Pak. J. Bot., 52(1): 9-15, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-1(16)

RHIZOBACTERIA AND SILICON SYNERGY MODULATES THE GROWTH, NUTRITION AND YIELD OF MUNGBEAN UNDER SALINE SOIL

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Abstract

Osmotic stress and imbalance nutrients uptake due to salinity stress are major threats that cause decline in growth and yield of crops. This problem is very common in arid and semi-arid regions because of low precipitation and bad quality of irrigation water. Plants also produced higher level of stress generating ethylene via 1-aminocyclopropane-1-carboxylic (ACC) acid when cultivated under limited supply of water. However, inoculation of ACC deaminase producing plant growth promoting rhizobacteria (PGPR) can grant resistance against salinity to crops. On the other hand, silicon (Si) application also increase the productivity of the crop under salt induced stress. This study was conducted to examine the influence of sole and combined application of ACC-deaminase containing PGPRs and Si on mungbean under salinity stress. Prior to sowing, seeds were inoculated with peat based bacterial culture. Silicon was applied at 150 kg ha^{-1} at the time of sowing using the source as $CaSiO_4$ (Calcium Silicate). After two and half month's crop was harvested. Combined application of PGPR + Si caused a significant improvement in growth and physiological attributes of mungbean under salinity. A significant improvement in K (2.30, 0.33 and 0.52-fold) and reduction in Na concentration (0.66, 0.55 and 0.56-fold) in leaves, shoot and seeds, respectively, validated the efficacious role of combine application of PGPR and Si regarding mitigation of salinity stress in mungbean. Significant increase in number of nodules, leaves and grain yield with PGPR + Si over control confirmed the ameliorative effect on mungbean under salinity stress. In conclusion, PGPR + Si is more effective compared sole application for enhancing growth, nutrients concentration and yield in mungbean under salinity stress.

Key words: Growth, PGPR, Salinity, Silicon, Mungbean, Yield.

Introduction

In tropical and subtropical regions, cultivation of mungbean (*Vigna radiata* L.) after wheat harvest is a common practice. Where mungbean is advantageous due to its short duration, low input requirements and wide adaptability, therefore, cultivated on more than 6 Mha (Nair *et al.,* 2012). It is considered nutrition security for poor man and widely consumed as food grains especially in developing countries of the world (Dhingra *et al.,* 1991; Singh and Singh, 2011).

Mungbean carries low levels of oligosaccharides and good source of highly digestible protein (~23%) making it most suitable diet for young babies (Ihsan *et al.,* 2013). In addition, cultivation of mungbean as leguminous crop is effective in improving the health and fertility of marginal soils (Chadha, 2001). However, the susceptibility of mungbean towards various biotic and abiotic stresses under changing climate has made difficult to achieve its potential yield (Sehrawat *et al.,* 2013). As biotic and abiotic stresses are major constrains (Gong *et al.,* 2013) for the production of crops, yet in particular salinity, across various ecological zones abiotic stresses are more dominant regarding its negative effects on plants (Chaves *et al.,* 2003). Salinity is a major stress generating factor that decreases mungbean growth and productivity due to poor germination of seeds (Rozema & Flowers, 2008; Saha *et al.,* 2010) and crop growth. Higher level of salts in soil not only induce osmotic stress but also plays an imperious role in the development of ion toxicity in plants (Rahnama *et al.,* 2010; James *et al.,* 2011). Osmotic stress disturbs the nutrient balance and impairs the potential of plants to detoxify reactive oxygen species (ROS) which hamper the activity of photosynthesis (Munns & Tester, 2008; Pang *et al.,* 2010).

Furthermore, accumulation of sodium (Na^+) and chloride (Cl^-) ions restrict the uptake of potassium (K^+) ion leading to the death of cells and reduction in productivity of mungbean (Friedman *et al.,* 2006; Ahmad & Umar, 2011; James *et al.,* 2011). Low level of Rhizobium survival under salinity stress decreases the nodulation of mungbean ultimately reduce the nitrogen fixation (Mudgal *et al.,* 2010). Besides all above, accumulation of glutamine and proline under salinity stress increase the concentration of amino di-carboxylic acid (Soussi *et al.,* 1998; Munns *et al.,* 2002) that increases the synthesis of endogenous stress ethylene and plays significant role in reduced root elongation and low productivity of crops (Glick *et al.,* 1999).

