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***Research article***

# Bio-associative effect of rhizobacteria on nodulation and yield of mungbean (*Vigna radiata* L.) under saline conditions

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## HIGHLIGHTS

- Interactive effect of co-inoculants improves the symbiotic efficacy of mungbean in saline soils
- PGPR improves the nodulation and yield of mungbean by increasing number of root hairs
- Combined application of bacterial co-inoculants also improves the quality of soil health

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## ABSTRACT

A beneficial association between rhizobacteria and roots of leguminous plant may results in the development of more nodulation on the roots of legume. Therefore, co-inoculation of rhizobia and plant growth promoting rhizobacteria (PGPR) was tested for improving nodulation and yield of mungbean under salt stressed conditions. In this study, one pre-isolated strain of each *Rhizobium* (*Rhizobium phasoli*) and PGPR (*Pseudomonas putida* - $KB_3$) were tested as sole inoculation as well as in all possible combinations at three salinity levels (Original, 4.0 and 8.0 dS $m^{-1}$) with six replicates by using two factorial under Complete Randomized Design (CRD). The results demonstrated that co-inoculation increased the plant height, fresh biomass, straw yield, grain yield, 100-grain weight, number of nodules per plant, nodule fresh weight, nodule dry weight, root length, root dry weight significantly at different salinity levels under pot conditions. Biochemical analysis of plant samples (straw and grain) showed a significantly ($P<0.05$) higher protein content, N concentration in straw, P concentration in grain and straw at different salinity levels under pot conditions in comparison to respective control (uninoculated). Post-harvest soil analysis revealed that the integrated use of bacterial co-inoculants along with compost also improved the quality of soil health. The finding of this study imply that co-inoculation with *R. phasoli* and *P. putida* - $KB_3$ could be the most effective for achieving better nodulation and yield of mungbean under saline conditions.

**Key words:** Co-inoculation, PGPR, Rhizobium, Salinity, Mungbean



 

## 1. Introduction

Pakistan is an agricultural country producing a variety of crops. In legumes, pulses are important food crops with large protein content and used widely in feeding masses but its per acre yield is very low (Ashraf *et al.,* 2007). Population is increasing at alarming rate, which demands urgent boost up in the crop yield from existing area. Mungbean *(Vigna radiata* L.) is an important pulse crop in many Asian countries including Pakistan, where the diet is mostly cereal based. The area under mungbean crop in Pakistan is 140.8 x $10^3$ hectares and producing about 93.0 x $10^3$ tons of grains annually (Anonymous, 2013). This low grain yield of the mungbean crop (i.e. only 660.5 kg $ha^{-1}$) on an average as shown in the Agricultural Statistics of Pakistan (2013-2014). However, the nodulation in mungbean is very poor that is the main cause of its low yield under agro-ecological conditions of Pakistan. It can be enhanced by proper utilization of biological and genetic potential of microbial and plant species (Shahzad *et al.,* 2010).

There are a number of factors which affect the growth and nodulation in legumes. Among these, salinity is one of the most deleterious factors that affect plant growth by affecting plant physiological processes positively or negatively (Hamdia and Shaddad, 2010; Shahzad *et al.,* 2014). It is a very serious problem for crop production that suppresses plant growth, especially under arid and semi-arid regions (Parida and Das, 2005). Salinity negatively affects the growth and yield of plant by increasing production of ethylene. Increased concentration of ethylene due to exogenous application of 1-aminocyclpropane-1-carboxylic acid (ACC) or salinity can reduce root growth (Li *et al.,* 2013, Shahzad *et al.,* 2014). The biosynthesis of ethylene in excess quantity due to biotic and abiotic stresses inhibits the growth and thus yield of plants. It affects the symbiosis between legume–rhizobium by reducing survival of rhizobium, inhibiting the infection process, affecting nodule formation and function, thus reducing plant growth (Al-Falih, 2002). It is documented that growth and nodulation of pea was adversely affected by salinity and nodulation was more sensitive to salinity than plant growth (Naeem *et al.,* 2008; Sehrawat *et al.,* 2015).

Recently, some plant growth promoting rhizobacteria (PGPR) have shown great potential for improving plant growth by altering the biosynthesis of endogenous phytohormones through the production of specific enzymes. Among these enzymes, bacteria with ACC deaminase plays an important role in the regulation of the plant hormone, ethylene and therefore, affect the growth and development of plants (Glick *et al.,* 2007; Khalid *et al.,* 2009). Ethylene ($C_2H_4$) is a powerful plant hormone and its presence in very low concentration could have a huge impact on the growth of plants (Khalid *et al.,* 2006b; Shahzad *et al.,* 2010). Ethylene is needed for the germination of seeds of many plant species, and its production rate increases during germination and growth of seedlings (Abeles *et al.,* 1992). Low level $C_2H_4$ (as low as 10 mg $L^{-1}$) found to increase root initiation and growth, while higher levels (up to 25 mg $L^{-1}$) can lead to growth inhibition of roots (Ma *et al.,* 1998). Higher ethylene concentration was also known as an inhibitor of nodulation in various legumes, including both those that produce indeterminate nodules and those that produce determinate nodules (Nukui *et al.,* 2000; Arshad and Frankenberger, 2002; Shahzad *et al.,* 2013). In recent years, PGPR have received significant importance for sustainable agriculture tools because they are potential benefits to agriculture in the world, which is important for both under green house and field conditions (Arshad *et al.,* 2008; Contesto *et al.,* 2008). In addition to stimulate plant growth, PGPR ensure the availability of nutrients and nutrient use efficiency, and reduce biotic and abiotic stress (Lebeau *et al.,* 2008; Masoud and Abbas, 2008).

The microorganisms may be present in the rhizosphere, rhizoplane, root tissue and/or in a specialized root structure called a nodule. Very important and significant interactions were reported among plant, soil, and microorganisms present in the soil environment (Antoun and Prevost, 2005). These interactions may be beneficial, harmful and/or neutral, and can significantly influence plant growth and development (Adesemoye and Kloepper, 2009; Lau and Lennon, 2011). The microorganisms colonizing plant roots generally include bacteria, algae, fungi, protozoa and actinomycetes. Enhancement of plant growth and development by application of these microbial populations is well evident (Saharan and Nehra, 2011; Bhattacharyya and Jha, 2012), of different microbial populations present in the

rhizosphere, bacteria are the most abundant microorganisms (Kaymak, 2010). PGPR play a significant role in enhancing plant growth and development both under non-stress and stress conditions by a number of direct and indirect mechanisms (Glick *et al.,* 2007; Nadeem *et al.,* 2010). The mechanisms that promote plant growth include: nitrogen fixation, phosphorus solubilization, production of siderophores, plant growth regulators and organic acids as well as protection by enzymes like ACC-deaminase, chitinase and glucanase (Berg, 2009; Hayat *et al.,* 2010). Root growth promotion is one of the major markers by which the beneficial effect of PGPR is measured. Stimulation of root growth and biomass production of different plant species by inoculation with PGPR has been well documented (Arshad *et al.,* 2008; Patel *et al.,* 2008). Rapid growth and establishment of roots, whether by elongation of primary roots, is advantageous for young seedlings as this increases the ability of the plant to obtain more water and nutrients from the surrounded environment. The use of PGPR containing ACC-deaminase in co-inoculation with rhizobia could be more beneficial due to their multiple effects on plant through different growth enhancing mechanisms. Furthermore inoculation benefits can be enhanced by maintaining high densities of effective bacteria around the roots. Thus, integrated use of PGPR having trait ACC-deaminase and rhizobia could be highly effective approach in improving yield and nodulation of mungbean crop. Keeping in view, the present study was designed to evaluate the efficacy of co-inoculation with *Rhizobium* and PGPR at different salinity levels for improving nodulation and yield of mungbean under pot conditions.

