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POTENTIAL OF PHOSPHATE SOLUBILIZING *BACILLUS* STRAINS FOR IMPROVING GROWTH AND NUTRIENT UPTAKE IN MUNGBEAN AND MAIZE CROPS

Maqshoof Ahmad[1,*], Zainab Adil[1], Azhar Hussain[1], Muhammad Zahid Mumtaz[2], Muhammad Nafees[3], Iqra Ahmad[1] and Moazzam Jamil[1]

[1]Department of Soil Science, University College of Agriculture and Environmental Sciences, the Islamia University of Bahawalpur, Bahawalpur-63100, Pakistan; [2]Institute of Molecular Biology and Biotechnology, the University of Lahore, Pakistan; [3]Department of Horticultural Sciences, University College of Agriculture and Environmental Sciences, the Islamia University of Bahawalpur, Bahawalpur-63100, Pakistan
[*]Corresponding author's e-mail: maqshoof_ahmad@yahoo.com

Phosphate-solubilizing bacteria (PSB) improve plant nutrition by solubilizing insoluble phosphorus compounds in alkaline calcareous soils thus improve plant growth of both leguminous and non-leguminous crops. In the present study, two promising PSB strains *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 were evaluated for their potential to improve plant growth and nutritional status of mungbean and maize crops. The experiment was conducted in pots under wire house conditions following completely randomized design (CRD) with three replications. The results revealed that co-inoculation with *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 exhibited an increased nodulation in mungbean which was measured in terms of nodules number, and nodules fresh and dry weight as compared to sole inoculation. This co-inoculation of bacterial strains also increased plant growth and nutritional status of mungbean and maize crops. It increased nitrogen (N) concentration up to 142 and 18%, phosphorus (P) concentration up to 90 and 43%, and potassium (K) concentration up to 71 and 44%, in shoots of mungbean and maize crops, respectively. It is concluded that the tested PSB strains *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 have the potential to be used as promising bio-inoculants to maximize plant growth and nutritional status of the crop for sustainable production.
Keywords: Plant nutrition, *Vigna radiate,* atmospheric N_2, rhizobia, bio-inoculant, co-inoculation

INTRODUCTION

Maize (*Zea mays*) is the third most important cereal crop after wheat and rice around the globe (Gerpacio and Pingali, 2007; Zulfiqar *et al*., 2017a,b); however, it is an exhaustive crop that needs more nutrients from soil. Mungbean (*Vigna radiata* L.) on the other hand, is not only important in human nutrition due to its high protein contents but also improves soil fertility through fixing atmospheric nitrogen (N_2) by symbiotic association with rhizobia (Arain, 2012). The incorporation of mungbean into existing cropping systems as restorative crop could be beneficial even for following crop. Maize-legume cropping system is becoming popular over the globe and is considered important for sustainable crop production systems. In Pakistan, mungbean and maize have low yields due to low fertility status of soil and high cost of synthetic fertilizers (Azam *et al*., 2017; Wasaya *et al*., 2017). Synthetic fertilizers although improve growth and development of crops, yet its constant use is hazardous not only for plants but also for the environment. Therefore, an alternate agricultural approach could be the inoculation with plant growth promoting rhizobacteria (PGPR) that can improve crop growth and yield by improving soil fertility and biological control (Fernando *et al*., 2005; Dubey *et al*., 2018; Zahir *et al*., 2018). The PGPR inoculation is economical and environment friendly.

Phosphorus (P) is the second most essential macronutrient required for plant growth and development after nitrogen. Its deficiency in soils is well-known and constant P-fertilizers use is desired to sustain crop production. Plants can use only a small amount of applied P-fertilizers and rest is immobilized into sparingly soluble compounds (Wang *et al*., 2015). In calcareous soils, P makes a complex with calcium and a very small fraction of readily available P is accessible to plants due to low solubility and high fixation in these soils (Mahdi *et al*., 2011; Wang *et al*., 2015). The high application rate of P-fertilizers due to low efficiency is economically and environmentally unsustainable and affects microbial diversity, soil fertility and causes a reduction in crop yield (Harvey *et al*., 2009). Among naturally abundant rhizospheric microorganisms, phosphate solubilizing bacteria (PSB) have the ability to dissolve insoluble P compounds in soil and help plants in accessing P required for growth and provide an alternative approach for sustainable agriculture (Lavakusha *et al*., 2014). The bacterial strains related to genera *Pseudomonas*, *Bacillus*, and *Rhizobium* are the most studied strains which have the ability to solubilize P by lowering the pH of the medium (Mumtaz *et al*., 2017), production of organic acids like lactic, citric, acetic, gluconic, malonic, and succinic acids (Wei *et al*., 2018), and phosphatase enzymes (Kapri and Tewari, 2010).

