Spanish Journal of Agricultural Research
17 (1), e0801, 12 pages (2019)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2019171-13828
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, O.A, M.P. (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Growth promotion and yield enhancement of barley cultivars using ACC deaminase producing *Pseudomonas fluorescens* strains under salt stress

Mitra Azadikhah[1], Fatemeh Jamali[2], Hamid-Reza Nooryazdan[1] and Fereshteh Bayat[1]

[1]*Persian Gulf Univ., Fac. Agr. Nat. Resour., Dept. Plant Breeding, Bushehr, P. O. Box 75169-13817, Iran.* [2]*Persian Gulf Univ., Fac. Agr. Nat. Resour., Dept. of Plant Protection, Bushehr, P. O. Box 75169-13817, Iran.*

Abstract

Plant growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme reduce the level of stress, ethylene and stimulate plant growth under various biotic and abiotic stress conditions. The present study aims at characterizing efficient salt-tolerant, ACC deaminase containing *Pseudomonas fluorescens* strains with plant growth-promoting activity isolated from the rhizosphere of barley plants and evaluating the influence of potent plant growth-promoting rhizobacteria (PGPR) isolates on growth and yield of five barley cultivars under salinity stress. Plant growth and yield in barley cultivars following inoculation with salt-tolerant, ACC deaminase producing PGPR strains under salt stress were quantified. Results indicated that under various levels of salinity (50, 100 and 150 mM NaCl) inoculation with PGPRs had positive impact on growth parameters and yield of barley cultivars including plant height, spike length, weight and number, peduncle length, number of grains per spike, 1000-grain weight and grain yield, comparing to uninoculated control plants under salinity stress. Inoculation of barley cultivars with bacteria ameliorated the negative effects of salinity and resulted in increase in growth and yield. Besides, as the salinity levels increased, growth and yield of barley cultivars decreased; however, cultivars showed different responses to salt stress. This study demonstrates the vital role of rhizobacteria containing ACC deaminase for increasing salt tolerance and consequently improving the growth and yield of barley plants under salinity stress.

Additional keywords: plant growth-promoting rhizobacteria; salinity stress; indole acetic acid.

Abbreviations used: ACC (1-aminocyclopropane-1-carboxylate); CFU (colony forming unit); DF (Dworkin and Faster minimal medium); DMRT (Duncan's Multiple Range Test); IAA (indole acetic acid); KB (King's medium B agar); LB (Luria–Bertani broth); MGW (1000-grain weight); PGP (plant growth-promoting); PGPR (plant growth-promoting rhizobacteria); TSB (tryptic soy broth).

Authors' contributions: Conceived and designed the work: FJ. Wrote and revised the paper: FJ and MA. All authors performed the experiments, analyzed the data, read and approved the final manuscript.

Citation: Azadikhah, M.; Jamali, F.; Nooryazdan, H. R.; Bayat, F. (2019). Growth promotion and yield enhancement of barley cultivars using ACC deaminase producing *Pseudomonas fluorescens* strains under salt stress. Spanish Journal of Agricultural Research, Volume 17, Issue 1, e0801. https://doi.org/10.5424/sjar/2019171-13828

Received: 13 Aug 2018. **Accepted**: 26 Feb 2019.

Copyright © 2019 INIA. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC-by 4.0) License.

Funding: The authors gratefully acknowledge the College of Agriculture and Natural resources, Persian Gulf University, Bushehr, Iran, for the support in the current study.

Competing interests: The authors have no competing interests to declare.

Correspondence should be addressed to Fatemeh Jamali: jamali@pgu.ac.ir

Introduction

Soil salinity is among the most significant environmental stresses in agriculture, suppressing plant growth and productivity of crops worldwide (Hu & Schmidhalter, 2005). Under high salinity conditions, plant growth and photosynthesis are adversely affected due to the increased amount of ethylene in root, ionic imbalance and hyper-osmotic condition in plants (Niu *et al*., 1995; Mayak *et al*., 2004). Accumulation of salts in the soil decreases the osmotic potential, thereby interferes with water absorption by roots and affects cell growth and related metabolism. Additionally, high salinity levels are toxic to the plants due to the formation of reactive oxygen species (ROS) causing oxidative damage and as a result, decreasing plant development (Munns & Tester, 2008).

