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Research Paper

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

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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 World wide 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

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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 plans (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 Sgroy *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; 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 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 (OD_{540} = 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 (OD_{540} = 0.45; 10^7-10^8 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% $HgCl_2$ 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 thrice. 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 $m^{-2}s^{-1}$) 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 (OD_{540})], 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 (OD_{540} > 0.75), ten strains having medium growth (OD_{540}: 0.50-0.75) were grouped as medium ACC utilizing strains and the remaining having less growth *i.e.* OD_{540} < 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 (OD_{540}) 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	IAA production (mg L^{-1})	
	Without L- TRP	With L-TRP
Mk1	6.89 d	9.26 b
Mk3	3.26 e	9.47 b
Mk5	1.90 f	6.09 c
Mk8	8.20 bc	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 e-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 kl	0.339 f-l
Mk15	0.749 ab	0.370 e-l	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 ($p \leq 0.05$)		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 e-k	0.625 d-f	0.373 e-l	0.354 g-l
M3	0.808 cd	0.632 de	0.379 e-l	0.293 j-m
M4	0.610 d-g	0.502 e-k	0.401 e-k	0.263 k-m
M5	0.582 d-i	0.434 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 e-j	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 cd	0.443 e-k	0.418 e-k
M10	1.179 a	0.249 k-m	0.082 m	0.115 lm
LSD value ($p \leq 0.05$)		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	Control	4 dS m^{-1}	6 dS m^{-1}	4 dS m^{-1}	6 dS m^{-1}
	Total dry matter (g $plant^{-1}$)			Salt tolerance index	
Control	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-e	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.

ever, the effect of the strain M6 was significant in comparison with respective un-inoculated control.

Inoculation with rhizobacteria and *Rhizobium* had very promising results for improving the salt tolerance index at all salinity levels. Maximum increase in salt tolerance index was observed with rhizobacterial strain Mk1, both at low as well as high salinity level (Table 5 However, in case of *Rhizobium* inoculation, maximum increase in salt tolerance index (1.6 fold) over the un-inoculated control was observed with *Rhizobium* strain M6, at 4 dS m^{-1}, while M6 gave most promising results at 6 dS m^{-1} (Table 6).

Table 6 - Effect of rhizobial inoculation on total dry matter and salt tolerance index of mung bean seedlings under salt-stressed axenic conditions.

Treatment	Control	4 dS m^{-1}	6 dS m^{-1}	4 dS m^{-1}	6 dS m^{-1}
	Total dry matter (g $plant^{-1}$)			Salt tolerance index	
Control	0.030 l-o	0.029 l-o	0.013 o	0.99 f	0.43 c
M1	0.072 a-e	0.057 d-i	0.028 m-o	1.95 cd	0.96 ab
M2	0.081 ab	0.028 m-o	0.013 o	0.93 f	0.44 c
M3	0.068 b-g	0.068 b-f	0.028 m-o	2.32 b	0.96 ab
M4	0.080 ab	0.053 e-j	0.025 m-o	1.79 de	0.86 b
M5	0.064 b-h	0.059 c-i	0.028 m-o	1.98 cd	0.95 ab
M6	0.088 a	0.077 a-c	0.036 j-n	2.61 a	1.23 a
M7	0.049 f-k	0.058 c-i	0.026 m-o	1.98 cd	0.90 b
M8	0.049 h-k	0.047 h-l	0.022 no	1.58 e	0.74 b
M9	0.076 a-d	0.066 b-h	0.031 k-o	2.24 bc	1.04 ab
M10	0.040 i-n	0.045 i-m	0.022 no	1.52 e	0.75 b
LSD value	0.0163			0.2850	

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-ino-

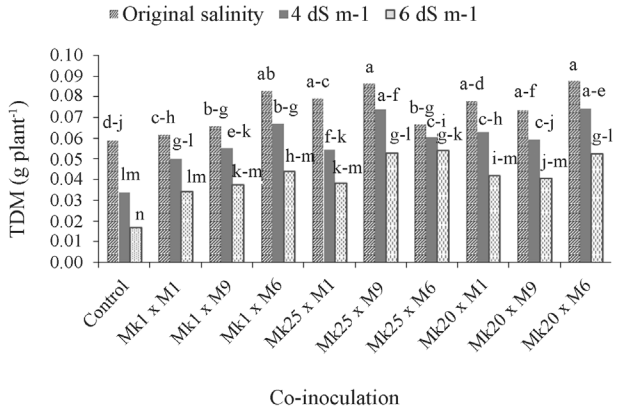

Figure 1 - Effect of co-inoculation of *Rhizobium* and *Pseudomonas* strains on total dry matter of mung bean seedlings under salt-stressed axenic conditions. Bars sharing same letters are statistically at par at 5% level of probability. n = 3. Mk1, *Pseudomonas syringae*; Mk20, *Pseudom*onas fluorescens; Mk25, *Pseudomonas fluorescens* Biotype G; M1, M2, M3, *Rhizobium phaseoli*.

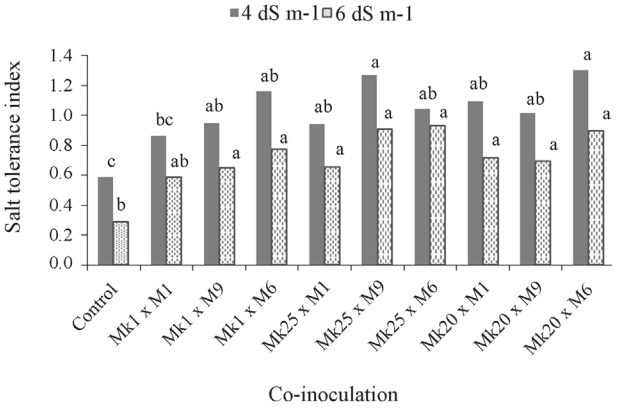

Figure 2 - Effect of co-inoculation of *Rhizobium* and *Pseudomonas* strains on salt tolerance index of mung bean seedlings under salt-stressed axenic conditions. Bars sharing same letters are statistically at par at 5% level of probability. n = 3. Mk1, *Pseudomonas syringae*; Mk20, *Pseudomonas fluorescens*; Mk25, *Pseudomonas fluorescens* Biotype G; M1, M2, M3, *Rhizobium phaseoli*.

culated 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. It 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 Gomaa, 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-ino-

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 salt tolerance index in mung bean under pot and field conditions.

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