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Efficacy of Phosphate Solubilizing Bacteria Isolated from Vertisols on Growth and Yield Parameters of Sorghum

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Abstract: Phosphorus is one of the major plant macronutrients and plays an important role in plant metabolism ultimately reflecting on crop yields. Although vertisols contain appreciable amounts of total P, it is not available for crop uptake in adequate amounts due to its insoluble nature. Phosphate Solubilizing Bacteria (PSB) are a group of organisms that solubilize fixed form of P and make it available to plants. In the present study the ability of 16 PSB isolates to promote growth and yield parameters of sorghum was tested under greenhouse conditions. Among all the treatments tested, inoculation with PSBV-1 recorded the highest earhead weight, grain yield, P content and P uptake in root and grain of sorghum plants. Inoculation of sorghum plants with PSBV-2 recorded the highest shoot length, shoot dry matter and P uptake in shoot. Almost all the PSB isolates tested in the present study were able to improve the growth and yield parameters of sorghum significantly compared to absolute control, rock phosphate control and single super phosphate control. The efficient strains from this study should be tested in field conditions before they can be exploited in a commercial set up. The success of these strains will at least solve some of the problems of P fixation in vertisols.

Key words: Vertisols, phosphate solubilizing bacteria, phosphorus uptake, plant growth promoting substances

INTRODUCTION

Phosphorus is a vital nutrient required for the growth and development of both plants as well as microorganisms. Majority of soils contain substantial reserves of total P but most of it remains relatively inert with only less than 10% of soil P entering the plant-animal cycle (Kucey *et al*., 1989). Consequently P deficiency is widespread and there is a need for application of P fertilizers to maintain crop production. Among the amounts of P fertilizers applied, only a small portion of it is utilized by the plant while the remaining portion is converted to insoluble complexes in soil (Vassilev and Vassileva, 2003). Soil contains a group of microorganisms called phosphate solubilizers which are capable of solubilizing insoluble phosphates through production of organic acids and chelating oxo acids from sugars (Asea *et al*., 1988; Halvorson *et al*., 1990).

Considerable work on phosphate solubilizing microorganisms has been done earlier and the results invariably indicated that inoculation of crops with phosphate solubilizing bacteria improved growth and increased the yield and P uptake in a variety of crop plants (Khamparia, 1995; Jisha and Alagawadi, 1996; Defreitas *et al*., 1997; Kumar *et al*., 2001; Sundara *et al*., 2002; Zaida *et al*., 2003; Hameeda *et al*., 2006a). Vertisols are soils which have higher content of expanding clay, free calcium carbonate and high pH. Although they contain appreciable amounts of P, it is not released in adequate amounts for crop growth. Majority of mineral P in vertisols is present in the form of poorly soluble calcium mineral phosphates (Ae *et al*., 1991). To date there are many phosphate solubilizing microorganisms developed elsewhere but they have not been very consistent in their performance everywhere due to their poor adaptability to the changing soil and agroclimatic conditions

(Alagawadi *et al*., 1992). This provides an opportunity to study P solubilizers and develop locality specific strains which can be used in vertisols. Phosphate solubilizing bacteria isolated from vertisols of Northern Karnataka were used in this study. The present investigation was undertaken to study the influence of different strains of phosphate solubilizing bacteria on growth, yield and P uptake in sorghum under greenhouse conditions.

MATERIALS AND METHODS

Soil Type, Seeds and Fertilizer

The soil used for pot culture experiment was medium black collected from 0-15 cm depth from E block, plot number 125 of Main Research Station (MRS), University of Agricultural Sciences, Dharwad. The collected soil was mixed thoroughly, sieved and then filled in earthen pots of 30 cm diameter and 30 cm in height at the rate of 18 kg per pot. The required quantity of farm yard manure (90 g/pot) was weighed separately for each pot and mixed thoroughly with 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. Sorghum seeds of DSH-3 variety obtained from Senior Sorghum Breeder, University of Agricultural Sciences, Dharwad were used in the pot culture experiment. Recommended dose of fertilizer for sorghum was (100:75:37.5 kg NPK ha^{-1}) applied. N in the form of urea, P in the form of single superphosphate or Mussoorie rock phosphate as per the treatment schedule and K in the form of muriate of potash were applied in calculated quantities as basal dose at the time of sowing. The experimental treatments were absolute control (no inoculation and no fertilizer), 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 19 treatments which were replicated three times.

