Ann Microbiol (2012) 62:1321–1330
DOI 10.1007/s13213-011-0380-9

ORIGINAL ARTICLE

The combined application of rhizobial strains and plant growth promoting rhizobacteria improves growth and productivity of mung bean (*Vigna radiata* L.) under salt-stressed conditions

Maqshoof Ahmad · Zahir Ahmad Zahir · Hafiz Naeem Asghar · Muhammad Arshad

Received: 4 July 2011 / Accepted: 24 October 2011 / Published online: 15 November 2011

Abstract Salinity stress induces higher levels of ethylene in plants in arid and semi-arid regions. This increased concentration of ethylene can be inhibited by using plant growth promoting rhizobacteria (PGPR) containing ACC-deaminase. A pot trial was conducted under salt-stressed conditions to evaluate the potential of combined application of *Rhizobium phaseoli* (M6 and M9), and PGPR (*Pseudomonas syringae*, Mk1; *Pseudomonas fluorescens*, Mk20 and *Pseudomonas fluorescens* Biotype G, Mk25) to improve the productivity of mung bean. The results showed that salinity stress decreased significantly mung bean growth, yield and physiological parameters but inoculation with either rhizobia or PGPR alone enhanced these parameters significantly. However, the combined application of rhizobia and PGPR was more effective for reducing the depressing effect of salinity on mung bean. Co-inoculation increased the shoot fresh weight (145%), root fresh weight (173%), number of pods plant^{-1}(150%), pod fresh weight (182%), total dry matter (269%), relative water content (19%), water use efficiency (51%), potassium concentration in leaves (33%), sodium concentration in leaves (56%) and nitrogen concentration in grains of mung bean (99%), compared with the uninoculated control. The results imply that combined application of *Rhizobium* and *Pseudomonas* strains can improve the productivity of mung bean. Thus, these strains could be evaluated in intensive field trials for developing biofertilizers to improve the productivity of mung bean under salt-affected conditions.

M. Ahmad · Z. A. Zahir (✉) · H. N. Asghar · M. Arshad
Institute of Soil and Environmental Sciences,
University of Agriculture,
Faisalabad 38040, Pakistan
e-mail: zazahir@yahoo.com

Keywords ACC-deaminase · Ionic balance · *Pseudomonas* · *Rhizobium* · Water use efficiency

Introduction

Mung bean (*Vigna radiata* L.) is an important conventional pulse crop in Pakistan and is grown world-wide. Its importance is due not only to its high nutritional value but also because it improves soil fertility by fixing atmospheric nitrogen (Elahi et al. 2004). However, under the agro-ecological conditions of Pakistan, mung bean yields are low. One of the major factors contributing to its low yield is salinity. Salinity is a serious production problem for crops as saline conditions are known to suppress plant growth, particularly in arid and semi-arid regions (Parida and Das 2005). Pakistan is located in such an arid/semi-arid region where rainfall is low as well as irregular and, under such conditions, the most detrimental natural problems for crop growth are salinity and sodicity. The total cultivated area of Pakistan is 23.80 million ha, of which 6.18 million ha is severely affected by salinity/sodicity (Anonymous 2010). Salinity induces osmotic stress by limiting absorption of water from soil (Mayak et al. 2004) and ionic stress resulting from high concentrations of potentially toxic ions within plant cells.

Salinity induces the imbalance of mineral nutrients and their distribution within plants (Glenn et al. 1999). Salt stress also affects the morphology, anatomy, ultrastructure and metabolism of plant species (Prat and Fathi-Ettai 1990; Silveira et al. 2003), thus resulting in an overall decrease in plant growth and yield. The decreased photosynthetic rate in plants due to salinity stress leads to a decrease in the relative water content of plants (Ben-

Asher et al. 2006). Salinity stress adversely affects total dry matter and plant growth, as most of the energy available is used in to make osmotic adjustments by the plant (Munns and Termaat 1986). Salinity adversely affects plant growth and yield by enhancing ethylene production. Increased production of ethylene due to exogenous application of 1-aminocyclopropane-1-carboxylic acid (ACC) or salinity can decrease root growth (Madhaiyan et al. 2007).

The survival and distribution of rhizobia in soil and the rhizosphere is affected by salinity (Tate 1995). These bacterial strains have variable ability to tolerate salt stress (Lloret et al. 1995). Rhizobial inoculation has been reported to promote plant growth and development by multiple mechanisms such as N_2 fixation, production of plant growth regulators and disease suppression (Naz et al. 2009).

The use of rhizobacteria is one of the most acceptable approaches to reduce the effect of stress-induced ethylene on plants. These plant growth promoting rhizobacteria (PGPR) contain the enzyme ACC-deaminase, which hydrolyzes ACC (immediate precursor of ethylene) to yield ammonia and α-ketobutyrate (Mayak et al. 1999). PGPR can boost plant growth, particularly under stressed conditions, by regulating accelerated ethylene production in response to a multitude of abiotic and biotic stresses (Belimov et al. 2009; Ahmad et al. 2011). Inoculation with PGPR containing ACC-deaminase can increase growth, yield, nutrient concentrations and ionic balance within the plants due to lowering of the ethylene content of these strains (Yue et al. 2007; Nadeem et al. 2009). Inoculation/co-inoculation with *Rhizobium* and PGPR strains containing ACC-deaminase decreased the intensity of the classical triple response by increasing the seedling length of inoculated mung bean seedlings and decreasing the stem diameter—a typical response to the dilution in a classical triple response bioassay (Ahmad et al. 2011). Different PGPR strains differ in their ability to promote plant growth due to differences in ACC deaminase activity (Shaharoona et al. 2006; Nadeem et al. 2009; Ahmad et al. 2011). This difference may also be due to the presence of other growth-promoting characters i.e., chitinase activity, phosphate solubilization, root colonization, etc., in addition to ACC-deaminase activity (Ahmad et al. 2011).

