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Development and evaluation of phosphate solubilising microbial inoculants for fodder production in problem soils

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Abstract
A total of 70 phosphate solubilising bacterial (PSB) isolates and 90 phosphate solubilising fungal (PSF) isolates were obtained from 145 rhizosphere and non-rhizosphere soil samples collected from different parts of India representing problem soils. They were screened for P solubilisation, plant growth promoting substances (IAA, GA) production and abiotic stress (salt and acid) tolerance and plant growth support with 50% reduction in P fertilizer. Ten phosphate solubilising microorganisms (PSMs) were selected based on their abiotic stress tolerance ability and their plant growth promoting potential in normal and problem soils *in vitro*. They were further evaluated for their efficiency in enhancing biomass of fodder cowpea (*cv*. BL 2) in normal soil under field condition. The treatments details were T_1: Control [Uninoculated (UIC) + unfertilized]; T_2: RDF (UIC + 100% RDF); T_3: PSF12(1); T_4: PSF47(1); T_5: PSF48(3); T_6: PSF48(4); T_7: PSF131(1); T_8: PSB9a(2); T_9: PSB26(2); T_{10}: PSB68(3); T_{11}: PSB103(1) and T_{12}: PSB136(1). But treatments T_3 to T_{12} had PSM inoculated seeds with 100% of N and K, and 50% of recommended dose of P. The experiment was conducted in randomized block design with 3 replications. PSB136(1) recorded about 5 t/ha green fodder yield higher than RDF (20.2 t/ha) and maximum dry fodder yield (6.3 t/ha) followed by T_5, T_6 and T_{10} which were at par with RDF in normal soil. However maximum seed yield was recorded by T_7 (3.78 q/ha). Treatments were non-significant for the plant height, root length and nodule count. The same experiment was replicated for cowpea (BL-2) production in acid soil (pH 5.4). PSB103(1) recorded maximum GFY and DFY (35.2 and 8.1 t/ha) followed by PSB136(1), PSF12(1), PSB26(2) which were at par with RDF (32.0 and 7.4 t/ha). Higher crude protein yield and CP content were also recorded, which were at par with RDF and but significantly more than control (781 kg/ha and 12.7%, respectively). It was concluded that selected PSMs could be used as suitable phosphate solubilising bioinoculants in fodder crops.

Keywords: Acid soil, Biomass, Fodder crops, Normal soil, Phosphate solubilising microorganisms

Introduction

Phosphorus (P) is one of the essential elements that are necessary for plant development and growth; it makes up about 0.2% of a plant's dry weight. It is second only to nitrogen (N) among mineral nutrients most commonly limiting the growth of crops. On average, the P content of soil is about 0.05% (w/w); however, only 0.1% of this P is available for plant use (Zhu *et al.*, 2011). Phosphorous deûciency in soil is addressed by the application of P fertilizers. Large amounts of P applied as fertilizer enter into the immobile pools through precipitation reactions with highly reactive Al^{3+} and Fe^{3+} in acidic and Ca^{2+} in calcareous or normal soils (Gyaneshwar *et al.*, 2002). Soil pH between 6 and 7.5 is ideal for P-availability; beyond this range P-availability is reduced. In India, out of the 157 Mha of the cultivated area 49 Mha is acidic, of which 26 Mha is having pH <5.6 and the rest vary from 5.6 to 6.5 and area under salt-affected soils is 6.73 M ha. Efficiency of applied P fertilizer throughout the world is around 10-25 % (Isherword, 1998), and concentration of bioavailable P in soil is very low, reaching the level of 1.0 mg kg^{-1} soil (Goldstein, 1994). Many microorganisms in the soil and rhizosphere are effectively releasing P for the growth of plants from total soil P through solubilisation and mineralization (Bhattacharyya and Jha, 2012). For better utilization of the P accumulated in soils, PSMs that are capable of transforming insoluble P to soluble forms can function as biofertilizers. They increase the soluble P content (Zhu *et al.*, 2012). Most of the PSMs are capable of producing plant growth promoting substances (PGPS) like indole acetic acid (IAA), gibberellic acid (GA) *etc*. in addition to solubilizing insoluble phosphates. However, in problematic soil (acidic, saline and alkaline), efficiency of biofertilizers decreases, thus application of biofertilizers becomes inefficient (Srinivasan *et al*., 2014; Anon., 2015). But the

salt-tolerant or halophilic soil microorganisms that exhibit the ability to solubilise insoluble P facilitate the development of saline-alkali soil-based agriculture (Zhu *et al*., 2011; Srinivasan *et al*., 2012a). Although availability of MPS microorganisms isolated from problem soils suitable for acid and salt-affected soils is scarce. Therefore, this study was carried out to develop mineral phosphate solubilising biofertilizers suitable for fodder production with reduced P fertilizer application in acid and normal soils.

