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Improving growth and yield of maize through bioinoculants carrying auxin production and phosphate solubilizing activity

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Abstract

In this study, effect of three rhizobacteria, Bacillus megaterium (K2), Bacillus subtilis (K4) and Bacillus sp. Cp-h6 (K6) varying in P solubilization, ACC deaminase activity and auxin production were evaluated for promotion of shoot and root growth, yield and P uptake by maize plant under different P sources (control, rock phosphate and DAP). All the three strains used in this study had different phosphate solubilization potential but as phosphate solubilization potential increased from K2 to K6 (from 180 to 698 µg mL^{-1}), the auxin production potential decreased accordingly (from 48.1 to 8.2 µg mL^{-1}). Bacillus sp. Cp-h6 showed more enhancement effect on the tested parameters compared to Bacillus megaterium and Bacillus subtilis with all three P sources. Furthermore, in uninoculated plants, DAP was more effective than rock phosphate, but DAP response (percent increase) was least under inoculation. Maximum increase was recorded in plant parameters where rock phosphate and bioinoculants were applied together. In pot study, shoot length of maize plants increased from 89.8 to 125 cm with DAP (39% increase) while with rock phosphate, it increased from 49 to 97.7 cm (99% increase) through inoculation. Likewise, percent increase over uninoculated control caused by the bacterial inoculation in shoot dry matter was 77 and 134 in pot study while 26 and 65 under field conditions with DAP and rock phosphate, respectively. Similarly, substantial increment in the root dry matter yield, root length, cob weight, kernel yield, P uptake in stover and P uptake in kernel were recorded with inoculation of rhizobacterial strains. In conclusion, rock phosphate could be effectively used as P source in the presence of these bioinoculants, particularly Bacillus sp. Cp-h6. The bioinoculant could be used for developing biofertilizer for field application to improve yield and quality of maize kernels.

Keywords: *PGPR, Bacillus, P sources, Zea mays, stover*

Introduction

During the last couple of decades, the work on utilizing plant growth promoting rhizobacteria (PGPR) for sustainable agriculture has increased tremendously. These PGPR are known to enhance plant growth through different mechanisms such as synthesis of plant growth promoting substances (auxin, cytokinins, gibberellins, absicic acid and ethylene), phosphate solubilization, biological N_2 fixation, siderophore production, ACC deaminase activity, stabilization of soil aggregates, acting as biocontrol agents, cyanide production, chelating Fe required for pathogens and induction of systemic resistance in plants (Solano *et al.*, 2008a; Solano *et al.*, 2008b; Spaepen *et al.*, 2009; Lugtenberg and Kamilova, 2009). Among these, a group of PGPR, collectively known as PSM (phosphate solubilizing microbes), has been extensively studied to enhance bioavailability of phosphorus (P) in soil. In spite of good indigenous total soil P content, plant available fraction (1.0 mg kg^{-1}) is too low (Goldstein, 1994) to support normal growth and yield of plants. Phosphate solubilizing microbes (PSM) improve plant growth and productivity by improving bioavailable P in soil through solublization of indigenous soil P as well as reducing precipitation of applied fertilizer P. These PSM increase bioavilabe soil P content through production of various organic acids (Khan *et al.*, 2007), proton extrusion, microbial respiration and phosphatase activity (Jorquera *et al.*, 2008). Organic acids produced by PSM complex Ca, Fe and Al cations, lower soil pH and compete for adsorption sites with phosphate ions (Nahas, 1996; Stevenson, 2005). Similarly, another PGPR group has been investigated for its ability to have positive impact on plant growth because of productivity of secondary metabolites such as plant growth regulators in the

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rhizosphere for plant uptake (Arshad and Frankenberger, 1998). Numerous PGPR have been reported capable of producing auxin, an established plant hormone (Biswas *et al*., 2000; Erturk *et al*., 2010; Bal *et al*., 2013; Sukumar *et al*., 2013; Cassan *et al*., 2014). Studies have demonstrated that the PGPR can stimulate plant growth through the production of auxins (Erturk *et al*., 2010; Sadeghi *et al*., 2012). Likewise, during the recent years, several reports appeared in the published literature on PGPR, possessing ACC deaminase activity to improve plant growth because of their ability to suppress accelerated production of ethylene in plant roots (Saleem *et al.*, 2007; Arshad *et al.*, 2008; Shaharoona *et al.*, 2008; Zahir *et al.*, 2008; Baig *et al.*, 2012). This suppression of ethylene by PGPR helps plant to eliminate adverse impacts of high levels of ethylene in plant tissues. High concentration of ethylene severely reduces root elongation and increases pathogen infection (Glick, 2004).