Scientists are working on different strategies for improving the growth and productivity of plants under salinity stress in all over the world. Inoculation of plant

growth promoting rhizobacteria (PGPR) is considered an effective and environment friendly strategy for overcoming salinity stress (Ilangumaran & Smith, 2017). These PGPR secret multiple enzymes i.e., ACC deaminase and metabolites that can alleviate abiotic stresses in plants (Dardanelli *et al.,* 2008; Ilangumaran & Smith, 2017; Zafar-ul-Hye *et al.,* 2018; Danish *et al.,* 2019; Danish & Zafar-ul-Hye, 2019; Zafar-ul-Hye *et al.,* 2019). Furthermore, ACC-deaminase producing PGPR are remarkable in decreasing stress ethylene (Glick *et al.,* 1999) and mitigation of salinity stress (Yang *et al.,* 2016; Kataoka *et al.,* 2017; Mishra *et al.,* 2018). Furthermore, the application of silicon (Si) has been found effective to alleviate salinity stress on crop plants (Khan *et al.,* 2018). It improves the activity of roots that facilitate nutrients uptake, decreases transpiration losses and osmotic stress. Improvement in ATPase and PPase in the plasma membrane by application of Si enhance the uptake of K^+ while decreasing Na^+ (Tuna *et al.,* 2008).

Use of Si and PGPR is well reported for the alleviation of salinity stress. However, the current study was conducted with the aim to compare the sole and combined application of Si and PGPR containing ACC deaminase on growth and yield of mungbean under salinity. It was hypothesized that the combined application of PGPR containing ACC deaminase and Si may have better potential to improve growth and yield of mungbean under salinity stress.

Materials and Methods

Collection and sterilization of seeds: The seeds of mungbean (BWP-2016) were purchased from certified seed dealer of Punjab Government from the local market. Initially broken and damaged seeds were separated manually from healthy seeds. After that seeds were sterilized with $HgCl_2$ (0.1%) by dipping for 5 min. Finally, seeds were washed three times with autoclaved water (Sadiq & Ali, 2013).

Collection of PGPR and seed inoculation: Peat based inoculum of 1-aminocyclopropane 1-carboxylic acid (ACC) deaminase containing strain *Pseudomonas putida* (Nadeem *et al.,* 2007, 2009) was collected from Soil Microbiology and Biochemistry Laboratory, University of Agriculture, Faisalabad. Seeds were inoculated with PGPR by pouring inocula of PGPR, 10% sugar solution and coating the seed with peat and clay mixture. Seeds of control treatments were also coated with 10% sugar solution, peat and clay mixture without PGPR inocula (Ahmed *et al.,* 2012).

Soil collection and characterization: For cultivation of mungbean plough layer (30 cm) was collected. The soil was characterized as dark brown and saline in with JAKHAR soil series. The soil texture was assessed by hydrometer method (Gee and Bauder, 1986) which was loam (USDA triangle). Soil pH and EC was examined by following the methodology of Schofield and Taylor, (1955) and USDA, (1954), respectively. The organic matter in soil was determined by Walkley and Black method (Jackson, 1975). Organic nitrogen in the soil was determined using the equation:

$$\text{Organic N (\%)} = \text{Soil Organic Matter} / 20$$

For extractable soil P determination, Olsen and Sommers method was used (Olsen & Sommers, 1982). However, the extractable K in soil was determined according to the method described by Nadeem *et al.,* (2013). The physio-chemical characteristics of soil are provided in Table 1.

Table 1. Physio-chemical properties of soil.