The plants secret root exudates which mediate plant-microbes interactions. The P-solubilizing rhizobacterial and *Rhizobium* strains can colonize in root nodules, and promote plant growth and development through N fixation, uptake of nutrients by solubilization of insoluble P, K, and zinc sources, production of siderophores, phytohormones, antibiotics, ACC deaminase activity, and enzymatic activity (Ghosh and Basu, 2006; Mumtaz *et al*., 2017; Zahir *et al*., 2018). The P-solubilizing strains are involved in N_2 fixation through fixing atmospheric N_2 into NH_3 which is restricted to legume crops and can be utilized for non-leguminous crops through efficient crop rotation systems. The N_2 fixing flora in non-leguminous rhizosphere could be an alternative source of N-fertilizers (Bhattacharjee *et al*., 2008). The production of exopolysaccharides (EPS) by PSB strains also demonstrated P-solubilization (Yi *et al*., 2008; Mumtaz *et al*., 2017). However, literature reports limited information related to the relationship between EPS and P-solubilization ability of these bacteria.

The PSB based biofertilizers can help plants to grow under nutrient deficient soil conditions. These biofertilizers can act as supplements of chemical fertilizers and can reduce the burden of pesticides and agrochemicals (Vessey, 2003). Considering the importance of PSB for beneficial effects on plant growth and developments, the current pot experiment is an attempt to investigate the potential of phosphate solubilizing *Bacillus* strains to promote plant growth and nutrients uptake by maize and mungbean crops.

MATERIALS AND METHODS

***Collection of bacterial strains*:** Two phosphate solubilizing *Bacillus* strains i.e. *Bacillus aryabhattai* S10 (Accession # KX788862) and *Bacillus subtilis* ZM63 (Accession # KX788861) were taken from the gene bank of Soil Microbiology and Biotechnology Laboratory, Department of Soil Science, University College of Agriculture & Environmental Sciences, the Islamia University of Bahawalpur, Pakistan (Najam-ul-Sehar *et al*., 2015; Mumtaz *et al*., 2017).

***Preparation of inoculum and seed treatment*:** The inoculum was prepared by growing the bacterial strains in modified Dworkin and Foster (DF) minimal media (Dworkin and Foster, 1958) amended with insoluble zinc (Zn) source zinc oxide (ZnO) as described by (Fasim *et al*., 2002). Before seeds treatment, seeds were sterilized by dipping in ethanol for 2 minutes followed by dipping in 0.2% $HgCl_2$ for 4 minutes and then rinsed several time with sterilized distilled water. The seeds of maize and mung bean were treated with *Bacillus* strains by dipping in respective inoculum for 20 minutes. Bacterial strains were co-inoculated by using 1:1 ratio of respective strains. The uninoculated control was maintained by dipping seeds in control DF minimal media amended with ZnO. A set of four treatments composed of T_1 = uninoculated control, T_2 = *Bacillus aryabhattai* S10, T_3 = *Bacillus subtilis* ZM63 and T_4 = *Bacillus aryabhattai* S10 + *Bacillus subtilis* ZM63.

***Pot experiment*:** A pot experiment was conducted in the wirehouse to evaluate the potential of phosphate solubilizing bacteria for improving plant growth and nutrient uptake in maize and mungbean under natural conditions. Pots were filled with 12 kg air dried soil passed through 0.2 mm sieve having pHs 7.63, EC 1.32 dS m^{-1}, organic matter 0.44%, N 0.02%, available P 17.2 ppm, and extractable K 95 ppm. The inoculated seeds of mungbean variety NM-11 were sown in 1^{st} week of March. To evaluate the effectiveness of growing mungbean on following crop, inoculated seeds of maize variety DK-6142 (Monsanto, Pakistan) were sown in 1^{st} week of August in the same soil in pots which was used for mungbean experiment. Mungbean was grown up to physiological maturity while maize was harvest after 55 days of growth initiation. The experiments were laid out in complete randomized design (CRD) with five replications of each treatment. The recommended dose of NPK for maize @ 120, 100, 100 kg ha^{-1} respectively and for mungbean @ 40, 75 and 60 kg ha^{-1} were applied in the form of Urea, Diammonium Phosphate (DAP), and Sulphate of Potash (SOP). Pots were irrigated with good quality irrigation water. All agronomic practices were carried out according to requirement.

