Effectiveness of halo-tolerant, auxin producing *Pseudomonas* and *Rhizobium* strains to improve osmotic stress tolerance in mung bean (*Vigna radiata* L.)

Maqshoof Ahmad1,2, Zahir A. Zahir1, Farheen Nazli3, Fareeha Akram1, Muhammad Arshad1, Muhammad Khalid1

1Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan.
2University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan.
3Pesticide Quality Control Laboratory, Bahawalpur, Pakistan.

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Abstract

Halo-tolerant, auxin producing bacteria could be used to induce salt tolerance in plants. A number of *Rhizobium* and auxin producing rhizobacterial strains were assessed for their ability to tolerate salt stress by conducting osmoadaptation assay. The selected strains were further screened for their ability to induce osmotic stress tolerance in mung bean seedlings under salt-stressed axenic conditions in growth pouch/jar trials. Three most effective strains of *Rhizobium* and *Pseudomonas* containing ACC-deaminase were evaluated in combination, for their ability to induce osmotic stress tolerance in mung bean at original, 4, and 6 dS m⁻¹ under axenic conditions. Results showed that sole inoculation of *Rhizobium* and *Pseudomonas* strains improved the total dry matter up to 1.4, and 1.9 fold, respectively, while the increase in salt tolerance index was improved up to 1.3 and 2.0 fold by the *Rhizobium* and *Pseudomonas* strains, respectively. However, up to 2.2 fold increase in total dry matter and salt tolerance index was observed due to combined inoculation of *Rhizobium* and *Pseudomonas* strains. So, combined application of *Rhizobium* and *Pseudomonas* strains could be explored as an effective strategy to induce osmotic stress tolerance in mung bean.

Key words: ACC-deaminase, *Pseudomonas*, *Rhizobium*, osmotic stress, salt tolerance index.

Introduction

Salinity is one of the major yield limiting factors worldwide that hinders plant growth by affecting a number of physiological processes positively or negatively. It is a serious production problem for crops as saline conditions are known to suppress plant growth, particularly in arid and semiarid regions (Parida and Das, 2005). 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. Salinity stress adversely affects total dry matter and plant growth as most part of the energy is used in making osmotic adjustments by the plant (Munns and Termaat, 1986). 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) and consequently growth of the plant.

The ethylene which is produced in excess due to salinity may be controlled by a number of chemical and biological approaches. However, in most cases, chemical approach may become expensive, less feasible or potentially harmful to the environment. The use of rhizobacteria containing ACC-deaminase becomes one of the most widely acceptable approaches to reduce the effect of stress-induced ethylene on plants. These plant growth promoting rhizobacteria (PGPR) contain an enzyme ACC-deaminase which hydrolyzes ACC (immediate precursor of ethylene) into ammonia and α-ketobutyrate (Mayak et al., 1999; Tahir et al., 2006). These PGPR boost the plant growth, particularly under stressed conditions, by the regulation of accelerated ethylene production in response to a
multitude of abiotic and biotic stresses (Belimov et al., 2009a, 2009b). The increase in growth of plants under normal conditions has been reported due to inoculation with Indole acetic acid (IAA) producing bacteria (Malhotra and Srivastava, 2006). As IAA plays a vital role to induce salinity tolerance in plants (Azooz et al., 2004) hence, the use of auxin producing PGPR containing ACC deaminase activity may be more economical, environmental friendly and feasible in a natural soil and plant system.

Bacterial strains have variable ability to tolerate the salt stress (Lloret et al., 1995) and some salt tolerant Rhizobium strains can grow at NaCl concentration up to 500 mM. The salt-tolerant rhizobia go through some morphological, metabolic and structural modifications to muddle through the salt stress. Higher salt tolerance by bacterial strains was also reported by Mensah et al. (2006) and Skorupska, 1993; Dashti et al. (2009). Similarly, Hafeez et al. (1988) reported that most of the Rhizobium strains were salt tolerant and performed better for growth promotion of Vigna radiata under salt-stressed conditions. So, it is imperative to screen indigenous strains of rhizobia that are more efficient to fix atmospheric nitrogen under stressed conditions (Woldeyohannes et al., 2007).