Materials and Methods

***Sample collection and isolation of PSMs*:** About 145 rhizosphere and non-rhizosphere soil samples (0-15 cm depth) from the salt-affected areas of Haryana (Karnal), and West Bengal (Kolkata, Sundarbans), acid soils from Himachal Pradesh (Palampur district), Kerala (Vellayani, Trivandrum, Mancompu, Alleppey, Kumarakom, Vytella, Calicut), Karnataka (Madikeri, Appangala, Puttur, Sirsi, Hubli, Dharwad) were collected and used for isolation of PSMs. The soil samples were analyzed for pH, electrical conductivity (EC), exchangeable sodium percentage (ESP) following standard procedures (Jackson, 1973; Baruah and Barthakur, 1997). Collected soil samples representing crop plants and grasses from different regions of India were then analysed for total bacterial, total fungal populations and total phosphate solubilising bacterial and fungal populations. Isolation of PSMs was carried out by standard serial dilution and plating method using Pikovskaya's media (Pikovskaya, 1948). Both phosphate solubilising bacterial (PSB) and fungal (PSF) colonies forming solubilising zone around them were picked, purified and preserved for further studies.

Screening for phosphate solubilising activity and PGPS production: Individual PSM isolates (1.0 ml overnight grown culture of PSB and 3-day-old homogenized culture of PSF) were inoculated in 50 ml Pikovskaya's broth in four replicates with an equal number of uninoculated controls. The flasks were incubated on an orbital shaker at 150 rpm at 28±2°C for 10 days. The amount of Pi released in the broth in duplicate flasks was estimated at 5 and 10 days after inoculation (DAI) in comparison with a set of uninoculated controls. The broth cultures of PSB were centrifuged at 8,000 rpm for 20 min in a refrigerated centrifuge (REMI, India) to separate the supernatant from the cell debris and insoluble phosphate. In the case of fungal isolates, the cultures were filtered through Whatman No. 1 filter paper and the filtrate was used for estimation of Pi released. The available P content in the supernatant/ filtrate was estimated using the phosphomolybdic blue color method (Jackson, 1973). The pH of the supernatant was also recorded using a digital pH meter. Simultaneously, PSMs were spotted on Pikovskaya's agar plates and size of solubilizing zones was measured. PSMs were also screened for indole acetic acid (IAA; Gordon and Weber, 1951) and Gibberellic acid (GA; Holbrook *et al*., 1961; Tien *et al*., 1979) production by colorimetric methods.

Screening for acidity and salt tolerance of PSB and PSF isolates: Ability of selected PSB and PSF isolates to tolerate abiotic stresses *viz*., salt and acid tolerances were studied *in vitro*. The isolates were exposed to stress and growth in the presence of different concentrations of NaCl and in different acidic pH was examined separately by estimating growth. The overnight grown bacterial cultures were inoculated into 50 ml LB broth containing 0.5 (control), 2, 4, 6, 8 and 10% NaCl and pH 7, 6, 5 and 4 and incubated at room temperature. The growth was observed at an interval of 24 h up to three days by measuring the cell density at 600 nm wavelength using double beam UV-vis spectrophotometer (A_{600}). Similarly, three days old fungal cultures were homogenized and inoculated into 50 ml of potato dextrose broth containing 0.5 (control), 2, 4, 6, 8 and 10% NaCl and incubated at 28 ± 2 °C for seven days. After incubation the cultures were filtered through pre-weighed Whatman No. 1 filter papers and dried to constant weight at 60 °C in a hot air oven. The dry mycelial weight was then recorded for each culture.

Seed treatment and evaluation of PSMs for fodder crop growth: Seed inoculation is a suitable technique in establishing the inoculated microorganisms in the immediate vicinity of the emerging roots after germination. Charcoal based PSM formulations were prepared and fodder sorghum, oat and cowpea seeds used in this study were treated with bioformulation and dried in shade and were used for sowing in pots and fields.

Ten each PSF and PSB isolates were tested for their plant growth promoting potential of different fodder crops in pots with 50% P in three soils *viz*., normal soil (pH 7.1; EC 0.3 dS/m), acid soil (pH 5.6; EC 1.2 dS/m) and saline-alkali soil (pH 8.8; EC 4.8; ESP 15.8). A field experiment (with selected five each PSF and PSB isolates) was also conducted in two soils at two locations (normal soil: Central Research Farm, ICAR-IGFRI, Jhansi and acid soil: University Farm, AAU, Jorhat) in randomized block

design with 3 replications and 30 m^2 plot size. For field evaluation, fodder cowpea (*cv*. BL-2) was used at a seed rate of 35 kg/ha with recommended dose of fertilizer (RDF) of NPK @ 20:60:30 kg/ha. The treatments details were T_1: Control [Uninoculated (UIC) + unfertilized]; T_2: RDF (UIC + 100% RDF); T_3: PSF12(1); T_4: PSF47(1); T_5: PSF48(3); T_6: PSF48(4); T_7: PSF131(1); T_8: PSB9a(2); T_9: PSB26(2); T_{10}: PSB68(3); T_{11}: PSB103(1) and T_{12}: PSB136(1). But treatments T_3 to T_{12} had PSM inoculated seeds with 100% of N and K, and 50% of recommended dose of P. Growth parameters of cowpea plants *viz*., shoot and root length, fresh and dry weight, number of branches, plant height and nodule count were observed.