## 2. Materials and methods

The present study was conducted to evaluate the effectiveness of bacterial co-inoculants (nodule bacteria and free living rhizobacteria) on symbiotic efficiency and yield of mungbean (*Vigna radiate* L.) at three different salinity levels under pots conditions at the research area of University College of Agriculture (UCA), University of Sargodha (UOS), Sargodha, Pakistan.

### 2.1. Pot experiment

A pot experiment was conducted to assess the efficacy of co-inoculation with *rhizobium* and PGPR having trait of ACC-deaminase for improving nodulation and yield of mungbean at three different salinity levels under natural conditions. For pot experiment, inocula of *Rhizobium* and rhizobacteria having trait of ACC-deaminase ($10^7$-$10^9$ CFU $mL^{-1}$, 0.6 of OD600) was prepared as described by Shahzad *et al.* (2010), and was injected into sterilized peat (100 mL $kg^{-1}$, 1:1 w/w seed to pea ratio). Seeds were inoculated by mixing with peat and sugar solution (10%) @ 100 mL $kg^{-1}$ peat while control was consisted of the seeds treated with peat having nutrient broth and 10% sugar solution only. Treated seeds were dried under shade for 8 hour. For pot study, sandy loam soil (surface layer up to 15 cm) was taken from the research area of UCA, UOS, and passed through a sieve (60 meshes, size 2mm), before filling the pots. Pre and post analyses of soil was done for physicochemical characteristics (Table 1). Each pot was filled with 12 kg soil receiving nutrients N, P and K @ 25, 60 and 25 kg $ha^{-1}$ as urea, single super phosphate (SSP) and sulfate of potash (SOP), respectively. While full doses of PK were applied by mixing them uniformly with soil before filling the pots. Three salinity levels, 1.39 (i.e. original), 4 and 8 dS $m^{-1}$ were used. The salinity was developed by the addition and thoroughly mixing of the calculated amount of NaCl salt in each pot. Five seeds of mungbean were sown in each pot at soil moisture level (water holding capacity) of 70%. One seedling was maintained in each pot after germination. Uprooted four seedlings were incorporated into soil of the same pot. The pots of each treatment were arranged randomly with six replications at ambient light and temperature in net house. Moisture level between 55 and 65% was maintained in each pot by using canal water. At flowering stage, three replications from each treatment were harvested and roots were observed to record number of nodules and nodule dry weight per plant. At maturity, yield and

yield contributing parameters were taken from the remaining three replications of each treatment. Pre and post soil analysis was done for soil physicochemical properties.

## 2.2. Characterization of bacterial isolates for growth promoting traits

The bacterial strains were characterized for different plant growth promoting characteristics such as ACC-deaminase activity, chitinase activity, siderophores production and root colonizing ability (Table 2). ACC deaminase activity in cell was determined as reported by Duan *et al.* (2008). Bacterial strains were grown in tetracycline (TY) supplemented with 20 µg $mL^{-1}$ tetracycline when required for 2-3 days at 28±1 °C. Cells were washed two times with 0.1M Tris-hydrochloride (pH 7.5) and then were resuspended in modified M9 minimal medium with 5 mM concentration of ACC. Cells were incubated in a shaker at 28 °C for 40 h. After induction, ACC-deaminase activity was estimated by determining ά-ketobutyrate resulting from ACC cleavage by ACC deaminase enzyme (Penrose and Glick 2003). The total protein content of cells was determined by the Bradford reagent protocol (Bradford 1976). Final ACC deaminase activity was expressed in nmol α-ketobutyrate $mg^{-1}$ protein $h^{-1}$. Chitinase activity was quantified using the protocol developed by Cattelan *et al.* (1999). Siderophore production assay for bacterial strains were performed by the methods of Schwyn and Neilands (1987). Root colonization potential by selected bacterial strains was examined under axenic conditions (Simons *et al.,* 1996).

## 2.3. Growth and yield parameters

### 2.3.1. Plant analysis

Plants were harvested at physiological maturity stage. Harvested plants representing each treatment were sampled to measure growth and yield components. Harvested plant biomass (shoot and grain) was dried to constant weight at 75°C. To determine shoot and grain N and P concentrations, the dried samples (0.5g) were milled and subsequently digested with 5 mL of conc. $H_2SO_4$ and $H_2O_2$ (Wolf, 1982). Protein content of grain samples was estimated indirectly by determining the total amount of N (Kjeldahl 1883). While, P concentrations in shoot and grain samples were determined by using the method of Allen *et al.* (1986).

## 2.4. Statistical analysis

The data were statistically analyzed (Steel *et al.,* 1997). The means values were compared at $P< 0.05$ by Duncan's multiple range test (Duncan, 1955).

## 3. Results

The results showed that the plant height of mungbean was reduced significantly ($p\leq0.05$) by increasing salinity levels and minimum plant height (22.98 cm) was observed at 8 dS $m^{-1}$ (Table 3). However, bacterial co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* reduced the inhibitory effect of salinity and increase the plant height with different degree of efficacy. Maximum increase in plant height (42.6%) was observed as a result of co-inoculation with *P. putida* - $KB_3$ x *R. phaseoli* at 8 dS $m^{-1}$ over uninoculated control (8 dS $m^{-1}$). In most of the cases, the increase in plant height due to sole inoculation was statistically non significant ($p\leq0.05$) as compared to uninoculated control. Under normal conditions, maximum increase in plant height (18%) over control was observed due to co-inoculation with *P. putida* - $KB_3$ x *R. phaseoli* at 1.19 dS $m^{-1}$ followed by sole inoculation with *R. phaseoli* and *P. putida* - $KB_3$ that was 4.8 and 6.9% higher over uninoculated control, respectively. Similar trend of bacterial inoculants was also observed at 4 dS $m^{-1}$ to increase plant height under salt stressed conditions. Maximum fresh biomass was obtained as a result of co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* that was 23.1% higher than uninoculated control. While sole inoculation also showed significant ($p\leq0.05$) increase in fresh biomass that was up to 11.5% more than control. At 4.0 dS $m^{-1}$, highest increase in fresh biomass (21.6% more over control) was observed because of co- inoculation with *P. putida* - $KB_3$ and *R.*

*phaseoli* that was at par to *P. putida* - $KB_3$ (Table 3). Lowest fresh biomass was obtained with *R. phaseoli* and respective control.

At high salinity (8 dS $m^{-1}$), maximum increase in fresh biomass (35.5%) was recorded by co-inoculation with *P. putida* - $KB_3$ x *R. phaseoli* while rest of the treatments also showed increase in fresh biomass of mungbean up to 17.1% than uninoculated control.