Co-inoculation of legumes with Pseudomonas sp. and rhizobia has also been reported to stimulate plant growth by affecting some physiological functions (Derylo and Skorupska, 1993; Dashli et al., 1997). It improves plant growth by reduction in ethylene level (Shaharooma et al., 2006), direct stimulation of rhizobial growth/survival in the soil, enlargement of the root system by hormone production for enhanced nutrient uptake and increase in the number of potential colonization sites by Rhizobium (Gull et al., 2004; Barea et al., 2005). So, the present study was conducted to screen the auxin producing, halo-tolerant Rhizobium and Pseudomonas strains for improving osmotic stress tolerance index in mung bean.

Materials and Methods

Collection of bacterial strains

Twenty five strains of rhizobacteria and ten Rhizobium strains were isolated from rhizosphere and nodules of mung bean growing in salt-affected fields. The Rhizobium strains were coded as M1-M10 and rhizobacterial strains as Mk1-Mk25 (Ahmad et al., 2011).

ACC-metabolism assay (Qualitative)

The ability of rhizobacteria to utilize ACC as sole nitrogen source was assayed qualitatively as described by Jacobson et al. (1994).

Auxin production assay

Auxin production by the rhizobacterial strains in the presence and absence of L-tryptophan was estimated by using colorimetric method in terms of IAA equivalents by following the method of Sarwar et al. (1992).

Osmoadaptation assay

Osmoadaptation assay of rhizobial and rhizobacterial strains was carried out as described by Zahir et al. (2010), to assess their salinity tolerance at original, 4, 8 and 12 dS m⁻¹ salinity levels. Four salinity levels i.e. original, 4, 8 and 12 dS m⁻¹ were developed in yeast extract mannitol (YEM) and general purpose media (GPM) for rhizobial and rhizobacterial strains, respectively. Fifteen milliliters of the respective broth were taken in test tubes. The sterilized broth in tubes were inoculated with the uniform population of the respective rhizobial or rhizobacterial strains (OD₅₄₀ = 0.3). Tubes were incubated at 28 ± 1 °C and absorbance was measured by using spectrophotometer at 540 nm wavelengths after 3 days of incubation.

Preparation of inocula for osmoadaptation and pouch trials

Inocula were prepared in flasks by using YEM and DF minimal salt medium containing ACC as substrate (N source), without agar 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 hours under shaking (100 rpm) conditions. After incubation, optical density was measured and uniform population (OD₅₄₀ = 0.45; 10⁶-10⁷ cfu mL⁻¹) was achieved by dilution with sterilized water prior to seed inoculation.

Screening of rhizobacterial and rhizobial strains for inducing osmotic stress tolerance

The growth pouch and jar experiments were conducted in growth room for the screening of rhizobacteria and Rhizobium, respectively, under gnotobiotic conditions. Broths were prepared by DF minimal salt medium containing ACC as substrate and YEM medium for rhizobacteria and Rhizobium, respectively, as described earlier.

Mung bean seeds were surface-sterilized by dipping in 95% ethanol for few moments followed by 0.2% HgCl₂ solution for three minuets, and thoroughly washing with sterilized water. Three surface-sterilized seeds were dipped in the respective inocula of rhizobacteria or Rhizobium (prepared as described above) for ten minutes and placed in the autoclaved growth pouches (MEGA-International, West St. Paul, USA) or jars for rhizobacteria and Rhizobium, respectively. Sterilized broths were used for the control treatment. Each treatment was replicated three. Three salinity levels (original, 4 and 6 dS m⁻¹) were maintained by using NaCl in sterilized Hoagland solution (1/2 strength) and modified N-free, Hoagland solution for rhizobacteria and Rhizobium, respectively, (Fahraeus, 1957). In the growth room, the temperature was maintained at 28 ± 1 °C and 10 hours of light (275 μmol m⁻²s⁻¹) alternated with
14 hours darkness. The rhizobacterial inoculated trial was harvested after 21 days while the Rhizobium inoculated trial was harvested after 60 days and data regarding seedlings growth were recorded.