***Physiological attributes*:** After eight weeks of germination, leave samples of mung bean and maize were collected and analyzed for relative water contents and chlorophyll contents. The relative water content (RWC) of the fully expanded youngest leaf was observed by recording fresh, turgid, and dry weight as described by Mayak *et al*. (2004). Turgid weight was taken after overnight soaking of leaves in distilled water while dry weight was recorded after drying in an oven at 67°C for 24 h. chlorophyll "a" and chlorophyll "b" was determined by the following method as described by Hiscox and Israelstam (1979) and Arnon (1949), respectively.

***Growth and yield parameters*:** Nodulation in mung bean in terms of a number of nodules, nodules fresh and dry weight was observed at the time of harvesting. Growth attributes e.g. plant height, root length, shoot dry weight, and root dry weight was recorded both in maize and mung bean. The number of pods, pods fresh and dry weight of mung bean while the number of grain $plant^{-1}$ of both maize and mung bean were recorded.

***Plant analysis*:** At maturity, after harvesting leaves sample from maize and leaves as well as grain sample from mung bean were analyzed for N, P and potassium (K). Plant dried leaves and grain samples were wet digested by following the methods as described by Wolf (1982). Nitrogen contents were determined by the Kjeldahl method as described by Ryan (2017) and multiplied by a factor of 6.25 for determination of protein contents (Thimmaiah, 2004). Phosphorus and K contents were determined according to a method of Ryan (2017).

***Statistical analysis*:** The data reported in the current study are the means of five replicates. The analysis of variance technique (ANOVA) was performed by using statistical software Statistix 8.1 separately for mung bean and maize. The values were compared for the significance of the difference between the treatments through Least Significant Difference (LSD) test (Steel *et al*., 2007).

RESULTS AND DISCUSSION

***Nodulation in mungbean*:** The nodulation in PSB treated mungbean plants was significantly higher than uninoculated control (Table 1). Highest increase in number of nodules (153%), nodules fresh weight (116%) and nodules dry weight (128%) with comparison to uninoculated control was observed in the treatments where *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 were used in combination.

***Physiological attributes of mungbean and maize crops*:** The sole as well as co-inoculation with PSB strains significantly improved the relative water contents, chlorophyll "a" and chlorophyll "b" contents in mungbean and maize crops (Table 2). Sole inoculation with *Bacillus aryabhattai* S10 showed better performance for improving the physiological attributes as compared to *Bacillus subtilis* ZM63. Co-inoculation with *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 reported the maximum increase in relative water contents (19% in mungbean and 32% in maize), chlorophyll "a" and chlorophyll "b" contents in mungbean and maize crops as compared to respective uninoculated control.

Table 1. Effect of phosphate solubilizing bacterial strains on nodulation in mungbean.

Treatment	Number of nodules (pot^{-1})	Nodules fresh weight (g pot^{-1})	Nodules dry weight (g pot^{-1})
Uninoculated control	15 d	0.19 d	0.07 c
Bacillus aryabhattai S10	33 b	0.35 b	0.15 a
Bacillus subtilis ZM63	26 c	0.22 c	0.10 b
B. aryabhattai S10 + *B. subtilis* ZM63	38 a	0.41 a	0.16 a
LSD ($p \leq 0.05$)	2.5963	0.0270	0.0313

Means sharing different letters are statistically different at 5 % level of probability (n = 5)

***Growth attributes of mungbean and maize crops*:** Mungbean and maize plants treated with PSB strains showed improvement in growth attributes at $p \leq 0.05$ in comparison to uninoculated control. All PSB treatments in mungbean and maize crop either sole inoculation with *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 or combined use of *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 showed significantly higher growth attributes over uninoculated

Table 2. Effect of phosphate solubilizing bacterial strains on physiological attributes of mung bean and maize crops.

Treatment	Mungbean			Maize		
	RWC (%)	Chlorophyll a (µg/g)	Chlorophyll b (µg/g)	RWC (%)	Chlorophyll a (µg/g)	Chlorophyll b (µg/g)
Uninoculated control	65.4 d	1.34 d	0.46 c	71.7 d	3.67 d	5.04 d
Bacillus aryabhattai S10	75.0 b	2.49 b	0.76 b	85.3 b	6.25 b	9.99 b
Bacillus subtilis ZM63	68.2 c	1.88 c	0.72 b	79.5 c	5.54 c	8.46 c
B. aryabhattai S10 + *B. subtilis* ZM63	77.8 a	2.87 a	0.90 a	94.7 a	8.34 a	11.91 a
LSD ($p \leq 0.05$)	2.6982	0.1523	0.0779	3.1957	0.8041	1.4034

Means sharing different letters are statistically different at 5 % level of probability (n = 5); RWC: relative water contents

Table 3. Effect of phosphate solubilizing bacterial strains on growth attributes of mung bean and maize crops.