Seed Inoculation and Sowing

Healthy bold seeds of sorghum were sown at the rate of 10 seeds/pot to which 1 mL of bacterial inoculum/seed 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 harvest. After harvest, plant growth and yield parameters like height, root length, dry matter content, earhead weight, grain yield, thousand grain weight, phosphorus content (Jackson, 1973), phosphorus uptake and available phosphorus content in soil (Jackson, 1973) were recorded. Population of all P solubilizing bacterial cultures at the time of sowing was to the range of 10^8 cfu mL^{-1}. Statistical analysis of data obtained in the present study was carried out by completely randomized design as described by Panse and Sukhatme (1985). The PSB strains, PSBV-1 to PSBV-16 used in the experiment were obtained from the culture collection of Department of Agricultural Microbiology, UAS, Dharwad. The pot culture experiment was conducted under greenhouse conditions in the Department of Agricultural Microbiology, UAS, Dharwad.

RESULTS

Shoot and Root Length of Sorghum Plants

Among the 16 strains of Phosphate Solubilizing Bacteria (PSB) used in this study, about ten of them showed significant increase in shoot length of sorghum plants over absolute control (152.70 cm) (Table 1). All strains of PSB showed more shoot length over RP control but only PSBV-2 was

Table 1: Growth and yield parameters of sorghum plants at harvest as influenced by inoculation of efficient isolates of phosphate solubilizing bacteria

Treatments	SL (cm)	RL (cm)	EW (g plant^{-1})	GY (g plant^{-1})	GTW (g)
Absolute control	152.7	23.00	35.09	30.52	27.36
SSP control	190.7	29.33	67.29	64.16	31.66
RP-control	173.5	24.66	45.19	41.76	28.81
PSBV-1+RP	194.5	29.50	72.28	69.19	32.25
PSBV-2+RP	212.5	29.67	68.13	64.44	30.36
PSBV-3+RP	208.5	30.00	66.53	63.39	31.33
PSBV-4+RP	186.7	30.83	64.92	61.82	30.40
PSBV-5+RP	192.0	28.17	69.23	65.85	30.51
PSBV-6+RP	206.2	28.50	64.82	61.68	30.11
PSBV-7+RP	186.5	27.83	61.75	58.74	29.85
PSBV-8+RP	196.0	30.33	68.37	65.22	30.22
PSBV-9+RP	205.0	30.00	66.72	64.26	30.06
PSBV-10+RP	204.8	28.50	68.03	64.88	30.41
PSBV-11+RP	181.7	30.67	62.11	59.37	29.92
PSBV-12+RP	190.7	29.33	64.76	61.52	31.69
PSBV-13+RP	204.3	28.33	70.11	66.87	32.20
PSBV-14+RP	182.0	32.33	67.20	64.05	31.70
PSBV-15+RP	190.7	29.50	67.28	64.16	30.19
PSBV-16+RP	192.3	28.00	62.63	60.26	29.89
SEM±	10.06	1.48	2.74	0.49	0.07
CD at 1%	38.47	5.66	10.46	1.89	0.26

SL: Shoot Length; RL: Root Length; EW: Earhead Weight; GY: Grain Yield; GTW: Grain Test Weight (weight of 1000 grains); SSP: Single Super Phosphate; RP: Rock Phosphate

significantly superior over RP control. In the present study there was no significant difference between strains of PSB with respect to shoot length. PSBV-2 recorded maximum shoot length (212.5 cm) followed by PSBV-3 (208.5 cm), PSBV-6 (206.2 cm), PSBV-9 (205.0 cm), PSBV-10 (204.8 cm) and PSBV-13 (204.3 cm), all of which showed marked increase in shoot length over SSP control. The root length of sorghum plants at harvest was significantly increased in SSP control treatment (29.33 cm) over absolute control (23.00 cm) but was on par with RP control (24.66 cm) (Table 1). PSBV-4 (30.83 cm), PSBV-8 (30.33 cm), PSBV-11 (30.67 cm) and PSBV-14 (32.33 cm) showed significant increase in root length over RP control. Although nine strains of PSB recorded higher root length than SSP control, none of them were significantly superior to SSP control. The PSB strains showed no significant difference among themselves with respect to root length. Maximum root length was recorded by strain PSBV-14 while it was least in the case of PSBV-7 (27.83 cm).