***Statistical analyses*:** Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used to compare the differences between mean values for each attribute using statistical software WASP 2.0.

Results and Discussion

Characterization and isolation of PSMs: All 145 rhizosphere and non-rhizosphere soil samples collected from Himachal Pradesh, Haryana, Uttar Pradesh, Kerala, Karnataka and West Bengal were used for isolation of phosphate solubilising microorganisms (bacteria and fungi). All soil samples from HP were acidic and 90% samples from Haryana were sodic, saline to saline-alkaline in nature. Total bacterial population ranged from $0.4x10^8$ - $32x10^8$ CFU/g and fungi population from $0.1x10^4$ - $80x10^4$ CFU/g, PSB from $5x10^4$ - $301x10^4$ CFU/g and PSF from $1x10^4$ - $38x10^4$ CFU/g soil. In 2 soil samples, PSB was either absent or very low to count, while PSF was either absent or very low to count in 16 samples. About 84% soil samples collected from Kerala and Karnataka belonged to acid soils. Total bacteria ranged from $1x10^7$ - $138x10^7$ CFU/g and fungi from $1x10^4$ - $38x10^4$ CFU/g, PSB from $5x10^4$ - $322x10^4$ CFU/g and PSF from $1x10^4$ - $80x10^4$ CFU/g soil. PSB was either absent or very low to count in two samples, while PSF was in 6 soil samples. About 78% samples from West Bengal were salt affected soils. Total bacterial population ranged from 72.5 x 10^8 - 126.5 x 10^8 CFU/g and fungi from $1x10^4$ - $41x10^4$ CFU/g, PSB from $6.5x10^4$ - $109x10^4$ CFU/g and PSF from $0.5x10^4$ - $31x10^4$ CFU/g soil. These findings were in line with the earlier reports of Pal (1998) and Srinivasan *et al.* (2012a). After counting population, both bacterial (70 PSB) and fungal (90 PSF) colonies forming solubilising zone around them were isolated, purified and preserved. Isolation of PSB from acid soil was also reported by Pal (1998), from alkaline soil by Nautiyal *et al.* (2000), halophilic bacterium from Daqiao saltern of China (Zhu *et al.*, 2011), both PSB and PSF from salt affected soils (Srinivasan *et al.*, 2012a). Kawai *et al.* (2000) reported even higher numbers of acid-tolerant microorganisms from the tea field (pH of soil, 3.3-3.9) when compared to fields of wheat, barley, rice or vegetables (pH of soil, 6.0-6.2).

Screening for phosphate solubilising activity and PGPS production: All 160 isolates were screened for mineral phosphate solubilising (MPS) activity in Pikovskaya's agar plates. About 26 isolates had more than 5 mm zone of solubilization, ten isolates had more than 15 mm zone of solubilization size after 3 days incubation. Similar phosphate solubilising activity was previously reported (Park *et al.*, 2010; Rahman *et al.*, 2017). Screening for P solubilisation was conducted at 5 DAI and 10 DAI intervals in Pikovskaya's medium with tricalcium phosphate and Udaipur rock phosphate as P source. Among the tested isolates, 20 isolates had more than 5 mg/ml solubilising activity. PSB (58%) and PSF (92%) isolates produced acids and lowered the pH of growth medium from neutral to 3.7-4.2 after three days of inoculation. The acidity remained stable in the medium for up to 10 days. The reduction in pH from neutral clearly indicated the production of organic acids, which is considered to be responsible for P solubilization. It was suggested that microorganisms which decrease the medium pH during growth are efficient P solubilizers (Kpomblekou and Tabatabai, 1994). Deepa *et al.* (2010) also reported that P-solubilizing activity was coincided with a concomitant decrease in pH of the medium (pH 7.0 - < 3.0). IAA produced by PSB ranged from 6.4 to 41.9 µg/ml and the maximum was recorded by PSB136(1), while IAA produced by PSF ranged from 9.1 to 32.6 µg/ml. Rahman *et al.* (2017) also observed a range of 10.90 to 63.35 µg/ml IAA production by PSB isolates. Similar trends were also observed earlier (Deepa *et al.*, 2010; Gupta *et al.*, 2012; Mohan *et al.*, 2017). GA produced by selected PSB ranged from 1.1 to 8.5 µg/ml and by PSF from 2.4 to 11.2 µg/ml. Vikram *et al.* (2007) reported that all 30 PSB isolated from the crops grown in vertisols were able to produce both IAA and GA and the concentration ranged from 1.1 to 28.0 and 0.6 to 9.8 µg/25 ml of broth, respectively. Saleemi et al. (2017) also reported that all seven selected PGPR exhibited indole acidic acid production, whereas five isolates produced gibberellic acid ranging from 5.5 to 30.6 and 10.0 to 14.8 mg/L, respectively.