Treatment	Mungbean				Maize			
	Plant height (cm)	Root length (cm)	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})	Plant length (cm)	Root length (cm)	Plant dry weight (g)	Root dry weight (g)
Uninoculated control	18.5 d	31.8 d	9.3 d	1.5 c	92.7 d	4.9 d	28.2 d	3.1 d
Bacillus aryabhattai S10	22.9 b	35.9 b	14.3 b	2.1 b	112.1 b	6.6 b	36.9 b	5.1 b
Bacillus subtilis ZM63	20.5 c	33.6 c	11.3 c	1.7 c	105.2 c	5.3 c	33.4 c	3.9 c
B. aryabhattai S10 + *B. subtilis* ZM63	29.9 a	38.8 a	20.2 a	3.5 a	120.4 a	7.1 a	38.9 a	6.9 a
LSD ($p \leq 0.05$)	1.4280	0.8931	0.9650	0.3794	3.3932	0.2077	1.4728	0.7560

Means sharing different letters are statistically different at 5 % level of probability (n = 5

Table 4. Effect of phosphate solubilizing bacterial strains on yield attributes of mung bean.

Treatment	Number of pods ($plant^{-1}$)	Number of grains ($plant^{-1}$)	Pod fresh weight ($g\ plant^{-1}$)	Pod dry weight ($g\ plant^{-1}$)
Uninoculated control	7 d	34.3 c	9.63 d	2.90 c
Bacillus aryabhattai S10	11 b	41.2 ab	13.49 ab	4.21 b
Bacillus subtilis ZM63	9 c	40.8 ab	11.58 c	3.20 c
B. aryabhattai S10 + *B. subtilis* ZM63	13 a	44.4 a	14.03 a	5.08 a
LSD ($p\leq0.05$)	1.4221	5.4708	1.0396	0.4541

Means sharing different letters are statistically different at 5 % level of probability (n = 5)

Table 5. Effect of phosphate solubilizing bacterial strains on nutrient concentration in shoot of mungbean and maize crops.

Treatment	Mungbean				Maize			
	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Protein (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Protein (%)
Uninoculated control	1.04 c	0.10	1.07 d	6.49 c	1.21 c	0.12 d	1.19 d	7.54 c
Bacillus aryabhattai S10	1.84 b	0.16	1.54 b	11.48 b	1.40 ab	0.17 b	1.50 b	8.18 ab
Bacillus subtilis ZM63	1.51 b	0.13	1.32 c	9.44 b	1.35 b	0.15 c	1.34 c	8.41 b
B. aryabhattai S10 + *B. subtilis* ZM63	2.52 a	0.19	1.83 a	15.74 a	1.43 a	0.19 a	1.71 a	8.96 a
LSD ($p\leq0.05$)	0.3871	0.0255	0.1070	2.4226	0.0852	0.0104	0.0589	0.5327

Means sharing different letters are statistically different at 5 % level of probability (n = 5)

Table 6. Effect of phosphate solubilizing bacterial strains on nutrient concentration in grains of mungbean.

Treatment	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Protein (%)
Uninoculated control	1.38 c	0.30 d	1.63 d	8.65 c
Bacillus aryabhattai S10	2.17 b	0.51 b	2.05 b	13.57 b
Bacillus subtilis ZM63	1.98 b	0.39 c	1.96 c	12.37 b
B. aryabhattai S10 + *B. subtilis* ZM63	2.71 a	0.59 a	2.15 a	16.96 a
LSD ($p\leq0.05$)	0.2146	0.0462	0.0738	1.3364

Means sharing different letters are statistically different at 5 % level of probability (n = 5)

control plants (Table 3). Co-inoculation with *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 reported the highest increase in growth attributes of mungbean and maize crops. In mungbean, co-inoculation reported 62, 22, 117, 133% more plant height, root length, shoot dry weight, and root dry weight as compared to respective un-inoculated control. Co-inoculation in maize crop resulted in 30, 45, 37 and 122% increase in plant height, root length, shoot dry weight, and root dry weight as compared to uninoculated control.

Yield components of mungbean crop**:** The PSB strains had a variable effect on yield and yield contributing parameters of mungbean crop. The sole inoculation of PSB strains caused a significant increase in pods number, pods fresh and dry weight and number of grains in mungbean as compared to uninoculated control (Table 4). However, co-inoculation with *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 showed more promising results and the increase in pods fresh weight and number of grains in pods of mungbean was up to 46 and 29% as compared to uninoculated control.

