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PHYSIOLOGICAL RESPONSE OF MUNG BEAN TO RHIZOBIUM AND PSEUDOMONAS BASED BIOFERTILIZERS UNDER SALINITY STRESS

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Plant growth, physiology, and quality parameters are affected by higher levels of ethylene under salinity stress. Certain plant growth promoting rhizobacteria (PGPR) can successfully be used to alleviate the detrimental effects of salinity-stress induced ethylene by degrading its immediate precursor 1-aminocyclopropane-1-carboxylic acid (ACC), through the activity of ACC-deaminase enzyme. A pot experiment was conducted to evaluate the effectiveness of *Rhizobium* and *Pseudomonas* containing ACC-deaminase for their ability to reduce the negative impact of salinity stress on physiology and quality parameters of mung bean. Results showed that salinity stress adversely affected the CO_2 assimilation, stomatal conductance, photosynthetic rate, and chlorophyll contents in mung bean however; inoculation with either *Rhizobium* or *Pseudomonas* significantly reduced the adverse effect of salinity. It has been observed that co-inoculation of *Rhizobium* and *Pseudomonas* was the most effective treatment to reduce the inhibitory effect of salinity on CO_2 assimilation rate, stomatal conductance, photosynthetic rate, and chlorophyll content. Co-inoculation also improved the nutrient balance and increased the phosphorus and protein concentration in grain of mung bean. The results suggested that such strains could be effective for reducing the deleterious effects of salinity on growth, physiology and quality of mung bean.

Keywords: *Pseudomonas*, *Rhizobium phaseoli*, salt tolerance, physiology, quality

INTRODUCTION

Among pulses, mung bean (*Vigna radiata* L.) is an important crop grown in Pakistan and all over the globe. It is important in human diet due to its high nutritional value in addition to its role for improving soil fertility by fixing atmospheric nitrogen (Elahi *et al.*, 2004). Salinity is a serious production problem for crops particularly in arid and semi-arid regions due to high temperature and low rainfall (Parida and Das, 2005).

In saline environment, plant growth is affected by complex interaction of hormones, osmotic effects, specific ion effect and nutritional imbalances, probably all occur simultaneously. Salinity affects the morphology and metabolism (Silveria *et al.*, 2003) as well as nutrient balance in plants (Glenn *et al.*, 1999) which results in poor yield and quality of the produce. It is well documented that increasing Na^+ contents in soil cause an increase in Na^+ uptake and in general, decrease in K^+ contents of plant (Ashraf, 2004). Therefore, massive uptake of particular nutrient results in nutrient imbalance and deficiencies of certain other nutrients. The salinity adversely affects the plant growth and physiology by enhancing the ethylene production. Increased production of ethylene due to salinity can decrease root growth (Madhaiyan *et al.*, 2007).

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 survival and distribution of rhizobia in soil and the rhizosphere is affected by salinity (Tate, 1995). They have variable ability to tolerate salt stress (Lloret *et al.*, 1995).

The use of rhizobacteria containing ACC-deaminase is one of the most acceptable approaches to reduce the effect of stress-induced ethylene on plants. These PGPR contain an enzyme ACC-deaminase which hydrolyzes ACC (immediate precursor of ethylene) into ammonia and α-ketobutyrate (Mayak *et al.*, 1999). The PGPR boost the plant growth, physiology, and nutrient balance, particularly under stressed conditions, by the regulation of accelerated ethylene production in response to a multitude of abiotic and biotic stresses (Belimov *et al.*, 2009; Nadeem *et al.*, 2009; Ahmad *et al.*, 2011). Inoculation/co-inoculation with *Rhizobium* and PGPR strains containing ACC-deaminase decreased the intensity of the classical triple response by increasing the seedlings length of inoculated mung bean seedlings and decreasing the stem diameter, which is 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 difference in ACC deaminase activity (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 reduction in ethylene level (Shaharoona *et al.*, 2006), direct stimulation of rhizobial growth/survival in the soil, enlargement of the root system by hormone production for enhanced nutrient uptake (Barea *et al.*, 2005). It has been reported that the root elongation rate, mineral N, P and K and microelements absorption and uptake are consequently improved after microbial inoculation (Dobbelaere and Okon, 2007). This could result in a general better mineral nutrition of the plant (Burdman *et al.*, 1998). The impact of co-inoculation with Rhizobium and PGPR containing ACC-deaminase has been studied in previous study on mung bean under axenic conditions (Ahmad *et al.*, 2011); however, there is little information about their impact on physiological and quality parameters. So, the present study has been conducted to evaluate the potential of *Rhizobium* and *Pseudomonas* strains to alleviate the deleterious effects of salinity on physiological and quality parameters of mung bean.