Shoot, Root and Total Dry Matter

Among the three control treatments (which did not receive inoculation), SSP control recorded maximum shoot dry matter followed by RP control and absolute control (Fig. 1). Among the PSB strains, PSBV-2, PSBV-3, PSBV-9 and PSBV-10 recorded significant increase in shoot dry matter over absolute control and RP control. Although most PSB strains recorded higher shoot dry matter than SSP control, none of them were significantly superior over SSP control. The strains of PSB did not differ significantly with each other with respect to shoot dry matter content. PSBV-2 recorded highest shoot dry matter whereas PSBV-14 recorded least dry matter (Fig. 1).

The root dry matter content of sorghum plants did not increase significantly over absolute control either due to SSP or RP application (Fig. 1). PSBV-15 and PSBV-16 recorded significantly higher root dry matter over both RP control and SSP control. PSBV-16 recorded highest root dry matter (Fig. 1) closely followed by PSBV-15 and both of them were significantly superior over PSBV-2, PSBV-6, PSBV-13 and PSBV-14.

There was no significant difference between three control treatments with respect to total dry matter content of sorghum plants at harvest (Fig. 1). PSBV-2, PSBV-3, PSBV-9, PSBV-10, PSBV-15,

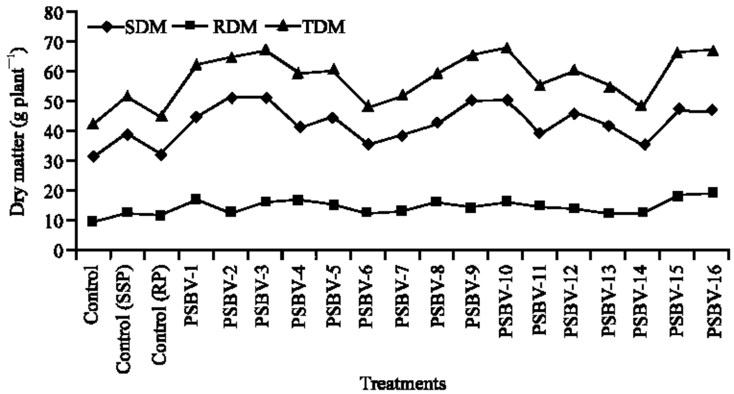

Fig. 1: Dry matter content of sorghum plants at harvest as influenced by efficient isolates of phosphate solubilizing bacteria

and PSBV-16, recorded significantly higher total dry matter over RP control while rest of them were on par with it. About 14 strains of PSB recorded higher total dry matter than SSP control but all of them were statistically on par with SSP control. Significant differences in total dry matter content were also observed between all strains of PSB. Highest total dry matter was recorded from the treatment receiving inoculation of PSBV-10 (Fig. 1).

Earhead Weight, Grain Yield per Plant and Test Weight of Sorghum Seeds

All the inoculation treatments and SSP control (67.29 g) recorded significantly higher earhead weight than absolute control (35.09 g) and RP control (45.19 g) (Table 1). PSBV-1 recorded highest earhead weight (72.28 g) and was on par with other PSB strains except PSBV-7 (61.75 g). PSBV-1 recorded earhead weight which was significantly superior compared to PSBV-7. PSB strains increased the earhead weight of sorghum plants but they were not significantly superior over SSP control.

All the inoculation treatments as well as RP control and SSP control recorded significantly higher grain yield/ plant over absolute control (Table 1). All PSB strains showed significant increase in grain yield over RP control while only PSBV-1 (69.19 g) and PSBV-13 (66.87 g) were able to record significantly higher grain yield than SSP control. PSBV-1 recorded highest grain yield which was significantly superior over all other PSB strains. PSBV-13 was able to record significantly higher grain yield than all other PSB strains except PSBV-1, PSBV-5 (65.85 g) and PSBV-8 (65.22 g).