Treatment of plant seeds and seedlings with plant growth-promoting rhizobacteria (PGPR) is a new approach that has been developed in recent years to alleviate the adverse effects of salinity. PGPR are a group of free-living bacteria living in the plant rhizosphere, known for their beneficial impacts on growth and health of host plants (Lucy *et al*., 2004; Qin *et al*., 2011; Grönemeyer *et al*., 2012). These bacteria can either directly or indirectly enhance the growth of plants (Glick, 1995). In direct growth stimulation, these bacteria provide soluble phosphate, fix nitrogen,

produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase and phytohormones like indole acetic acid (IAA) and increase the bioavailability of iron through bacterial siderophore for plants (Lucy *et al*., 2004; Glick *et al*., 2007a). Indirect promotion of growth occurs when these bacteria protect plants from detrimental effects of plant pathogens, *e.g.* by competition, antibiosis and hyperparasitism (Raaijmakers *et al*., 2009). PGPRs are also capable of increasing plant resistance to biotic and abiotic environmental stresses. One of the main mechanisms by which rhizobacteria exert positive effects on plants under abiotic stresses is production of ACC deaminase and regulation of ACC, a precursor to stress-induced ethylene in host plants (Glick *et al*., 1998; Glick *et al*., 2007a,b). The effectiveness of rhizobacteria with ACC deaminase activity has been reported in reducing stress ethylene, conferring beneficial effects to plants under various environmental stressors such as pathogen and insect infection, high salinity, drought and heavy metal contamination (Mayak *et al*., 2004; Yildirim *et al*., 2006; Glick *et al*., 2007a,b; Qin *et al*., 2014).

Previous research has revealed that inoculation with PGPR can alleviate the salinity stress effects in several plant species. PGPR inoculants examined on wheat, maize, tomato as well as cotton under salt stress has shown to ameliorate deleterious effects of salt stress (Mayak *et al*., 2004; Egamberdieva, 2007, 2009; Yao *et al*., 2010).

Considering the importance of salinity stress in Iran, the objectives of the present study were: (1) to study four native *Pseudomonas fluorescens* strains isolated from barley rhizosphere in Iran for ACC deaminase activity and tolerance to NaCl induced salinity under *in vitro* conditions, (2) to evaluate other bacterial plant growth-promoting activities including production of siderophore and indole acetic acid (IAA) and solubilization of phosphate, and (3) to determine the potential of PGPR strains to enhance growth and yield of five barley cultivars under different salinity levels in natural soil by conducting pot experiments.

Material and methods

Microorganisms, plants, and culture conditions

Four *Pseudomonas fluorescens* strains (B10, B2-10, B2-11 and B4-6) isolated from the rhizosphere of barley plants in Bushehr province, Iran, were obtained from a culture collection of Biological Control Lab., Persian Gulf University, Iran. Bacteria were routinely cultivated at 27°C on King's B medium agar (KB) (peptone 20 g, K_2HPO_4 1.5 g, $MgSO_4$-7 H_2O 1.5 g, glycerol 20 g, and agar 15 g, pH 7.0) (King *et al*., 1954) and Luria-Bertani broth (LB; Difco, Detroit, MI, USA). Glycerol stock (20% w/v) of bacterial strains was prepared and stored at -80°C until further use. Seeds of barley (*Hordeum vulgare* L.) cvs. 'Desert', 'Mid-day', 'Zehak', 'South' and 'Karun', obtained from the Seed and Plant Improvement Institute (Karaj, Iran), were surface-disinfected in ethanol 70% (v/v), followed by 10 min in 10% (v/v) H_2O_2, rinsed thoroughly with sterile double-distilled water, and pre-germinated for three days on 0.85% water agar at 25°C in darkness.

ACC deaminase activity assay

The ability of bacterial strains to utilize ACC was evaluated based on Dell'Amico *et al*. (2005) with slight modifications. Bacteria were grown on tryptic soy broth medium (TSB) (TSB, Merck) for 48 hours. Thereafter, 50 µL of bacterial suspension was transferred to 20 mL of DF (Dworkin and Faster) minimal medium (Dworkin & Faster, 1958) containing 3 mM L^{-1} ACC as the selective medium, DF containing 2 g L^{-1} $(NH4)_2SO_4$ as positive control and DF minimal medium with no amendments (as negative control). After 48 h incubation at 27 ºC in shaker incubator with 120 rpm, an optical density at 405 nm was determined using spectrophotometer for each suspension. The ability of a strain to utilize ACC was verified by comparing the optical density (OD) of each bacterial suspension with that of negative control. The absence of growth in negative control confirmed the utilization of ACC as N source.

Salt tolerance assay

Salt tolerance test was conducted based on Qin *et al*. (2014) with slight modifications. Bacteria were grown on modified mineral-based nutrient agar ($CaSO_4$ 0.1 g, K_2HPO_4 0.2 g, peptone 1 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, agar 15 g in 1000 mL distilled water) amended with increasing concentrations of NaCl (0–10%, w/v) at intervals of 1% at 27 °C.

Determination of other plant growth-promoting traits

Isolates were screened for siderophore and IAA production and solubilization of phosphate based on Islam *et al*. (2009). Siderophores were detected on Chrome Azural S (CAS) agar plates by the formation of orange halos around bacterial colonies after incubation for 24 h at 27 °C. P-solubilization activity was tested on Pikovaskaya's agar medium containing 2.5% tricalcium phosphate. The formation of transparent halo zone around bacterial colonies after incubation for 24-48

h at 28 °C was observed. Ability to produce IAA was detected using the colorimetric method of Bric *et al*. (1991). The IAA secreted in the culture was determined using a calibration curve of pure IAA (Sigma-Aldrich, St. Louis, MO, USA) as a standard following the regression analysis. IAA production assay was conducted in a completely randomized design with three replications. The experimental data were analyzed statistically using SAS program and means were separated by Duncan's Multiple Range Test (DMRT).