Co inoculation of legumes with rhizobacteria and rhizobia has been reported to stimulate plant dry matter and grain yield by affecting some physiological functions in different crops (Derylo and Skorupska 1993; Dashti et al. 1997). It improves plant growth by reducing ethylene levels (Shaharoona et al. 2006), directly stimulating rhizobial growth/survival in the soil, enlarging the root system by hormone production for enhanced nutrient uptake, and increasing the number of potential colonization sites by rhizobium, phosphate solubilization and pathogen suppression due to the production of antibiotics (Gull et al. 2004; Barea et al. 2005). Root elongation rate, mineral N, P and K and microelements absorption and uptake are reported to be consequently improved after *Azospirillum* inoculation (Dobbelaere and Okon 2007). This could result in a general better mineral nutrition of the plant phosphorous, iron and molybdenum essential for rhizobia-nodule formation and nitrogen fixation activities (Burdman et al. 1998). Co-inoculation has also been reported to increase water use efficiency (WUE) in plants under stress (Vivas et al. 2003). The impact of co-inoculation with Rhizobium and PGPR containing ACC-deaminase has been established under normal conditions. However, we have hypothesized that this approach could be used more effectively under salt-affected conditions. In a series of experiments under axenic (Ahmad et al. 2011) and natural conditions, the efficacy of three strains of PGPR (*Pseudomonas syringae*, Mk1; *Pseudomonas fluorescens*, Mk20 and *Pseudomonas fluorescens* Biotype G, Mk25) and two strains of *Rhizobium phaseoli* (M6 and M9) was studied. The present paper describes the potential of these selected strains to improve the productivity of mung bean under salt-affected conditions in pot trials under ambient conditions.

Materials and methods

Collection of bacterial strains

Three strains of PGPR (*Pseudomonas syringae*, Mk1; *Pseudomonas fluorescens*, Mk20 and *Pseudomonas fluorescens* Biotype G, Mk25) and two strains of *Rhizobium phaseoli* (M6 and M9) were isolated from the rhizosphere and nodules of mung bean growing in salt-affected conditions (EC_e=4.1–6.7 dS m^{-1}), respectively, (Ahmad et al. 2011). These rhizobacterial strains, which contain ACC-deaminase activity, and *Rhizobium* were assessed for their ability to tolerate salt stress by conducting an osmoadaptation assay (data not given) and were evaluated alone and in combination for their potential to improve the productivity of mung bean under salt-stressed conditions in pot trials.

Preparation of inocula

Inocula were prepared in flasks by using yeast extract mannitol (YEM) and DF minimal salt medium (Dworkin and Foster 1958) without agar containing ACC as substrate (N source) for rhizobial and rhizobacterial strains, respectively. Each flask containing broth was inoculated with respective strains of rhizobia or rhizobacteria and incubated at 28±1°C for 72 h under shaking (100 rpm) conditions. After incubation, optical density was measured at 540 nm

for *Rhizobium* and *Pseudomonas* strains, and uniform population (OD_{540}=0.45; 10^7–10^8 CFU mL^{-1}) was achieved by dilution with sterilized water prior to seed inoculation.

Pot trial

The PGPR and *Rhizobium* strains were evaluated alone and in combination for their potential to improve the productivity of mung bean under salt-stressed conditions in a pot trial.

The experiment was conducted with different set of treatments, i.e., inoculation either with *Rhizobium* and rhizobacteria alone, or combinations of these strains in the presence of recommended levels of chemical fertilizers. The soil used in the pots was analyzed for physical and chemical characteristics (Table 1) according to standard protocols as described by Ryan et al. (2001). Mung bean seeds were inoculated with respective bacterial strains by using slurry prepared with sterilized peat, broth culture (OD_{540}=0.45; 10^7–10^8 CFU mL^{-1}) and sterilized sugar solution (10%) in the ratio 5:4:1. For co-inoculation, broth cultures of PGPR and *Rhizobium* were used in a 1:1 ratio for preparation of slurry. In the case of the un-inoculated control, seeds were coated with sterilized (autoclaved) peat treated with sterilized broth.

Three salinity levels (original, i.e., 1.41; 4; 6 dS m^{-1}) were used. These levels were chosen keeping in mind the salinity status of the soils in which mung bean is cultivated in Pakistan. We surveyed the area where mung bean is cultivated and collected soil and root samples for isolation of bacterial strains and the assessment of soil salinity status. These soil samples were analyzed for EC_e and it was observed that these fields had EC_e levels ranging from 4.1 to 6.7 dS m^{-1}. The pots were lined with polythene sheets before adding soil and there was no leaching provision in the pots as the only hole at the bottom was plugged with cork. Three salinity levels, 1.41 (i.e. original), 4 and 6 dS m^{-1} were generated by using a calculated amount of NaCl salt in each pot and mixing it using a mechanical mixer. Ten inoculated seeds of mung bean were sown in each pot containing 12 kg pot^{-1} soil. There were six replications of each treatment. Similarly, three pots (1.41, 4 and 6 dS m^{-1}) were maintained as reference pots to check the effect of irrigation water on salinity levels. EC_e was monitored regularly in the reference pots and from the experimental pots at the end. There was no significant change in the salinity levels at the end. Pots were arranged in a wire cage under ambient light and temperature according to completely randomized design. The recommended dose of P and K fertilizers (60 kg ha^{-1} each) as diammonium phosphate and sulfate of potash, respectively, and half of the recommended dose for N (20 kg ha^{-1}) as urea was applied to each pot. All fertilizers were applied as a basal dose at the time of sowing. Plants were irrigated with good quality irrigation water meeting the irrigation quality criteria for the crop (Ayers and Westcot 1985). After germination, thinning was carried out to maintain a uniform plant population. Plants from three replications were uprooted at the flowering stage to assess nodulation while the remaining three replications were taken to maturity for harvesting. Data on growth and yield parameters were collected at harvest upon physiological maturity.

Table 1 Physical and chemical characteristics of soil used for pot trials

Characteristic	Unit	Value
Sand	%	53.1
Silt	%	27.5
Clay	%	19.4
Textural class	–	Sandy clay loam
Saturation percentage	%	32.0
pH_s	–	7.7
EC_e	dS m^{-1}	1.41
Available phosphorus (Olson)	mg kg^{-1}	3.30
Extractable potassium (NH_4OAC)	mg kg^{-1}	84
Organic matter	%	0.71
Total nitrogen	%	0.05

Plant analyses

After 60 days, fresh leaf samples were collected and analyzed for sodium (Na^+) and potassium (K^+) concentration by flame photometer as described by Ryan et al. (2001). Grain samples were digested according to the method of Wolf (1982) and nitrogen was determined by the Kjeldhal method.

Measurement of WUE

The photosynthetic (*A*), and transpiration (*E*) rates were measured by portable infra-red gas analyzer [IRGA (LCA-4; Analytical Development Company, Hoddeson, UK)]. These parameters were taken in the morning (8:00–10:00 a.m.) at a photosynthetic photon flux density of 1,200–1,400 μmol m^{-2} s^{-1} (Ben-Asher et al. 2006). Two fully expanded leaves from one plant in each pot were selected for the measurement of the data regarding above parameters. WUE is the ratio of *A* to *E* and was calculated as

$$\text{Water use efficiency (A/E)} = \frac{\text{Photosynthetic rate (A)}}{\text{Transpiration rate (E)}}$$

Relative water content

The relative water content (RWC) of shoots was determined by using the following formula as described by Mayak et al. (2004).

$$\text{Relative water content (RWC)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fully turgid weight} - \text{Dry weight}}$$

The fully turgid weight of leaf was taken after putting it in 100% humidity in the dark at 4 °C for 48 h.