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 selected from the culture bank of Soil Microbiology and Biochemistry Lab., Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. These *Pseudomonas* spp. containing ACC-deaminase activity and *Rhizobium* strains were evaluated alone and in combination for their potential to reduce the inhibitory effects of salinity on physiology and quality of mung bean in pot experiment.

***Preparation of inocula*:** Inocula were prepared in flasks by using yeast extract mannitol (YEM) and DF minimal salt medium without agar (Dworkin and Foster, 1958) containing ACC as substrate (N source), for *Rhizobium* and *Pseudomonas* sp., respectively. Each flask containing broth was inoculated with respective strains of *Rhizobium* and *Pseudomonas* and incubated at 28±1°C for 72 hours 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 pot trial was conducted with different sets of treatments i.e. inoculation either with *Rhizobium* and PGPR (*Pseudomonas*) alone, or the combinations of these strains in the presence of recommended levels of chemical fertilizers (N:P:K @ 20:60:60 kg ha^{-1}). Mung bean seeds were inoculated with 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 *Pseudomonas* and *Rhizobium* were used in 1:1 ratio for preparation of the slurry. In case of un-inoculated control, seeds were coated with the sterilized (autoclaved) peat, sugar solution, treated with sterilized broth.

Three salinity levels, 1.41 i.e. original soil EC_e, 4 and 6 dS m^{-1} were used. These levels were chosen keeping in view the salinity status of the soils, where mung bean is cultivated. 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. The salinity was developed by using a calculated amount of NaCl salt and thoroughly mixing with a mechanical mixer. Ten inoculated seeds of mung bean were sown in each pot containing 12 kg pot^{-1} soil. Similarly, three pots (1.41, 4 and 6 dS m^{-1}) were maintained as reference pots, one for each level, to check the effect of irrigation water on salinity levels and EC_e was monitored regularly in the reference pots and in the experimental pots at the end. There was no significant change in the salinity levels at the end. The experiment had a 3 × 2 × 3 factorial completely randomized design (CRD) with six replications (Steel *et al.*, 1997). The factors were 3 bacterial strains (*Pseudomonas syringae*, Mk1; *Pseudomonas fluorescens*, Mk20 and *Pseudomonas fluorescens* Biotype G, Mk25), 2 *Rhizobium phaseoli* strains (M6 and M9) along with their combinations (Mk1 x M6; Mk1 x M9; Mk20 x M6; Mk20 x M9; Mk25 x M6; Mk25 x M9) and 3 levels of salt affected soil (1.41, 4 and 6 dS m^{-1}). Pots were arranged in a wire house at ambient light and temperature according to above mentioned layout. The recommended dose of P and K fertilizers (60 kg ha^{-1} each) as diammonium phosphate and sulphate of potash respectively, and half of the recommended dose for N (20 kg ha^{-1}) as urea were applied in each pot. All fertilizers were applied as basal dose at the time of sowing. The plants were irrigated with good quality irrigation water meeting the irrigation quality criteria for crop (Ayers and Westcot, 1985). After germination, thinning was done to maintain a uniform plant population. Plants from three replications were uprooted at flowering stage to assess nodulation while the remaining three replications were taken to maturity for harvesting. The data on growth and yield parameters were collected at harvest upon physiological maturity.

***Plant analyses*:** Grain samples were digested according to the method of Wolf (1982) and nitrogen was determined by Kjeldahl method. Phosphorus was determined by spectrophotometer (Shimadzu, Japan) as described by Chapman and Prat (1961). Crude protein was calculated by multiplying the grain nitrogen content with a factor of 6.25 (Thimmaiah, 2004).