Plant growth-promotion activity of *Pseudomonas* strains under salinity stress

The impacts of four effective *Pseudomonas* strains were evaluated on barley yield and growth under greenhouse conditions. Bacterial strains were grown in LB broth overnight. Cultures were washed with sterile 0.9% NaCl solution and adjusted to an OD at 600 nm of 0.125 that corresponds to cell density of about 10^8 colony forming units (CFU) mL^{-1}. Before performing greenhouse experiment, root tip colonization of bacterial strains on barley cultivars under salinity (50, 100 and 150 mM NaCl) was assessed under gnotobiotic conditions as described by Egamberdieva (2011) with some modifications. It was revealed that our bacterial strains were capable of colonizing the rhizosphere of barley cultivars at the highest saline conditions (150 mM) (unpublished data).

For greenhouse experiment, after soaking pre-germinated barley seeds in the bacterial suspensions for 1 h, 25 seeds were transferred to plastic pots filled with 8 kg of sterilized soil. Soil sample for the study was collected from the experimental field at Persian Gulf University, Bushehr, Iran, air-dried, sieved (2 mm, 10 mesh) and analyzed for physico-chemical characteristics. The soil was clay loam having pH 6.2, organic matter 1.15%, total nitrogen 0.1%, total P 18 mg kg^{-1}, and electrical conductivity 5.45 mS m^{-1}.

Plants were watered when needed after emergence, and no fertilizers were used. Salinity conditions at four-leaf stage were established by adding 50, 100 and 150 mM NaCl into the irrigation water (sterile distilled water). Electrical conductivity (EC) of these solutions was 5 dS m^{-1} for 50 mM NaCl, 10 dS m^{-1} for 100 mM NaCl and 15 dS m^{-1} for 150 mM NaCl. Sterile distilled water with no addition of NaCl was considered as non-stressed controls. Plants were grown in the greenhouse at 25ºC and at a 16/8 day/night regime. After 15 days, thinning was done to leave 15 uniform seedlings in each pot. Morphological parameters like plant height, peduncle length, spike length, spike weight, number of grains per spike, 1000-grain weight (MGW) and grain yield were measured at physiological maturity stage.

Survival of bacterial strains in barley rhizosphere

The effect of salinity levels on the survival of inoculated strains in the rhizosphere of barley cultivars was determined under greenhouse conditions which were described above.

At harvest, plants were removed from the pots and gently shaken to discard loosely adhering soil. Roots with tightly adhering soil were weighted, placed in 10 mL sterile saline solution (0.9%), and shaken for 20 min at 420 rpm. The number of bacteria in the resulting suspensions was determined as CFU by plating serial dilutions on KB agar plates.

Experimental setups and statistical analyses

A pot experiment was performed in a split-split plot design based on randomized complete block with three replicate pots per treatment. Main plots, subplots and sub-sub plots were consisted of salinity (at three levels of 50, 100 and 150 mM), five varieties ('Karun', 'Zehak', 'Mid-day', 'Desert' and 'South'') and four bacterial strains, respectively. In the greenhouse experiment, controls were consisted of plants either not watered with NaCl solutions or not inoculated with bacterial strains. Each experimental unit was a pot consisted of 15 plants. Data were subjected to statistical analysis using SAS 9.1 (SAS Inst., Inc., Cary, NY, USA). Means were subsequently compared using the DMRT.

Results

ACC deaminase production

All selected strains from the rhizosphere of barley were able to grow on DF minimal medium amended with ACC, indicating that they have the ACC deaminase activities. Optical densities of *Pseudomonas* suspensions in minimal medium without nitrogen source (negative control), amended with 2 g of $(NH_4)_2SO_4$ L^{-1} (positive control), or with ACC (3 mM L^{-1}) are listed in Table 1.

Characterization of PGP properties of the bacteria and their tolerance to NaCl

The bacterial strains were screened for their plant growth-promoting (PGP) traits and the results are summarized in Table 2. All the four isolates produced IAA and siderophores and also had phosphate solubilizing activity. The NaCl tolerance of bacterial strains was tested, and the results revealed that all strains could tolerate 8% of NaCl stress after which

Table 1. ACC deaminase activity of *Pseudomonas* strains.

Isolate	Optical density[a]		
	DF+$(NH_4)_2SO_4$[b]	DF+ACC[c]	DF[d]
B10	3.048±0.021	1.673±0.039	0.272±0.018
B2-10	2.962±0.038	2.051±0.02	0.353±0.024
B2-11	2.907±0.055	2.156±0.028	0.289±0.039
B4-6	3.049±0.024	1.828±0.01	0.334±0.022

[a]Optical density (405 nm) of bacterial strains. [b]DF minimal medium amended with ammonium sulphate as a positive control. [c]DF minimal medium amended with 1-aminocyclopropane-1-carboxylate as a selective medium. [d]DF minimal medium as a negative control (DF). Values are means ± SE.

no growth was observed. Results also showed that as the salt concentration increased, the bacterial growth decreased. Since the four strains showed high tolerance to 8% NaCl, they were selected to study their effects on barley yield and growth under saline stress.