The treatments receiving inoculation of PSB strains, RP control and SSP control recorded significantly higher test weight than absolute control (Table 1). All PSB strains recorded significantly higher test weight than RP control while only PSBV-1 (32.25 g) and PSBV-13 (32.20 g) were able to record significantly higher test weight than SSP control. PSBV-1 recorded highest test weight closely followed by PSBV-13 and both of them were significantly superior over all other strains of PSB.

Phosphorus Content in Shoot, Root and Grain

All the treatments including RP control and SSP control showed significantly higher P content in shoot than absolute control (Fig. 2). The treatments receiving PSB inoculations and SSP control recorded significantly higher P content in shoot than RP control. PSBV-1 recorded highest shoot P content which differed significantly over SSP control and all other strains of PSB except PSBV-13. All PSB strains, RP control and SSP control significantly enhanced P content in roots of sorghum plants over absolute control (Fig. 2). Majority of PSB strains recorded higher root P content than SSP control while PSBV-5, PSBV-8 and PSBV-13, were significantly superior to SSP control. PSBV-1 recorded highest root P content and was significantly superior over all other PSB strains

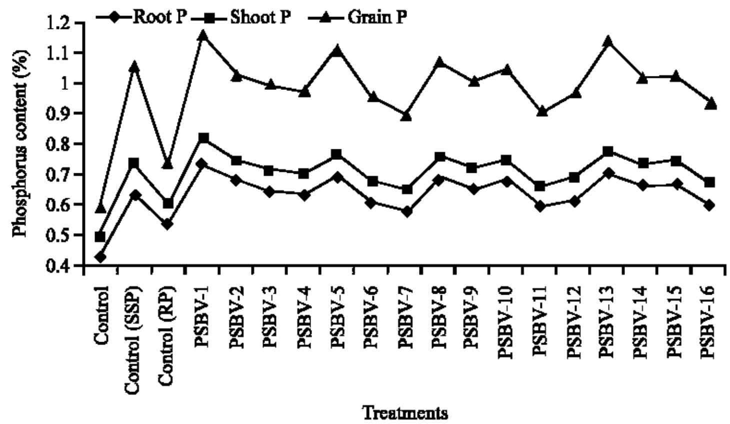

Fig. 2: Phosphorus content of sorghum plants at harvest as influenced by efficient isolates of phosphate solubilizing bacteria

except PSBV-5 and PSBV-13. The treatments receiving PSB inoculation, SSP control and RP control recorded significantly higher P content in grains over absolute control (Fig. 2). PSBV-1, PSBV-5 and PSBV-13 recorded significantly higher P content in grain over SSP control. The highest grain P content was recorded from PSBV-1 which was on par with PSBV-5 and PSBV-13 while differing significantly with remaining strains of PSB.

Phosphorus Uptake in Shoot, Root and Grain

All strains of PSB excluding PSBV-6, PSBV-7, PSBV-11 and PSBV-14, recorded significantly higher P uptake in shoot compared to absolute control (Table 2). Although 12 strains of PSB recorded higher shoot P uptake than SSP control (286.72 mg), none of them differed significantly with it. The highest shoot P uptake among PSB strains was recorded by PSBV-2 (379.32 mg) which was significantly superior over PSBV-6 (239.98 mg), PSBV-7 (250.58 mg), PSBV-11 (260.36 mg) and PSBV-14 (253.78 mg).

All the treatments except RP control recorded significantly higher P uptake in roots over absolute control (Table 2). PSBV-1, PSBV-3, PSBV-4, PSBV-5, PSBV-8, PSBV-10, PSBV-15 and PSBV-16 recorded significant increase in P uptake of roots over RP control. Most of the PSB strains recorded higher P uptake in root than SSP control while only PSBV-1 (128.10 mg), PSBV-15 (124.90 mg) and PSBV-16 (118.78 mg) were significantly superior over SSP control. The highest P uptake in root was recorded by PSBV-1 closely followed by PSBV-15 (124.90 mg) and both of them recorded significantly higher P uptake in roots than PSBV-2 (87.41 mg), PSBV-6 (77.27 mg), PSBV-7 (78.47 mg), PSBV-11 (89.81 mg), PSBV-13 (89.41 mg) and PSBV-14 (82.95 mg).