Identification of selected strains

The selected rhizobacterial strains were identified by using the BIOLOG® identification system (Microlog System release 4.2 Biolog Inc., USA). The Biolog® identification system has been found equally as reliable for identification as 16s RNA (Flores-Vargas and O, Hara, 2006). The rhizobacterial strains were Pseudomonas syringae, (Mk1); Pseudomonas fluorescens, (Mk20) and Pseudomonas fluorescens Biotype G, (Mk25). The similarity values obtained from the Biolog assay were 94, 89 and 88% for Pseudomonas syringae, (Mk1); Pseudomonas fluorescens, (Mk20) and Pseudomonas fluorescens Biotype G, (Mk25), respectively. The confirmation of the Rhizobium strains was carried out by isolation of the Rhizobium from the nodules of mung bean and upon re-inoculation these strains nodulated the mung bean seedlings (Ahmad et al., 2011).

Screening of effective combinations of Rhizobium and Pseudomonas strains containing ACC-deaminase for inducing osmotic stress tolerance

Screening of effective (Pseudomonas x Rhizobium) combinations was carried out under gnotobiotic conditions in the growth room. Broth cultures of selected Rhizobium and Pseudomonas strains were prepared as described earlier.

For co-inoculation, broth cultures of Pseudomonas strains and Rhizobium were used in the 1:1 ratio. Surface-sterilized mung bean seeds were dipped in this combined broth for ten minutes. Three co-inoculated seeds were sown in autoclaved growth pouches. In case of control, sterilized broths were used for seed dipping. Each treatment was replicated thrice. Three salinity levels [original (1.62), 4 and 6 dS m⁻¹] were maintained by using NaCl in sterilized Hoagland solution (1/2 strength). The temperature in the growth room was adjusted to 28 ± 1 °C with 10 hours of light (275 μmol m⁻² s⁻¹) and 14 hours dark period. Data regarding seedlings growth were recorded.

The experiments were conducted in the growth room and all operations were carried out aseptically in a Laminar flow-hood.

Salt tolerance index

Effect of bacterial inoculation on mung bean was calculated by taking the salt tolerance indices of inoculated and un-inoculated plants grown under stress and normal conditions according to Shetty et al. (1995) as:

\[ \text{Salt tolerance index (STI)} = \frac{\text{DWS or DWI}}{\text{DWC}} \]

where DWS = dry weight of stressed plants, DWI = dry weight of inoculated plants and DWC = dry weight of un-stressed and un-inoculated control.

Statistical analysis

Analysis of variance techniques (ANOVA) were 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

ACC metabolism assay for rhizobacterial strains

The rhizobacterial strains were screened for their ability to utilize ACC as a sole source of nitrogen and for this purpose a qualitative ACC metabolism assay was performed. The results of the bioassay showed that all the rhizobacterial strains had the ability to utilize ACC as a sole source of nitrogen but with variable degree of efficacy. So, all these strains possessed ACC-deaminase activity. On the basis of growth [optical density values at 540 nm (OD₅₄₀)], these strains were grouped into low, medium and high ACC utilizing strains (Table 1).

Out of twenty five strains, seven strains having the maximum ACC utilization were grouped as high ACC utilizing strains (OD₅₄₀ > 0.75), ten strains having medium growth (OD₅₄₀: 0.50-0.75) were grouped as medium ACC utilizing strains and the remaining having less growth i.e. OD₅₄₀ < 0.50 were grouped as low ACC utilizing strains.

On the basis of the results of ACC metabolism assay, ten strains having maximum cell growth on ACC substrate were selected for further experimentation.

Table 1 - Cell growth (OD₅₄₀) of the rhizobacteria on ACC substrate (Average of three replicates ± SE).

| Code name | OD Value    | Code name | OD Value    |
|-----------|-------------|-----------|-------------|
| Mk1*      | 0.76 ± 0.03 | Mk14      | 0.41 ± 0.02 |
| Mk2       | 0.59 ± 0.02 | Mk15*     | 0.71 ± 0.03 |
| Mk3*      | 0.95 ± 0.02 | Mk16      | 0.61 ± 0.02 |
| Mk4       | 0.43 ± 0.02 | Mk17      | 0.37 ± 0.03 |
| Mk5*      | 0.63 ± 0.03 | Mk18      | 0.47 ± 0.02 |
| Mk6       | 0.49 ± 0.03 | Mk19      | 0.55 ± 0.03 |
| Mk7       | 0.047 ± 0.02| Mk20*     | 0.79 ± 0.03 |
| Mk8*      | 0.61 ± 0.02 | Mk21      | 0.31 ± 0.03 |
| Mk9       | 0.60 ± 0.03 | Mk22*     | 0.75 ± 0.04 |
| Mk10*     | 0.77 ± 0.01 | Mk23      | 0.51 ± 0.02 |
| Mk11      | 0.44 ± 0.02 | Mk24      | 0.55 ± 0.03 |
| Mk12      | 0.53 ± 0.01 | Mk25*     | 0.91 ± 0.02 |
| Mk13*     | 0.77 ± 0.02 |           |             |