Influence of PGP strains on barley yield and growth under saline stress

Data analysis of variance showed that the main effects of salinity, variety and bacterial strains and the interaction between them were significant on most measured traits ($p \leq 0.05$) (Table 3). Generally, as salinity increased, all measured traits decreased; however, inoculation of seeds with bacterial strains ameliorated salinity stress and resulted in an increase in barley growth parameters and yield comparing to non-inoculated control.

Under normal condition and salinity of 50 mM, isolate B2-10 (with 10% increase), at 100 mM NaCl stress, all bacterial strains and at 150 mM salinity B4-6 and B2-10 elevated plant height in comparison with other treatments (Table 4). Data regarding peduncle length and spike length showed that treatment with B2-10 strain significantly promoted these traits by 26-46% and 26-30%, respectively, under normal condition and salt stress (Table 4). Similarly, inoculation with B2-10 strain caused the higher number of grains per spike (with 12-14% increase) under all salinity levels. Under normal condition and at 50 and 150 mM, B2-10 strain increased spike number (20, 19 and 12%, respectively) significantly over untreated control; however, at 100 mM, B10 and B4-6 strains performed better (Table 4). B2-10 and B4-6 were the most effective isolates which enhanced MGW up to about 14%. The maximum spike weight was obtained under normal condition following inoculation of seeds with B2-10 strain comparing to other treatments. Under normal condition and all salinity levels, B2-10 strain had the maximum impact on increasing grain yield, with no significant difference to B2-11 under normal condition and 100 mM salinity (Table 4).

Barley varieties showed variations in plant height during NaCl induced salinity stress (Table 5). Under all conditions, 'Karun' showed the maximum plant height comparing to other varieties. 'South' showed the maximum spike length and peduncle length under all salinity levels. 'Desert' had the highest spike number at all salinity levels which was not significantly different to 'Mid-day' at 50 mM. 'Mid-day' had the highest number of grains per spike at all salinity levels comparing to other varieties. In all salinity levels, 'Zehak' had the highest MGW, which was not significantly different to 'Karun' and 'Mid-day' varieties at 50 and 100 mM salinity. Considering spike number, under normal condition, 'Karun' had the highest spike number which was not significantly different to 'Desert'. Under salinity levels of 50, 100 and 150 mM, 'Desert' showed the maximum number of spikes, which was not significantly different to 'Mid-day'. Considering grain yield, under normal condition and salinity of 50 and 150 mM, 'Desert' had the highest grain yield. Under salinity level of 150 mM, 'Mid-day' showed the maximum grain yield, which was not significantly different to 'Desert' and 'Karun' varieties (Table 5).

Inoculation of barley varieties with tested PGPR strains caused an increase in different measured traits

Table 2. Plant growth promotion traits of *Pseudomonas* strains isolated from barley rhizosphere.

Isolate	Phosphate solubilization	IAA (mgL^{-1})	Siderophore	Salt tolerance range%
B10	+	1c	+	0-8
B2-10	+	2.4ab	+	0-8
B2-11	+	1.76b	+	0-8
B4-6	+	2.42a	+	0-8

Considering IAA production, means with the same letters are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Table 3. Analysis of variance of impacts of bacterial strains on barley yield and yield components under different salinity levels.

Source changes	df	Plant height (cm)	Peduncle length (cm)	Spike length (cm)	Spikes number	Number of grains/ spike	1000-grain weight (g)	Spike weight (g)	Grain yield (g)	Rhizosphere colonization ($\times 10^7$ CFU g^{-1} rhizosphere)
Block	2	160.81**	48.11**	17.15**	175.57**	181.64**	58.95**	5.74**	0.042*	2.35*
Salinity	3	718.68**	104.7**	27.23**	403.31**	654.18**	185.87**	0.82**	0.71**	1348.4**
Error 1	6	38.43	13.9	0.1	1.28	7.52	3.65	0.033	0.12	1.33
Variety	4	2971.05**	9.13**	28.94**	153.21**	4506.1**	284.84**	0.21**	0.27**	47.8 **
Salinity × Variety	12	4.36ns	72.67ns	0.067*	1.44*	2.69ns	0.4ns	0.006ns	0.015ns	2.53**
Error 2	32	1.04	0.083	0.009	0.2	1.76	0.81	0.003	0.016	0.6
Bacteria	4	164.70**	136.65**	18.52**	72.48**	236.21**	122.02**	0.23**	0.19**	600.5**
Salinity × Bacteria	12	9.48**	5.02**	0.19**	1.53*	5.41**	3.48**	0.022*	0.007ns	86.3**
Variety × Bacteria	16	16.53**	6.16**	1.84**	6.48**	23.42**	85.86**	0.25**	0.067**	5.19**
Salinity × Variety × Bacteria	48	1.70ns	0.32ns	0.8**	0.93ns	4.78**	0.5ns	0.005ns	0.018**	0.71**
Error 3	160	3.32	1.24	0.04	0.82	2.51	1.31	0.01	0.009	0.87
Coefficient of variation		3.54	1.33	2.93	3.89	3.20	3.28	4.27	4.78	14.8

ns, *, **: non-significant, significant at 5% and 1% level of probability, respectively.