Statistical analysis

Analysis of variance techniques (ANOVA) were applied to analyze the data (Steel et al. 1997) using a completely randomized design, and means were compared by Duncan's multiple range test (Duncan 1955).

Results

The data (Table 2) revealed that salinity stress had an inhibitory effect on shoot fresh weight of mung bean. However, co-inoculation decreased the inhibitory effect of salinity on shoot fresh weight and improved it. However, sole inoculation of either *Rhizobium* or PGPR was non-significant in comparison with the respective un-inoculated control at all salinity levels. The maximum improvement in shoot fresh weight (145%) compared with respective un-inoculated control was observed due to co-inoculated combination Mk1×M9 at 6 dS m^{-1}. However, the combinations Mk25×M6 and Mk25×M9 were at par with the respective un-inoculated control. Under normal conditions, maximum improvement in shoot fresh weight (33%) compared with the respective un-inoculated control was observed due to co-inoculation of PGPR strain Mk20 and *Rhizobium* strain M6. At 4 dS m^{-1}, all the treatments were statistically similar to the corresponding un-inoculated control.

The data in Table 2 reveal that root fresh weight improved upon inoculation with either *Rhizobium* or PGPR containing ACC-deaminase alone, which otherwise decreased with salinity. But co-inoculation was more effective for reducing the effect of salinity on root fresh weight. Maximum improvement in root fresh weight (173%) compared with the respective un-inoculated control was observed due to the co-inoculated combination Mk1×M6 at 6 dS m^{-1}. However, the co-inoculated combination Mk25×M6 was non-significant compared with the corresponding un-inoculated control. At 4 dS m^{-1}, the increase in root fresh weight due to co-inoculation ranged from 58% to 95%, while under normal conditions this improvement was up to 105% compared with un-inoculated controls. However, the combinations Mk25×M9 and Mk1×M6 gave a non-significant increase in root fresh weight over the corresponding un-inoculated control. Sole inoculation with PGPR containing ACC-deaminase and *Rhizobium* strain M9 was also effective under salinity stress. However, the effect of *Rhizobium* strain M6 was non-significant in comparison with the respective un-inoculated control under normal conditions as well as at 4 dS m^{-1}. The PGPR strain Mk1 showed non-significant results when compared with un-inoculated control under normal conditions as well as under salinity stress.

Inoculation/co-inoculation had a promising effect on number of pods $plant^{-1}$ in mung bean growing in pot trials under salt-stressed conditions (Table 3). At 6 dS m^{-1}, maximum improvement in number of pods $plant^{-1}$ (150%)

Table 2 Effect of inoculation/co-inoculation on shoot and root fresh weight of mung bean under salt-stressed conditions in pot trials. Means with the same lower case letters are statistically at par at 5% level of probability; n = 3. Mk1 = *Pseudomonas syringae*; Mk20 = *Pseudomonas fluorescens*; Mk25 = *Pseudomonas fluorescens* Biotype G; M6 and M9 = *Rhizobium phaseoli*

Strain	Treatment		
	1.41 dS m^{-1}	4 dS m^{-1}	6 dS m^{-1}
Shoot fresh weight (g $plant^{-1}$)			
Control	35.06 b–g	30.53 e–g	12.44 i
Mk25	41.99 a–c	35.89 b–g	19.12 hi
Mk20	35.94 b–g	39.59 a–f	11.20 i
Mk1	35.48 b–g	29.76 fg	16.64 i
M9	41.06 a–d	38.31 a–f	18.65 hi
M6	36.42 b–g	33.75 c–g	20.41 hi
Mk25 x M9	30.75 e–g	34.80 c–g	18.91 hi
Mk25×M6	40.45 a–e	31.25 d–g	18.47 hi
Mk20×M9	44.86 ab	34.08 c–g	26.59 gh
Mk20×M6	46.49 a	35.99 b–g	29.88 fg
Mk1×M9	46.16 a	33.43 c–g	30.56 e–g
Mk1×M6	39.71a–f	35.69 b–g	30.11 fg
Root fresh weight (g $plant^{-1}$)			
Control	8.72 ef	4.47 l–n	1.90 s
Mk25	11.77 d	6.31 ij	4.87 lm
Mk20	13.78 c	8.18 fg	2.97 p–r
Mk1	8.56 ef	4.45 l–n	2.24 rs
M9	11.57 d	6.04 jk	3.63 n–p
M6	9.55 e	4.75 lm	3.84 m–p
Mk25×M9	8.75 ef	8.59 ef	4.07 m–o
Mk25×M6	12.34 d	7.53 gh	2.49 q–s
Mk20×M9	17.90 a	8.25 fg	3.44 n–q
Mk20×M6	15.83 b	8.72 ef	3.11 o–r
Mk1×M9	11.44 d	7.91 f–h	4.71 lm
Mk1×M6	9.47 e	7.07 hi	5.19 kl

Table 3 Effect of inoculation/co-inoculation on number of pods plant^{-1} and pod fresh weight plant^{-1} of mung bean under salt-stressed conditions in pot trials. Means sharing same letters are statistically at par at 5% level of probability (n=3)

Strain	Treatment		
	1.41 dS m^{-1}	4 dS m^{-1}	6 dS m^{-1}
Number of pods plant^{-1}			
Control	7.33 e–i	6.33 f–j	3.33 l
Mk25	10.67 ab	9.33 a–e	6.00 g–k
Mk20	7.33 e–i	8.00 c–g	6.00 g–k
Mk1	9.67 a–e	5.00 j–l	6.67 f–j
M9	9.67 a–e	6.00 g–k	5.33 i–l
M6	10.0 a–d	8.33 c–f	6.67 f–j
Mk25×M9	9.33 a–e	9.67 a–e	5.67 h–k
Mk25×M6	8.67 b–f	5.67 h–k	3.33 l
Mk20×M9	10.33 a–c	8.00 c–g	8.33 c–f
Mk20×M6	10.0 a–d	5.33 i–l	4.00 kl
Mk1×M9	11.0 a	8.67 b–f	8.33 c–f
Mk1×M6	8.33 c–f	6.67 f–j	7.67 d–h
Pods fresh weight (g plant^{-1})			
Control	5.53 g–m	6.42 e–j	2.31 o
Mk25	7.84 c–e	8.58 bc	4.66 k–n
Mk20	5.53 g–m	4.17 mn	3.94 mn
Mk1	6.97 c–h	4.05 mn	6.51 e–i
M9	8.39 b–d	3.42 no	6.32 e–k
M6	7.23 c–g	6.49 e–i	3.94 mn
Mk25×M9	7.40 c–f	10.83 a	4.80 i–n
Mk25×M6	7.04 c–h	5.40 h–m	2.32 o
Mk20×M9	7.95 b–e	6.69 d–h	4.71 j–n
Mk20×M6	6.92 c–h	4.48 l–n	4.67 k–n
Mk1×M9	9.51 ab	5.98 f–l	4.85 i–n
Mk1×M6	7.06 c–h	6.99 c–h	4.07 mn