*Strains selected for further experimentation.
Auxin production by rhizobacterial strains

The results of the auxin production assay (Table 2) showed that all rhizobacterial strains produced auxins measured in terms of IAA equivalents in the presence and absence of L-TRP but they varied in their ability to produce auxin. In the absence of L-TRP, the maximum auxin was produced by the strain Mk25 which gave significantly different results compared with control as well as other strains. While in the presence of L-TRP, the strains Mk20 and Mk25 were equally effective to produce auxin, and they were statistically different from control and other strains.

Osmoadaptation assay

Salt tolerance of selected rhizobacterial strains containing ACC-deaminase and *Rhizobium* was assessed by conducting osmoadaptation assay. The *Rhizobium* and rhizobacterial strains were grown at four salinity levels *i.e.* original, 4, 8 and 12 dS m⁻¹ and the optical density was measured. The results showed that the bacterial strains varied in their ability to tolerate the salt-stressed conditions.

### Osmoadaptation assay for rhizobacterial strains

To assess salt tolerance of rhizobacterial strains, these were grown at four salinity levels *i.e.* original (1.59), 4, 8 and 12 dS m⁻¹. The growth of strains (OD at 540 nm) was measured after 3 days of incubation. The results of the study revealed that growth of rhizobacterial strains decreased with increasing level of salinity and the strains varied in their ability to tolerate the higher level of salinity. At higher salinity level (12 dS m⁻¹), the maximum optical density was observed in case of strain Mk25 followed by Mk1, Mk8, and Mk20 (Table 3).

### Osmoadaptation assay for Rhizobium strains

The results (Table 4) of the osmoadaptation assay for *Rhizobium* strains showed that salinity stress had negative effect on the growth (cell density) of *Rhizobium* strains and the growth of these strains decreased with increasing level of salinity. But all the strains had variable growth at all salinity levels. Under normal conditions, the maximum cell density was observed by the strains M10. At 12 dS m⁻¹, maximum growth was observed in the case of M9 strain.

Screening of rhizobacteria and *Rhizobium* strains for inducing osmotic stress tolerance

The rhizobacteria containing ACC-deaminase activity and *Rhizobium* were screened for their ability to improve osmotic stress tolerance in mung bean under axenic conditions. The results imply that the salinity significantly reduced the total dry matter and salt tolerance index of mung bean seedlings but the inoculation with rhizobacteria containing ACC-deaminase and *Rhizobium*, significantly reduced the inhibitory effects of salinity and improved the total dry matter and salt tolerance index of mung bean seedlings.

Data (Table 5) showed that all the strains of rhizobacteria containing ACC-deaminase showed non-significant increase in total dry matter of mung bean seedlings except Mk1 which was statistically significant over un-inoculated control and it increased total dry matter the up to 1.4 fold over the un-inoculated control, at higher salinity level. Inoculation with *Rhizobium* also reduced the inhibitory effect of salinity with different degrees of efficacy (Table 6). Under original salinity level, maximum increase in total dry matter over the un-inoculated control was 192% with *Rhizobium* strain M6. At 6 dS m⁻¹, all the strains showed non-significant results with un-inoculated control, how-

### Table 2 - Auxin production (IAA equivalents) by rhizobacterial strains in the presence and absence of L-TRP.

| Strain | Without L-TRP (mg L⁻¹) | With L-TRP (mg L⁻¹) |
|--------|------------------------|---------------------|
| Mk1    | 6.89 d                 | 9.26 b              |
| Mk3    | 3.26 e                 | 9.47 b              |
| Mk5    | 1.90 f                 | 6.09 c              |
| Mk8    | 8.20 b                 | 11.42 a             |
| Mk10   | 6.70 d                 | 11.66 a             |
| Mk13   | 6.88 d                 | 9.76 b              |
| Mk15   | 6.56 d                 | 10.35 b             |
| Mk20   | 9.64 a                 | 12.41 a             |
| Mk22   | 7.61 c                 | 11.66 a             |
| Mk25   | 8.51 b                 | 12.43 a             |
| LSD (p < 0.05) | 0.6432 | 1.0626 |

Means sharing same letters are statistically at par at 5% level of probability. n = 3.