comparing to uninoculated control; however, varieties did not show identical response to inoculation with different strains (Table 6). Inoculation of 'Karun' with B2-11 resulted in the maximum plant height as compared to other treatments. Seed inoculation of 'Zehak' with B2-10 (with 46% increase), and 'South' with B4-6 and B2-10 (with 27 and 25.6% increase, respectively) increased peduncle length more significantly than the other treatments. The maximum spike length was observed following the inoculation of 'South' with B2-10, which was not significantly different to the treatment of 'Desert' with B4-6 strain. Inoculation of 'Mid-day' and 'Desert' with B2-10 resulted in the highest spike number as compared to other treatment. The highest increase in the number of grain per spike was observed in 'Mid-day' following seed inoculation with B2-11 strain. Inoculation of 'Zehak' with B10 caused the maximum MGW, which was significantly different to all treatments. The maximum increase in spike weight was seen in 'Mid-day' and 'Karun' after seed inoculation with B2-10 strain. Inoculation of 'Karun' with B2-10, B4-6 and B10 strains, 'Zehak', 'Mid-day' and 'South' with B2-10 and B2-11 and 'Desert' with B2-10, B2-11 and B4-6 caused the highest increase in grain yield of barley cultivars as compared to other treatments (Table 6).

Survival of bacterial strains in the barley rhizosphere

The survival of bacterial strains in the rhizosphere of five barley cultivars grown in different salinity levels was determined under greenhouse conditions. Analysis of variance revealed that the main effects of salinity, variety and bacterial strains and the interaction between them were significant on rhizosphere colonization ($p \leq 0.05$) (Table 3). All four bacterial strains were able to colonize and survive in the rhizosphere of barley cultivars. However, their colonization level was partly inhibited under salt stress. Under normal condition isolate B2-10, at 50 mM, isolates B2-10 and B2-11, at 100 mM NaCl stress, isolate B2-11 and at 150 mM salinity B4-6, B2-11 and B2-10 had higher rhizosphere colonization in comparison to other treatments (Table 4). Barley varieties showed variations in rhizosphere colonization by bacterial strains during NaCl induced salinity stress (Table 5). Under normal conditions, 'Mid-day' at 50 mM, 'South' at 100 mM and 150 mM all barley varieties, except 'Zehak', showed higher rhizosphere colonization. Barley genotype at the cultivar level had significant impact on rhizosphere colonization by bacterial strains under greenhouse conditions (Table 6). In 'Karun' the rhizosphere colonization by B2-10, and in 'Mid-day' the colonization by B2-11 were higher than by other strains. In 'Zehak', bacterial strains were not significantly different regarding rhizosphere colonization. In 'Desert' and 'South', B2-11 strain colonized the rhizosphere better than the others; however, the colonization level was not significantly different to B2-10 and B4-6 (in 'Desert') and B2-10 (in 'South').

Discussion

This study demonstrates the efficacy of *P. fluorescens* strains containing ACC deaminase activity for inducing

Table 4. Mean comparison of barley yield and yield components under salt stress with or without bacterial inoculations.