over the respective un-inoculated control was observed in the co-inoculated combination Mk20×M9 and Mk1×M9 , which otherwise was decreased by 55% due to salinity. Other treatments also showed significant effects when compared with the respective un-inoculated control except *Rhizobium* strain M9 and co-inoculated combinations Mk25×M6 and Mk20×M6 which gave non-significant results when compared with un-inoculated control. Under normal conditions as well as at 4 dS m^{-1}, most of the treatments showed non-significant effects when compared with the corresponding un-inoculated control.

The data in Table 3 show that salinity stress decreased pod fresh weight of mung bean but inoculation/co-inoculation improved it by reducing the inhibitory effect of salinity. Under normal conditions, the maximum pod fresh weight (9.51 g) was observed with the co-inoculated combination Mk1×M9, which was 72% more than the un-inoculated control, followed by 52%, 44% and 42% with the *Rhizobium* strain M9, co-inoculated combination Mk20×M9 and PGPR strain Mk25, respectively. However, most of the treatments were at par with the control. At 4 dS m^{-1}, all the treatments showed non-significant results compared with the corresponding un-inoculated control except PGPR strain Mk25 and co-inoculated combination Mk25×M9. Pod fresh weight was decreased by most of the treatments in comparison with controls. Higher salinity (6 dS m^{-1}) adversely affected pod fresh weight, with decreases of up to 57%. Inoculation/co-inoculation with *Rhizobium* or PGPR containing ACC-deaminase reduced this inhibitory effect. The maximum improvement in pod fresh weight (182%) over un-inoculated controls was observed upon inoculation with PGPR containing ACC-deaminase Mk1 at 6 dS m^{-1}, followed by 173% and 110% due to *Rhizobium* strain M9 and co-inoculated combinations Mk1×M9, respectively. There were non-significant results in case of co-inoculated combination Mk25×M6 at higher salinity level. While the sole inoculation of either *Rhizobium* or PGPR containing ACC-deaminase also showed significant increase in pod fresh weight at higher salinity levels, where a 70–173% increase in pod fresh weight was recorded.

It is evident from the data regarding total dry matter (Table 4) that inoculation with *Rhizobium* and PGPR containing ACC-deaminase reduced the effect of salinity significantly. But co-inoculation was more pronounced in reducing the effect of salinity on total dry matter of mung bean. Maximum improvement in total dry matter (269%) compared with the respective un-inoculated control was observed upon a co-inoculated combination at the 6 dS m^{-1} salinity level. However, the combination Mk25×M6 gave a non-significant increase in total dry matter compared with the respective un-inoculated control at 6 dS m^{-1} while the PGPR strain Mk1 showed non-significant results compared with the un-inoculated control at lower salinity levels but significantly higher than the corresponding un-inoculated control at 6 dS m^{-1}.

Salinity stress impaired grain development in mung bean, with development decreasing with increasing salinity level (Table 4). There was no grain development at higher salinity levels, i.e., 6 dS m^{-1}. However, inoculation/co-inoculation decreased the inhibitory effect of salinity and grain yield improved significantly with most treatments. Separate inoculation of PGPR containing ACC-deaminase significantly improved grain yield in mung bean. The PGPR strain Mk25 showed the best results when compared with other strains at all salinity levels. Maximum improvement in grain yield was observed by Mk25 inoculation at 6 dS m^{-1}. Under normal conditions as well as at 4 dS m^{-1}, the PGPR strain Mk25 led to increases in grain yield of 27% and 52% over the un-inoculated control. The other two PGPR strains gave significantly better results at higher salinity level but they decreased the grain yield at lower salinity levels. However,

Table 4 Effect of inoculation/co-inoculation on total dry matter and grain yield of mung bean under salt-stressed conditions in pot trial. Means sharing same letters are statistically at par at 5% level of probability (n=3)

Strain	Treatment		
	1.41 dS m^{-1}	4 dS m^{-1}	6 dS m^{-1}
Total dry matter (g $plant^{-1}$)			
Control	8.30 g–i	6.40 kl	2.07 r
Mk25	11.06 b	9.41 d–f	5.47 m–o
Mk20	9.76 de	8.75 f–h	3.46 q
Mk1	8.85 f–h	7.00 jk	3.72q
M9	10.56 bc	9.93 cd	4.78 op
M6	8.86 f–h	8.12 hi	4.10 pq
Mk25×M9	9.41 d–f	9.12 d–g	5.21 no
Mk25×M6	10.89 b	8.63 f–h	2.64 r
Mk20×M9	12.70 a	9.00 e–g	6.08 lm
Mk20×M6	12.23 a	8.00 hi	5.81 l–n
Mk1×M9	12.10 a	9.13 d–g	7.54 ij
Mk1×M6	9.72 de	8.79 f–h	7.64 ij
Grain yield (g $plant^{-1}$)			
Control	1.98 h–l	1.77 j–n	0.00 t
Mk25	2.51 b–g	2.68 b–e	1.81 j–m
Mk20	1.30 n–q	1.69 k–o	1.08 qr
Mk1	1.90 i–l	1.19 p–r	1.40 m–q
M9	2.91 a–c	1.95 i–l	1.26 o–q
M6	2.17 f–k	2.46 c–h	1.16 p–r
Mk25×M9	2.47 c–h	2.50 c–g	0.73 rs
Mk25×M6	3.30 a	2.37 d–i	0.52 s
Mk20×M9	2.89 a–c	2.59 b–f	2.15 f–k
Mk20×M6	2.05 g–l	2.08 g–l	0.47 s
Mk1×M9	3.29 a	3.01 ab	1.25 o–q
Mk1×M6	1.60 l–p	2.27 e–j	2.79 b–d

this reduction was non-significant in the case of Mk20 at 4 dS m^{-1}, while Mk25 showed a non-significant decrease in grain yield at original salinity level. The *Rhizobium* inoculation was also effective at higher salinity level but strain M9 was non-significant at 4 dS m^{-1}, and M6 gave non-significant results at the original salinity level. Co-inoculation with *Rhizobium* and PGPR containing ACC-deaminase was effective at all salinity levels; however, the effect was more pronounced at higher salinity levels. At 6 dS m^{-1}, the maximum grain yield was observed by the co-inoculated combination of Mk1×M6, followed by co-inoculated combinations Mk20×M9 and Mk1×M9, respectively. At 4 dS m^{-1}, the increase in grain yield over the un-inoculated control ranged from 35% to 86%, while under normal conditions this increase was 7% to 141%. However, the combinations Mk20×M6 and Mk1×M6 gave non-significant results compared with the corresponding un-inoculated controls.