Screening PSMs for abiotic stress tolerance: Ten isolates each of PSB and PSF were selected based on

their phosphate solubilizing activity and IAA and GA production to screen for abiotic stress tolerance. It was performed by quantifying the growth in medium with different acidity levels and salt concentrations. All the ten PSB isolates were capable of growing in acidic pH up to 4, but there was decline in the cell density at pH 4 as compared to neutral pH (Fig 1). PSB26(2), PSB9a(2) and PSB136(1) shown increased growth at pH 6, PSB26(2) and PSB136(1) at pH 6 and 5 and PSB136(1) at pH 6, 5 and 4 than pH 7. Assessment of acid tolerance of various PSB strains was also reported by Pal (1998), where growth of all the tested PSB strains in acidic pH (5.4-5.6) was observed with varied growth rates, but the strain PAS-2 isolated from waste land of pH 4.8 had highest acid tolerance.

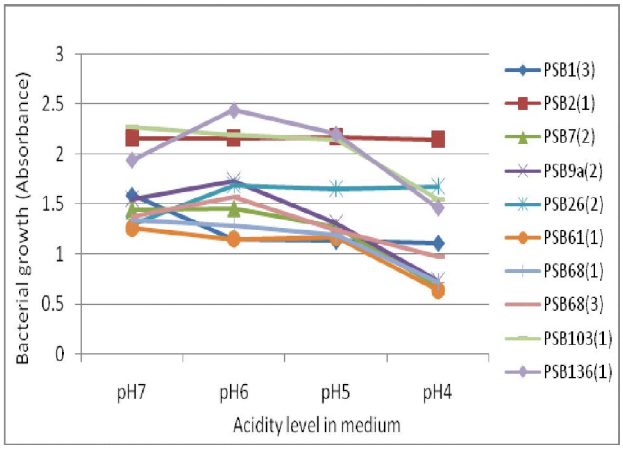

Fig 1. Screening of PSB growth at different acidity levels

All the 10 PSB isolates had shown growth in presence of all tested salt concentrations with decline in the cell density towards increasing salt concentration in eight PSB isolates (Fig 2). PSB103(1) recorded maximum cell density at 2% NaCl than control (0.5%). PSB2(1) and PSB103(1) had cell density at 2 and 4% NaCl equal to control. Mohan *et al*. (2017) also observed growth of PSB isolates up to 7% NaCl. Mendpara *et al.* (2013) reported that out of six isolates, two (GSD1-*Exiguobacterium* sp. and GSD2-*Serratia* sp.) showed salt tolerance up to 10% NaCl concentration. Srinivasan *et al.* (2012b) reported that among 12 PSB, *Aerococcus* sp. strain PSBCRG$_1$-1 recorded highest (12.15) log viable cell count at 0.4 M NaCl concentration after 7 DAI and the lowest log cell count (1.39) was recorded by *Pseudomonas aeruginosa* strain PSBI$_3$-1 at 2.0 M NaCl concentration after 24 h of incubation. Four phosphate solubilizing *Enterobacter* isolated from non-rhizospheric soil from Western Ghats exhibited growth at a wide range of pH 6-12, but it was optimum at pH 7.0 and tolerated up to 7% (w/v) salt concentration (Deepa *et al.*, 2010).

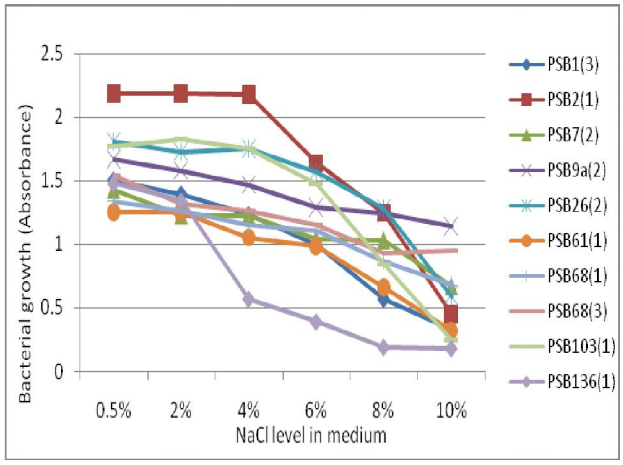

Fig. 2. Screening of PSB growth at different salt concentration

All ten PSF isolates were found to grow up to pH 4.0. In general, there was a reduction in the growth with increase in acidity (Fig 3), although PSF 29(1) recorded maximum growth (2.82 cm) at pH 4.0. About 50% isolates showed gradual decline to increasing acidity and they were considered more tolerant. Whereas PSF48(5) and PSF48(4) showed sharp decline beyond pH 5 were considered sensitive to acidity. Assessment of acid tolerance of PSB strains were also reported earlier (Pal, 1998). Kawai *et al.* (2000) reported that fungi and yeasts isolated from acid soils grew in a wide pH range of 2.5-7.0. F-13 (*Penicillium janthinellum*) and F-15 (*Trichoderma asperellum*) showed good growth even at pH 2.2.