In majority of the reported studies on PGPR, efforts have been focused on correlating the impact of PGPR to improve plant growth with any single growth promoting trait. However, it is highly likely that plant growth promotion in response to inoculation might be the result of collective impact of more than one growth promoting traits. This study is reporting the influence of PGPR possessing multiple growth promoting traits (P solubilizing, ACC deaminase activity, and auxin producing activity) on growth and yield of maize in the presence of rock phosphate (RP) and diammonium phosphate (DAP).

Materials and Methods

Isolation of rhizobacteria

For isolation, soil samples were collected from rhizosphere of maize and rhizobacteria were isolated by the dilution plate technique (Wollum II, 1982) using tryptic soy agar (TSA), a general purpose medium (Atlas, 2004). Thirty nine colonies exhibiteding prolific growth and distinguished morphologically were selected for streaking on fresh plates. Further, purification and multiplication of the isolates was done by streaking on fresh agar plates.

Characterization of selected isolates on the basis of plant growth promoting traits

Rhizobacterial isolates were characterized for phosphate solubilizing activity, ACC deaminase activity and auxin (indole acetic acid) production. For quantitative estimation of phosphate solubilizing activity, broth containing 20 mL of NBRIP medium taken in Erlenmeyer flasks (150 mL) was inoculated with isolates. Uninoculated broth was used as a control. The flasks were placed at 30 °C in shaking incubator at 180 rpm for two days. Supernatant was taken and phosphate content was determined using Ryan *et al.* (2001) protocol.

All the selected isolates (39) were tested for IAA (auxin) production using the protocol described by Khalid *et al.* (2004). ACC deaminase activity of rhizobacteria was tested according to modified methods of Honma and Shimomura (1978) and Penrose and Glick (2003).

Selection and identification of rhizobacteria

Out of 39 isolates, three rhizobacteria designated as K2, K4 and K6 were selected on the basis of phosphate solublizing activity, ACC deaminase activity and auxin production potential. Rhizobacterial isolates were identified by partial sequencing of the 16S rRNA gene as *Bacillus megaterium* (K2), *Bacillus subtilis* (K4) and *Bacillus* sp. Cp-h60 (K6). The 16S rRNA gene sequences were submitted to the GeneBank/EMBL/DDBJ databases and the accession numbers of the strains K2, K4 and K6 are AB6388886, AB6388888 and AB6388891, respectively. Interestingly, strain having highest P-solubilizing activity was found to carry minimum auxin production potential and vice versa.

Effect of selected rhizobacteria on maize

Axenic study

Growth promoting activity of selected rhizobacteria was assessed in a series of experiments such as jar experiment under axenic conditions, pot and field trials. To attain uniform population of isolates, optical density 0.6 (10^8-10^9 cfu mL^{-1}) of broth (inoculated) at λ 590 nm was developed. Surface disinfected seeds of maize were inoculated with the broth mixed with 10% sugar solution, peat and clay mixture. Peat to clay ratio was used as 1:1 w/w (Baig *et al*., 2012). The seeds were shaken well till fine coating appeared on seeds. Control was treated with sterilized peat plus clay mixed with sterilized broth and sugar solution. Inoculated seeds were placed over night for drying under lab conditions. Jar experiment was conducted to assess the potential of three selected rhizobacterial strains differing in phosphate solublizing activity, ACC deaminase activity and auxin production potential for improving growth of maize under axenic conditions. For this, sand was sieved through a 2 mm sieve, dipped in 5% HCl solution and washed thoroughly with distilled water. Glass jars were filled with 500 g sand and 1.0 g rock phosphate (33.4% P_2O_5) was applied as exclusive P source. After autoclaving at 121°C, three pre-germinated seeds of maize were transplanted to each jar. Seeds were inoculated with selected isolates while sterilized broth without inocula was used as uninoculated control. For providing nutrients to seedlings, sterilized Hoagland solution (without P) was

applied. Each treatment was replicated thrice using completely randomized design. Jars were placed in a growth chamber at 25±1 ^{o}C adjusted to 12 h light at relative humidity of 70%. Experiment was harvested after 3 weeks and data regarding root and shoot growth were recorded.