***Measurement of physiological parameters*:** The physiological parameters i.e. photosynthetic rate (A), stomatal conductance of water (gs) and sub-stomatal CO_2 concentration (Ci), were measured by portable infra-red gas analyzer [IRGA (LCA-4)]. These parameters were taken in the morning (08.00-10.00) at photosynthetic photon flux density of 1200-1400 µ mol m^{-2} s^{-1} (Ben-Asher *et al.*, 2006). Two fully-expanded leaves from one plant in each experimental unit were selected for the measurement of the data regarding above parameters. SPAD chlorophyll contents in leaf of mung bean plant were measured using SPAD chlorophyll meter SPAD-502 (Minolta, Minolta Co, Ltd, UK) as described by Hussain *et al.* (2000).

***Statistical analysis*:** Analysis of variance technique (ANOVA) was applied to analyze the data (Steel *et al.*, 1997) using completely randomized design and means were compared by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

The data (Table 1) showed that salinity caused a significant decrease in sub-stomatal CO_2 concentration. The higher salinity level was more destructive and it decreased the sub-stomatal CO_2 concentration up to 31%. Inoculation/co-inoculation was effective under normal as well as salt-stressed conditions for improving the sub-stomatal CO_2 concentration but the results were more pronounced at higher salinity level. The maximum increase in sub-stomatal

Table 1. Effect of inoculation/co-inoculation on substomatal CO_2 concentration (vpm) and stomatal conductance of water (mmol m^{-2} S^{-1}) of mung bean under salt-stressed natural conditions in pot trial

Treatment	1.41 dS m^{-1}	4 dS m^{-1}	6 dS m^{-1}
Substomatal CO_2 concentration (vpm)			
Control	247.33 e-h	198.33 l	171.00 m
Mk25	246.00 e-h	226.00 h-k	225.00 h-k
Mk20	246.33 e-h	251.33 e-h	238.00 f-j
Mk1	278.00 b-d	225.33 h-k	212.00 j-l
M9	238.00 f-j	232.00 g-j	242.33 e-h
M6	246.00 e-h	212.00 j-l	226.33 h-k
Mk25 x M9	299.00 ab	232.33 g-j	249.00 e-h
Mk25 x M6	317.00 a	258.33 d-g	196.33 l
Mk20 x M9	269.00 c-e	249.00 e-h	262.67 d-f
Mk20 x M6	288.00 bc	237.33 f-j	260.67 d-f
Mk1 x M9	294.00 a-c	257.00 d-g	239.33 f-i
Mk1 x M6	280.00 b-d	204.00 kl	213.33 i-l
LSD value ($p \leq 0.05$)	22.59		
Stomatal conductance of water (mmol m^{-2} S^{-1})			
Control	0.025 h	0.022 hi	0.008 i
Mk25	0.042 b-g	0.032 b-h	0.027 d-h
Mk20	0.046 a-d	0.038 b-g	0.025 e-h
Mk1	0.045 a-d	0.042 b-f	0.027 d-h
M9	0.046 a-d	0.037 b-g	0.030 c-h
M6	0.050 a-c	0.039 b-f	0.025 e-h
Mk25 x M9	0.044 a-e	0.047 a-d	0.035 b-g
Mk25 x M6	0.042 b-g	0.045 a-e	0.025 e-h
Mk20 x M9	0.047 a-d	0.039 b-f	0.036 b-g
Mk20 x M6	0.051 ab	0.044 a-f	0.030 d-h
Mk1 x M9	0.062 a	0.041 b-f	0.027 d-h
Mk1 x M6	0.045 a-e	0.040 b-f	0.024 f-h
LSD value ($p \leq 0.05$)	0.0163		

Means sharing same letters are statistically non-significant at 5 % level of probability. (n = 3)

Mk1, *Pseudomonas syringae;* Mk20, *Pseudomonas fluorescens;* Mk25, *Pseudomonas fluorescens* Biotype G; M6, and M9, *Rhizobium phaseoli*

CO_2 concentration was obtained with the co-inoculated combination Mk20 × M9 which caused 53% increase in sub-stomatal CO_2 concentration over the un-inoculated control at 6 dS m^{-1}. At 4 dS m^{-1}, the maximum increase (30%) was obtained with co-inoculated combination Mk25 × M6.