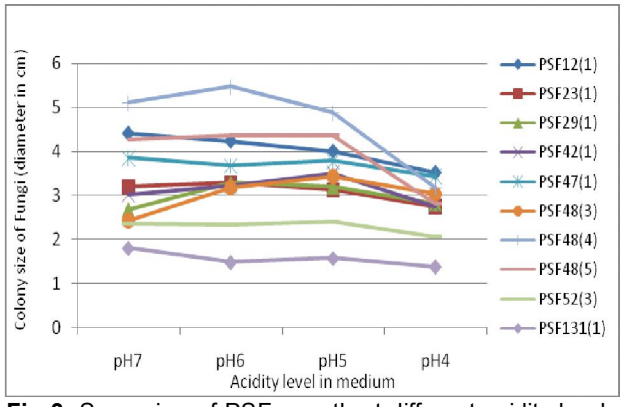

Fig 3. Screening of PSF growth at different acidity levels

Similarly, all ten PSF had shown growth up to 10% NaCl concentration in medium with decrease in size at higher salt concentrations (Fig 4). The maximum growth of all PSF was recorded at 2% salt stress except PSF 29(1) and PSF42(1) which recorded at 4% salt stress. Srinivasan *et al.* (2012b) observed the growth of PSF isolates up to 1.0M NaCl concentration, however, there

was a reduction in mycelial dry weight of fungi with increase in concentration of NaCl, and the reduction was significant at 0.8 and 1.0 M concentrations. New *et al.* (2013) found that among 12 soil yeasts isolates, four isolates (I1, I2, I3 and I4) tolerated NaCl up to 14% in plate screening.

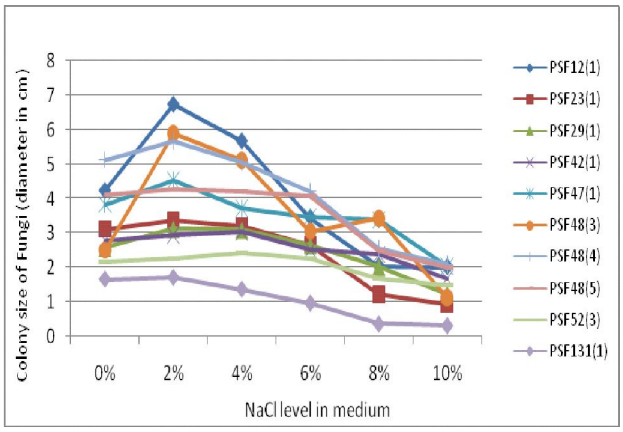

Fig 4. Screening of PSF growth at different salt concentration

Evaluation of PSF isolates for oat production in vitro: Ten PSF isolates were tested in normal soil, acid soil and saline-alkali soil to enhance biomass of fodder oats (*var.* JHO 822) with 50% reduced P fertilizer of RDF with the aim to save 50% P fertilizer application. These cultures were used as charcoal based bioinoculants and seeds were inoculated and sown in pots to test their influence on production of oats in three soil types separately (Table 1). Highest dry fodder weight was recorded at RDF treatment in case of normal soil (21 g) followed by PSF48(5), PSF42(1) and PSF47(1) which were statistically at par with RDF. In saline-alkali soil, the maximum dry fodder weight (16.6 g) was produced by PSF52(3) followed by PSF131(1), RDF, PSF42(1) and PSF48(5) which were significantly superior over all other treatments. Whereas in acid soil, PSF48(4) recorded the highest dry matter (22.4 g) production, followed by PSF29(1), PSF-48(3), PSF47(1) and PSF48(5) which were at par among themselves and with PSF48(4). Maximum number of seeds per plant produced and seed weight per pot were recorded in 100% RDF NPK treatment in normal (53.7 and 26.3) and saline-alkali (51.7 and 19.7) soil (Table 1). However, three PSF isolates in normal [PSF23(1), PSF47(1) and PSF48(3)] and three in saline-alkali soil [PSF48(4), PSF52(3) and PSF131(1)] produced number of seeds at par with RDF. In case of seed weight, PSF12(1), PSF23(1) and PSF47(1) in normal soil and PSF29(1), PSF48(4) and PSF48(5) were at par with RDF. All these treatments were significantly superior to control. Whereas, in acid soil PSF47(1) and PSF48(4) recorded significantly highest seed count (41.4 and 41.2) which was at par with RDF (35). The differences in seed weights recorded in acid soil were non-significant. Zhu *et al.* (2012) also reported that *Pichia farinose* strain FL7 spent mushroom substrate based biofertilizer significantly improved the whole dried plant weight of soybean in pot experiments.

Table 1. Evaluation of PSF for Oat production in normal and problem soils with reduced P *in vitro*

