serratia* sp.). These strains were obtained from the culture collection of Department of Agricultural Microbiology, UAS, Dharwad and maintained on tricalcium phosphate agar medium (Pikovskaya 1948).

Soil type, seeds and fertilizer

A field experiment was conducted on medium black clay soil with a pH of 7.5, organic carbon (0.40 %), available N (170 kg ha^{-1}), available P (30 kg ha^{-1}) and available K (290 kg ha^{-1}) at the Main Research Station, University of Agricultural Sciences, Dharwad, India. 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.

Green gram [*Vigna radiata* (L.) Wilczek] seeds of variety 'China mung' obtained from the Main Research Station, University of Agricultural Sciences, Dharwad were used in the experiment at a rate of 15 kg ha^{-1} (Desai 2004). Fertilizers were applied as per the recommended dosage for green gram (25: 50 kg NP ha^{-1}) (Thiyagarajan *et al*. 2003; Desai 2004). Nitrogen in the form of urea and phosphorus in the form of single super phosphate (SSP) or Mussourie rock phosphate (RP) as per the treatment requirements were applied as basal dose. The experiment consisted of the following treatments: SSP control (no inoculation with SSP as P source), RP control (no inoculation with RP as P source) and

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seven other treatments involving inoculation of seven different strains of PSB with recommended dose of P in the form of RP. These treatments were laid in randomized block design (RCBD) which consisted of four replications in 3 m × 3 m plots.

Seed inoculation and sowing

Seed inoculation was done with carrier based inoculum (10 g/kg seed) of different PSB isolates using gum Arabic solution as an adhesive. Disease-free, healthy and bold green gram seeds were sown by hand dibbling with a spacing of 30 cm between rows and 15 cm between the plants within each row. Thinning was done 15 days after sowing to maintain one plant hill^{-1} (180 plants plot^{-1}).

Plant protection

To check the weed growth, intercultivation was done at three and six weeks after sowing with the help of a blade hoe and two hand weedings were done at the 4th and 7th week after sowing. A spray of chloropyriphos 20 EC (0.04%) was given at three weeks after sowing to control aphids. At seven weeks after sowing, a spray of carbendazim 50 WP (0.05%) was given to control powdery mildew.

Experimental method and statistical analysis

Plants were harvested 70 days after sowing when it attained physiological maturity and grain and straw yields were recorded. The other observations like P content and uptake in grain and straw, population of PSB in the rhizosphere of green gram and available P content in soil at harvest were recorded. The P content was estimated by following the standard Vanadomolybdate phosphoric yellow color method of Jackson (1973) and available P content in soil by Olsen's method as described by Jackson (1973). The enumeration of PSB in the rhizosphere of green gram at harvest was done by the dilution plate technique using Pikovskaya's medium (Pikovskaya 1948). The statistical analysis of the field data was carried out by randomized block design using GLM procedure of SAS (version 8) and means of treatments were compared using LSD (least significant difference) at $P = 0.1$ to evaluate the effect of treatment (SAS 1999).

RESULTS

Grain and straw yield

The early vigor of green gram crop (15 days after sowing) as influenced by inoculation of different PSB strains can be noted in **Plate 1**. All the strains of PSB showed significantly higher grain yield than RP control. All strains of PSB except PSBV-7 recorded significantly higher grain yield than SSP control (**Fig. 1**). Highest grain (10.07 Q ha^{-1}) and straw yield (32.55 Q ha^{-1}) was recorded in PSBV-13 treatment and was statistically significant ($p<0.1$) over all other strains of PSB used in the study (**Fig. 1**). The straw yields recorded by all PSB strains were significantly higher than RP control and SSP control.

Phosphorus content and total P uptake

The treatments receiving PSB inoculation and SSP control recorded significantly higher P content in shoot, root and grain than RP control (**Fig. 2**). The highest root P content was recorded by PSBV-13 (0.99%) while highest shoot and