Mineral concentration in mung bean and maize**:** Results showed that inoculation with *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 significantly increased nutrients concentration both in mungbean and maize crops as compared to respective control (Table 5 & 6). Sole inoculation with *Bacillus aryabhattai* S10 reported better increased in N, P, K and protein contents in shoots of mungbean and maize as compared to *Bacillus subtilis* ZM63 (Table 5). The co-inoculation performed better then sole inoculation and showed highest increase in N concentration (142% in mungbean and 18% in maize), P concentration (90% in mungbean and 58% in maize), K concentration (71% in mungbean and 43% in maize) and protein contents (143% in mungbean and 19% in maize) in shoot over uninoculated control. The combined use also reported the maximum (96, 97, 32 and 96%) increase in nitrogen, phosphorus, potassium concentration and protein contents, respectively, in mungbean grains as compared to uninoculated control (Table 6).

DISCUSSION

Intercropping mungbean is important to improve the income of small landholders farming community as it ensures higher returns through better utilization of resources. Mungbean productivity is poor under arid and semi-arid climatic conditions in Pakistan due to poor nodulation that can be improved through effective symbiosis of *Rhizobium* and rhizobacterial stains. The application of efficient bacterial strains biofertilizers can increase mungbean nodulation and yield (Zahir *et al.*, 2018). The current study was conducted to evaluate the efficiency of two promising PSB strains *B. aryabhattai* S10 and *B. subtilis* ZM63 to improve plant growth and nutritional status of mungbean and maize crops in pots condition. The results showed that inoculation with *B. aryabhattai* S10 and *B. subtilis* ZM63 significantly improved nodulation in mungbean that might be due to the better symbiotic association of target strains with mungbean. The inoculation with PGPR produces phytohormones (IAA) that stimulates root growth of mungbean and improved nodules formation through increased surface area of rhizobial infection during nodulation (Ghosh and Basu, 2006; Ahmad *et al*., 2014). Sindhu and Dadarwal (2001) reported increase in nodulation due to co-inoculation linked with release of cellulases that helps rhizobia to infect root hair by rupturing cell wall.

Increased nodulation improved nitrogen availability, plant growth and yield and reduces their dependence on fertilizers inputs. In the present study, the inoculation with PSB strains improved crop physiology, growth, and yield of mungbean that might be due to microbial metabolites like production of indole acetic acid, siderophores, organic acids and microbial enzymes produced by inoculated strains helps to expand root surface area and improved nutrient bioavailability (Mumtaz *et al*., 2017; Wei *et al*., 2018). The results of our study are consistent with Dubey *et al*. (2018) and Zahir *et al*. (2018) who reported that multistrain biofertilizers improved mungbean nodulation, plant growth and grain yield under field conditions. Similarly, the increased in growth and yield due to better reproductive growth in mungbean and maize has also been reported in previous studies by Ahmad *et al*. (2014) and Mumtaz *et al*. (2018), respectively.

Phosphorus availability in calcareous soils is usually lower due to its fixation in poorly soluble phosphate mineral and is unavailable for plant uptake (Mahdi *et al*., 2011). Increased crop productivity under current study is the reflection of P minerals solubility. Application of PSB strains significantly improved P concentration in shoot and grains of mungbean that had direct effect on nitrogenase activity during nodulation, increasing root hair development, and nutrients availability which are helpful in reproductive growth and flower development (Selvaraj *et al*., 2008; Lavakusha *et al*., 2014). Rana *et al*. (2012) reported significant improvement in P concentration of wheat grains due to addition of bio-inoculants. Previously, a similar increase in P and N uptake in walnut seedlings due to co-inoculation of PSB strains was reported by Yu *et al*. (2012). More N accumulation represents the presence of nitrogenase in the tested strains that could facilitate N fixation and uptake in plants (Spaepen *et al*., 2007). Application of such kind of bio-inoculants is a potential low-cost alternative to expensive soluble fertilizers to increase yield and nutrient concentration in mungbean and cereals (Vessey, 2003; Bhattacharjee *et al*., 2008). Our study demonstrated that addition of phosphate solubilizing *Bacillus* strains could improve plant growth and nutrient concentration of mungbean and maize. More investigations will be required to evaluate target nutrients solubilizing *Bacillus* strains under different soil and agro-climatic conditions before launching the bio-inoculants for crop production.

***Conclusion*:** It is concluded that *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 are potential plant growth promoting P-solubilizing strains which significantly enhanced plant growth and nutritional value of mungbean and maize crops. These PSB strains may be evaluated in extensive field trials to evaluate their potential for use as biofertilizers.

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