All the treatments including SSP control and RP control showed significantly higher P uptake in grains compared to absolute control (180.68 mg) (Table 2). Similarly, SSP control (677.52 mg) and all treatments receiving PSB inoculation showed significant increase in P uptake in grains over RP control (305.68 mg). PSBV-1 (799.14 mg), PSBV-5 (728.30 mg) and PSBV-13 (758.31 mg) recorded significantly higher P uptake in grain than SSP control. The highest P uptake in grain was recorded by PSBV-1 which was significantly superior over rest of the PSB strains.

Total Phosphorus Uptake in Sorghum Plants

The total P uptake in sorghum plants was found to increase significantly due to inoculation of PSB strains, application of RP and SSP as compared to absolute control (Fig. 3). All PSB strains and

Table 2: Phosphorus uptake in sorghum plants at harvest as influenced by inoculation of efficient isolates of phosphate solubilizing bacteria

Treatments	P uptake in shoot (mg plant^{-1})	P uptake in root (mg plant^{-1})	P uptake in grain (mg plant^{-1})
Absolute control	156.13	41.58	180.68
SSP-control	286.72	81.34	677.52
RP-control	196.95	65.11	305.68
PSBV-1+RP	366.26	128.10	799.14
PSBV-2+RP	379.32	87.41	659.87
PSBV-3+RP	361.40	103.65	629.46
PSBV-4+RP	290.44	109.73	600.89
PSBV-5+RP	337.35	108.12	728.30
PSBV-6+RP	239.98	77.27	587.19
PSBV-7+RP	250.58	78.47	522.19
PSBV-8+RP	322.94	111.53	697.85
PSBV-9+RP	362.59	93.96	644.53
PSBV-10+RP	376.73	112.69	676.05
PSBV-11+RP	260.36	89.81	536.70
PSBV-12+RP	313.02	88.89	591.82
PSBV-13+RP	325.50	89.41	758.31
PSBV-14+RP	253.78	82.95	649.47
PSBV-15+RP	349.77	124.90	655.07
PSBV-16+RP	313.29	118.78	564.64
SEM±	30.98	9.02	9.38
CD at 1%	118.49	34.51	35.86

SSP: Single super Phosphate, RP: Rock Phosphate

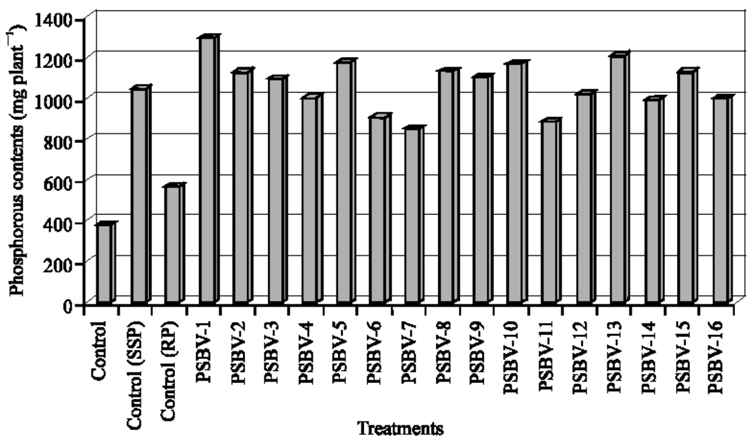

Fig. 3: Total P uptake in sorghum plants at harvest as influenced by inoculation of efficient isolates of phosphate solubilizing bacteria

SSP control showed significant increase in total P uptake over RP control. PSBV-1 and PSBV-13 recorded significantly higher total P uptake than SSP control while other strains of PSB were on par with them. PSBV-1 recorded highest total P uptake and was significantly superior over all other PSB strains excluding PSBV-5, PSBV-10 and PSBV-13.