Pot trial

A pot experiment was conducted to evaluate the effectiveness of selected rhizobacteria for improving growth of maize in a net house of the University of Agriculture, Faisalabad, Pakistan. Seeds of maize inoculated with selected rhizobacteria were sown in pots containing soil at the rate of 12 kg pot^{-1}. The soil used in the study was sandy clay loam having pH 7.6; electrical conductivity, 2.2 dS m^{-1}; organic matter, 0.52%; total nitrogen, 0.05%; available P, 7.3 mg kg^{-1} and extractable K, 96.0 mg kg^{-1} soil. Nitrogen and K were applied at the rate of 110 and 60 kg ha^{-1}, respectively, in all treatments. Half dose of N was applied at the time of sowing while remaining ½ N was applied at the time of first irrigation. Recommended dose of P (85 kg ha^{-1}) was applied at sowing either as RP or DAP. Fertilizer treatments were NK (control), NP(RP)K and NP(DAP)K. Effect of these fertilizer treatments was investigated with and without inoculation of rhizobacteria (*Bacillus megaterium*, *Bacillus subtilis* and *Bacillus* sp. Cp-h60). Same amount of NK was applied to all treatments. Each treatment was replicated thrice. Experiment was harvested after two months of sowing and data regarding growth parameters were collected.

Field trial

Field trial was conducted on same soil used for pot study with the same treatments matrix. Treatments were applied according to RCBD factorial design in field with four replicates and were irrigated with canal water. At maturity, data regarding growth and yield parameters were collected. Phosphorus content in grain and stover samples was determined by using standard method.

Statistical analysis

Data collected in axenic trial were analyzed by applying ANOVA, using Satatistix 8 (Version 8.1, Copyright©, 1985-2005) while of pot and field trials, factorial ANOVA was applied (Steel *et al*., 1997). The means were compared by least significant difference using Duncan's multiple range test (Duncan's, 1955).

Results

Inoculation with rhizobacterial strains carrying multiple growth promoting traits resulted in better shoot growth (shoot length and shoot dry matter) than uninoculated plants. However, efficacy of these strains varied considerably under each fertilizer treatment (Table 1 and Table 2). All the strains showed very much promising results in both axenic and pot trials in promoting plant height and shoot dry matter (SDM) production, but strain K6 was most effective. Under axenic condition, increase in shoot length was 48, 72 and 89% with K2, K4 and K6 strain, respectively over uninoculated control in the presence of RP as an exclusive source of P. Similar response of these strains was observed in pot study (Table 1 and Figure 1). These strains improved shoot length under all three fertilizer treatments (control, DAP and RP).

Table 1: Relative efficacy of *Bacillus* spp. for improving shoots length (cm) of maize

Isolate	Shoot Length			
	Axenic study	Pot study		
	RP	NK	N P(DAP) K	N P(RP) K
Uninoculated control	21.2 (00) d	45.3 (0) h	89.8 (0) de	49.0 (0) h
Bacillus megaterium (180, 1.27, 48.1)*	31.4 (48)† c	51.0 (12) h	104.0 (16) bc	84.7 (73) e
Bacillus subtilis (572, 0, 19.5)	36.4 (72) b	61.0 (35) g	107.0 (19) b	87.3 (78) e
Bacillus sp. Cp-h60 (698, 1.85, 8.2)	40.0 (89) a	74.7 (65) f	125.0 (39) a	97.7 (99) cd

*Value in parenthesis show P-solubilizing activity (µg mL^{-1}), ACC deaminase activity (µmol L^{-1}) and auxin producing potential (µg mL^{-1}) of *Bacillus* spp., respectively; †% Increase over respective uninoculated control; LSD (Axenic study):1.6144, LSD (pot study): 8.2762

Although maximum shoot length was recorded with DAP but increase (%) over respective uninoculated control was more in case of RP (Table 1). In uninoculated plants, impact of RP application (insoluble source of P) on plant height was negligible, but it increased on combined application of inoculation and RP as plant height increased up to 99% over uninoculated control.