In case of straw yield, highest increase (up to 23%) was recorded due to co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* at 1.19 dS $m^{-1}$ than uninoculated control (Table 3). Whereas, sole inoculation with *R. phaseoli* also gave significant ($p \leq 0.05$) increase in straw yield that was 10.2% higher over uninoculated control. Followed by *P. putida* - $KB_3$ that gave 4.1% increase over uninoculated control but this increase was at par with particular control. At 4.0 dS $m^{-1}$, highest increase in straw yield (16.5% higher over control) was observed as a result of co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* that was statistically similar to *P. putida* - $KB_3$. Lowest straw yield was produced with *R. phaseoli* in comparison to uninoculated control. At high salinity (8 dS $m^{-1}$), maximum increase in straw yield was recorded up to 29.4 % with co-inoculation of *P. putida* - $KB_3$ and *R. phaseoli* while rest of the treatments also showed increase in straw yield that up to 20.9% higher than uninoculated control.

Results regarding grain yield revealed that bacterial inoculants showed significant increase in grain yield under normal and salt stressed soil conditions (Table 3). At EC 1.19 dS $m^{-1}$, maximum increase in grain yield was 25.8% higher over uninoculated control due to co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli*. While sole inoculation also gave significant increase in grain yield that was 15.6% more over uninoculated control. At 4.0 dS $m^{-1}$, maximum increase in grain yield was observed (up to 27.1%) as a result of co-inoculation *P. putida* - $KB_3$ and *R. phaseoli* over untreated control. Minimum grain yield was obtained due to inoculation with *R. phaseoli* in comparison to control. At higher EC 8 dS $m^{-1}$, maximum increase (up to 80%) in grain yield was recorded due to co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* while rest of the treatments also showed increase in grain yield that up to 11% higher over respective control. Number of pods per plant also an important agronomic trait that play a significant role for increasing grain yield under normal and salt stressed conditions (Table 4). At EC 1.19 dS $m^{-1}$, maximum increase (45.4% higher over control) in number of pods per plant by co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli*. While sole inoculation also gave significant increase in number of pods per plant that ranged from 17.3 to 30.4% respectively due to sole inoculation with *P. putida* - $KB_3$ and *R. phaseoli* over respective control. At 4.0 dS $m^{-1}$, maximum increase in number of pods per plant was recorded (up to 114.8%) as a result of co-inoculation *P. putida* - $KB_3$ and *R. phaseoli* as compared to control. While sole inoculation also gave significant increase in number of pods per pot that was up to 56.5% higher as compared to uninoculated control. At higher EC 8 dS $m^{-1}$, maximum increase (up to 128.8%) in number of pods per pot was recorded due to co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* while rest of the treatments also showed increase in number of pods per plant that was ranging from 44.7 to 72.2 higher over respective control. Bacterial inoculation significantly increased the 100-grain weight under normal and salt stressed soil conditions.

Maximum increase in 100-grain weight was recorded up to 18.5% than uninoculated control by co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* at EC 1.19 dS $m^{-1}$ (Table 4) Sole inoculation also significantly increased the 100-grain weight that ranged from 10.9 to 12.7% due to *P. putida* - $KB_3$ and *R. phaseoli* as compared to control. At 4.0 dS $m^{-1}$, maximum increase in 100-grain weight was obtained (up to 7.1%) due to co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli*. While single inoculation also showed significant increase in 100-grain weight that was up to 2.9% higher over control. At higher EC 8 dS $m^{-1}$, highest increase in 100-grain weight was recorded up to 35.1% by combined use of *P. putida* - $KB_3$ and *R. phaseoli* while rest of the treatments also gave significant increase in 100-grain weight over respective control.Maximum increase (up to 50%) in root length was recorded as a result of co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* over control at EC 1.19 dS $m^{-1}$.

**Table 1:** Physicochemical characteristics of agricultural soils used in the study

| Soil properties | Soil analysis | | Reference |
|---|---|---|---|
| | Pre-experiment | Post experiment | |
| Mechanical analysis | | | |
| Sand (g $kg^{-1}$) | 591 | | Bouyoucos (1962) |
| Silt (g $kg^{-1}$) | 192 | | |
| Clay (g $kg^{-1}$) | 217 | | |
| Textural class | Sandy loam | | |
| Chemical analysis | | | |
| pH | 7.84 | 7.82 | US Salinity Laboratory Staff (1954) |
| ECe (dS $m^{-1}$) | 2.13 | 2.20 | |
| Soil organic matter (g $kg^{-1}$) | 7.91 | 7.97 | Walkley and Black (1934) |
| Total soil N (mg $kg^{-1}$) | 1527 | 1536 | Jackson (1962) |
| Soil available P (mg $kg^{-1}$) | 9.12 | 11.21 | (Watanabe and Olsen, 1965). |
| Soil available K (mg $kg^{-1}$) | 163.4 | 168.7 | US Salinity Laboratory Staff (1954) |

±: Standard error of means

**Table 2:** Characterization and identification of bacterial co-inoculants used in the study

| Bacterial strains | ACC-deaminase activity (nmol α-ketobutyrate $mg^{-1}$ protein $h^{-1}$) | Chitinase activity (qualitative) | Siderophores production | Phosphate solubilization | Root colonization (cfu $g^{-1}$) |
|---|---|---|---|---|---|
| *P. putida* – KB3 | 461 ± 11.3 | +ve | +ve | ++ve | $4.76 \times 10^5$ |
| *Rizobium phaseoli* | ND | -ve | +ve | +ve | $6.71 \times 10^4$ |

±: Standard error of means; ND: Not determine, Mean values are average of four replicates

**Table 3:** Effect of bacterial co-inoculation on growth and yield of mungbean under salt stressed pot conditions

| Bacterial co-inoculants | Salinity levels | | | Main effect of strains |
|---|---|---|---|---|
| | 1.19 dS $m^{-1}$ | 4.0 dS $m^{-1}$ | 8.0 dS $m^{-1}$ | |
| Plant height (cm) | | | | |
| Control (Uninoculated) | 40.84 gh | 30.72 de | 18.90 a | 30.15 A |
| *Pseudomonas putida* - $KB_3$ | 42.41 h | 32.77 ef | 24.29 bc | 33.16 AB |
| *Rhizobium phaseoli* | 43.28 hi | 33.78 ef | 21.78 ab | 32.95 AB |
| *P. putida* - $KB_3$ x *R. phaseoli* | 40.84 i | 37.01fg | 26.95 cd | 37.32 B |
| Main effect of salinity | 43.63 C | 33.57 B | 22.98 A | |
| Fresh biomass (g $plant^{-1}$) | | | | |
| Control (Uninoculated) | 62.37 f | 35.57 c | 25.36 a | 44.27 A |
| *Pseudomonas putida* - $KB_3$ | 66.46 g | 41.25 de | 29.70 b | 43.91 A |
| *Rhizobium phaseoli* | 69.54 g | 37.76 cd | 29.19 b | 44.22 A |
| *P. putida* - $KB_3$ x *R. phaseoli* | 77.12 h | 43.24 e | 34.37 c | 51.58 B |
| Main effect of salinity | 68.87 C | 39.45 B | 29.66 A | |
| Straw yield (g $plant^{-1}$) | | | | |
| Control (Uninoculated) | 47.65 g | 27.98 bc | 20.63 a | 32.08 A |
| *Pseudomonas putida* - $KB_3$ | 49.59 gh | 32.82 de | 24.07 ab | 35.49 AB |
| *Rhizobium phaseoli* | 52.52 h | 29.33 cd | 24.94 bc | 35.60 AB |
| *P. putida* - $KB_3$ x *R. phaseoli* | 58.59 i | 33.59 de | 26.70 b | 39.63 B |
| Main effect of salinity | 52.09 C | 30.93 B | 24.08 A | |
| Grain yield (g $plant^{-1}$) | | | | |