### Table 3 - Response of rhizobacterial strains to different levels of salinity after 3 days of incubation.

| Strain | Optical density at 540 nm |
|--------|---------------------------|
|        | 1.59 dS m⁻¹ | 4 dS m⁻¹ | 8 dS m⁻¹ | 12 dS m⁻¹ |
| Mk1    | 0.623 b-f | 0.574 b-i | 0.499 b-l | 0.468 c-l |
| Mk3    | 0.945 a   | 0.400 c-l | 0.247 l   | 0.293 i-l |
| Mk5    | 0.653 b-e | 0.752 ab  | 0.374 e-l | 0.353 f-l |
| Mk8    | 0.742 a-c | 0.467 c-l | 0.447 d-l | 0.438 d-l |
| Mk10   | 0.543 b-k | 0.293 i-l | 0.325 g-l | 0.288 j-l |
| Mk13   | 0.548 b-k | 0.595 b-g | 0.277 k-l | 0.339 f-l |
| Mk15   | 0.749 ab  | 0.370 c-i | 0.491 b-l | 0.235 l   |
| Mk20   | 0.437 d-l | 0.312 g-l | 0.639 b-e | 0.384 e-l |
| Mk22   | 0.691 a-d | 0.590 b-h | 0.315 g-l | 0.308 h-l |
| Mk25   | 0.935 a   | 0.565 b-j | 0.577 b-i | 0.487 b-l |
| LSD value (p ≤ 0.05) | 0.2298 |

Means sharing same letters are statistically at par at 5% level of probability. n = 3.
Table 4 - Response of rhizobial strain to different levels of salinity after 3 days of incubation.

| Strain | Optical density at 540 nm |
|--------|--------------------------|
|        | 1.61 dS m⁻¹ | 4 dS m⁻¹ | 8 dS m⁻¹ | 12 dS m⁻¹ |
| M1     | 0.894 bc     | 0.604 d-h  | 0.411 e-k  | 0.343 h-m  |
| M2     | 0.453 c-k     | 0.625 d-f  | 0.373 c-l  | 0.354 g-l  |
| M3     | 0.808 cd     | 0.632 de  | 0.379 c-l  | 0.293 j-m  |
| M4     | 0.610 d-g     | 0.502 c-k  | 0.401 e-k  | 0.263 k-m  |
| M5     | 0.582 d-i     | 0.433 e-k  | 0.440 e-k  | 0.299 j-m  |
| M6     | 0.998 a-c     | 0.639 de  | 0.465 e-k  | 0.359 f-l  |
| M7     | 0.541 c-e     | 0.234 k-m  | 0.301 j-m  | 0.273 j-m  |
| M8     | 1.130 ab     | 0.633 de  | 0.331 i-m  | 0.284 j-m  |
| M9     | 1.066 ab     | 0.809 ed  | 0.443 e-k  | 0.418 e-k  |
| M10    | 1.179 a      | 0.249 k-m  | 0.082 m   | 0.115 lm   |
| LSD value (p ≤ 0.05) | 0.2180 |

Means sharing same letters are statistically at par at 5% level of probability. n = 3.