Characteristic	Unit	Value
Sand	%	40
Silt	%	40
Clay	%	20
Textural class	-	Loam
Saturation percentage	%	44
pH_s	-	8.10
EC_e	dS m^{-1}	6.09
Extractable phosphorus	mg kg^{-1}	10.92
Extractable potassium	mg kg^{-1}	255
SAR	$cmol_c$ kg^{-1}	1.60
ESP	%	45.5

Pot preparation and nutrients: Clay pots (30 cm deep × 15 cm in diameter) having the capacity to carry 6 kg soil were used for the experiment. To fulfil macronutrients requirement, nitrogen (N), phosphorus (P) and potassium (K) were added at the rate of 20, 60 and 60 kg ha^{-1} respectively, as recommended dose at the time of sowing. The sources of nutrients were diammonium phosphate (DAP) and sulphate of potash (SOP). Silicon was applied at 150 kg ha^{-1} as per treatment plan at the time of sowing by using salt of calcium silicate (Xie *et al.,* 2015).

Sowing of seeds and irrigation: In each pot, five seeds were sown initially. After germination of seeds, thinning was done and two healthy seedlings were maintained in each pot. For irrigation of pots tube well was used as per crop demand.

Site of experiment and treatments: Experiment was carried out in the research area of Department of Soil Science, Bahauddin Zakariya University, Multan, Punjab, Pakistan. Climate of site was semi-arid with annual precipitation of 45 mm and mean annual temperature 29°C. There were 4 treatments with 6 replications following Completely Randomized Design (CRD). The treatments were control (no PGPR + no silicon), Silicon (Si = 150 kg ha^{-1}), PGPR and Si + PGPR.

Harvesting and data collection: At the time of maturity, plants were harvested for growth, nutrients and yield attributes. Shoot and root length, number of leaves, root and shoot fresh and dry weight was recorded. Meter rode was used for measuring shoot and root length. Top loading balance was used to examine the fresh weight of root and shoot. For dry weight determination, samples were dried at 65 ± 1°C till constant weight achieved. Data for nodulation was collected at flowering stage of mung bean.

Plant analysis: For determination of phosphorus (P), potassium (K), sodium (Na) and silicon (Si) in leaves, shoot and seeds samples were digested with di-acid mixture (HNO_3: $HClO_4$ in 2:1 ratio) as described by Chapman & Pratt (1961). Yellow colour methodology was adopted for determination of P in shoot, leaves and seeds on spectrophotometer at 420 nm (Jones *et al.*, 1991). For analysis of K, digested shoot, leaves and seeds samples were run on flame photometer as described by Nadeem *et al.* (2013). For nitrogen determination in shoot, leaves and seeds, H_2SO_4 digestion (Jones *et al.*, 1991) was distillate on Kjeldahl's apparatus (Van Schouwenberg & Walinge, 1973). However, Si of leaves, shoot and seeds was assessed by running dry ashing digested samples on atomic absorption spectrophotometer (AAS).

Results

Single and combined application of PGPR and Si significantly ($p \leq 0.05$) affected the root and shoot length, number of leaves (Table 2), roots and shoot fresh and dry weight (Table 3) of mungbean under salinity stress (Table 2). Minimum length of root and shoot, number of leaves, root and shoot fresh and dry weight was observed in control under salinity stress. Combined application of PGPR and Si significantly improved growth attributes in mungbean comparative to control under salinity stress (Table 2). A significant improvement in shoot length of mungbean comparative to Si and control under drought stress was also observed where PGPR was inoculated as sole amendment. However, for number of leaves, root/ shoot fresh and dry weight sole application of Si and PGPR remained statistically alike to each other. Maximum significant increase of 59.5% and 50.4% was noted in Si + PGPR comparative to control under salinity stress in root and shoot length of mungbean, respectively. Likewise, Si + PGPR gave 27.5, 135.0, and 126.9% higher number of leaves, roots and shoot fresh weight in mungbean plants comparative to control under salinity stress, respectively. Maximum increase of 163.8 and 150.2% in root and shoot dry weight was noted in Si + PGPR comparative to control under salinity stress, respectively.