Available P Content in Soil

The highest available P content in soil was recorded due to inoculation of sorghum plants with PSBV-4 (Fig. 4). PSBV-1, PSBV-2, PSBV-3, PSBV-4, PSBV-6, PSBV-9, PSBV-13 and PSBV-16 recorded significantly higher available P content in soil than SSP control while PSBV-5, PSBV-7, PSBV-8, PSBV-10, PSBV-11, PSBV-12, PSBV-14 and PSBV-15 were on par with it. Available P content recorded in soil inoculated with PSBV-4 differed significantly with all other PSB strains except PSBV-9.

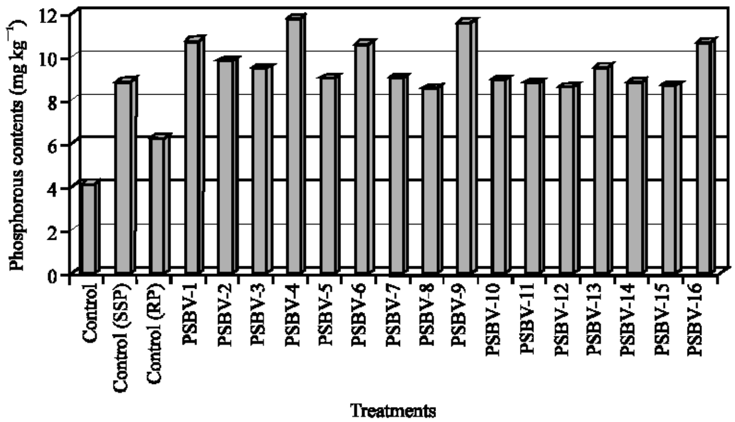

Fig. 4: Available P content in soil at harvest as influenced by inoculation of efficient isolates of phosphate solubilizing bacteria

DISCUSSION

Inoculation of phosphate solubilizing bacteria (PSB) to crop plants improves solubilization of fixed soil P and applied phosphates ultimately resulting in improved crop growth and higher yields (Gaind and Gaur, 1991; Pandey *et al*., 2006). The problem of P fixation is usually higher in vertisols wherein P is fixed as calcium phosphates and unavailable for plant uptake (Ae *et al*., 1991). In the present study, an attempt was made to use PSB isolates from vertisols and test their efficacy on growth and yield parameters of sorghum under pot culture conditions. The height of sorghum plants increased by 22.48, 20.17, 18.83, 18.16, 18.04 and 17.77% due to inoculation of PSBV-2, PSBV-3, PSBV-6, PSBV-9, PSBV-10 and PSBV-13, respectively over RP control. Similar increases in plant height due to inoculation of PSB have been reported in soybean, opium poppy, gram, canola, maize (Patil, 1990; Khamparia, 1995; Tomar *et al*., 1994; Dubey, 1996; Defreitas *et al*., 1997; Hameeda *et al*., 2006a). *Bacillus megaterium* when applied as soil drench to rhizosphere of potted tea plants showed that the percentage increase in height, number of leaves and lateral branches were considerably higher than control uninoculated tea plants (Chakraborty *et al*., 2006). The increased plant height could be a result of increased cell elongation and multiplication due to enhanced nutrient uptake by plants following inoculation with PSB. It can also be attributed to the production of plant growth promoting substances in the vicinity of roots by PSB. All efficient strains of PSB used in the present study were able to produce both IAA and GA though in different quantities (Vikram *et al*., 2007). Production of plant growth promoting substances by PSB contributed to their stimulatory effect on plant growth (Sattar and Gaur, 1987; Chakraborty *et al*., 2006; Hameeda *et al*., 2006b). It is well established that IAA and GA have a role in shoot and root elongation as well as in enhancing plant growth (Brown, 1975).