Salinity levels (mM)	Bacteria	Plant height (cm)	Peduncle length (cm)	Spike length (cm)	Spike number
Control	Control	71.5±1.78^{f}	13.81±0.27^{j}	5.98±0.21^{hi}	23.4±0.44^{e}
	B10	76.27±1.5^{c}	17.02±0.62^{b}	7.36±0.25^{b}	26.5±0.69^{b}
	B4-6	74.54±1.81^{d}	16.28±0.41^{d}	7.39±0.21^{b}	25.6±0.57^{c}
	B2-10	78.49±2.13^{a}	20.19±1.1^{a}	7.79±0.26^{a}	28.2±0.77^{a}
	B2-11	77.51±1.93^{b}	17.11±0.25^{b}	7.29±0.22^{b}	25.7±0.57^{c}
50	Control	69.59±1.74^{h}	13.19±0.25^{k}	5.65±0.2^{j}	22.1±0.49^{f}
	B10	72.46±1.59^{e}	14.78±0.38^{h}	6.77±0.24^{d}	24.3±0.39^{d}
	B4-6	72.89±1.76^{e}	15.49±0.41^{f}	7.11±0.22^{c}	24.1±0.48^{d}
	B2-10	73.11±1.89^{e}	16.85±0.37^{c}	6.78±0.2^{d}	26.3±0.9^{b}
	B2-11	72.93±1.7^{e}	16.31±0.24^{d}	6.6±0.18^{e}	24±0.53^{d}
100	Control	67.46±1.85^{i}	12.51±0.24^{l}	5.2±0.19^{l}	20.5±0.39^{h}
	B10	70.85±1.57f^{g}	14.34±0.37^{i}	6.26±0.22^{g}	22.3±0.39^{f}
	B4-6	70.49±1.79^{g}	14.75±0.45^{h}	6.67±0.22^{de}	22.3±0.46^{f}
	B2-10	70.36±1.87^{g}	15.65±0.32^{e}	6.43±0.2^{f}	23.3±0.69^{e}
	B2-11	70.89±1.81f^{g}	15.83±0.24^{e}	6.08±0.2^{h}	22.6±0.54^{f}
150	Control	65.31±1.77^{j}	11.85±0.27^{m}	4.84±0.19^{m}	18.8±0.4^{i}
	B10	67.45±1.58^{i}	13.01±0.38^{k}	5.63±0.24^{j}	20.5±0.38^{h}
	B4-6	69.13±1.89^{h}	14.15±0.47^{i}	6.2±0.23^{g}	20.5±0.42^{h}
	B2-10	68.93±1.92^{h}	15.11±0.35^{g}	5.91±0.18^{i}	21.1±0.55^{g}
	B2-11	68.15±1.74^{i}	14.27±0.27^{i}	5.4±0.17^{k}	20.7±0.62^{gh}

Table 4. Continued.

Salinity levels (mM)	Number of grains/spike	1000-grain weight (g)	Spike weight (g)	Grain yield (g)	Rhizosphere colonization ($\times 10^7$ CFU g^{-1} rhizosphere)
Control	46.07±2.08^{d}	33.94±1.09^{de}	2.43±0.05^{c}	2.02±0.09^{b}	ND
	49.8±2.33^{b}	37.56±0.77^{ab}	2.63±0.08^{b}	2.09±0.03^{b}	13.55±0.2^{c}
	49±1.94b^{c}	36.9±0.72^{b}	2.52±0.05^{b}	2.08±0.03^{b}	13.87±0.25^{c}
	52.8±2.22^{a}	38.67±1.03^{a}	2.74±0.1^{a}	2.17±0.03^{a}	15.89±0.42^{a}
	49.8±2.47^{b}	37.12±0.8^{b}	2.68±0.09^{ab}	2.14±0.06^{ab}	15.17±0.38^{b}
50	43.93±2.09^{e}	32.83±1.1^{ef}	2.35±0.05^{e}	1.91±0.05^{c}	ND
	46.07±2.21^{d}	35.59±0.72^{ef}	2.47±0.05^{b}	2.01±0.03^{b}	6.05±0.24^{e}
	46.53±2.03^{d}	36.29±0.62^{b}	2.46±0.05^{bc}	2.03±0.02^{b}	6.39±0.18^{e}
	49.4±2.04^{b}	35.79±0.87^{bc}	2.49±0.05^{b}	2.05±0.02^{b}	6.64±0.23^{d}
	48±2.28^{c}	35.73±0.68^{c}	2.44±0.05^{c}	2.01±0.02^{b}	7.06±0.2^{d}
100	41.47±2^{g}	31.82±1.08^{f}	2.3±0.05^{ef}	1.88±0.02^{cd}	ND
	43.73±2.18^{ef}	34.66±0.71^{d}	2.4±0.05^{cd}	1.94±0.04^{c}	3.16i±0.04^{i}
	44.53±2.03^{e}	35.28±0.65^{c}	2.43±0.05^{c}	1.94±0.03^{c}	3.65±0.06^{gh}
	46.4±2.04^{d}	35.09±0.81^{c}	2.42±0.05^{c}	2.03±0.02^{b}	3.82±0.09^{g}
	46.2±2.37^{d}	34.9±0.71^{cd}	2.38±0.05^{d}	1.96±0.02^{bc}	4.28±0.09^{f}
150	39.47±1.84^{h}	30.8±1.05^{f}	2.23±0.05^{f}	1.79±0.04^{e}	ND
	40.8±2.22^{g}	33.69±0.75^{e}	2.34±0.05^{e}	1.84±0.05^{c}	2.59±0.03^{j}
	42.43±2.13^{f}	34.25±0.67^{d}	2.38±0.05^{d}	1.84±0.03^{c}	3.17±0.04^{i}
	43.8±2.17$_{ef}$	33.14±0.85^{e}	2.38±0.05^{d}	1.97±0.03^{bc}	3.29±0.07^{hi}
	44.3±2.28$_{e}$	33.59±0.69^{e}	2.34±0.06^{e}	1.91±0.03^{c}	3.31±0.07^{hi}

Means followed by the same letters in columns are not significantly different according to DMRT ($p \leq 0.05$). Values are means ± SE (n = 15). ND: not detected.

Table 5. Effect of salinity levels on growth and yield of five barley cultivars.