Both PGPR and Si had significant effect on number of nodules, number of pods, pods fresh weight, pod dry weight and grain yield of mungbean under salinity stress (Table 4). It was observed that the Si + PGPR remained significantly better comparative to control for number of nodules, number of pods, pods fresh weight, pod dry weight and grain yield of mungbean under salinity stress. Sole inoculation of PGPR remained significantly higher comparative to control for number of nodules, pod dry weight and grain yield of mungbean under salinity stress. However, sole application of Si and control remained statistically alike to each other for number of pods, pods fresh weight, pod dry weight and grain yield of mungbean under salinity stress. Maximum increase of 0.73, 0.33, 1.14, 2.08 and 5.02-fold was noted in number of nodules, number of pods, pods fresh weight, pod dry weight and grain yield of mungbean, respectively, where Si + PGPR was done comparative to control under salinity stress.

Table 2. Effect of single and combined application of Si and PGPR on growth attributes of mungbean under salinity stress.

Treatments	Root length (cm)	Shoot length (cm)	Number of leaves (plant^{-1})
Control	13.84 ± 2.11 b	19.83 ± 0.73 d	9.67 ± 0.33 b
Si	16.01 ± 2.54 ab	24.24 ± 0.44 c	10.67 ± 0.88 ab
PGPR	17.48 ± 2.82 ab	27.17 ± 0.66 b	11.00 ± 0.58 ab
Si + PGPR	22.08 ± 2.43 a	29.83 ± 0.43 a	12.33 ± 0.33 a

Data represent the means ± SE of three replicates compared by LSD. Means having different letters are significantly different at $p \leq 0.05$

Table 3. Effect of single and combined application of Si and PGPR on fresh and dry weight of mungbean root and shoot under salinity stress.

Treatments	Root fresh weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)
Control	2.00 ± 0.36 b	6.27 ± 1.12 b	0.36 ± 0.07 c	2.47 ± 0.41 b
Si	3.50 ± 0.54 a	10.78 ± 1.56 ab	0.45 ± 0.04 bc	4.36 ± 0.51 ab
PGPR	3.80 ± 0.53 a	11.11 ± 2.19 ab	0.62 ± 0.06 b	5.50 ± 0.63 ab
Si+ PGPR	4.70 ± 0.36 a	14.23 ± 2.18 a	0.95 ± 0.09 a	6.18 ± 0.98 a

Data represent the means ± SE of three replicates compared by LSD. Means having different letters are significantly different at $p \leq 0.05$

Table 4. Effect of single and combined application of Si and PGPR on yield attributes of mungbean.

Treatments	Number of nodules	Number of pods	Pods fresh weight (g pot^{-1})	Pods dry weight (g pot^{-1})	Grain yield (g pot^{-1})
Control	10.0 ± 0.58 c	9 ± 0.58 b	3.00 ± 0.79 b	1.3 ± 0.20 c	0.50 ± 0.31 c
Si	13.0 ± 0.58 b	9 ± 0.58 b	4.27 ± 0.63 b	2.1 ± 0.29 bc	0.96 ± 0.08 c
PGPR	14.7 ± 0.88 b	11 ± 1.00 ab	4.88 ± 0.29 ab	2.4 ± 0.18 b	1.94 ± 0.42 b
Si+ PGPR	17.3 ± 0.88 a	12 ± 0.58 a	6.42 ± 0.64 a	4.0 ± 0.51 a	3.01 ± 0.15 a

Data represent the means ± SE of three replicates compared by LSD. Means having different letters are significantly different at $p \leq 0.05$

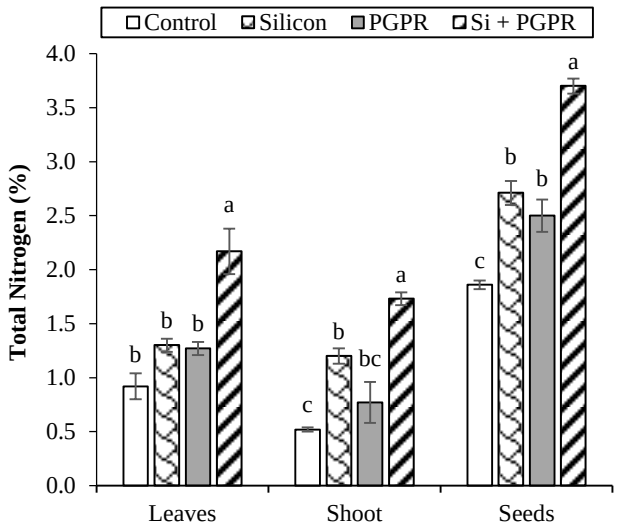

Fig. 1. Effect of single and combined application of Si and PGPR on nitrogen in leaves, shoot and seeds of mungbean.