An increase of 31.10, 25.02, 24.37 and 22.99% in root length of sorghum plants was noticed due to inoculation with PSBV-14, PSBV-4, PSBV-11 and PSBV-8, respectively over RP control and are comparable with results of Srivastava and Chaudhary (1993) and Hoeflich and Ruppel (1994). In accordance with root and shoot growth, the dry matter content in root and shoot as well as total dry matter content of sorghum plants was also enhanced due to inoculation of PSB along with RP application. The total dry matter content of sorghum plants due to inoculation of PSBV-10, PSBV-3, PSBV-15, PSBV-9 and PSBV-2 increased up to 50.34, 49.99, 47.75, 45.51 and 43.85%, respectively over RP control. These results are in agreement with those of Alagawadi and Gaur (1992) who obtained

30% increase in dry matter content of sorghum plants due to inoculation of *Pseudomonas striata*. Similar results on enhanced dry matter content of paddy, sorghum, maize and groundnut due to inoculation with PSB are also reported earlier (Datta *et al*., 1982; Jisha and Alagawadi, 1996; Pal, 1998; Mudalagiriyappa *et al*., 1997; Hameeda *et al*., 2006a; Pandey *et al*., 2006). The increased root and shoot dry matter yield of sorghum plants is a result of enhanced root and shoot growth and enhanced P uptake by plants receiving PSB inoculations.

The inoculation of PSB strains significantly increased the yield and yield parameters of sorghum plants over absolute control and RP control. The yield attributing characters like earhead weight, grain weight and test weight of sorghum were significantly enhanced in treatments receiving inoculation of PSB strains. PSBV-1, PSBV-13, PSBV-5, PSBV-8 and PSBV-10 further recorded an increase in grain yield per plant by 65.68, 60.12, 57.69, 56.18 and 55.36% over RP control. Seed treatment of maize with *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 increased the yield by 85 and 64% compared to uninoculated control (Hameeda *et al*., 2006a). Inoculation of sorghum with *Pseudomonas striata*, maize with *Bacillus* sp., potato with *P. striata* and paddy with *Bacillus firmus* have been shown to increase yield up to 41.00, 33.85, 60.00 and 34.73%, respectively over control (Jisha and Alagawadi, 1996; Pal, 1998; Datta *et al*., 1982). Similar increases in yield were obtained due to inoculation of *Pseudomonas fluorescens* and *Rhizobium leguminosarum* in peanut and maize plants, respectively (Chabot *et al*., 1996; Dey *et al*., 2004). The favorable effect of PSB on yield of sorghum can be attributed to better growth and yield attributing characters of sorghum plants in the presence of improved available P status of soil (Fig. 4) and also due to production of growth hormones like IAA and GA by PSB strains used in the study (Vikram *et al*., 2007).

The sorghum plants receiving PSB inoculation along with RP application showed significantly higher P content and P uptake over the ones receiving RP alone. The root P content in PSB inoculated plants ranged from 0.577-0.732% as compared to 0.535% in RP control and 0.634% in SSP control. Similarly, P content in grains of inoculated plants ranged from 0.889-1.155% whereas that in RP control and SSP control were 0.732-1.056%, respectively. These results indicate that all PSB strains enhanced P content over RP control while some PSB strains increased P content over SSP control. Similar trend was observed with respect to P uptake in shoot, root and grains as well as total uptake. These results are in conformity with earlier findings that P solubilizers increase P content and enhance P uptake in several crops (Gaur, 1990; Gaind and Gaur, 1991; Dubey, 1996; Anthoniraj *et al*., 1994). Pal (1998) reported that phosphate nutrition of maize, fingermillet, amaranthus and buckwheat were improved after seed inoculation of crops with phosphate solubilizing *Bacillus* sp. Inoculation of PSB like *Serratia marcescens*, *Pseudomonas fluorescens* and *Bacillus* spp. improved shoot and grain P uptake in maize and peanut plants (Dey *et al*., 2004; Sahin *et al*., 2004; Hameeda *et al*., 2006a). The high increase in P uptake and its consequent reflection on yield as an effect of PSB inoculation may be caused by the ability of PSB strains to solubilize insoluble inorganic phosphates as well as to produce necessary phytohormones (Alexander, 1977; Kumar *et al*., 2001; Khalid *et al*., 2004; Hameeda *et al*., 2006b). All PSB strains used in the present study produced considerable amounts of IAA and GA besides being efficient in solubilization of insoluble inorganic phosphates (Vikram *et al*., 2007). The positive influence of PSB isolates on growth and yield parameters of sorghum can be confirmed by testing their efficiency under field conditions. The success of these PSB isolates will improve availability of P in vertisols and pave the way for their utilization under commercial conditions.

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