Relative water content (RWC) was decreased significantly due to salinity stress (Table 5), while inoculation with *Rhizobium* and PGPR containing ACC-deaminase significantly improved RWC. However, co-inoculation with *Rhizobium* and PGPR containing ACC-deaminase was more effective. A variable response was observed by all combinations under normal as well as salt-stressed conditions. At 6 dS m^{-1}, maximum improvement in RWC was observed with the combination Mk1×M9 ,where the increase in RWC compared with its un-inoculated control was 19%.

In this study, water use efficiency (WUE) decreased significantly due to salinity but inoculation/co-inoculation reduced the adverse effect of salinity and improved WUE values (Table 5). Minimum WUE was observed in un-inoculated control at 6 dS m^{-1} salinity, where the decrease was up to 31% compared with the salinity control (1.41 dS m^{-1}). The *Rhizobium* and PGPR containing ACC-deaminase

Table 5 Effect of inoculation/co-inoculation on relative water content (RWC) and water use efficiency (WUE) of mung bean under salt-stressed conditions in pot trial. Means sharing same letters are statistically at par at 5% level of probability (n=3)

Strain	Treatment		
	1.41 dS m^{-1}	4 dS m^{-1}	6 dS m^{-1}
RWC (g kg^{-1})			
Control	700.0 hi	604.3 n	468.0 r
Mk25	762.3 bc	632.3 lm	529.0 pq
Mk20	728.3 d–g	622.0 l–n	515.3 pq
Mk1	717.0 e–h	623.7 l–n	531.7 o–q
M9	724.7 d–h	629.3 l–n	507.0 q
M6	747.3 b–d	648.3 kl	515.0 pq
Mk25×M9	771.7 b	620.3 mn	513.7 pq
Mk25×M6	773.0 b	639.0 k–m	511.3 q
Mk20×M9	807.7 a	713.0 f–h	540.7 op
Mk20×M6	742.3 c–e	680.7 ij	527.7 pq
Mk1×M9	798.0 a	703.0 g–i	556.0 o
Mk1×M6	731.3 d–f	662.7 jk	518.7 pq
WUE (μmol m^{-2} s^{-1}/ mmol m^{-2} s^{-1})			
Control	2.36 i–l	2.05 no	1.63 q
Mk25	2.61 d–j	2.29 k–n	1.66 pq
Mk20	2.63 d–i	2.69 d–g	1.89 o–q
Mk1	3.08 b	2.90 b–d	2.33 j–m
M9	3.04 bc	2.62 d–i	1.93 op
M6	2.77 c–f	2.69 d–h	1.68 pq
Mk25×M9	2.87 b–e	2.77 c–f	2.46 g–l
Mk25×M6	3.01 bc	3.34 a	1.75 pq
Mk20×M9	3.09 b	2.88 b–e	2.39 h–l
Mk20×M6	3.40 a	2.50 f–l	1.73 pq
Mk1×M9	2.64 d–i	2.49 f–l	2.25 l–n
Mk1×M6	2.54 f–k	2.60 e–j	2.07 m–o

decreased the adverse effects of salinity on WUE at all salinity levels significantly, while the results of *Rhizobium* strain M6 were non-significant compared with the respective un-inoculated control at 6 dS m^{-1}. Co-inoculation was more effective in improving WUE at all salinity levels than sole inoculation of *Rhizobium* and PGPR containing ACC-deaminase. However, a variable response was observed with all combinations under normal as well as salt-stressed conditions. At 6 dS m^{-1}, maximum increase in WUE (51%) over the respective un-inoculated control was observed due to co-inoculated combination Mk25×M9 where the increase was 47%. However, the combinations Mk25×M6 and Mk20×M6 gave non-significant results compared with un-inoculated control.

The data shown in Table 6 show that the potassium concentration in mung bean leaves decreases significantly with increased salinity levels. However, sole and combined inoculation of *Rhizobium* and PGPR containing ACC-deaminase significantly reduced the adverse effect of salinity, thus improving the potassium concentration. Under normal conditions, all treatments were equally effective for improving potassium concentration. The increase in potassium concentration over the un-inoculated control ranged from 21% to 32%. As observed under normal conditions, bacterial inoculation was also effective under salinity stress and all strains and their combinations significantly improved potassium concentration over the corresponding un-inoculated control. At 6 dS m^{-1}, Mk1×M9 gave a maximum increase in potassium concentration (33%) over the un-inoculated control, and was non-significant when compared with other treatments but significantly higher than the un-inoculated control.

Sodium competes with potassium under salinity stress; therefore, its concentration increased when the mung bean plants were subjected to salinity stress (Table 6). The maximum sodium concentration in mung bean leaves (9.5 g kg^{-1}) was observed when plants were grown at 6 dS m^{-1} salinity. However, inoculation with *Rhizobium* and PGPR containing ACC-deaminase decreased the effect of salinity, hence decreasing the sodium concentration in mung bean leaves. However, co-inoculation was more effective than sole inoculation with *Rhizobium* and PGPR containing ACC-deaminase at all salinity levels. Under normal conditions, the reduction in sodium concentration due to inoculation/co-inoculation ranged from 10% to 33%, and the minimum sodium concentration (4.6 g kg^{-1}) was observed due to combination Mk25×M9. Inoculation/co-inoculation was more effective for reducing the sodium concentration under salinity conditions. At 6 dS m^{-1}, maximum reduction (56% less than the un-inoculated control) in sodium concentration was observed with combination Mk20×M9.