Data regarding shoot dry matter (SDM) yield indicates that SDM yield of maize increased in response to inoculation in axenic, pot and field experiments (Table 2). Under axenic conditions, inoculating the seeds with K2, K4 and K6 increased the SDM from 0.091g to 0.124, 0.139 and

0.155 g, respectively. Under pot conditions, in case of DAP, inoculation increased the SDM yield from 19.9 to 35.1 g pot^{-1}, whereas with RP it increased from 13.6 to 31.8 g pot^{-1}. Maximum increase of 134% was recorded with strain K6 in the presence of RP over uninoculated control. Further, it was observed that in case of uninoculated treatment, SDM yield was almost two third in the presence of RP than that recorded in case of DAP, but this gap in yield was reduced when seeds were inoculated with PGPR having multiple growth promoting traits. Similarly, under field conditions, without inoculation, SDM yield with RP was much less than DAP, but inoculation increased the SDM yield up to greater extent.



Figure 1: Relative efficacy of *Bacillus* spp. for improving growth of maize grown in pots amended with rock phosphate

Like shoot growth, PGPR inoculation also promoted root growth under both axenic and pot conditions. Each PGPR strain proved quite effective but with different degree of efficacy (Table 3). Root length incremented from a control of 9.2 to 14.4, 13.2 and 16.4 cm with inoculation of K2, K4 and K6 strain, respectively, under axenic conditions. In pot study, when P sources were compared, without inoculation, DAP was more effective in promoting root length (6 fold increase in root length over NK control) than RP. However, in case of PGPR inoculation, increase with RP (3.92 fold) was more than recorded with DAP (0.73 fold). Similarly, root dry matter (RDM) yield of maize also varied with source of P, inoculation and type of inoculant. Although, appreciable increase in RDM yield was recorded with all rhizobacterial strains (K2, K4 and K6), but inoculation of K6 was most effective. It was observed that in case of RP amended soil, response of inoculation was stronger in promoting RDM yield compared to that recorded in the presence of DAP. Effect of inoculation on root growth in the presence of RP is also very obvious in Figure 2. Strain K6 enhanced RDM yield up to 159 and 34% over respective uninoculated controls in the presence of RP and DAP, respectively. Further, it is noteworthy that with no P application, the effect of inoculation was promising as inoculation caused up to 198.3% increase in RDM yield over respective uninoculated control.

Table 2: Relative efficacy of *Bacillus* spp. for improving shoot dry matter yield of maize

Isolate	Shoot dry matter								
	Axenic study(g)			Pot study (g pot^{-1})			Field study (ton ha^{-1})		
	RP	NK	N P(DAP) K	N P(RP) K	NK	N P(DAP) K	N P(RP) K		
Uninoculated control	0.091 (0) d	12.3 (0) i	19.9 (0) f	13.6 (0) h	13.1 (0) g	20.33 (0) cd	14.3 (0) fg		
Bacillus megaterium* (180, 1.27, 48.1)	0.124 (35) † c	17.3 (40) g	25.3 (27) d	22.2 (64) e	15.0 (14.5) efg	22.1 (8.7) abc	19.7 (37.7) d		
***Bacillus subtilis* (572, 0, 19.5)**	0.139 (53) b	19.4 (58) f	28.8 (45) c	24.7 (82) d	15.7 (19.8) ef	22.8 (12.1) ab	19.8 (38.46) d		
***Bacillus* sp. Cp-h60 (698, 1.85, 8.2)**	0.155 (70) a	25.4 (106) d	35.1 (77) a	31.8 (134) b	16.86 (28.5) e	23.96. (17.8) a	21.1 (54.5) bcd		