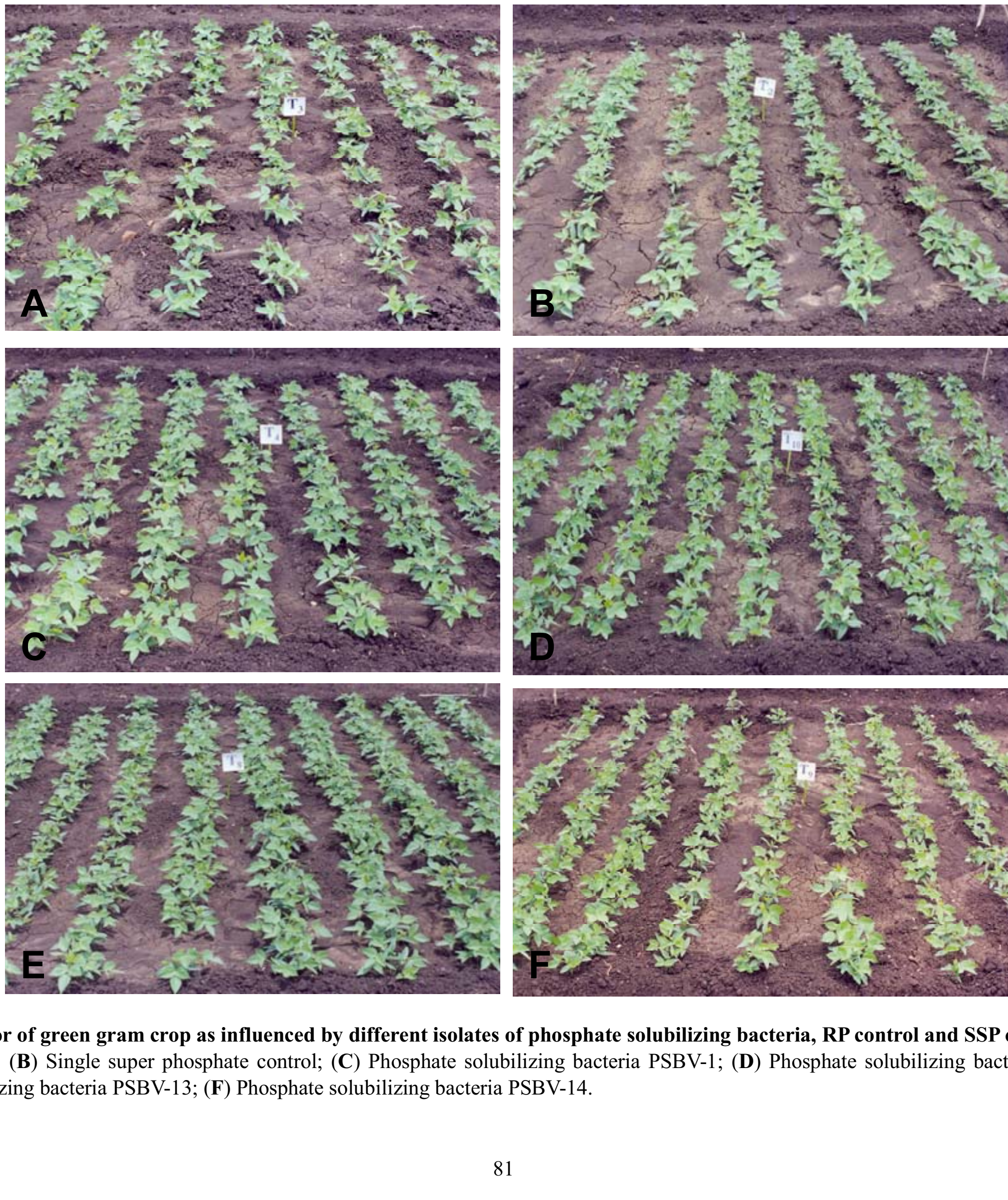

Plate 1 Early vigor of green gram crop as influenced by different isolates of phosphate solubilizing bacteria, RP control and SSP control. (**A**) Rock phosphate control; (**B**) Single super phosphate control; (**C**) Phosphate solubilizing bacteria PSBV-1; (**D**) Phosphate solubilizing bacteria PSBV-5; (**E**) Phosphate solubilizing bacteria PSBV-13; (**F**) Phosphate solubilizing bacteria PSBV-14.