| | | | | |
|---|---|---|---|---|
| Control (Uninoculated) | 14.72 d | 7.59 b | 4.26 a | 8.86 A |
| *Pseudomonas putida* - $KB_3$ | 16.86 e | 8.40 b | 5.63 ab | 10.03 BA |
| *Rhizobium phaseoli* | 17.02 ef | 8.43 bc | 4.73 a | 10.06 B |
| *P. putida* - $KB_3$ x *R. phaseoli* | 18.52 f | 9.65 c | 7.67 b | 11.95 C |
| Main effect of salinity | 16.78 C | 8.52 B | 5.57 A | |

Values are means of three replicates. Values sharing different letters, in a column, differ significantly from each other at $p < 0.05$ (Duncan's multiple range test)

While sole inoculation also significantly increased the root length that ranged from 15.2 to 21.3% with *R. phaseoli* and *P. putida* - $KB_3$ respectively, than control. At 4.0 dS $m^{-1}$, maximum increase in root length was recorded (up to 76%) through co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* over control. While sole inoculation also showed significant increase in root length that was up to 30.4% more over uninoculated control. At higher EC 8 dS $m^{-1}$, maximum root length was 115.3% higher over respective control due to co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* while rest of the treatments also gave significant increase in root length that was up to 60% higher over control. Similar to root length, at EC 1.19 dS $m^{-1}$, maximum (up to 19.8%) increase in root dry weight was obtained as a result of co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* as compared to control. While sole inoculation also increased the root dry weight that ranged from 3.2 to 8.1% due to inoculation with *R. phaseoli* and *P. putida* - $KB_3$ respectively, over respective control. At 4.0 dS $m^{-1}$, maximum increase in root dry weight was recorded (up to 101%) through co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* over control. While single inoculation also showed significant increase in root dry weight that was up to 76.1% more over control. At higher EC 8 dS $m^{-1}$, maximum root dry weight was 377.5% higher over respective control by combined use of *P. putida* - $KB_3$ and *R. phaseoli* while rest of the treatments also depicted significant increase in root dry weight that ranged from 209 to 266.3% due to inoculation with *R. phaseoli* and *P. putida* - $KB_3$ respectively, over respective control.

## 3.1. Nodulation and protein content

Bacterial inoculation significantly increased the number of nodules per plant under normal and salt stressed soil conditions (Table 5). At EC 1.19 dS $m^{-1}$, maximum number of nodules per plant was recorded (up to 43) as compared to uninoculated control with application of *P. putida* - $KB_3$ and *R. phaseoli*. While sole inoculation also gave significant increase in number of nodules per plant that ranged from 32 to 36% higher because of inoculation with *P. putida* - $KB_3$ and *R. phaseoli* over respective control. At 4.0 dS $m^{-1}$, maximum increase in number of nodules per plant was obtained (up to 26) by combined application of *P. putida* - $KB_3$ and *R. phaseoli* over control. While sole inoculation also gave significant increase in number of nodules per plant that was 13 as compared to control. At higher EC 8 dS $m^{-1}$, maximum nodules per plant was recorded up to 12 by integrated application of *P. putida* - $KB_3$ and *R. phaseoli* while rest of the treatments also showed significant increase in number of nodules per plant that range from 6 to 7 as compared to control treatment. Bacterial sole inoculation/co-inoculation showed significant increase in nodule fresh weight per plant under normal and salt stressed soil conditions (Table 5). At 1.19 dS $m^{-1}$, maximum nodule fresh weight per plant was obtained up to 160.4% more over uninoculated control by co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli*. While sole inoculation also gave significant improvement in nodule fresh weight per plant that was up to 109.5% due to sole inoculation as compared to respective control. At 4.0 dS $m^{-1}$, maximum increase in nodule fresh weight per plant was recorded (up to 243.1%) through co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* over control. While single inoculation also gave significant increase in nodule fresh weight per plant that was up to 85.7% more over control. At higher EC 8 dS $m^{-1}$, highest nodule fresh weight per plant was recorded up to 440.8% higher over respective control by application of *P. putida* - $KB_3$ and *R. phaseoli* while remaining treatments also exhibited

significant increase in nodule fresh weight per plant that was ranging from 157.2 to 329.8% more over control treatment. Maximum nodule dry weight per plant (168.7% higher over control) was recorded due to co-inoculation when tested at EC 1.19 dS $m^{-1}$. But sole inoculation also showed promising increase in nodule dry weight per plant that ranged from 97.3 to 116.7% because of inoculation with *P. putida* - $KB_3$ and *R. phaseoli* respectively, over control. At 4.0 dS $m^{-1}$, maximum nodule dry weight per plant was recorded up to 244% because of co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* over control. While sole inoculation also gave significant increase in nodule dry weight per plant that were up to 85.4% more over uninoculated control. At EC 8 dS $m^{-1}$, maximum nodule dry weight per plant was recorded up to 442% higher over respective control by co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* while rest of the treatments also gave significant increase in nodule dry weight per plant that was ranging from 157.3 to 240% higher than control. Results regarding protein content in grain showed that inoculation/or co-inoculation had shown promising increase in protein content in grain under normal and salt stressed soil conditions (Table 5). At EC 1.19 dS $m^{-1}$, maximum increase in protein content in grain was 20.4% due to co-inoculation with *P. putida* - $KB_3$ x *R. phaseoli* over uninoculated control. While sole inoculation also gave better increase in protein content in grain that was up to 11.8% over control. At EC 4.0 dS $m^{-1}$, maximum protein content in grain was recorded up to 20.8% through co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* over control. While sole inoculation also gave significant increase in protein content in grain that was up to 15.4% more than uninoculated control.

**Table 4:** Effect of bacterial co-inoculation on number of pods per plant, 100-grain weight and root growth of mungbean under salt stressed pot conditions