* Value in parenthesis show P-solubilizing activity (µg mL^{-1}), ACC deaminase activity (µmol L^{-1}) and auxin producing potential (µg mL^{-1}) of *Bacillus* spp., respectively; †% Increase over respective uninoculated control; LSD (axenic study): 0.009728, LSD (pot study): 1.1769, LSD (field study): 2.2395



Bacillus megaterium Uninoculated control | *Bacillus subtilis* Uninoculated control | *Bacillus* sp. Cp-h60 Uninoculated control

Bacterial strains

Figure 2: Relative efficacy of *Bacillus* spp. for improving root growth of maize grown in pots amended with rock phosphate

Cob weight recorded in field trial revealed that selected PGPR promoted the parameter to a greater extent as evident from data presented in Table 4. It was noticed that K6 strain possessing high P solubilizing and ACC deaminase activity and low auxin production than others (K2 and K4), stood the most promising in enhancing cob weight. With K6 inoculation, cob weight was improved by almost 1.7, 1.4 and 2.0 fold with cotrol DAP and RP treatments, respectively, over respective uninoculted controls. Although cob weight was almost 2 times less with RP compared to DAP, but it is noteworthy that with inoculation, the results were comparable with DAP.

Data regarding the effect of inoculation on kernel yield of field grown maize plants supplied with three P sources (control, DAP and RP) is presented in Table 4. Strain K6 was more effective in promoting kernel yield than K2 and K4. It increased the yield up to 50 and 141% in the presence of DAP and RP over their respective uninoculated controls. With no P application, impact of K6 inoculation was also very promising; kernel yield was improved from 5.4 to 9.8 ton ha^{-1} (91% increase over respective control). Although, K2 and K4 enhanced kernel yield compared to uninoculated control but they were statistically at par in all three fertilizer treatments (Table 4).

Results of pot trial regarding the response of PGPR inoculation on P uptake in stover showed that all the three selected PGPR strains increased uptake in stover under all three fertilizer treatments over their respective uninoculated controls, but with varying degree of effectiveness (Table 5). K6 strain was the most potent while K2 was least effective. P uptake in stover of uninoculated plants was very low (5.80 mg pot^{-1}) under no exogenous P application. However, P uptake was increased up to 68.2 mg pot^{-1} with PGPR

Table 3: Relative efficacy of *Bacillus* spp. for improving root dry biomass and root length of maize grown under axenic conditions and pots

Isolate	Root Dry Matter				Root Length				
	Axenic study (g)	Pot study (g pot^{-1})			Axenic study (cm)	Pot study (cm)			
	RP	NK	N P(DAP) K	N P(RP) K	RP	NK	N P(DAP) K	N P(RP) K
Uninoculated control	0.080 (0) d	3.0 (0) k	16.3 (0) e	7.0 (0) i	9.2 (0) d	9.9 (0) h	37.1 (0) e	11 (0) h
Bacillus megaterium* (180, 1.27, 48.1)	0.098 (23)† b	5.3 (73) j	19.5 (20) b	14.3 (104) f	14.4 (57) b	20.2 (104) f	53.1(43) b	44.4 (303) d
***Bacillus subtilis* (572, 0, 19.5)**	0.092 (16) c	5.0 (56) j	18.1 (11) c	12.2 (74) g	13.2 (43) c	16.6 (67) g	47.8 (29) c	36.3 (230) e
***Bacillus* sp. Cp-h60 (698, 1.85, 8.2)**	0.125 (57) a	9.6 (198.3) h	21.9 (34) a	17.2 (146) d	16.4 (78) a	34.3 (246) e	64 (73) a	54.2 (392) b

* Value in parenthesis show P-solubilizing activity (µg mL^{-1}), ACC deaminase activity (µmol L^{-1}) and auxin producing potential (µg mL^{-1}) of *Bacillus* spp., respectively; †% Increase over respective uninoculated control; LSD(root dry matter, axenic study): 0.004809), LSD(root dry matter, pot study):0.74, LSD (root length, pot study: 3.2102

Table 4: Relative efficacy of *Bacillus* spp. for improving cob weight and kernel yield of maize grown under field conditions