Treatments	Dry fodder weight (g/pot)			Seed count (No./plant)			Seed weight (g/pot)		
	Normal soil	Acid soil	Saline-alkali soil	Normal soil	Acid soil	Saline-alkali soil	Normal soil	Acid soil	Saline-alkali soil
Control	12.2d	15.0bc	8.5c	26.2d	30.5cd	25.3c	14.2f	15.0	12.0c
RDF (NPK)	21.0a	13.5bc	15.7a	53.7a	35.0abcd	51.7a	26.3a	20.8	19.7a
PSF-12(1)	15.7bcd	14.1bc	13.2ab	43.2bc	30.6cd	33.2bc	21.9ab	15.1	13.2bc
PSF-23(1)	15.6bcd	14.4bc	14.1ab	47.9ab	38.3ab	35.4bc	21.5abc	19.9	12.0c
PSF-29(1)	14.3cd	18.6ab	12.4abc	37.9c	38.9ab	36.3bc	14.4ef	19.5	16.9ab
PSF-42(1)	19.3ab	16.0bc	15.3a	41.7bc	30.7d	36.2bc	15.7cdef	17.3	14.1bc
PSF-47(1)	18.2abc	17.8abc	10.7bc	47.5abc	41.4a	36.5bc	20.8abcd	17.2	14.6bc
PSF-48(3)	16.0bcd	18.3abc	10.2bc	46.1abc	37.3abc	38.7b	20.1bcde	15.8	13.5bc
PSF-48(4)	15.6bcd	22.4a	14.2ab	38.9bc	41.2a	42.3ab	16.6bcdef	17.1	16.2ab
PSF-48(5)	19.5ab	17.6abc	15.1a	39.5bc	33.8bcd	36.8bc	15.0def	18.5	16.9ab
PSF-52(3)	15.7bcd	13.3c	16.6a	42.3bc	39.5ab	41.3ab	15.4def	16.0	13.7bc
PSF-131(1)	15.4bcd	14.3bc	16.5a	38.6bc	40.4ab	45.3ab	18.9bcdef	17.6	15.2bc
SEm	1.6	1.7	1.1	3.3	2.5	4.3	2.0	1.5	1.3
CD (0.05)	4.7	5.1	4.4	9.7	7.3	12.6	5.8	NS	3.8

Means bearing different superscripts in a column differ significantly (P<0.05)

Table 2. Evaluation of PSB for fodder sorghum production in normal and problem soils with reduced P *in vitro*

Treatments	Plant height (cm)			Green fodder yield (g/pot)			Dry fodder yield (g/pot)		
	Normal soil	Acid soil	Saline-alkali soil	Normal soil	Acid soil	Saline-alkali soil	Normal soil	Acid soil	Saline-alkali soil
Control	190.8d	203.0c	165.8e	123.5c	122.5e	106.8e	43.3f	47.3e	34.5e
RDF (NPK)	221.0ab	220.5abc	218.7a	142.2abc	158.7bcd	142.3b	50.0cdef	59.5bcd	47.5bc
PSF-12(1)	210.5bc	227.0ab	202.8abc	135.8bc	147.8d	168.2a	46.3ef	51.8de	55.1ab
PSF-23(1)	215.8abc	231.3ab	182.7cde	131.1bc	147.8d	112.5de	44.8f	59.3bcd	43.5cd
PSF-29(1)	224.3a	234.7ab	208.7ab	136.7bc	153.5cd	121.2de	47.8def	62.7bc	41.5cde
PSF-42(1)	222.5a	240.5a	219.0a	148.7ab	176.3abc	182.7a	57.3abc	67.2abc	56.6a
PSF-47(1)	210.2bc	235.5ab	184.3cde	162.5a	183.7ab	108.2e	63.6a	65.0abc	35.8de
PSF-48(3)	215.0abc	230.5ab	200.2abcd	142.0abc	151.0d	126.3cd	46.3ef	51.9de	40.5cde
PSF-48(4)	218.8ab	216.7bc	180.2de	150.2ab	155.0cd	114.3de	53.3bcde	58.5cd	33.9e
PSF-48(5)	214.8abc	235.0ab	191.5bcd	152.3ab	162.0bcd	137.2bc	58.2ab	63.7abc	42.7cd
PSF-52(3)	210.2bc	223.7ab	183.2cde	153.2ab	193.8a	122.3cde	54.7bcd	72.1a	40.3cde
PSF-131(1)	206.7c	218.7bc	193.3bcd	164.7a	188.2a	115.3de	61.2ab	68.2ab	35.9de
SEm	3.9	6.9	7.2	7.9	8.6	5.4	2.8	3.1	2.8
CD (0.05)	11.4	20.2	21.03	22.9	25.2	15.8	8.1	9.0	8.1

Means bearing different superscripts in a column differ significantly (P<0.05)

Table 3. Field evaluation of PSMs for fodder cowpea (*cv* BL-2) production in normal soil

Treatments	GFY (t/ha)	DFY (t/ha)	Plant height (cm)	Seed yield (q/ha)	Root length (cm)	Nodule count	Nodule fresh weight (g/plant)	Nodule dry weight (g/plant)
T_1	14.90d	4.17d	66.3	2.15f	18.2	31.9	0.45g	0.07f
T_2	20.23abc	5.57abc	72.5	2.44ef	24.9	32.5	0.53fg	0.08f
T_3	17.43cd	4.73cd	85.1	2.53def	25.2	40.9	0.59efg	0.11e
T_4	18.63cd	4.83cd	92.8	2.76cdef	23.6	47.0	0.95b	0.18bc
T_5	24.00ab	6.10ab	82.6	3.47abc	23.0	40.8	0.95b	0.17bcd
T_6	21.23abc	5.47abc	79.0	2.80cdef	22.8	40.7	0.94bc	0.17cd
T_7	19.23bcd	4.87cd	85.5	3.78a	23.0	32.7	0.72def	0.15d
T_8	18.23cd	4.57cd	82.6	3.25abcd	21.0	44.2	0.73de	0.15d
T_9	18.80cd	4.70cd	85.1	3.60ab	22.9	43.2	0.83bcd	0.19b
T_{10}	20.90abc	5.27abc	74.8	2.78cdef	22.6	52.2	1.15a	0.28a
T_{11}	19.53bcd	5.07bcd	74.9	3.31abcd	22.7	39.3	0.76cde	0.16cd
T_{12}	25.23a	6.27a	88.1	2.94bcde	24.7	42.7	0.61efg	0.15d
SEm	1.74	0.37	3.7	0.27	1.32	4.6	0.06	0.01
CD (0.05)	5.094	1.1	NS	0.791	NS	NS	0.189	0.02