| Bacterial co-inoculants | Salinity levels | | | Main effect of strains |
|---|---|---|---|---|
| | 1.19 dS $m^{-1}$ | 4.0 dS $m^{-1}$ | 8.0 dS $m^{-1}$ | |
| Number of pod per plant | | | | |
| Control (Uninoculated) | 23.61 e | 12.32 c | 5.83 a | 13.92 A |
| *Pseudomonas putida* - $KB_3$ | 27.71 fg | 17.45 d | 7.99 ab | 17.72 B |
| *Rhizobium phaseoli* | 30.79 g | 19.28 d | 10.04 bc | 20.04 B |
| *P. putida* - $KB_3$ x *R. phaseoli* | 34.90 h | 26.47 ef | 13.34 c | 24.91 C |
| Main effect of salinity | 29.25 C | 18.88 B | 9.30 A | |
| 100-grain weight (g) | | | | |
| Control (Uninoculated) | 5.14 cd | 4.17 b | 3.42 a | 4.24 A |
| *Pseudomonas putida* - $KB_3$ | 5.70 def | 5.25 de | 4.33 b | 5.09 B |
| *Rhizobium phaseoli* | 5.79 ef | 5.29 de | 4.30 b | 5.12 B |
| *P. putida* - $KB_3$ x *R. phaseoli* | 6.09 f | 5.50 def | 4.62 bc | 5.41 B |
| Main effect of salinit | 5.68 C | 5.05 B | 4.17 A | |
| Root fresh weight (g $plant^{-1}$) | | | | |
| Control (Uninoculated) | 52.39 e | 22.35 b | 7.45 a | 27.40 A |
| *Pseudomonas putida* - $KB_3$ | 98.85 g | 31.93 c | 19.16 b | 49.98 B |
| *Rhizobium phaseoli* | 108.29 h | 41.51 d | 25.32 bc | 58.38 C |
| *P. putida* - $KB_3$ x *R. phaseoli* | 134.65 i | 76.69 f | 40.29 d | 83.88 D |
| Main effect of salinit | 98.55 C | 43.12 B | 23.06 A | |
| Root dry weight (g $plant^{-1}$) | | | | |
| Control (Uninoculated) | 7.39 d | 3.30 b | 1.10 a | 3.93 A |
| *Pseudomonas putida* - $KB_3$ | 14.58 f | 4.71 bc | 2.83 ab | 7.37 B |
| *Rhizobium phaseoli* | 16.02 f | 6.12 cd | 3.74 b | 8.63 B |
| *P. putida* - $KB_3$ x *R. phaseoli* | 19.86 g | 11.35 e | 5.97cd | 12.39 C |
| Main effect of salinit | 14.46 C | 6.37 B | 3.41 A | |

Values are means of three replicates. Values sharing different letters, in a column, differ significantly from each other at $p < 0.05$ (Duncan's multiple range test)

At higher EC 8 dS $m^{-1}$, maximum protein content in grain was obtained up to 34.5% more over control by application of *P. putida* - $KB_3$ and *R. phaseoli* while rest of the treatments also showed significant increase in protein content in grain that was up to 22% over respective control.

## 3.2. Biochemical parameters

Maximum (up to 22.8% over control) increase of N content in straw was obtained as a result of co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli*. Sole inoculation also exhibited a promising increase in N content in straw that ranged from 10 to 14.1% as compared to control. At 4.0 dS $m^{-1}$, maximum increase in N content in straw was recorded (up to 30%) through co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* over control. While sole inoculation also gave significant increase in N content in straw that was up to 24% more over control. At higher EC 8 dS $m^{-1}$, maximum N content in straw was 47.1% higher over respective control by application of *P. putida* - $KB_3$ and *R. phaseoli* while rest of the treatments also depicted a significant increase in N content in straw that was up to 32.3% more over uninoculated control. In case of phosphorus (P) content in grain, maximum increase in grain was recorded as up to 27.7% by co-inoculation with *P. putida* - $KB_3$ x *R. phaseoli* over control. While sole inoculation also gave better increase in P content in grain that ranged from 14.2 to 18.5% due to sole inoculation with *R. phaseoli* and *P. putida* - $KB_3$ respectively, over respective control. At 4.0 dS $m^{-1}$, maximum increase in P content in grain was recorded up to 30.1% through co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* over control.

**Table 5:** Effect of bacterial co-inoculation on nodulation and protein content of mungbean under salt stressed pot conditions

| Bacterial co-inoculants | Salinity levels | | | Main effect of strains |
|---|---|---|---|---|
| | 1.19 dS $m^{-1}$ | 4.0 dS $m^{-1}$ | 8.0 dS $m^{-1}$ | |
| Number of nodule per plant | | | | |
| Control (Uninoculated) | 15.77 | 7.00 | 2.77 | 8.51 A |
| *Pseudomonas putida* - $KB_3$ | 32.00 | 10.00 | 6.00 | 16.00 B |
| *Rhizobium phaseoli* | 36.00 | 13.00 | 7.93 | 18.98 B |
| *P. putida* - $KB_3$ x *R. phaseoli* | 43.00 | 26.00 | 12.00 | 27.00 C |
| Main effect of salinity | 31.69 C | 14.00 B | 7.18 A | |
| Nodule fresh weight (g $plant^{-1}$) | | | | |
| Control (Uninoculated) | 52.39 e | 22.35 b | 7.45 a | 27.40 A |
| *Pseudomonas putida* - $KB_3$ | 98.85 g | 31.93 c | 19.16 b | 49.98 B |
| *Rhizobium phaseoli* | 108.29 h | 41.51 d | 25.32 bc | 58.38 C |
| *P. putida* - $KB_3$ x *R. phaseoli* | 134.65 i | 76.69 f | 40.29 d | 83.88 D |
| Salinity levels | 98.55 C | 43.12 B | 23.06 A | |
| Nodule dry weight (g $plant^{-1}$) | | | | |
| Control (Uninoculated) | 7.39 d | 3.30 b | 1.10 a | 3.93 A |
| *Pseudomonas putida* - $KB_3$ | 14.58 f | 4.71 bc | 2.83 ab | 7.37 B |
| *Rhizobium phaseoli* | 16.02 f | 6.12 cd | 3.74 b | 8.63 B |
| *P. putida* - $KB_3$ x *R. phaseoli* | 19.86 g | 11.35 e | 5.97cd | 12.39 C |
| Salinity levels | 14.46 C | 6.37 B | 3.41 A | |
| Protein content (%) | | | | |
| Control (Uninoculated) | 20.01 ef | 17.47 cd | 12.15 a | 16.54 A |
| *Pseudomonas putida* - $KB_3$ | 21.55 efg | 19.67 de | 14.83 b | 18.68 AB |
| *Rhizobium phaseoli* | 22.37 fg | 20.17 ef | 14.26 ab | 18.93 AB |
| *P. putida* - $KB_3$ x *R. phaseoli* | 24.09 g | 21.11 ef | 16.34 bc | 20.51 B |
| Salinity levels | 22.01 B | 19.60 B | 14.39 A | |

Values are means of three replicates. Values sharing different letters, in a column, differ significantly from each other at $p < 0.05$ (Duncan's multiple range test) While sole inoculation also showed significant increase in P content in grain that was up to 25% more than uninoculated control. At higher EC 8 dS $m^{-1}$, maximum P content in grain was 56.5% higher over respective control by application of *P. putida* - $KB_3$ and *R. phaseoli* while rest of the treatments also showed significant increase in P content in grain that was up to 43.4% higher over respective control. Results about P content in straw showed that maximum increase (up to 29.3% over control) of P content in straw was obtained due to co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli.* While sole inoculation also gave increase in P content in straw that was up to 20% by sole inoculation as compared to control. At 4.0 dS $m^{-1}$, maximum increase in P content in straw was recorded up to 26.3% higher through co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* over control. While single inoculation also showed significant increase in P content in straw that was up to 21.1% more over control. At EC 8 dS $m^{-1}$, maximum P content in straw was recorded up to 58.3% higher over respective control by co-inoculation with *P. putida* - $KB_3$ and *R. phaseoli* while rest of the treatments also showed significant increase in P content in straw that ranged from 41.7 to 33.3% because of sole inoculation more over uninoculated control.