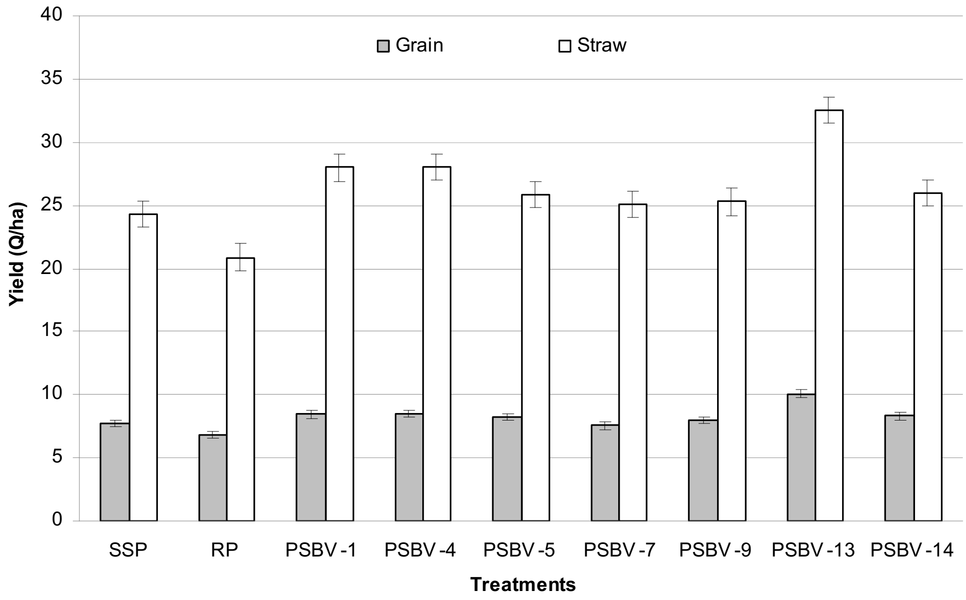

Fig. 1 Yield of green gram as influenced by inoculation of different isolates of phosphate solubilizing bacteria. Values are the means of four replicates. Mean separation was achieved using least significant difference (LSD) at $P = 0.1$. Error bars represent standard error of the mean.

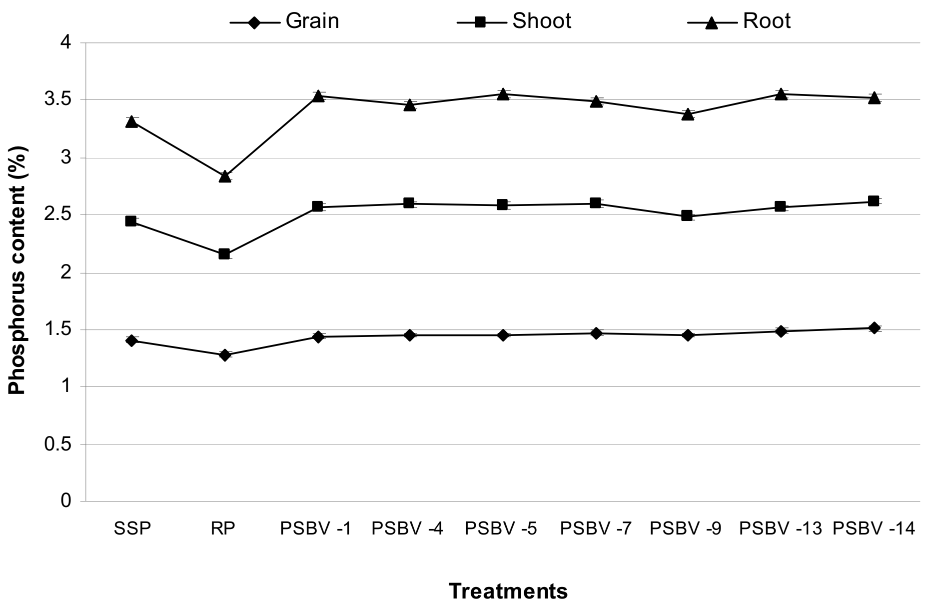

Fig. 2 Phosphorus content in green gram crop at harvest as influenced by inoculation of different isolates of phosphate solubilizing bacteria. Values are the means of four replicates. Mean separation was achieved using least significant difference at $P = 0.1$. Error bars represent standard error of the mean.

grain P contents were recorded by PSBV-4 (1.14%) and PSBV-14 (1.51%), respectively (**Fig. 2**). The root P contents from all PSB strains were significantly higher than SSP control (0.87%) except for PSBV-4 (0.87%) while the shoot P content of all PSB strains were significantly superior than the SSP control (1.03%) with the exception of PSBV-9 (1.03%) (**Fig. 2**). All treatments receiving inoculation of PSB recorded significantly higher P contents in grains compared to SSP control.

Highest total P uptake was recorded by PSBV-13 (50.78 kg ha^{-1}) which was significantly different from all other treatments (**Table 1**). All strains of PSB recorded significantly higher total P uptake than SSP (35.87 kg ha^{-1}) and RP control (26.39 kg ha^{-1}) (**Table 1**).