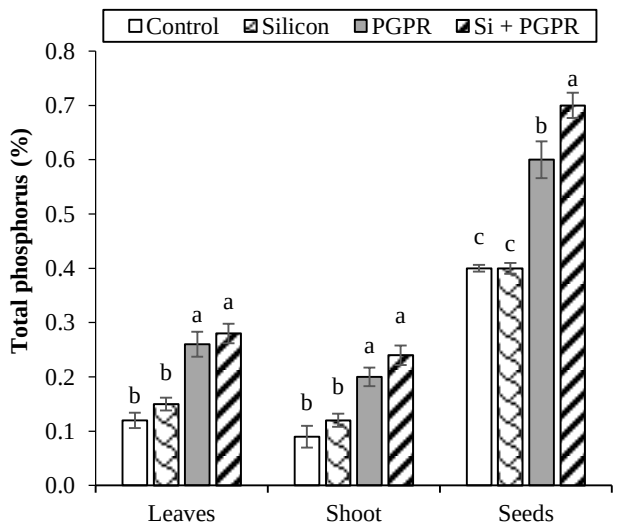

Fig. 2. Effect of single and combined application of Si and PGPR on phosphorus in leaves, shoot and seeds of mungbean.

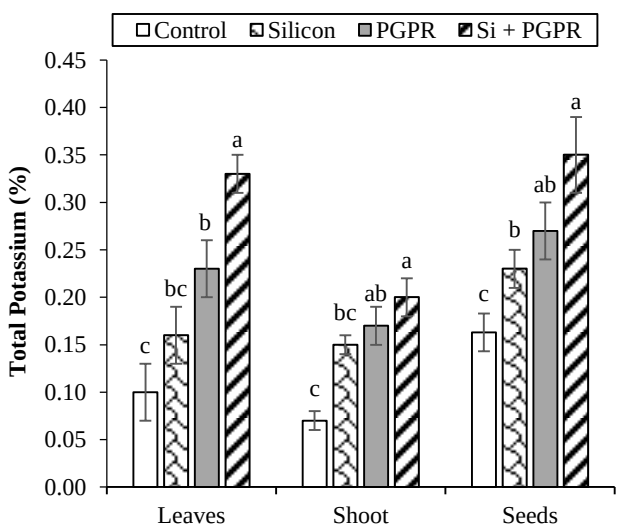

Fig. 3. Effect of single and combined application of Si and PGPR on potassium in leaves, shoot and seeds of mungbean.

Application of PGPR and Si differed significantly ($p \leq 0.05$) for N concentration in leaves, shoot and seeds of mungbean under salinity stress (Fig. 1). It was observed that Si + PGPR showed significantly higher N concentration comparative to control in leaves, shoot and seeds of mungbean under salinity. Single application of Si and PGPR remained statistically alike to each other but differed significantly for N concentration in seeds. However, in shoot of mungbean, application of Si performed significantly better comparative to control for N concentration. No significant change was noted in N concentration of mungbean of leaves comparative to control where Si and PGPR were applied as sole amendment. Maximum increase of 1.36, 2.33 and 0.99 fold was noted in N concentration of mungbean leaves, shoot and seeds, respectively, where Si + PGPR was done comparative to control under salinity stress.

Both PGPR and Si significantly ($p \leq 0.05$) affected P concentration in leaves, shoot and seeds of mungbean under salinity stress (Fig. 2). Application of Si + PGPR performed significantly better comparative to control for P concentration in leaves, shoot and seeds of mungbean under salinity. Single application of PGPR remained statistically alike to Si + PGPR for P concentration in leaves and shoot. However, Si + PGPR showed significantly higher P concentration in seeds comparative to PGPR. No significant change was noted in P concentration of mungbean leaves, shoot and seeds comparative to control where Si was applied alone. Maximum increase of 1.33, 1.67 and 0.75-fold was noted in P concentration of mungbean leaves, shoot and seeds, respectively, where Si + PGPR was done comparative to control under salinity stress.