Salinity stress adversely affected the stomatal conductance of water (Table 1). Inoculation with *Rhizobium* and *Pseudomonas* improved the stomatal conductance of water under normal as well as salt-stressed conditions. The co-inoculation was even more effective for improving the stomatal conductance of water. However, variable response was observed by all the combinations. Under normal conditions, the combination Mk1 × M9 was most effective and resulted in up to 149% increase in stomatal conductance of water over the un-inoculated control. While at 4 dS m^{-1}, the combination Mk25 × M9 gave better results where the increase in stomatal conductance of water was up to 112%. At higher salinity level i.e. 6 dS m^{-1}, maximum increase in stomatal conductance of water over un-inoculated control was observed due to co-inoculated combination Mk20 × M9.

The data (Table 2) showed that inoculation/co-inoculation significantly increased the chlorophyll contents of mung bean leaves and decreased the adverse effects of salinity stress. The combination Mk20 × M9 was the most efficient under salinity stress and it improved the SPAD chlorophyll value up to 26 and 28% over the un-inoculated control at 4 and 6 dS m^{-1}. The sole inoculation of *Rhizobium* and *Pseudomonas* also significantly improved the SPAD chlorophyll value under normal conditions as well as salt-stressed conditions.

Data (Table 2) revealed that the inoculation/co-inoculation significantly improved the photosynthetic rate in mung bean plant and reduced the adverse effects of salinity. The maximum photosynthetic rate 14.01 µmol m^{-2} S^{-1} was observed with the combination Mk20× M6 under normal conditions which was 31% more than un-inoculated control,

Table 2. Effect of inoculation/co-inoculation on SPAD chlorophyll value and photosynthetic rate (µmol m^{-2} S^{-1}) of mung bean under salt-stressed natural conditions in pot trial

Treatment	1.41 dS m^{-1}	4 dS m^{-1}	6 dS m^{-1}
SPAD chlorophyll value			
Control	36.91 i	29.21 o	23.61 t
Mk25	41.53 e	31.28 m	24.43 s
Mk20	40.28 f	31.27 m	25.27 qr
Mk1	38.35 h	31.18 m	24.81 rs
M9	39.03 g	30.23 n	25.70 q
M6	40.28 f	31.61 m	26.21 p
Mk25 x M9	45.53 a	35.87 j	26.33 p
Mk25 x M6	44.08 b	33.18 l	25.07 r
Mk20 x M9	45.13 a	36.87 i	30.31 n
Mk20 x M6	43.45 c	32.91 l	26.35 p
Mk1 x M9	45.57 a	34.20 k	29.21 o
Mk1 x M6	42.15 d	33.11 l	25.75 q
LSD value ($p \leq 0.05$)	0.4577		
Photosynthetic rate (µmol m^{-2} S^{-1})			
Control	10.72 e-h	9.62 i	8.13 j
Mk25	14.00 a	11.48 d-f	9.91 hi
Mk20	11.00 ef	11.08 ef	10.57 f-h
Mk1	10.88 e-g	11.56 de	10.77 e-h
M9	12.79 c	10.76 e-h	10.08 g-i
M6	13.43 a-c	11.11 d-f	10.75 e-h
Mk25 x M9	12.86 bc	12.87 bc	10.05 g-i
Mk25 x M6	13.96 a	13.35 a-c	9.23 i
Mk20 x M9	13.72 ab	13.69 a-c	11.05 e-h
Mk20 x M6	14.01 a	11.99 d	11.64 de
Mk1 x M9	11.99 d	12.96 bc	11.31 d-f
Mk1 x M6	12.89 bc	13.64 a-c	10.93 e-g
LSD value ($p \leq 0.05$)	0.7724		

Means sharing same letters are statistically non-significant at 5 % level of probability. (n = 3)

Mk1, *Pseudomonas syringae;* Mk20, *Pseudomonas fluorescens;* Mk25, *Pseudomonas fluorescens* Biotype G; M6, and M9, *Rhizobium phaseoli*

while the maximum improvement in photosynthetic rate was by the same combination at 6 dS m^{-1}, and by Mk20 × M9 at 4 dS m^{-1}. The sole inoculation of *Rhizobium* and *pseudomonas* also gave significant results at all salinity levels

Crude protein content was significantly improved with inoculation/co-inoculation which otherwise was significantly decreased with increasing level of salinity. Co-inoculation was more efficient for improving the crude protein content in grains of mung bean (Table 3). Co-inoculation with *Rhizobium* and *Pseudomonas* was more efficient than sole inoculation. Under normal conditions, the combination Mk20 × M6 was more efficient and the increase was up to 89% over control. At 4 dS m^{-1}, the maximum increase was 86% with the combination Mk25 × M6 while at 6 dS m^{-1}, the combination Mk1 × M9 increased the crude protein up to 99% over the un-inoculated control.