Isolate	Cob weight (ton ha^{-1})			Kernel yield (ton ha^{-1})		
	NK	N P(DAP) K	N P(RP) K	NK	N P(DAP) K	N P(RP) K
Uninoculated control	7.2 (0) g	14.8 (0) cd	8.1 (0) g	5.4 (0) g	11.0 (0) d	6.0 (0) g
Bacillus megaterium* (180, 1.27, 48.1)	9.6 (34) † f	17.6 (19) b	14.0 (57) d	7.6 (41) f	13.6 (24) bc	10.9 (82) d
***Bacillus subtilis* (572, 0, 19.5)**	10.1 (40) f	18.2 (23) b	14.9 (67) cd	8.0 (48) f	14.0 (28) b	11.6 (93) d
***Bacillus* sp. Cp-h60 (698, 1.85, 8.2)**	12.4 (71.8) e	20.6 (38.5) a	16.0 (97) c	9.8 (83) e	16.0 (46) a	12.7 (111) c

*Value in parenthesis show P-solubilizing activity (µg mL^{-1}), ACC deaminase activity (µmol L^{-1}) and auxin producing potential (µg mL^{-1}) of *Bacillus* spp., respectively; †% Increase over respective uninoculated control; LSD (cob weight):1.2259, LSD (kernel yield):0.9576

inoculation. It was recorded that P uptake in stover of both inocualted and uninoculated plants was maximum in case where P was applied through DAP. Oppositely, there was more percent increase in P uptake in case of RP compared to DAP application. With K6 strain, uptake increased almost 19 fold in the presence of RP while in the presence of DAP it increased about 4 fold. Similar to pot study, in field grown maize, almost similar trend in P uptake in maize stover due to fertilizer treatments was observed. Moreover, PGPR treatment also improved P uptake as it was increased from 1.2 to 5.4 mg m^{-2}, 3.2 to 11.5 mg m^{-2} and 1.5 to 8.4 mg m^{-2} over uninoculated control in DAP and RP treatments, respectively. Noteworthy, with no inoculation, difference in P uptake was negligible with RP compared to no P treatment but inoculation created a big difference (Table 5). P uptake in maize kernel was also differed with inoculation and source of P. Results showed that PGPR inoculation promoted P uptake in maize kernel by following the same trend as observed in case of P uptake in stover.

Discussion

In this study the impact of three *Bacillus* spp. differing in phosphate solubilization, ACC deaminase activity and auxin production potential were compared under no P application or P added as RP or DAP on growth and yield of maize. The results of axenic, pot and field trials revealed that all three *Bacillus* spp. promoted shoot and root growth, yield parameters (cob weight and kernel yield) and P uptake with different degree of efficacy. This promotion by *Bacillus* spp. could be related to increase in soluble/bioavilable fraction of P in soil as well as due to better root growth through their auxin production potential and ACC deaminase activity. Primarily increase in phosphate solubilization and bioavailable fraction occurred due to inoculating PGPR resulted in enhanced growth and yield of inoculated plants. Very interestingly, all the three strains used in this study had different P solubilization potential but as P solubilization potential increased from K2 to K6 (from 180 to 698 µg mL^{-1}), the auxin production potential decreased accordingly (from 48.1 to 8.2 µg mL^{-1}). Likewise, EL-Azeem *et al.* (2007) observed that isolates PC1 and BM1 both had P solubilization and auxin production potential. The strain PC1 had high phosphate solubilization activity (362.05 mg L^{-1}) and low auxin production (17.82 mg L^{-1}). Oppositely, strain BM1 having low phosphate solubilization activity (102.79 mg L^{-1}) carried high auxin production potential (25.40 mg L^{-1}). Among the fertilizer treatments, DAP proved to be a better source of P, but efficacy of inoculation was minimum in this treatment. Most likely because of being the readily available source of P. Maximum efficacy of inoculants was recorded in case of RP, most likely due to the ability of

these PGPR to convert RP into soluble form and this process occurred gradually and became the continuous source of plant available P. This premise is supported by in shoot dry weight with RP and only 16.36% with DAP over respective uninoculated control which is in strong agreement with our results. Like SDM yield, shoot length