Means bearing different superscripts in a column differ significantly (P<0.05)

Evaluation of PSB isolates for fodder sorghum production in vitro: Ten PSB isolates were evaluated in above three soils for biomass production of fodder sorghum (*var*. MP chari) in pots (Table 2). The germination percentage was found better in PSB treatments as compared to control. PSB7(2) and PSB9a(2) in general recorded maximum plant height and stem girth in all normal, acid and saline-alkali soils. All the PSB of salt-affected soil origin increased shoot length and root length of sorghum plants significantly over the reference bacterial strain (*P. striata*) and control in pots in salt-affected soil (Srinivasan *et al*. 2012a). However, PSB136(1) and PSB103(1) in acid soil, PSB1(3) and PSB9a(2) in saline-alkali soil, PSB26(2) and PSB136(1) in normal soil recorded significantly higher green fodder yield and dry matter yield. PSB strains JY17 (*Bacillus aryabhattai*) and JY22 (*B. aryabhattai*) showed strong phosphate-solubilizing activity under stress conditions of high pH, high salt and high temperature, and their inoculation significantly facilitated the growth of *V. vinifera* (*cv.* Cabernet Sauvignon) under greenhouse conditions and these two isolates can be used as biofertilizers in

saline-alkaline soils (Liu *et al*., 2016). Srinivasan *et al.* (2012a) reported that all PSMs of salt-affected soils except *P. striata* and one PSB isolate *Pseudomonas* sp. PSBI3-1, recorded a significant increase in the total dry matter content of sorghum plants in salt-affected soil. Dual inoculation with AMF (*Glomus manihotis*) and PSB (*Pseudomonas* sp.) resulted in highest growth of sorghum plants, increased total dry weight of sorghum 112 times (372%) compared to the uninoculated treatment in acid soil (Widada *et al.,* 2003).

Field evaluation of PSMs for fodder cowpea* (*cv* BL-2) *production in normal soil: Among the 10 PSMs evaluated for fodder cowpea (*cv* BL-2) production in normal soil, T_{12} [PSB136(1)] recorded maximum GFY and DFY (25.23 and 6.27 t/ha) followed by T5 (24.0, 6.1), T_6 (21.23 and 5.47) and T_{10} (20.9 and 5.27 t/ha) which were at par with RDF (20.23 and 5.57 t/ha). However maximum seed yield (3.78 q/ha) was recorded by T_7 [PSF131(1)]. Treatments were non-significant for the plant height, root length and nodule count. Maximum number of nodules was recorded at T_{10} [PSB68(3)], which was also significantly superior in nodule fresh weight and dry weight over other treatments. Deepa *et al.* (2010) demonstrated that PSB inoculation resulted in significant increment in root and shoot biomass and bacterial counts in the rhizosphere of cowpea under greenhouse conditions. It was suggested that PSMs can be used with or without insoluble P sources (like rock phosphates) for few years since soil has enough supply of insoluble P for solubilisation. Soybean plant growth was improved by using PSM biofertilizer with or without insoluble P, indicating PSM could be used alone with high efficiency without extra insoluble P (Zhu *et al.,* 2012). Application of poultry manure (PM) and PSB with rock phosphate (RP) in 1/2 RP+1/2 PM + PSB combination showed a remarkable effect and induced growth, yields and P uptake comparable to that recorded under the DAP treatment in chilli (Abbasi *et al*., 2016). However, some isolates performed differently in broth medium, pot culture evaluation and field evaluation. This might be due to differential adaptability to variations in the environment. PSB isolates UAGC 17, 19 and 65 were the best solubilizers in culture media; but they did not demonstrate the same efficiency when inoculated on cowpea (Souza *et al.,* 2016).