The data (Table 3) showed that phosphorus concentration in grains of mung bean decreased with increasing level of salinity. However, inoculation/co-inoculation improved the phosphorus concentration at all levels of salinity. Under normal conditions, the maximum phosphorus concentration (0.34% P) was obtained due to co-inoculation of *Pseudomonas* strain Mk20 and *Rhizobium* strain M9 which was 56% greater than un-inoculated control and was statistically different from all other strains. At 4 dS m^{-1}, the improvement in phosphorus concentration was up to 28–54% over the un-inoculated control while at 6 dS m^{-1}, the increase over the un-inoculated control ranged from 32–66%. The combination Mk20 × M9 was the most efficient which improved the phosphorus concentration up to 54 and 66% compared with the un-inoculated control at 4 and 6 dS m^{-1} salinity.

Table 3. Effect of inoculation/co-inoculation on protein and phosphorus concentration in grains of mung bean under salt-stressed natural conditions in pot trial

Treatment	1.41 dS m^{-1}	4 dS m^{-1}	6 dS m^{-1}
	Protein (%)		
Control	10.73 pq	8.77 rs	7.42 s
Mk25	11.63 m-p	10.50 pq	9.77 qr
Mk20	17.13 bc	12.88 k-n	11.54 n-p
Mk1	16.46 cd	14.92 d-h	15.60 d-g
M9	13.46 h-l	10.85 pq	11.31 op
M6	17.28 bc	15.21 d-g	11.56 n-p
Mk25 x M9	14.58 f-j	14.37 g-k	11.40 n-p
Mk25 x M6	18.28 b	16.29 c-e	13.06 j-m
Mk20 x M9	15.54 d-g	14.58 f-j	13.33 i-l
Mk20 x M6	20.28 a	15.17 d-g	12.65 l-o
Mk1 x M9	14.44 g-j	14.21 g-k	14.77 e-i
Mk1 x M6	16.12 c-f	15.18 d-g	8.78 rs
LSD value ($p \leq 0.05$)	1.342		
	P concentration in grain (%)		
Control	0.22 mn	0.18 p	0.15 q
Mk25	0.27 d-f	0.23 k-m	0.20 op
Mk20	0.27 d-f	0.25 h-k	0.23 lm
Mk1	0.24 i-l	0.26 e-h	0.22 mn
M9	0.29 bc	0.26 f-i	0.23 j-m
M6	0.30 b	0.28 c-f	0.20 no
Mk25 x M9	0.30 b	0.23 lm	0.22 m
Mk25 x M6	0.27 e-h	0.25 g-j	0.23 lm
Mk20 x M9	0.34 a	0.28 c-f	0.25 h-k
Mk20 x M6	0.29 bc	0.26 e-h	0.23 lm
Mk1 x M9	0.29 bc	0.25 h-k	0.24 i-l
Mk1 x M6	0.29 b-d	0.23 k-m	0.20 op
LSD value ($p \leq 0.05$)	0.0163		

Means sharing same letters are statistically non-significant at 5 % level of probability. (n = 3)

Mk1, *Pseudomonas syringae;* Mk20, *Pseudomonas fluorescens;* Mk25, *Pseudomonas fluorescens* Biotype G; M6, and M9, *Rhizobium phaseoli*

DISCUSSION

In a pot trial, three strains of *Pseudomonas* and two strains of *Rhizobium phaseoli* were evaluated alone and in combination for their potential to reduce the inhibitory effects of salinity on physiology and quality of mung bean.

In our study, the physiological parameters were negatively affected by salinity. The reduction in substomatal CO_2 concentration may be due to damage caused by salinity to photosynthetic tissue, to stomatal closure and consequently restricted availability of CO_2 for carboxylation thus leading to accelerated senescence (Pessarakli, 1994; Misra *et al.*, 2002). This reduction in photosynthetic rate, stomatal conductivity and substomatal CO_2 concentration has also been reported in bean plants (Brugnoli and Lauteri, 1991). The decreased photosynthetic rate due to decrease in CO_2 assimilation caused by decreased relative water content and stomatal conductivity has also been reported by Lawlor and Cornic (2002) and Ben-Asher *et al.* (2006). They reported salt-induced changes in photosynthesis due to reduction in stomatal conductivity.