It is evident from the data (Table 7) that inoculation/co-inoculation significantly improved the nitrogen concentration in mung bean grains. Co-inoculation with *Rhizobium* and PGPR containing ACC-deaminase gave very promising results. However, the results were variable at all salinity levels. Maximum improvement in nitrogen concentration (99%) compared with the respective un-inoculated control was observed by the combination Mk1×M9 at 6 dS m^{-1}. But the results of Mk1×M6 were non-significant when compared with the corresponding un-inoculated control. Sole inoculation with *Rhizobium* and PGPR containing ACC-deaminase also significantly improved the nitrogen concentration in grains, while the results with *Rhizobium* strain M9 were at par with the corresponding un-inoculated control at original and 4 dS m^{-1}, and the PGPR strain Mk25 gave statistically similar results compared with the respective un-inoculated control at all salinity levels.

Table 6 Effect of inoculation/co-inoculation on potassium and sodium concentration in leaves of mung bean under salt-stressed conditions in pot trials. Means sharing same letters are statistically at par at 5% level of probability (n=3)

Strain	Treatment		
	1.41 dS m^{-1}	4 dS m^{-1}	6 dS m^{-1}
Potassium in leaves (g kg^{-1})			
Control	17.6 f–h	16.0 h	13.4 i
Mk25	22.1 a–d	20.9 cd	16.5 h
Mk20	22.3 a–d	20.5 de	16.6 h
Mk1	21.2 b–d	21.2 b–d	16.9 gh
M9	22.6 a–c	19.1 e–f	16.7 h
M6	22.4 a–d	22.8 a–c	17.1 gh
Mk25×M9	22.3 a–d	21.9 a–d	17.0 gh
Mk25×M6	22.7 a–c	22.8 a–c	16.8 gh
Mk20×M9	22.4 a–d	23.1 ab	17.4 f–h
Mk20×M6	23.2 ab	22.4 a–d	16.8 gh
Mk1×M9	23.2 a	23.1 ab	17.8 f–h
Mk1×M6	23.0 ab	18.7 fg	16.5 h
Sodium in leaves (g kg^{-1})			
Control	6.8 c	8.1 b	9.5 a
Mk25	5.7 e–h	6.2 de	6.8 c
Mk20	6.1 d–f	5.9 d–g	6.3 cd
Mk1	6.0 d–g	5.9 d–g	5.9 d–g
M9	6.0 d–g	5.3 h–k	6.2 d–f
M6	5.9 d–h	5.6 f–i	6.2 de
Mk25×M9	4.6 l–n	4.7 k–n	4.9 j–l
Mk25×M6	5.1 i–l	5.0 j–l	4.9 j–l
Mk20×M9	4.7 k–n	4.8 k–m	4.1 n
Mk20×M6	5.0 j–l	5.1 i–l	4.7 k–n
Mk1×M9	5.1 i–l	4.7 k–n	4.2 mn
Mk1×M6	5.5 g–j	5.6 f–i	4.7 k–n

Table 7 Effect of inoculation/co-inoculation on nitrogen concentration in grains of mung bean under salt-stressed conditions in pot trials. Means sharing same letters are statistically at par at 5% level of probability (n=3)

Strain	Treatment		
	1.41 dS m^{-1}	4 dS m^{-1}	6 dS m^{-1}
Nitrogen (g kg^{-1})			
Control	17.2 j–l	14.0 lm	11.9 m
Mk25	18.6 h–l	16.8 j–l	15.6 k–m
Mk20	27.4 bc	20.6 f–k	18.5 h–l
Mk1	26.3 b–d	23.9 c–g	25.0 b–g
M9	21.5 d–j	17.4 j–l	18.1 i–l
M6	27.6 bc	24.3 b–g	18.5 h–i
Mk25×M9	23.3 c–i	23.0 c–i	18.2 i–l
Mk25×M6	29.2 ab	26.1 b–e	20.9 e–j
Mk20×M9	24.9 b–g	23.3 c–i	21.3 d–j
Mk20×M6	32.4 a	24.3 b–g	20.2 g–k
Mk1×M9	23.1 c–i	22.7 c–i	23.6 c–h
Mk1×M6	25.8 b–f	24.3 b–g	14.1 lm

Discussion

In a series of experiments under axenic conditions (Ahmad et al. 2011), three strains of PGPR and two strains of *Rhizobium phaseoli* were chosen to study their efficacy under natural conditions. These strains were evaluated for their potential to improve the productivity of mung bean under salt-affected conditions in pot trial under ambient conditions.

The results of our study in pot trials revealed that salinity significantly reduced the seedling growth of mung bean, but root growth was affected relatively more than shoots. It is very likely that root metabolic activity and physiological processes were more affected than those of shoots, or, alternatively, the difference might be due to the more intimate contact of roots with salt solution as compared to shoot (Mayak et al. 2004). The decreased growth may also be attributed needing the majority of the available energy for the making osmotic adjustments by the plant, thus decreasing plant growth and total dry matter (Munns and Termaat, 1986). Increased salinity in the rhizosphere decreases the osmotic potential of the root zone soil solution (Chartzoulakis et al. 2002), resulting in reduced availability of water to plants. It has been reported that increased production of ethylene due to exogenous application of ACC or salinity decreases root growth (Madhaiyan et al. 2007).

However, in our studies, inoculation with PGPR containing ACC-deaminase increased root and shoot growth as compared to un-inoculated controls. This could be due to lowering of ethylene levels by these strains, thus improving plant growth. It is very likely that exogenous application of ethylene and/or salinity had an adverse effect on plant growth but inoculation with PGPR containing ACC-deaminase improved plant growth by decreasing the ethylene concentration (Shaharoona et al. 2006; Nadeem et al. 2009; Ahmad et al. 2011).

It was also observed in this study that PGPR strains improved the efficiency of *Rhizobium* for improving the growth of mung bean but with different degrees of efficacy. PGPR containing ACC-deaminase have been reported to eliminate, or at least reduce, the stress-induced ethylene-mediated negative impact on plants (Glick 2005; Safronova et al. 2006). The effect of these PGPR containing ACC deaminase in boosting plant growth, particularly under stressed conditions, has also been studied by the regulation of accelerated ethylene production in response to a multitude of abiotic and biotic stresses (Zahir et al. 2008; Belimov et al. 2009; Ahmad et al. 2011). Co-inoculation with *Rhizobium* and *Pseudomonas* strains containing ACC-deaminase increased plant height, total dry matter and pod fresh weight by 269% and 162%, respectively, compared to the un-inoculated control in our study, but in a study conducted by Nadeem et al. (2007) on salt-affected conditions in pot trials, the separate application of ACC-deaminase strain *Pseudomonas fluorescens* increased the biomass, cob mass and grain yield of maize by 51%, 40% and 50%, respectively, compared to the un-inoculated control. It has also been reported that inoculation/co-inoculation improved the transpiration rate and other physiological processes (Vivas et al. 2003; Gaballah and Gomaa 2005), thus decreasing the effect of salinity on plant growth, and leading to increased total dry matter.