Field evaluation of PSMs for fodder cowpea* (*cv* BL-2) *production in acid soils: The same 10 PSMs were also evaluated for cowpea production in acid soil. T11 [PSB103(1)] recorded maximum GFY and DFY (35.2 and 8.1 t/ha) followed by T12 [PSB136(1): 35.1 and 8.08], T3 [PSF12(1): 34.4 and 7.92] and T9 [PSB26(2): 33.5 and 7.7 t/ha, respectively] which were at par with RDF (32.0 and 7.4 t/ha). These PSMs also recorded higher crude protein yield and CP% which were at par with RDF and significantly more than control (7.8 q/ha and 12.7%, respectively). Four PSMs recorded plant height at par with RDF. Similar results reported in different crops corroborated with the present findings. Application of phosphate solubilizing microbes significantly improved the yield of maize in Ultisol Jatinangor, but did not significantly affect on soil P, available soil P, phosphatase and P uptake of plants (Fitriatin *et al*., 2014). Application of a mixture of PSB and PSF gave better effect on soil available P and yield of maize. PSM with 50% phosphate fertilizer gave better effect on soil P and yield of maize (Fitriatin *et al*., 2014). Young *et al.* (1990) reported inoculation of PSM in two sub-tropical-acidic soils (Hualain soil: pH 4.2 and Yuanchang soil: pH 5.4) of Taiwan significantly enhanced peanut yield (by 20 and 73%, respectively) over control, which were at par with rock phosphate application @ 660 kg/ha. They also reported 24% increased growth of leucaena in Hinshe acidic soil (pH 5.0). Application of PSM biofertilizer *Pseudomonas cepaceae, P. mallei*, *Aspergillus niger* and *Penicillium* sp. increased soil phosphate and yield of maize on Ultisols. Indeed, solid carrier biofertilizers increased available soil P higher than liquid biofertilizers (Fitriatin *et al*., 2017). Kongpun *et al.* (2011) reported that cowpea growing in low P acidic soil was directly benefited from the local arbuscular mycorrhizal fungi. The cowpea line Ubon Ratchathani was grown in acidic (pH 5) and non acidic (pH 6.7) soils with and without AMF inoculation and total dry weight of inoculated cowpea was not affected by soil acidity whereas it was depressed in un-inoculated plants. Singh *et al.* (2011) reported from field study that the PSB isolate RPB3 helped to proliferate lentil field emergence, root length, plant height, branching, nodulation number, pod per plant, number of seed per pod and plant, l000-seed weight and finally seed yield by 15.5, 41.4, 23.0, 35.3, 47.7, 23.9, 5.3, 31.0, 16.5 and 30.3 per cent, respectively over control in an acid soil. The differences between isolates in their ability to colonize and perform in acid soil could be readily related to their inherent acidity tolerance. The PSM isolated from soils of higher pH ranges failed to show their beneûcial effect at lower pH ranges, the mechanism of which needs to be investigated further. The PSB isolated from soils of higher pH ranges (6.2- 7.1) also failed to show their beneûcial effect at lower pH ranges (Pal, 1998). However, the response of seed inoculation

Table 4. Field evaluation of PSMs for fodder cowpea (*cv* BL-2) production in acid soils

Treatments	Plant height (cm)	GFY (t/ha)	DMY (t/ha)	CP (%)	CP yield (kg/ha)
T_1	135.9c	26.8bcd	6.15bc	12.67e	781.4cd
T_2	157.2a	32.0abc	7.36ab	13.60d	1002.3abc
T_3	166.9a	34.4ab	7.92ab	14.37ab	1137.9ab
T_4	132.2c	27.1bcd	6.23bc	13.97bcd	868.3bcd
T_5	149.6abc	30.2abc	6.95ab	13.97bcd	970.4abc
T_6	136.6c	28.2abcd	6.49abc	14.20abc	923.7abcd
T_7	137.6bc	26.5cd	6.11bc	14.47ab	885.5bcd
T_8	130.7c	21.7d	5.00c	13.77cd	688.0d
T_9	156.0ab	33.5abc	7.70ab	14.60a	1125.1ab
T_{10}	157.8a	20.8d	4.79c	13.60d	653.7d
T_{11}	156.9a	35.2a	8.10a	14.57a	1178.6a
T_{12}	158.0a	35.1a	8.08a	14.57a	1178.5a
SEm	6.6	2.7	0.62	0.19	92.7
CD (0.05)	19.25	7.88	1.81	0.56	271.9

Means bearing different superscripts in a column differ significantly (P<0.05)

in accentuating vegetative and grain yield, and improvement of phosphate nutrition was variable in different crop genotypes. This might be due to differential rhizosphere effect of crops (Alexander, 1983; Gaind and Gaur, 1991).

PSMs also showed additive beneficial effects when co-inoculated with other plants friendly microorganisms. In acidic sandy loam soil (pH of 5.2), dual inoculation of PSB + *Rhizobium* with fertilizer (P_2O_5) significantly increased plant height, number of green leaves, branching, plant biomass, green pod yield, soil available N, P, K status and root nodulation (Heisnam *et al.* 2017). Nadeem *et al*. (2017) also reported that combined inoculation of seed with *Rhizobium* + PSB along with 40 kg P/ha significantly increased the stem girth (1.84 cm), total dry matter (13.91 g/plant), green pod yield (196.4 g/ plant and 120.9 q/ha), soil nutrient status in acid soil (pH 6.2) over rest of treatment combinations.

Conclusion

It was concluded that cultures *viz*. PSB136(1), PSF48(3), PSF48(4) and PSB68(3) for normal soil and PSB103(1), PSB136(1), PSF12(1) and PSB26(2) for acid soil, could be used as suitable phosphate solubilising bioinoculants with 50% reduction in phosphatic fertilizer application without compromising yield as well as quality in fodder crops.

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