Salinity levels (mM)	Variety	Plant height (cm)	Peduncle length (cm)	Spike length (cm)	Spike number
Control	Karun	83.34±0.93^{a}	17.2±0.72^{b}	6.43±0.24^{g}	23.47±0.67^{fg}
	Zehak	75.02±0.97^{e}	16.45±0.84^{d}	7.31±0.23^{c}	25.13±0.41^{d}
	Desert	75.6±1.35^{e}	15.01±0.63^{hi}	7.46±0.2^{b}	28.13±0.59^{a}
	Mid-day	78.78±0.92^{c}	17.27±0.67^{b}	6.57±0.21^{f}	27.27±0.52^{b}
	South	65.56±1.59^{j}	18.46±0.82^{a}	8.03±0.18^{a}	25.33±0.63^{d}
50	Karun	80.64±0.48^{b}	15.47±0.38^{f}	5.92±0.19^{ij}	22.6±0.35^{i}
	Zehak	72.03±0.37^{g}	15.3±0.56^{fg}	6.71±0.18^{e}	23.27±0.41^{gh}
	Desert	71.43±0.59^{g}	13.87±0.38^{l}	6.98±0.19^{d}	26.2±0.66^{c}
	Mid-day	75.6±0.51^{e}	15.18±0.29^{gh}	5.82±0.17^{j}	25.87±0.53^{c}
	South	61.26±0.48^{k}	16.77±0.42^{c}	7.48±0.14^{b}	22.93±0.51^{hi}
100	Karun	78.7±0.5^{c}	14.53±0.41^{jk}	5.46±0.18^{k}	20.53±0.31^{k}
	Zehak	69.23±0.44^{h}	14.68±0.51^{j}	6.29±0.2^{h}	21.4±0.41^{j}
	Desert	69.5±0.66^{h}	13.21±0.31^{m}	6.36±0.23^{gh}	24.13±0.47^{e}
	Mid-day	73.72±0.32^{f}	14.47±0.25^{k}	5.48±0.16^{k}	23.73±0.43^{ef}
	South	58.9±0.48^{l}	16.17±0.38^{e}	7.06±0.16^{d}	21.33±0.47^{j}
150	Karun	76.81±0.56^{d}	13.73±0.43^{l}	5.03±0.17^{l}	18.47±0.38^{n}
	Zehak	66.62±0.45^{i}	13.26±0.51^{m}	5.51±0.18^{k}	19.13±0.37^{m}
	Desert	67.3±0.61^{i}	12.63±0.3^{n}	5.99±0.23^{i}	22.6±0.39^{i}
	Mid-day	72.1±0.37^{g}	13.81±0.29^{l}	5.1±0.15^{l}	21.53±0.32^{j}
	South	56.11±0.44^{m}	14.94±0.41^{i}	6.35±0.14^{gh}	19.8±0.47^{l}

Table 5. Continued.

Salinity levels (mM)	Number of grains/spike	1000-grain weight (g)	Spike weight (g)	Grain yield (g)	Rhizosphere colonization (×10^{7} CFU g^{-1} rhizosphere)
Control	49.47±1.29^{e}	37.97±0.67^{b}	2.66±0.08^{a}	2.2±0.03^{a}	12.22±0.23^{b}
	46.53±0.71^{g}	38.52±0.79^{a}	2.48±0.08^{de}	1.97±0.06^{de}	9.30 ±0.4^{c}
	47.6±1.49^{f}	37.03±0.7^{c}	2.65±0.08^{ab}	2.08±0.02^{bc}	12.23 ±0.23^{b}
	63.47±0.69^{a}	37.54±1.03^{b}	2.61±0.07^{b}	2.13±0.02^{b}	12.69±0.37^{a}
	40.4±0.95^{j}	33.13±0.97^{j}	2.57±0.09^{c}	2.13±0.09^{b}	12.03±0.2^{b}
50	46.47±0.72^{g}	36.41±0.33^{d}	2.45±0.05^{ef}	2.09±0.02^{bc}	5.39±0.18^{e}
	43.4±0.42^{h}	36.53±0.97^{cd}	2.33±0.05^{jk}	1.93±0.05^{ef}	3.43±0.15^{f}
	45.73±0.66^{g}	35.6±0.51^{f}	2.51±0.05^{d}	2±0.02^{de}	5.60±0.23^{e}
	61.07±0.6^{b}	36.27±0.79^{de}	2.48±0.05^{de}	2.04±0.02^{cd}	5.59±0.23^{e}
	37.27±0.69^{k}	31.41±0.77^{k}	2.41±0.05^{gh}	1.95±0.02^{g}	6.11±0.14^{d}
100	43.6±0.68^{h}	35.58±0.37^{f}	2.38±0.06^{hi}	1.98±0.02^{de}	3.29±0.2^{f}
	40.93±0.34^{ij}	35.77±0.89^{ef}	2.28±0.05^{l}	1.86±0.04^{g}	1.81±0.03^{i}
	43.6±0.57^{h}	34.66±0.54^{gh}	2.45±0.05^{fg}	1.97±0.03^{de}	3.41±0.24^{f}
	58.93±0.79^{c}	35.32±0.76^{f}	2.43±0.05^{fg}	1.99±0.02^{de}	3.30±0.2^{f}
	35.27±0.63^{l}	30.42±0.77^{l}	2.37±0.05^{ij}	1.94±0.02^{e}	3.09±0.2^{fg}
150	41.13±0.64^{ij}	33.97±0.4^{i}	2.34±0.06^{jk}	2±0.02^{de}	2.69±0.09^{gh}
	38.07±0.33^{k}	34.76±1.01^{g}	2.21±0.05^{m}	1.77±0.04^{h}	1.46±0.03^{i}
	41.6±0.54^{i}	33.25±0.54^{j}	2.41±0.05^{hi}	1.87±0.03^{fg}	2.87±0.26^{gh}
	57.2±0.86^{d}	34.18±0.69^{hi}	2.39±0.05^{hi}	1.85±0.04^{fg}	2.76±0.22^{gh}
	33.13±0.6^{lm}	29.32±0.72^{m}	2.30±0.05^{kl}	1.85±0.03^{g}	2.58±0.1^{h}