Table 5: Relative efficacy of *Bacillus* spp. for improving P uptake in stove and kernel of maize under pot and field conditions

Isolate	NK	N P(DAP) K	N P(RP) K
	Pot Study [P uptake in stover (mg pot^{-1})]		
Uninoculated control	5.80 (0) k	48.7 (0) g	7.33 (0) k
Bacillus megaterium (180, 1.27, 48.1)*	19.7 (170) †j	63.4 (30) e	38.3 (320) h
Bacillus subtilis (572, 0, 19.5)	30.1 (314) i	77.0 (58) c	55.7 (512) f
Bacillus sp. Cp-h60 (698, 1.85, 8.2)	68.2 (837) d	172.0 (253)) a	125.0 (1607) b
	Field Study [P uptake in stover (g m^{-2})]		
Uninoculated control	1.2 (0) g	3.2 (0) ef	1.5 (0) g
Bacillus megaterium (180, 1.27, 48.1)*	2.27 (64) fg	5.6 (75) d	3.4 (126) e
Bacillus subtilis (572, 0, 19.5)	3.3 (169) ef	7.0 (119) c	4.2 (180) e
Bacillus sp. Cp-h60 (698, 1.85, 8.2)	5.4 (348) cd	11.5 (261) a	8.4 (606) b
	Field Study [P uptake in kernel (g m^{-2})]		
Uninoculated control	1.4 (0) h	5.2 (0) f	2.0 (0) h
Bacillus megaterium (180, 1.27, 48.1)*	3.1 (118) g	7.1 (36) d	5.0 (120) f
Bacillus subtilis (572, 0, 19.5)	3.9 (175) g	8.1 (56) c	6.2 (210) e
Bacillus sp. Cp-h60 (698, 1.85, 8.2)	6.4 (360) de	14.6 (182) a	9.4 (370) b

*Value in parenthesis show P-solubilizing activity (µg mL^{-1}), ACC deaminase activity (µmol L^{-1}) and auxin producing potential (µg mL^{-1}) of *Bacillus* spp., respectively; †% Increase over respective uninoculated control; LSD [P uptake in stover(mg pot^{-1})]: 3.21, LSD [P uptake in stover (g m^{-2})]: , LSD [P uptake in kernel (g m^{-2})], 0.8768

the fact that in uninoculated plants supplied with RP, growth and yield of maize crop was not significantly increased over control (NK only). This may imply that in the absence of inoculation, P from RP was not available for plant uptake. In case of control, inoculation still had a positive impact most likely due to mobilization of unavailable/insoluble P. This premise is further supported by the P uptake which showed substantial increase in P uptake in response to inoculation with selected PGPR. So it is highly likely that the impact of three *Bacillus* spp. might be the combined result of multiple traits in the promotion of growth and yield of maize. Phosphate solubilizing, ACC deaminase and auxin production by PGPR are very critical for root growth. *Bacillus* sp. Cp-h60 (K6) promoted root growth more followed by *Bacillus megaterium* and *Bacillus subtilis*, this might have occurred due to difference in P-solubilizing, ACC deaminase activity and auxin production potential.

All three *Bacillus* strains differed in their efficacy under the three fertilizer treatments i.e.; no P, RP and DAP. This might have occurred due to slow release of P from RP and reduced rate of P fixation of released P under inoculated conditions. This increased bioavailable P fraction in soil which resulted in better shoot growth of inoculated plants. The results of Turan *et al.* (2007) showed that bacterial inoculation caused 21.94% increase of inoculated plants was also tremendously increased with all three *Bacillus* strains. However, maximum promotion in shoot length was recorded with *Bacillus* sp. Cp-h60 (K6) followed by *Bacillus megaterium* (K2) and *Bacillus subtilis* (K4), respectively. Similar variation in microbial response having auxin production potential and P solubilizing activity was observed by Shahab *et al.* (2009) as shoot length of mungbean was increased up to 150, 133 and 100% with *Bacillus thuringienesis* (CMG860), *Bacillus thuringienesis* (CMG857) and *Pseudomonas aerouginosa* (CMG854). Further, it was noted that response of inoculation varied with type of P source as percent increment in shoot length was more in case of RP followed by native P and DAP-P. In potato plants, Dawwam *et al.* (2013) recorded higher shoot length of plants inoculated with rhizobacteria capable of auxin production and phosphate solubilization than uninoculated control.