## 4. Discussion

In the present study, pre-isolated strains of PGPR containing ACC-deaminase activity (*P. putida* - $KB_3$) and *Rhizobium* (*R. phaseoli*) were tested alone as well as in combination at three different salinity levels (i.e. 1.19, 4.0 and 8.0 dS $m^{-1}$) to evaluate their effects on symbiotic efficacy and yield of mungbean under pot conditions.

### 4.1. Effect of PGPR inoculation on growth, nodulation and yield of mungbean under pot conditions

In this study, the salinity levels significantly ($p \leq 0.05$) reduced the growth of mungbean seedlings but root growth was relatively more affected in comparison to shoot. It is highly likely that the metabolic activity of the root was affected more than shoot. Moreover, direct exposure of growing roots to salt stress reduced root length and development (Mayak *et al.,* 2004). Elevated salinity in the rhizosphere reduces root zone osmotic potential (Chartzoulakis *et al.,* 2002), thus decreases the availability of water for plants. It is well documented that stress induced higher ethylene accumulation would result into reduced root growth (Madhaiyan *et al.,* 2007). Similarly, negative reports of salt stress on legume-*Rhizobium* symbiosis has been documented by many researchers (Cheng *et al.,* 2007; Shahzad *et al.,* 2014). In the current study, inoculation with PGPR containing ACC-deaminase increased the

**Table 6:** Effect of bacterial co-inoculation on biochemical attributes of mungbean under salt stressed conditions

| Bacterial co-inoculants | Salinity levels | | | Main effect of strains |
|---|---|---|---|---|
| | 1.19 dS $m^{-1}$ | 4.0 dS $m^{-1}$ | 8.0 dS $m^{-1}$ | |
| Nitrogen content in straw (%) | | | | |
| Control (Uninoculated) | 0.60 c | 0.50 b | 0.34 a | 0.48 A |
| *Pseudomonas putida* - $KB_3$ | 0.66 cd | 0.60 c | 0.45 b | 0.57 B |
| *Rhizobium phaseoli* | 0.68 de | 0.62 cd | 0.44 b | 0.58 B |
| *P. putida* - $KB_3$ x *R. phaseoli* | 0.74 e | 0.65 cd | 0.50 b | 0.63 B |
| Main effect of salinity | 0.67 C | 0.59 B | 0.43 A | |
| Phosphorus content in grain (%) | | | | |
| Control (Uninoculated) | 0.42 d | 0.36 c | 0.23 a | 0.34 A |
| *Pseudomonas putida* - $KB_3$ | 0.48 gh | 0.43 de | 0.33 b | 0.41 B |
| *Rhizobium phaseoli* | 0.49 h | 0.45 ef | 0.32 b | 0.42 B |
| *P. putida* - $KB_3$ x *R. phaseoli* | 0.53 i | 0.47 fg | 0.36 c | 0.45 C |
| Salinity levels | 0.48 C | 0.43 B | 0.31 A | |

| | | | | |
|---|---|---|---|---|
| Phosphorus content in straw (%) | | | | |
| Control (Uninoculated) | 0.21 d | 0.19 c | 0.12 a | 0.17 A |
| *Pseudomonas putida* - $KB_3$ | 0.25 fg | 0.22 de | 0.17 bc | 0.21 B |
| *Rhizobium phaseoli* | 0.26 gh | 0.23 def | 0.16 b | 0.22 BC |
| *P. putida* - $KB_3$ x *R. phaseoli* | 0.27 h | 0.24 efg | 0.19 c | 0.23 C |
| Salinity levels | 0.25 C | 0.22 B | 0.16 A | |

Values are means of three replicates. Values sharing different letters, in a column, differ significantly from each other at $p < 0.05$ (Duncan's multiple range test)

root length and weight compared to un-inoculated control. This could be affirmative of ethylene lowering in plant by bacterial ACC deaminase, thereby improving plant growth. The consistent higher ACC deaminase activity of PGPR thus indicates its potential for plant growth promotion over a range of stressful conditions that would cause the ethylene accumulation and inhibit root growth (Cheng *et al.,* 2007; Nadeem *et al.,* 2009). While, in this study, sole inoculation of *P.* PGPR containing ACC-deaminase enzyme cleaves ACC; the immediate precursor of ethylene and ammonia in the α-ketobutyrate (Glick *et al.,* 1998). PGPR inoculated plants showed higher plant height, fresh biomass and grain yield. These results clearly suggests that selected microorganisms can alter the morphology of the root system by enhancing root length and number of lateral roots, resulting in enhanced water uptake and increased shoot and root biomass (Martínez-Viveros *et al.,* 2010), which may be considered as a mechanism of salinity tolerance. The higher root length of inoculated plants is probably due to the availability of IAA hormone in the medium secreted by both inoculating bacteria. Additionally, IAA-producing PGPR strain colonization with plant roots seems to be responsible for the higher root length and nutrient uptake under hydroponic conditions (Shukla *et al.,* 2012). Several other researchers also reported that PGPR strains show IAA activity, and the interaction of IAA with plants could help in increased root growth under salinity, which may be an adaptive response to stresses, and also can contribute to maintaining leaf growth which is considered as a primary response of plant productivity under conditions of salinity (Albacete *et al.,* 2008).

## 4.2. Effect of Rhizobium inoculation on growth, nodulation and yield of mungbean under pot conditions

In our study, *Rhizobium* inoculation significantly increased the growth and yield of mungbean grown in salt stressed conditions under pot conditions. This might be because of the multifarious plant growth promoting mechanisms such as $N_2$-fixation, production of growth regulators and suppression of plant diseases. These results are further supported by the previous work of Naz *et al.* (2009); Shahzad *et al.* (2014) reported that strains of rhizobia vary in their ability to tolerate salt stress and subsequent plant growth promotion. Similarly, Hafeez *et al.* (1988) reported that most strains of *Rhizobium* were tolerant of salt and better results for the advancement of *Vigna radiate* growth under salt stressed conditions. In general, legumes plants are sensitive to environmental constraints (Zahran, 1999). However, some recent studies related to rhizobia had shown salt tolerance for legume host (Abdelsalam *et al.,* 2010). Therefore, it is imperative to characterize native *rhizobial* strains that are more efficient to fix atmospheric $N_2$ under salt stress conditions (Woldeyohannes *et al.,* 2007; Shahzad *et al.,* 2008). In this study, number of nodules and fresh biomass of mungbean was affected by salinity. Early nodule initiation is very sensitive towards specific stress factor such as salinity. The negative effect of salt stress on N2-fixation by mungbean could be ascribed through three different responses, mainly affecting rhizobial infection of legumes, the effect on growth and development of nodules and finally a direct effect on the activity of nodule $N_2$-fixation (Bouhmouch *et al.,* 2005).