Application of PGPR and Si showed significant ($p \leq 0.05$) differences for K concentration in leaves, shoot and seeds of mungbean under salinity stress (Fig. 3). Application of Si + PGPR performed significantly better comparative to Si and control for K concentration in leaves, shoot and seeds of mungbean under salinity. Single application of PGPR remained statistically alike to Si + PGPR for K concentration in shoot and seeds. However, Si + PGPR differed significantly better for K concentration in leaves comparative to PGPR. No significant change was noted in K concentration of mungbean leaves and shoot but significant in seeds comparative to control where Si was applied alone. Maximum increase of 2.30, 0.33 and 0.52-fold was noted in K concentration of mungbean leaves, shoot and seeds, respectively, where Si + PGPR was done comparative to control under salinity stress.

Both PGPR and Si remained significantly ($p \leq 0.05$) different for Na concentration in leaves, shoot and seeds of mungbean under salinity stress (Fig. 4). It was noted that Si + PGPR differed significantly better comparative to control for low Na concentration in leaves, shoot and seeds of mungbean under salinity. Inoculation of PGPR remained statistically alike to Si + PGPR for Na concentration in leaves, shoot and seeds. However, Si application showed significantly lower Na concentration in leaves, shoot and seeds comparative to control. A significant reduction of 0.66, 0.55 and 0.56-fold was noted in Na concentration of mungbean leaves, shoot and seeds, respectively, where Si + PGPR was done comparative to control under salinity stress.

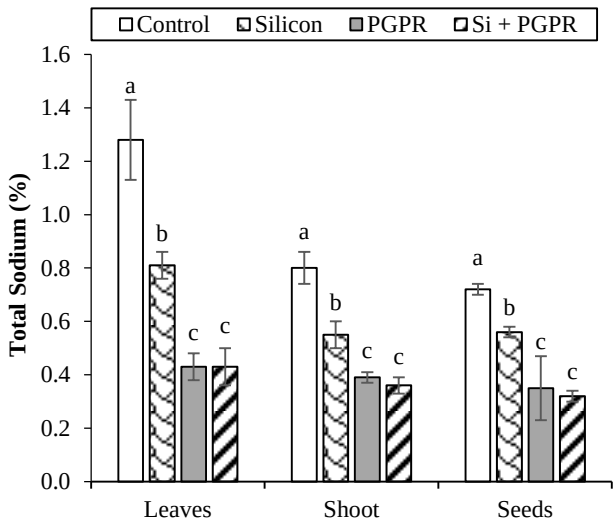

Fig. 4. Effect of single and combined application of Si and PGPR on sodium in leaves, shoot and seeds of mungbean.

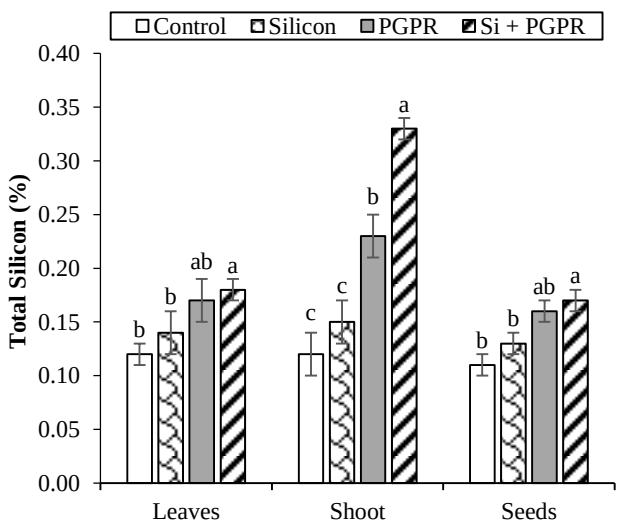

Fig. 5. Effect of single and combined application of Si and PGPR on silicon in leaves, shoot and seeds of mungbean.