Table 5 - Effect of rhizobacterial inoculation on total dry matter and salt tolerance index of mung bean seedlings under salt-stressed axenic conditions.

| Treatment | Total dry matter (g plant⁻¹) | Salt tolerance index |
|-----------|-----------------------------|----------------------|
|           | Control (4 dS m⁻¹) | 6 dS m⁻¹ | 4 dS m⁻¹ | 6 dS m⁻¹ |
|           | 0.037 a-g | 0.024 d-h | 0.014 h  | 0.66 c  | 0.40 e  |
| Mk1       | 0.051 a  | 0.043 a-d  | 0.034 a-g  | 1.18 a  | 0.93 a  |
| Mk3       | 0.042 a-f  | 0.038 a-g  | 0.023 d-h  | 1.04 ab  | 0.64 cd  |
| Mk5       | 0.046 a-c  | 0.037 a-g  | 0.023 e-h  | 1.02 ab  | 0.64 cd  |
| Mk8       | 0.044 a-d  | 0.039 a-f  | 0.028 b-h  | 1.07 ab  | 0.77 a-c  |
| Mk10      | 0.046 ab  | 0.033 a-h  | 0.022 f-h  | 0.92 b  | 0.61 cd  |
| Mk13      | 0.042 a-f  | 0.037 a-g  | 0.026 c-h  | 1.03 ab  | 0.71 bc  |
| Mk15      | 0.044 a-d  | 0.034 a-h  | 0.025 d-h  | 0.92 a  | 0.70 cd  |
| Mk20      | 0.051 a  | 0.043 a-c  | 0.032 a-h  | 1.17 a  | 0.87 ab  |
| Mk22      | 0.043 a-e  | 0.039 a-f  | 0.019 gh  | 1.07 ab  | 0.53 de  |
| Mk25      | 0.050 a  | 0.040 a-f  | 0.033 a-h  | 1.10 ab  | 0.91 a   |
| LSD value | 0.0163 | 0.1561 |

Means sharing same letters are statistically at par at 5% level of probability. n = 3.

Screening of effective combinations of Rhizobium and Pseudomonas containing ACC-deaminase for inducing osmotic stress tolerance

The most effective strains in the above trials were screened for their combined effect to improve salt tolerance in mung bean seedlings under salt-affected axenic conditions. The results showed that co-inoculation of Rhizobium and Pseudomonas strains containing ACC-deaminase activity significantly reduced the effect of salinity on total dry matter and salt tolerance index of mung bean seedlings.

It was revealed from the data (Figure 1) that co-inoculation with Rhizobium and Pseudomonas strains containing ACC-deaminase improved the total dry matter of mung bean seedlings which otherwise was decreased by salinity. At 6 dS m⁻¹, all the combinations showed significant increase in total dry matter compared with respective un-inoculated control and the maximum increase (2.2 fold) was observed by the combination Mk25 x M6. At 4 dS m⁻¹, maximum increase in total dry matter (1.2 fold) over respective un-inoculated control was observed with combination Mk20 x M6 followed by 1.18 fold increase in total dry matter over the un-inoculated control with the combination Mk25 x M9.

The salt tolerance index of mung bean seedlings (Figure 2) was improved due to co-inoculation with Rhizobium and Pseudomonas strains containing ACC-deaminase. At 6 dS m⁻¹, increase in salt tolerance index due to co-inoculation ranged from 1.0 to 2.2 fold over respective un-inoculated control. Maximum increase in salt tolerance index (2.2 fold) was observed by the combination Mk25 x M6. At 4 dS m⁻¹, increase in salt tolerance index due to co-inoculation was also significantly higher than respective un-inoculated control.
Maximum increase in salt tolerance index (1.2 fold) over un-inoculated control was observed with co-inoculated combination Mk20 x M6.

Discussion

Some PGPR are capable of lowering stress-induced ethylene levels through an enzyme ACC-deaminase (Zahir et al., 2010). This results in better growth and nodulation in legumes. It is very likely that these bacterial strains could be used to improve plant growth in stressed conditions. In the present study, it was observed that all the rhizobacterial strains possess ACC-deaminase activity as evident from the results of ACC metabolism assay. When grown on ACC, all the strains showed growth but variable cell density was observed. It means these strains have variability in their efficiency to utilize ACC as sole source of nitrogen. This difference in ACC utilization rate by these strains might be due to difference in their ACC-deaminase activity. Some PGPR strains contain an enzyme ACC-deaminase which cleaves the ACC; the immediate precursor of ethylene into ammonia and α-ketobutyrate (Glick et al., 1998) and use the released ammonia for their metabolism.