### 4.3. Effect of co-inoculation with PGPR and rhizobium on growth, nodulation and yield of mungbean under pot conditions

The results of this study showed that the co-inoculation with rhizobia PGPR containing ACC-deaminase activity was more effective in improving growth, nodulation and yield of mung bean as sole inoculation of *Rhizobium* or rhizobia under salt stressed conditions. Most likely containing ACC-deaminase activity PGPR promoted growth by reducing ethylene levels in roots (Arshad *et al.,* 2008). It is very likely that these rhizobacteria may also have stimulated nodulation indirectly through increased root growth of rhizobia provide multiple sites of infection for nodulation. Similarly, Mishra *et al.* (2009) also reported the improved nodule occupancy of *bradyrhizobium* in soybean due to the co-inoculation of *P. fluorescens*. It was also shown that the co-inoculation of PGPR containing ACC deaminase with rhizobia improved plant growth by reducing the level of ethylene (Shaharoona *et al.,* 2006). This could be due to the active synergy between PGPR and rhizobia with elevated plant growth promoting expression.

## 5. Conclusions

The bacterial inoculants used in this study were tested alone as well as in combination for evaluating their ability to promote growth, nodulation and yield of mungbean under salt stressed conditions. While, co-inoculation of *Rhizobium* and PGPR containing ACC-deaminase activity save the plant from harsh effects of stress environment, so far, some aspects still need further assessment. The well-focused approach keeping in view the molecular and physiological aspect of stress tolerance is required to facilitate crop production on problem soils. Therefore, further work is needed to evaluate the efficacy of this technology under salt-affected field conditions and the following aspects may particularly be explored. The centered approach considering the molecular and physiological aspects of stress tolerance is necessary to facilitate the production of crops in problematic soils. Therefore, further work is needed to assess the effectiveness of this technology in field conditions affected by salt.

## References

Abdel-Salam, M.S., S.A. Ibrahim, M.M. Abd-ElHalim, F.M. Badawy and S.E.M. Abo-Aba. 2010. Phenotypic characterization of indigenous Egyptian Rhizobial strains for abiotic stresses performance. J. Am. Sci., 6: 498-503.

Abeles, F.B., P.W. Morgan and M.E. Saltveit. 1992. Ethylene in plant biology. 2nd Edn. San Diego, Academic Press

Adesemoye, A.O. and J.W. Kloepper. 2009. Plant–microbes interactions in enhanced fertilizer-use efficiency. Appl. Microbiol. Biotechnol., 85: 1-12.

Albacete, A., M.E. Ghanem, C. Martinez-Andujar, M. Acosta, J. Sanchez-Bravo, V. Martinez, S. Lutts, I.C. Dodd and F. Perez-Alfocea. 2008. Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. J. Exp. Bot., 59: 4119-4131.

Al-Falih, A.M. 2002. Factors affecting the efficiency of symbiotic nitrogen fixation by *Rhizobium*. Pak. J. Biol Sci., 5: 1277-1293.

Allen, S.E., H.M. Grimshaw, A.P. Rowland. 1986. Chemical analysis. Moore, P.D., S.B. Chapman (Eds.), Methods in Plant Ecology, Blackwell Scientific Publication, Oxford, London, 285–344.

Anonymous. 2013. Agricultural Statistics of Pakistan. Ministry of Food, Agriculture and Livestock. Economic Wing, Government of Pakistan, Islamabad.

Antoun, H. and D. Prevost. 2005. Ecology of plant growth promoting rhizobacteria. In: Siddiqui, Z.A. (Ed.), PGPR: biocontrol and biofertilization, Springer, Dordrecht, 1–38.

Arshad, M. and W.T. Frankenberger. 2002. Ethylene: Agricultural Sources and Applications. Kluwer Academic Publishers, New York, 342 pp.

Arshad, M., B. Shaharoona and T. Mahmood. 2008. Inoculation with plant growth promoting rhizobacteria containing ACC-deaminase partially eliminates the effects of water stress on growth,

yield and ripening of (*Pisum sativum* L.). Pedosphere, 18: 611-620.

Ashraf, R. and T.A. Ali. 2007. Effect of heavy metals on soil microbial community and mung beans seed germination. Pak. J. Bot., 39: 629-636.

Berg, G. 2009. Plant microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl. Microbiol. Biotechnol., 84: 11-8.

Bhattacharyya, P.N. and D.K. Jha. 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol. Biotech., 28:1327-1350.

Bouhmouch, I., B. Souad-Mouhsine, F. Brhada and J. Aurag. 2005. Influence of host cultivars and Rhizobium species on the growth and symbiotic performance of Phaseolus vulgaris under salt stress. J. Plant Physiol., 162: 1103-1113.

Bouyoucos, G.J. 1962. Hydrometer method improved for making particle size analysis of soils. Agron. J., 54: 464-465.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72: 248-254.

Cattelan, A.J., Hartel, P.G. and J.J. Fuhrmann. 1999. Screening for plant growth promoting rhizobacteria to promote early soybean growth. Am. J. Soil Sci., 63: 1670-1680.

Chartzoulakis, K., M. Loupassaki, M. Bertaki and I. Androulakis. 2002. Effects of NaCl salinity on growth, ion content and CO< sub> 2</sub> assimilation rate of six olive cultivars. Sci. Horti., 96: 235-247.

Cheng, Z., Park, E. and Glick, B.R. 2007. 1-Aminocyclopropane-1-carboxylate (ACC) deaminase from Pseudomonas putida UW4 facilitates the growth of canola in the presence of salt. Can J Microbiol., 53: 912-8.

Contesto, C., G. Desbrosses, C. Lefoulon and F.G. Bena. 2008. Effects of rhizobacterial ACC-deaminase activity on Arabidopsis indicate that ethylene mediates local root responses to plant growth promoting rhizobacteria. Plant Sci., 175: 178-189.

Duan, J., K.M. Müller, T.C. Charles, S. Vesely and B.R. Glick. 2008. 1-aminocyclopropane-1-carboxylate (ACC) deaminase genes in Rhizobia: Isolation, characterization and regulation. 7th International PGPR Workshop, Amsterdam.

Duncan, D.B. 1955. Multiple rang and F test. Biometrics, 11: 1-42.

Glick, B.R., D.M. Penrose and J.A. Li. 1998. Model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J. Theor. Biol., 190: 3-68.

Glick, B.R., Z. Cheng, J. Czarny, Z. Cheng and J. Duan. 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur. J. Plant Pathol., 119: 329-39.

Hafeez, F.Y., Z. Aslam and K.A. Malik. 1988. Effect of salinity and inoculation on growth, nitrogen fixation and nutrient uptake of *Vigna radiata* (L.) Wilczek. Plant Soil, 106: 3-8.

Hamdia, M.A. and M.A.K. Shaddad. 2010. Salt tolerance of crop plants. J. Stress Physiol. Biochem., 6: 64-90.

Hayat, R., S. Ali, U. Amara, R. Khalid and I. Ahmed. 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. Ann. Microbiol., 60: 579-98.

Kaymak, D.C. 2010. Potential of PGPR in agricultural innovations. In: Maheshwari DK, editor. Plant growth and health promoting bacteria. Berlin Heidelberg, Germany: Springer Verlag.

Khalid, A., M. Arshad, B. Shaharoona and T. Mahmood. 2009. Plant growth promoting rhizobacteria (PGPR) and sustainable agriculture. Microbial Strategies for Crop Improvement. Springer-Verleg, Germany.

Khalid, A., M.J. Akhtar, M.H., Mahmood and M. Arshad. 2006. Effect of substrate dependent microbial produced ethyleneon plant growth. Microbiol., 75: 231-236.