Population of PSB in rhizosphere and available P content in soil

Application of SSP or RP alone did not influence the population of PSB in the rhizosphere of green gram. However, treatments receiving inoculation with PSB strains recorded a 2–4 times increase in the population of PSB in the rhizosphere. All PSB strains recorded significantly higher population of PSB in rhizosphere over RP control (3 × 10^{3} cfu g^{-1} soil) while all strains of PSB differed significantly over SSP control (4 × 10^{3} cfu g^{-1} soil) with regard to population of PSB in rhizosphere except PSBV-5 (5 × 10^{3} cfu g^{-1} soil) (**Fig. 3**).

The treatments receiving PSB inoculation and SSP control showed significantly higher available P in soil compared to RP control. The highest available P content in soil was recorded by PSBV-4 (21.04 kg ha^{-1}) which was significantly different from all other treatments. All strains of PSB recorded significantly higher available P than SSP control (15.38 kg ha^{-1}) except for PSBV-7 (15.43 kg ha^{-1}) (**Table 1**).

Table 1 Total phosphorus uptake in green gram plants and available P content in soil at harvest as influenced by inoculation of different isolates of phosphate solubilizing bacteria.

Treatments	Total P uptake (kg ha^{-1})[1]	Available P (kg ha^{-1})[1]
SSP	35.87 g	15.38 f
RP	26.39 h	10.34 h
PSBV-1	44.05 c	19.30 c
PSBV-4	44.73 b	21.04 a
PSBV-5	40.01 e	15.75 e
PSBV-7	40.01 e	15.43 f
PSBV-9	37.93 f	19.99 b
PSBV-13	50.78 a	16.56 d
PSBV-14	40.3 d	15.21 g

[1]Each value is the mean of four replicates. Within each column, means followed by same letter are not significantly different from each other at $P = 0.1$. Mean separation was achieved using least significant difference (LSD) test.

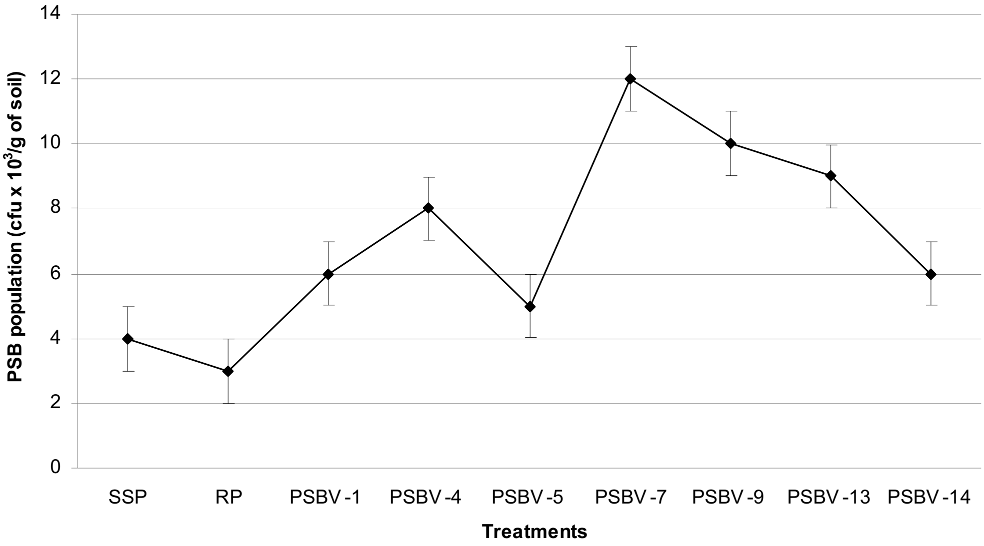

Fig. 3 Population of phosphate solubilizng bacteria (PSB) as influenced by inoculation of different isolates of PSB. Values are the means of four replicates. Mean separation was achieved using least significant difference at $P = 0.1$. Error bars represent standard error of the mean.

DISCUSSION

In vertisols, because of the problem of P fixation, there is no response to phosphatic fertilizers even at higher doses. Soil microorganisms play an important role in making P available to plants by releasing Pi from P-bearing organic compounds. They also bring about solubilization of insoluble inorganic phosphates mainly by production of organic acids (Gaur 1990). A large number of field experiments have been conducted in India and elsewhere on the effect of P-solubilizing microorganisms in improving P nutrition and crop yields. These effects have been reviewed critically by Cooper (1959), Kucey *et al.* (1989) and Gaur (1990) who pointed out that statistically significant yield increases were recorded in about 25-30% of the experiments and were associated with inconsistencies. The poor performance/inconsistencies of the strains are probably due to various factors including the soil, climate, crop plants and the genetic make up of the strain. Another important consideration could be the use of locally developed efficient strains for different soils and agroclimatic conditions. Such strains may have better adaptability and survival in soil and hence may perform better. So in the present study the efficacy of PSB strains to improve the yield and P uptake in green gram was tested under field conditions.