Some combinations of rhizobial and rhizobacterial isolates could not improve growth and yield parameters compared to un-inoculated controls, which might be due to certain compounds produced by the bacteria. Bacterial strains produce certain antibiotics, which may suppress other strains (Gull et al. 2004). It has also been reported that co-inoculation with PGPR and *Rhizobium* decreased plant growth due to the production of antibiotics and competition for attachment sites on root surfaces (Mirza et al. 2007).

In our study, WUE and RWC were negatively affected by salinity. This might be due to stomatal closure in plants caused by salinity stress, which decreases RWC (Lawlor and Cornic 2002; Ben-Asher et al. (2006) and physiological efficiency in plants. The decrease in physiological activity results in declined plant growth leading to reduced grain yield.

In our study, inoculation/co-inoculation decreased the effect of salinity on WUE and RWC, thus improving the yield of mung bean. This is an indication of enhanced water uptake due to inoculation/co-inoculation under salinity stress. It may be due to the longer roots of plants inoculated/co-inoculated with *Rhizobium* and PGPR containing ACC-deaminase under stress (Ahmad et al. 2011)

which might have helped the plants to uptake relatively more water from deeper soil under stressed conditions (Hamdia et al. 2004; Gaballah and Gomaa 2005).

In our study, salinity increased Na^+ concentration significantly while decreasing K^+ concentration within the mung bean leaves. This might be due to the fact that plants take up more Na^+ when they are subjected to salinity in order to maintain the turgor pressure within the plants (Ashrafet al. 2004), which might have reduced K^+ uptake. It has been reported in previous studies that uptake of Na^+ increases with increase in the Na^+ content in soil, which decreases the uptake of K^+ and Ca^{2+} by plants (Pervaiz et al. 2002; Ashraf et al. 2004).

In our study, nitrogen concentration in mung bean seeds was also adversely affected by salinity. This might be due to the reduced root growth with the increase in salinity-induced ethylene production, which decreased the root area thus making it insufficient to explore soil for required nutrient uptake (Gaballah and Gomaa 2005; Nadeem et al. 2009). However, inoculation/co-inoculation reversed the pattern and improved the nutrient balance and nitrogen concentration in mung bean seeds. This might be due to a reduction in ethylene production due to inoculation/co-inoculation with *Rhizobium* and PGPR containing ACC-deaminase, thus reducing the inhibitory effect of ethylene on root growth leading to greater proliferation of roots. The competency of co-inoculation for reducing the effect of salinity due to the reduction in ethylene level through ACC-deaminase activity was proved by conducting the classical triple response assay (Ahmad et al. 2011). The increased root area might have facilitated the plants to explore more soil for nutrient absorption (Hamdia et al. 2004; Nadeem et al. 2009).

The strains used in this experiment varied in their ability to decrease the effect of salinity on plant growth and the maximum response was observed when *Pseudomonas fluorescens* (Mk20) was co-inoculated with *Rhizobium phaseoli*. It is very likely that PGPR strains varied in their ACC-deaminase ability along with some other characters (Ahmad et al. 2011) that contributed differently for growth promotion. This difference may also be due to the presence of other growth-promoting characters, i.e., chitinase activity, phosphate solubilization, root colonization, etc. in addition to ACC-deaminase activity (Ahmad et al. 2011). The co-inoculation of these strains with *Rhizobium* might have enhanced the survival efficacy and proliferation of *Rhizobium*, which might be due to the solubilization of indigenous phosphorus (Ahmad et al. 2011) in the soil by *Pseudomonas* leading to improved plant growth. The proliferated roots and healthy plant might be a source of better nutrient supply for *Rhizobium*, thus improving their growth and efficacy. The more root growth enhances the root exudation, the more root colonization by microbes in the nutrient-rich environment.

In our studies, co-inoculation with *Rhizobium* and *Pseudomonas* strains containing ACC-deaminase increased the shoot fresh weight, root fresh weight, number of pods $plant^{-1}$, pods fresh weight, total dry matter, RWC, WUE, potassium concentration in leaves, sodium concentration in leaves and nitrogen concentration in grains of mung bean by 145%, 173%, 150%, 182%, 269%, 19%, 51%, 33%, 56% and 99%, respectively, compared with the un-inoculated control, while in previous studies conducted by Nadeem et al. (2007) under salt-affected conditions in pot trials, the separate application of *Pseudomonas fluorescens* increased the cob mass, total dry matter and grain yield of maize by 40%, 51% and 50%, respectively. So, co-inoculation should be preferred over separate inoculation of *Pseudomonas* strains containing ACC-deaminase for sustainable production of mung bean under salt-affected conditions. Moreover, under field conditions in Pakistan, high temperature and low rainfall results in net upward movement of salts, which results in secondary salinization, with higher levels of ethylene being produced (Mayak et al. 2004). In such circumstances, the use of PGPR containing ACC-deaminase in combination with *Rhizobium* should be preferred over single strain inoculation.

Thus, it can be concluded from the results of this study that combined application of *Rhizobium phaseoli* and *Pseudomonas* strains is more efficient for improving the productivity of mung bean under salt-stressed conditions when compared with separate inoculation of *Rhizobium* and *Pseudomonas* strains containing ACC-deaminase. However, it is imperative to evaluate these strains in combinations for the development of biofertilizers under field conditions to improve the productivity of mung bean under salt-affected conditions.

Acknowledgments We are highly thankful to Higher Education Commission, Pakistan for providing the financial support for this research project and H.M. Haroon for his Technical Services during the project.