Kjeldahl, J. 1883. Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern. Z. Anal. Chem. 22: 366-382

Lau, J.A. and J.T. Lennon. 2011. Evolutionary ecology of plant–microbe interactions: soil microbial structure alters selection on plant traits. New phytologist., 192: 215-224.

Lebeau, T., A. Braud and K. Jezequel. 2008. Performance of bioaugmentation-assisted

phytoextraction applied to metal contaminated soils: A review. Environ. Poll., 153: 497-522.

Li, J., C. Sun and F.F. Jin. 2013. NAO implicated as a predictor of Northern Hemisphere mean temperature multidecadal variability. Geophy. Res. Lett., 40: 5497-5502.

Ma, J.H., L. Yao, D. Chen and B. Morris. 1998. Ethylene inhibitors enhance vitro formation from apple shoot culture. Plant Cell Rep.,17: 211-214.

Madhaiyan, M., S. Poonguzhali and T. Sa. 2007. Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (*Lycopersicon esculentum* L.). Chemosphere, 69: 220-228.

Martínez-Viveros, O., M.A. Jorquera, D.E. Crowley, G. Gajardo and M.L. Mora. 2010. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J. Soil Sci. Plant Nutr., 10: 293-319.

Masoud, A. and S.T. Abbas. 2008. Evaluation of fluorescent pseudomonas for plant growth promotion, antifungal activity against *Rhizoctonia solanion* common bean, and bio-control potential. Biol. Cont., 48: 101-107.

Mayak, S., T. Tirosh and B.R. Glick. 2004. Plant growth promoting bacteria that confer resistance in tomato plants to salt stress. Plant Physiol. Biochem., 42: 565-72.

Mishra, P.K., S. Mishra, G. Selvakumar, S. Kundu and H. Gupta. 2009. Enhanced soybean (*Glycine max* L.) plant growth and nodulation by *Bradyrhizobium japonicum*-SB1 in presence of *Bacillus thuringiensis*-KR1. Acta Agri. Scand. Section–B Soil Plant Sci., 59: 189-196.

Nadeem, S.M., Z.A. Zahir, M. Naveed and M. Arshad. 2009. Rhizobacteria containing ACC deaminase confer salt tolerance in maize grown on salt affected soils. Can. J. Microbiol., 55: 1302-1309.

Nadeem, S.M., Z.A. Zahir, M. Naveed and M. Arshad. 2010. Microbial ACC-deaminase: prospects and applications for inducing salt tolerance in plants. Crit Rev Plant Sci., 29: 360-93.

Naeem, F., K.A. Malik and F.Y. Hafeez. 2008. *Pisum sativum*–Rhizobium interactions under different environmental stresses. Pak. J. Bot., 40: 2601-2612.

Naz, I., A. Bano and T. Ul-Hassan. 2009. Isolation of phytohormones producing plant growth promoting rhizobacteria from weeds growing in Khewra salt range, Pakistan and their implication in providing salt tolerance to *Glycine max* L. Afri. J. Biotech., 8: 5762-5766.

Nukui, N., H. Ezura, K. Yohsshi, T. Yasutaand and T. Minamisawa. 2000. Effect of ethylene precursor and inhibitors for ethylene biosynthesis and perception on nodulation in *Lotus japonicas* and *Macroptilium atropurpureum*. Plant Cell Physiol., 41: 893-897.

Parida, A. and A.B. Das. 2005. Salt tolerance and salinity effects on plants. Ecotoxicol. Environ. Safety, 60: 324-349.

Patel, D.K., G. Archana and G.N. Kumar. 2008. Variation in the nature of organic acid secretion and mineral phosphate solubilization by *Citrobacter* sp. in the presence of different sugars. Cur. Microbiol., 56: 168-174.

Penrose, D.M. and B.R. Glick. 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol Plant., 118: 10-15

Saharan, B.S. and V. Nehra. 2011. Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res., 21: 1-30.

Schwyn, B. and J.B. Neilands. 1987. Universal chemical assay for the detection and determination of siderophore. Ann. Biochem. 169: 47-56.

Sehrawat, N., M. Yadav, K.V. Bhat, R.K. Sairam and P.K. Jaiwal. 2015. Effect of salinity stress on mungbean [*Vigna radiata* (L.) wilczek] during consecutive summer and spring seasons. J. Agri. Sci., 60: 23-32.

Shaharoona, B., M. Arshad and Z.A. Zahir. 2006. Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation of mungbean (*Vigna radiata*). Lett Appl. Microbiol., 42: 155-159.

Shahzad, S.M, A. Khalid, M. Arshad and Kalil-ur-Rehman. 2010. Screening rhizobacteria containing ACC-deaminase for growth promotion of chickpea seedlings under axenic conditions. Soil Environ., 29: 38-46

Shahzad, S.M., A. Khalid, M. Arshad, M. Khalid and I. Mehboob. 2008. Integrated use of plant growth promoting bacteria and P-enriched compost for improving growth, yield and nodulation of chickpea. Pak. J. Bot. 40: 1735-1441.

Shahzad, S.M., A. Khalid, M.S. Arif, M. Riaz, M. Ashraf, Z. Iqbal and T. Yasmeen. 2014. Co-inoculation integrated with P-enriched compost improved nodulation and growth of chick pea (*Cicer asietinum* L.) irrigated and rainfed forming systems. Biol. Fert. Soils, Doi: 10.1007/s 00374-013-0826-2.

Shahzad, S.M., Arif, M. S., Riaz, M., Iqbal, Z., and Ashraf, M. (2013). PGPR with varied ACC-deaminase activity induced different growth and yield response in maize (*Zea mays* L.) under fertilized conditions. Eur. J. Soil Biol., 57: 27-34.

Shukla, A., A. Kumar, A. Jha, O.P. Chaturvedi, R. Prasad and A. Gupta. 2009. Effects of shade on arbuscular mycorrhizal colonization and growth of crops and tree seedlings in Central India. Agroforest. Syst., 76: 95-109

Simons, M., A.J. Van der Bij, I. Brand, L.A. de Weger, C.A. Wijffelman and B.J.J. Lugtenberg. 1996. Gnotobiotic system for studying rhizosphere colonization by plant growth promoting Pseudomonas bacteria. Mol. Plant Microb. Inter., 9: 600-607.

Steel, R., J. Torrie and D. Dicky. 1997. Principles and procedures of statistics; a biometrical approach. 3rd Ed. W.C.B/Mc Graw-Hill, New York.

US Salinity Laboratory Staff. 1954. Diagnosis and Improvement of Saline and Alkali Soils. L. A. Richards, Ed. U.S. Dept. of Agriculture, Washington, D.C. USA, P 160.

Walkley, A. and I.A. Black. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37: 29-37.

Watanabe, F.S. and S.R. Olsen. 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO3 extracts from soil. Soil Sci. Soc. Am. J., 29: 677-678.

Woldeyohannes, W.H., M.C. Dasilva and M. Gueye. 2007. Research note: Nodulation and nitrogen fixation of Stylosanthes hamata in response to induced drought stress. Arid Land Res. Manage., 21: 157-163.

Wolf, B. 1982. The comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. Commun. Soil Sci. Plant Anal., 3: 1035-1059.

Zahran, H.H. 1999. Rhizobium–legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol. Mol. Biol. Rev., 63: 968-89.