Similar to shoot growth, inoculation of *Bacillus* spp. resulted in a tremendous increase in SDM yield and root elongation under axenic conditions as well as in pot grown maize. It was noticed that strain K4 which performed better in case of SDM production than K2, produced less RDM yield not only under axenic study but in pot study as well. It is highly likely that it might have occured due to ACC deaminase activity of K4. Shahzad *et al.* (2010) observed that strains having better ACC utilization potential

improved RDM more than strains having poor ACC utilization potential. It is noteworthy that maximum root growth was observed in the presence of DAP but inoculation promoted root growth more in the presence of RP than DAP which is an agreement with the findings of Turan *et al.* (2007). They observed more pronounced impact of inoculation on root dry matter yield in the presence of RP than DAP as root dry matter yield was increased 13.8% with RP and only1.5% with DAP over respective uninoculated controls. Shaharoona *et al.* (2006) observed higher root elongation with PGPR inoculation containing ACC deaminase activity than uninoculated plants.

Similar to other studied parameters, cob weight and yield were increased significantly over uninoculated control. Strain possessing highest P solubilization and least auxin activity showed better results than others. Inoculation of *Bacillus* M-13 having P solubilizing activity promoted sunflower seed yield from 4.93 to 5.67 t ha^{-1} by solubilizing native soil P (Ekin, 2010). Similarly, Dey *et al.* (2004) reported that four *Pseudomonas* spp. (PGPR1, PGPR2, PGPR4 and PGPR7) positive for indole acetic acid production and P solubilizing activity promoted yield of peanut to greater extent over uninocualted control plants. Further, results obtained with DAP-P and RP-P (insoluble source) were significantly higher than control. In uninocualted plants difference in cob weight and kernel yield was more between plants receiving DAP (soluble P) and RP (insoluble P). However results were comparable with inoculation. This is in strong agreement with the findings of Tyagi *et al.* (2003) as they observed that with inoculation, grain yield of pea was improved from 9.19 to 13.19 and 13.47 kg ha^{-1} in the presence of SSP (soluble P) and RP (insoluble P), respectively.

Inoculation of *Bacillus* strains possessing multiple growth promoting traits promoted P uptake in stover and maize kernel with and without P application. This might have occurred due to their impact on P solubilization and better root growth resulting in more nutrient use efficiency. Chabot *et al.* (1996) reported up to 8% more P uptake in inoculated plants than uninoculated plants in maize plants under field conditions. According to Egamberdiyeva *et al.* (2007), inoculated plants accumulated more P than uninocualted control plants. Under axenic, pot and field studies bacteria possessing P solubilizing activity promoted growth by solubilizing indigenous P and also reduce fixation of applied P which results in better P use efficiency (Abbasi *et al.*, 2011).

Conclusion and Future Perspectives

The results of this research clearly show that the PGPR carrying multiple growth promoting traits (phosphate solubilization, ACC deaminase activity and auxin production) are highly useful to increase the growth, yield and P uptake by maize plants. Response of inoculation with *Bacillus megaterium*, *Bacillus subtilis* and *Bacillus* sp. Cp-h60 was significant on various parameters studied, however, *Bacillus* sp. Cp-h60 possessing highest P solubilization and lowest auxin production capability was the most efficient PGPR. Among the fertilizer treatments, maximum improvement in growth, yield and P uptake was observed in case of DAP, but efficacy of inoculation was minimum in this treatment. Inoculation of all three PGPR was the most effective in case of RP. Future efforts should focus on utilizing such PGPR with multiple growth promoting traits for improving nutrient use efficiency particularly of P from exogenously applied rock phosphate. These superior PGPR may have tremendous application in achieving sustainability in crop production through multifarious mechanisms. Moreover, specific PGPR could help in utilization of rock phosphate as an efficient source of P which is becoming major constraint to agriculture in developing countries.

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