References

Ahmad M, Zahir ZA, Asghar HN, Asghar M (2011) Inducing salt tolerance in mung bean through co-inoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate-deaminase. Can J Microbiol 57:578–589

Anonymous (2010) Economic Survey of Pakistan 2009–2010. Ministry of Food and Agriculture, Federal Bureau of Statistics, Islamabad

Ashraf M, Berge SH, Mahmood OT (2004) Inoculating wheat seedling with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. Biol Fertil Soils 40:157–162

Ayers RS, Westcot DW (1985) Water quality for agriculture. FAO Irrigation and Drainage Papers 29 (Rev.1), Rome

Barea J, Pozo MJ, Azcon R, Azcon-Aguilar C (2005) Microbial co-operation in the rhizosphere. J Exp Bot 56:1761–1778

Belimov AA, Dodd IC, Hontzeas N, Theobald JC, Safronova VI, Davies WJ (2009) Rhizosphere bacteria containing ACC-deaminase increase yield of plants grown in drying soil via both local and systemic hormone signaling. New Phytol 181:413–423

Ben-Asher J, Tsuyuki I, Bravdo BA, Sagih M (2006) Irrigation of grape vines with saline water, I. leaf area index, stomatal conductance, transpiration and photosynthesis. Agric Water Manage 83:13–21

Burdman S, Vedder D, German M, Itzigsohn R, Kigel J, Jurkevitch E, Okon Y (1998) Legume crop yield promotion by inoculation with *Azospirillum*. In: Elmerich C, Kondorosi A, Newton WE (eds) Biological Nitrogen Fixation for the 21st Century. Kluwer, Dordrecht, pp 609–612

Chartzoulakis K, Loupassaki M, Bertaki M, Androulakis I (2002) Effects of NaCl salinity on growth, ion content and CO_2 assimilation rate of six olive cultivars. Sci Hortic 96:235–247

Dashti N, Zhang F, Hynes R, Smith DL (1997) Application of plant growth-promoting rhizobacteria to soybean (*Glycine max* L. Merr.) increases protein and dry matter yield under short season conditions. Plant Soil 188:33–41

Derylo M, Skorupska A (1993) Enhancement of symbiotic nitrogen fixation by vitamin-secreting fluorescent *Pseudomonas*. Plant Soil 54:211–217

Dobbelaere S, Okon Y (2007) The plant growth promoting effect and plant responses. In: Elmerich C, Newton WE (eds) Associative and endophytic nitrogen-fixing bacteria and cyanobacterial associations. Kluwer, Netherlands, pp 1–2

Duncan DB (1955) Multiple range and multiple F-test. Biometrics 11:1–42

Dworkin M, Foster J (1958) Experiments with some microorganisms which utilize ethane and hydrogen. J Bacteriol 75:592–601

Elahi NN, Mustafa S, Mirza JI (2004) Growth and nodulation of mung bean (*Vigna radiata* L.) as affected by sodium chloride. J Res Sci 15:139–143

Gaballah MS, Gomaa AM (2005) Interactive effect of *Rhizobium* inoculation, sodium benzoate and salinity on performance and oxidative stress in two faba bean varieties. Int J Agric Biol 7:495–498

Glenn EP, Brown JJ, Blumwald E (1999) Salt tolerance and crop potential of halophytes. Crit Rev Plant Sci 18:227–55

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251:1–7

Gull M, Hafeez FY, Saleem M (2004) Phosphorus uptake and growth promotion of chickpea by co-inoculation of mineral phosphate solubilising bacteria and a mixed rhizobial culture. Aust J Exp Agric 44:623–628

Hamdia MA, Shaddad MAK, Doaa MM (2004) Mechanism of salt tolerance and interactive effect of *Azospirillum bransilense* inoculation on maize cultivars grown under salt stress conditions. Plant Growth Regul 44:165–174

Lawlor DW, Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficit in higher plants. Plant Cell Environ 25:255–294

Lloret J, Bolaños L, Lucas MM, Peart JM, Brewin NJ, Bonilla I, Rivilla R (1995) Ionic stress and osmotic pressure induce different alterations in the lipopolysaccharide of a *Rhizobium meliloti* strain. Appl Environ Microbiol 61:3701–3704

Madhaiyan M, Poonguzhali S, Sa TM (2007) Characterization of 1-Aminocyclopropane-1-carboxylate deaminase (ACC) deaminase containing *Methylobacterium oryzae* and interactions with auxins and ACC regulation of ethylene in canola (*Brassica campestris*). Planta 226:867–876

Mayak S, Tirosh T, Glick BR (1999) Effect of wild-type and mutant plant growth promoting rhizobacteria on the rooting of mung bean cuttings. J Plant Growth Regul 18:49–53

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572

Mirza BS, Mirza MS, Bano A, Malik KA (2007) Co-inoculation of chickpea with *Rhizobium* isolates from roots and nodules and phytohormone-producing *Enterobacter* strains. Aust J Exp Agric 47:1008–1015

Munns R, Termaat A (1986) Whole-plant responses to salinity. Aust J Plant Physiol 13:143–160

Nadeem SM, Zahir ZA, Naveed M, Arshad M (2009) Rhizobacteria containing ACC deaminase confer salt tolerance in maize grown on salt affected soils. Can J Microbiol 55:1302–1309

Nadeem SM, Zahir ZA, Naveed M, Arshad M (2007) Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. Can J Microbiol 53:1141–1149

Naz I, Bano A, Tamoor-ul-Hassan (2009) Morphological, biochemical and molecular characterization of rhizobia from halophytes of Khewra Salt Range and Attock. Pak J Bot 41:3159–3168

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Saf 60:324–349

Pervaiz Z, Xiaoe YA, Ancheng L (2002) Selection criteria for salt-tolerance in wheat cultivars at seedling stage. Asian J Plant Sci 1:85–87

Prat D, Fathi-Ettai RA (1990) Variation in organic and mineral components in young *Eucalyptus* seedlings under saline stress. Physiol Plant 79:479–86

Ryan J, Estefan G, Rashid A (2001) Soil and Plant Analysis Laboratory Manual, 2nd edn. International Center for Agriculture in Dry Areas (ICARDA), Syria

Safronova VI, Stepanok VV, Engqvist GL, Alekseyev YV, Belimov AA (2006) Root-associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. Biol Fertil Soils 42:267–272

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

Silveira JA, Viegas Rde A, da Rocha IM, Moreira AC, Moreira Rde A, Oliveira JT (2003) Proline accumulation and glutamine synthase activity are increased by salt induced proteolysis in cashew leaves. J Plant Physiol 160:115–123

Steel RGD, Torrie JH, Dicky DA (1997) Principles and procedures of statistics—a biometrical approach, 3rd edn. McGraw Hill, Singapore

Tate RL (1995) Soil microbiology (symbiotic nitrogen fixation). Wiley, New York, pp 307–333

Vivas A, Marulanda A, Ruiz-Lozano JM, Azcón R (2003) Influence of a *Bacillus* sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG-induced drought stress. Mycorrhiza 13:249–256

Wolf B (1982) The comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. Commun Soil Sci Plant Anal 13:1035–1059

Yue H, Mo W, Li C, Zheng Y, Li H (2007) The salt stress relief and growth promotion effect of RS-5 on cotton. Plant Soil 297:139–145

Zahir ZA, Munir A, Asghar HN, Shahroona B, Arshad M (2008) Effectiveness of rhizobacteria containing ACC-deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. J Microbiol Biotechnol 18:958–963