Both PGPR and Si application showed significant ($p \leq 0.05$) differences for Si concentration in leaves, shoot and seeds of mungbean under salinity stress (Fig. 5). Application of Si + PGPR differed significantly better comparative to control for Si concentration in leaves, shoot and seeds of mungbean under salinity. Application of PGPR remained statistically alike to Si + PGPR for Si concentration in shoot and seeds but differed significantly in leaves. However, Si application differed significantly better for Si concentration in leaves comparative to control. Maximum increase of 0.50, 1.75 and 0.55-fold was noted in Si concentration of mungbean leaves, shoot and seeds, respectively, where Si + PGPR was done comparative to control under salinity stress.

Discussion

In the current experiment, salinity stress significantly decreased the growth attributes (Table 2) of mungbean possibly due to less elongation of roots and division of cells in shoot. It is documented that both death and division of cells are major factors responsible to reduction in the growth of plants under salinity stress (Ogawa *et al.,* 2006) and cause restricted shoot and root length, fresh and dry weight as observed in the current experiment. According to Hasegawa *et al.,* (2000) salts concentration beyond the threshold limit in rhizosphere makes extraction of water difficult by the roots of plants that resulted in the restriction of shoot growth. A significant reduction in leaves, shoot and seeds NPK concentrations (Figs. 1-3) was possibly due to salinity stress that decreased the uptake of water by damaging the roots of plants and changing soil water potential. Reduction in the uptake of NPK is very common under damaged root system and osmotic stress developed by salts (Zhang *et al.,* 2010). The limited availability of NPK and osmotic stress developed by salts were linked with significant reduction in fresh and dry weight of root and shoot of mungbean, particularly in control. Higher levels of salts in rhizosphere also boosts the synthesis of stress ethylene that plays an imperative role in the loss of membrane integrity and activation of chlorophyllase (chlase) gene that degrades chlorophyll (Matile *et al.,* 1997). Nonetheless, Si + PGPR counteracted the adverse impacts of salinity on mungbean i.e. shoot and root length, fresh and dry weight of shoot and root (Table 2). This improvement in growth attributes of mungbean was possibly due to reduction in stress ethylene and secretion of indole acetic acid (IAA) by PGPR that not only promoted root elongation but also improved NPK concentration in leaves, shoot and seeds. Glick *et al.,* (1999) argued that enzyme ACC-deaminase secreted by PGPR claves 1-aminocyclopropane-1-carboxylic acid (ethylene precursor) into α-ketobutyrate and NH_3. Low levels of rhizospheric ethylene become balanced due to the movement of inside roots accumulated ACC to outside in rhizosphere along a concentration gradient. Validation of fact that low ethylene concentration significantly improved root elongation as documented by many scientists (Naz *et al.,* 2013; Zafar-ul-Hye *et al.,* 2018). Additionally, the application of Si with PGPR decreased the Na concentration (Fig. 4) significantly in leaves, shoot and seeds of mungbean compared to sole application of Si. In the current study, low concentration of Na in mungbean leaves, shoot and seeds by Si + PGPR was linked with simultaneous improvement of K concentration (Fig. 3) by better uptake of Si (Chen *et al.,* 2016) and possibly binding of Na with exopolysaccharides secreted by PGPR (Ashraf *et al.,* 2004; Kohler *et al.,* 2006; Nadeem *et al.,* 2010). Khan *et al.,* (2018) suggested that improvement in enzymatic activity by the application of Si also played an imperative role to grant resistance against salinity stress. Furthermore, Si application increased uptake of N (Singh *et al.,* 2005) and P (Subramanian & Gopalswamy, 1991) that are allied factors in mitigation of salinity stress.

Conclusion

Present research suggested that the PGPR and Si synergistically improved mungbean growth, grain yield and nutrient concentration in saline soil. But further investigations are required to explain more of its benefits and relation of Si and microbes under salinity. From the present experiment, it can be concluded that Si + PGPR

can improve and promote the growth of mungbean, so it might be used in a farming system to reduce the effects of salinity stress. It may also be suggested that this strategy might be useful in different crops to ameliorate the impacts of salinity stress.

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(Received for publication 18 June 2018)