Indole acetic acid is produced by many microbes including PGPR which is an important plant growth regulator. Therefore, there is a close interaction between auxin producing PGPR and plants (Malhotra and Srivastava, 2006; Anjum et al., 2011). In our laboratory study, it was observed that all rhizobacterial strains have the ability to produce auxin but with different efficacy and this auxin production was increased by the addition of L-TRP. The difference in auxin producing ability has also been reported by Fuentes-Ramirez et al. (Fuentes-Ramirez et al., 1993). Increased auxin production by different bacterial strains in the presence of L-TRP has also been reported by De and Basu (1996).

In our study, it was observed that growth of Rhizobium and rhizobacterial strains was adversely affected by salinity. Data showed that Rhizobium growth was optimum under normal conditions but under salt-stressed conditions the strains varied in their growth. Some strains showed more growth even at higher concentrations. Variable ability of bacterial strains to tolerate the salt stress has been reported by Lloret et al. (1995). They reported that some salt tolerant Rhizobium strains can grow at NaCl concentration up to 500 mM. Higher salt tolerance by bacterial strains was also reported by Mensah et al. (2006) and Sgroy et al. (2009).

Higher seedling biomass may increase salt tolerance index of plants thus making the plant better withstand salinity stress. In the present study, salinity significantly reduced the total dry matter and salt tolerance index of mung bean plants under axenic conditions. This might be due to the effect of salinity on plant metabolic and physiological processes or it might be due to the use of most part of energy in making osmotic adjustments by the plant thus decreasing plant growth and total dry matter (Munns and Termaat, 1986).

In our study, inoculation/co-inoculation with Rhizobium and auxin producing Pseudomonas containing ACC-deaminase improved the total dry matter and salt tolerance index of mung bean plants under axenic conditions. This might be due to the reduction of adverse effects of stress-induced ethylene on plant physiology. It has been reported that inoculation/co-inoculation improved the transpiration rate and other physiological processes (Vivas et al., 2003; Gaballah and Goma, 2005; Zahir et al., 2009), thus reducing the effect of salinity on plant growth leading to increased total dry matter and salt tolerance index. This might also be due to reduction in ethylene production due to inoculation/co-inoculation.
culation with *Rhizobium* and PGPR containing ACC-deaminase thus reducing the inhibitory effect of ethylene on root growth leading to more proliferation of roots. The competency of co-inoculation for reducing the effect of salinity due to reduction in ethylene level through ACC-deaminase activity has been proved by conducting the classical triple response assay (Ahmad et al., 2011).

Auxins are produced in excess amounts by the plants subjected to stress (Vaidyanathan et al., 1999; Yurekli et al., 2004) as a plant metabolic strategy to cope with the stressed conditions by shortening the life cycle (Yurekli et al., 2004). This is an adaptive mechanism in plants. But the production of IAA in plants requires an additional amount of energy along with other normal plant metabolic processes. This additional energy use in auxin production reduces the plant metabolism thus decreasing plant growth. So, inoculation with auxin producing PGPR may improve the plant growth (Zahir et al., 2010) thus decreasing the energy requirements. However, inoculation of plants under stress with PGPR that have dual character i.e. auxin production as well as ACC-deaminase activity might have decreased the stress-induced ethylene production as additional amount of IAA produced by these bacteria might be taken up by plants which activates the enzyme ACC synthase thus more ACC production. This ACC may come out due to concentration gradient and induce the enzyme ACC-deaminase in the PGPR which cleaves the ACC into ammonia and α-keto butyrate (Glick et al., 1998) thus lowers the ethylene production and improves the plant growth (Zahir et al., 2008).

The strains varied in their ability to reduce 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 vary in their ACC deaminase ability along with some other characters (Ahmad et al., 2011) that contributed differently for growth promotion. Similarly, it was reported in the previous findings that strains differ in their ability to promote plant growth due to difference in ACC deaminase activity (Shaharoona et al., 2006; Nadeem et al., 2007, 2009). This difference may also be due to the presence of other growth promoting characters, in addition to ACC-deaminase activity (Ahmad et al., 2011).

Our results imply that combined application of *Rhizobium* and *Pseudomonas* strains improved the osmotic stress tolerance in mung bean seedlings under axenic conditions. However, this approach could be explored as an effective strategy to improve salinity tolerance index in mung bean under pot and field conditions.

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