The results revealed significant increase in the seed and straw yield of green gram due to inoculation of PSB along with application of RP. The inoculation of PSB strains PSBV-13, PSBV-4, PSBV-1 and PSBV-14 recorded increase in the grain yield by 21-47% over RP control whereas the straw yield was increased by 24-34% over RP control by the same four strains of PSB. Dubey (1996) also observed an increase of 13% in the grain yield and 24% in the straw yield of soybean due to inoculation of *Pseudomonas striata* over control. Increased seed and straw yield due to inoculation of P solubilizers with or without the application of phosphatic fertilizers has been reported in chickpea and other crops by various research groups (Ahmad and Jha 1977; Gaur and Singh 1982; Tomar *et al.* 1994; Khamparia 1995; Mishra *et al.* 1995; Pal 1998) and the results of present investigation is consistent with these reports. The dry matter content of green gram plants increased by 27.66, 27.29, 27.11 and 23.81% over the RP control due to inoculation of PSBV-14, PSBV-9, PSBV-13 and PSBV-5, respectively in pot trials at 45 days after inoculation (DAI) (Vikram and Hamzehzarghani 2008). Increased seed and straw yield of green gram may be due to the fact that inoculated PSB strains, after establishing in the rhizosphere, released greater amounts of available P from the RP and this enabled the plants to take up more P resulting in improved nodulation, growth and yield. P-solubilizing microorganisms are known to produce plant growth regulators like indole-3-acetic acid (IAA) and gibberellic acid (GA_3) (Sattar and Gaur 1987; Lal 2002; Ponmurugan and Gopi 2006; Chakraborty *et al.* 2006; Hameeda *et al.* 2006). PSB strains used in the present study were able to produce both indole-3-acetic acid and gibberellic acid and this might have also contributed to the enhanced growth and yield of green gram (Vikram *et al.* 2007). Growth and yield of crop plants are influenced by the presence of sufficient quantities of nutrients in available form for plant uptake (Babannavar 1990). In the present study higher seed and straw yield of green gram in treatments receiving PSB inoculation and RP application can be ascribed to increased P availability and its uptake by the crop.

The phosphorus content and uptake in green gram plants and in seeds was also significantly increased in RP control compared to the PSB inoculated treatments. While the P content in shoots increased by 29-45% with different strains, the root P content increased by 17-30% and that in grains by 12-17% over the RP control. The increase in total P uptake by green gram due to PSB strains ranged from 43-92% over the RP control. Similarly, seed inoculation of maize, amaranthus, fingermillet and buckwheat with phosphate solubilizing *Bacillus* sp. improved the phosphate nutrition in these crops (Pal 1998). In pot trials the seed inoculation of green gram with PSBV-13, PSBV-14 and PSBV-5 increased the total P uptake of green gram plants by 65.36, 61.67 and 50.75%, respectively over RP control at 45 DAI (Vikram and Hamzehzarghani 2008). A similar increase in the P content and P uptake was noticed by inoculation of gram with *Bacillus megaterium* and *B. circulans* (Ahmad and Jha 1977), soybean with *Pseudomonas striata* (Dubey 1996), peanut with *Pseudomonas fluorescens* (Dey *et al.* 2004) and maize with *Serratia marcescens* and *Pseudomonas* sp. (Hameeda *et al.* 2008). All the PSB isolates tested in the present study were able to significantly increase the available P content in soil compared to the RP control. In a pot trial conducted earlier with green gram, all the 16 PSB isolates significantly increased the available P content in soil when compared to the control added with rock phosphate (Vikram and Hamzehzarghani 2008). The inoculated PSB strains increased the available P content of soil (**Table 1**) by establishing well in the rhizosphere which probably resulted in increased P uptake by plants.

Six of the seven strains used in the field experiment with green gram showed higher grain yield over SSP control indicating their potential to enhance the economic yield with cheaper insoluble inorganic P source like RP. Among the strains, PSBV-13 performed best by increasing the grain yield by 30.10% and straw yield by 34.01% over the SSP control. This strain could be used as a biofertilizer after extensive field trials. The performance of this strain along with others can also be tested in vertisols with different crops and different locations and agro climatic conditions of the state to confirm their performance.

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