In this study, inoculation/co-inoculation reduced the effect of salinity on physiological parameters thus improving the photosynthetic rate which led to increased growth and yield of mung bean. These results are also supported by the previous work in which improved physiology due to bacterial inoculation has been reported (Gaballah and Gomaa, 2005). The improvement in gaseous exchange enhanced photosynthetic rate in plants. Co-inoculation has been reported to increase the photosynthetic rate and stomatal conductance of water in plants under stress. However, variable response to gaseous exchange was observed (Vivas *et al.*, 2003). This increase in microbial activity might have increased the physiological parameters ultimately leading to increased yield.

It was also observed in the present study that chlorophyll contents of mung bean were increased due to inoculation/co-inoculation with *Rhizobium* and PGPR containing ACC-deaminase which otherwise were decreased due to salinity. Senescence of plant leaves is one of the major symptoms of accelerated ethylene levels (Arshad and Frankenberger, 2002). It is very likely that inoculation/co-inoculation with *Rhizobium* and PGPR containing ACC-deaminase suppressed synthesis of ethylene, thus reducing the effect of salinity-induced ethylene on chlorophyll decay. It was found in the previous studies that chlorophyll content in the plant shoot increased when inoculated with *Pseudomonas* stains having ACC- deaminase activity (Nadeem *et al.*, 2009). This increase in chlorophyll content may also be due to the increased photosynthetic leaf area of plant by inoculation/co-inoculation compared to un-inoculated control where leaf area was reduced due to salinity stress. Similarly, reduction in leaf area has been reported due to the death of old leaves caused by ion-toxicity. This may prevent the supply of nutrients and hormones to young leave (Munns, 1993) leading to reduced chlorophyll content. Improvement in chlorophyll content has also been reported in different plants inoculated with PGPR under salinity (Hamdia and El-Komy, 1998; Ashraf, 2004).

In our study, nutrients concentration in mung bean plants has also been adversely affected by salinity. This might be due to the reduced root growth with increase in salinity-induced ethylene production which decreased the root area thus making it insufficient to explore soil for required nutrient uptake. Decrease in nutrients might be due to high salt stress has also been reported in previous work (Nadeem *et al.*, 2009). However, inoculation/co-inoculation reversed the pattern and improved the nutrient concentration and improved phosphorus and crude protein concentration in mung bean seeds. This might be due to reduction in ethylene production due to inoculation/co-inoculation with *Rhizobium* and PGPR containing ACC-deaminase (Ahmad *et al.*, 2011) thus reducing the inhibitory effect of ethylene on root growth leading to more proliferation of roots. The increased root area might have facilitated the plants to explore more soil for nutrients absorption. This might also be due to the solubilization of indigenous phosphorus by these bacteria due to their phosphate solubilization activity (Ahmad *et al.*, 2011). Protein synthesis might be inhibited due to high ethylene concentrations while inoculation/co-inoculation with *Rhizobium* and PGPR containing ACC-deaminase decreased ethylene synthesis thus reducing the inhibitory effect on protein synthesis. Similarly, enhanced uptake of N, P and K, and increased protein concentration in plants under salt stress, due to bacterial inoculation has also been reported in previous studies (Nadeem *et al.*, 2009).

The strains used in this experiment varied in their ability to decrease the effect of salinity on plant physiology, and maximum response was observed when *Pseudomonas fluorescens* (Mk20) was co-inoculated with *Rhizobium phaseoli*. 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 PGPR with *Rhizobium* enhanced the survival efficacy and proliferation of *Rhizobium* might be due to the solubilization of indigenous phosphorus (Ahmad *et al.*, 2011).

It can be concluded from the results of the study that combined application of *Rhizobium phaseoli* and *Pseudomonas* strains is more efficient to reduce the inhibitory effects of salinity on physiology and quality of mung bean. Thus, these strains could be explored for their potential as effective biofertilizer for mung bean under salt-affected conditions.

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