Means followed by the same letters in columns are not significantly different according to DMRT ($p \leq 0.05$). Values are means ± SE (n = 15).

salt tolerance and consequently improving the growth of barley plants under salinity stress conditions. ACC deaminase activity has been found more widely present in soil bacteria belonging to several species of *Pseudomonas* and to the genera *Alcaligenes*, *Bacillus*, *Variovorax* and *Rhodococcus* (Belimov *et al*., 2005; Bal *et al*., 2013; Akhgar *et al*., 2014). Root associated bacteria possessing ACC deaminase activity assist plants to withstand biotic and abiotic stresses by decreasing the level of stress ethylene (Mayak *et al*., 2004; Dimkpa *et al*., 2009).

The four strains were also screened for their tolerance to increasing levels of NaCl. All the bacterial strains could tolerate up to 8% (w/v) NaCl concentrations on modified mineral-based NA plates. Besides, while the NaCl concentration increased, the growth of bacteria was noticed to decrease. Increase in salt concentration outside cell membrane enhances osmotic potential which is suggested to be one of the main reasons for reduced growth of PGPRs (Tank & Saraf, 2010).

The ACC deaminase producing isolates were also screened for multiple PGP traits, including solubilization of phosphate and production of IAA and siderophore. *In vitro* screening for characteristics commonly associated with plant growth promotion revealed that all bacterial strains were able to produce IAA in a range of 1-3.4 mg L^{-1}, indicating variability among barley isolates for IAA production. The ability of *Pseudomonas* strains to produce IAA indicates their potential to use as growth hormones or growth regulators. In addition to other factors positively influence plant growth, biosynthesis of IAA by bacterial strains is suggested as a principal means of attaining growth enhancement (Deepa *et al*., 2010). IAA plays major roles in root initiation and cell division and growth; it increases surface area of roots, and access to soil nutrients by enhanced root formation (Gray & Smith, 2005). These results suggest that the selected strains can be beneficial in enhancing growth of barley and other host plants by providing nutrients like iron

Table 6. Mean comparison of yield and yield components of barley varieties inoculated with different *Pseudomonas* strains.

Variety	Bacteria	Plant height (cm)	Peduncle length (cm)	Spike length (cm)	Spike number
Karun	Control	76.49±0.72^{e}	12.55±0.37^{n}	4.53±0.15^{o}	19.42±0.51^{h}
	B10	79.52±0.94^{c}	15.4±0.63f^{g}	6.37±0.25^{hi}	22.08±0.58^{ef}
	B4-6	79.85±0.47^{c}	15.07±0.4^{hi}	6.13±0.18^{j}	21.5±0.56^{f}
	B2-10	81.11±0.83^{b}	17.1±0.77^{b}	5.98±0.18^{j}	22.58±1.11^{e}
	B2-11	82.39±1.11^{a}	16.05±0.25^{cd}	5.57±0.19^{l}	20.75±0.63^{fg}
Zehak	Control	68.65±0.79^{l}	12.3±0.23^{n}	5.34±0.17^{m}	20.58±0.76^{g}
	B10	71.11±0.93^{jk}	13.93±0.3^{l}	6.63±0.23^{g}	23.67±0.72^{de}
	B4-6	72.16±0.62^{hi}	14.8±0.25^{gk}	7.16±0.16^{e}	22.25±0.6^{e}
	B2-10	70.6±1.35^{k}	18.01±0.76^{a}	6.85±0.29^{f}	23.33±0.81^{de}
	B2-11	71.09±1.29^{jk}	15.59±0.24^{ef}	6.3±0.18^{i}	21.33±0.78^{f}
Desert	Control	66.72±0.81^{m}	11.87±0.23^{o}	5.8±0.17^{k}	23.25±0.63^{de}
	B10	73.04±1.01^{h}	13.27±0.45^{m}	7.3±0.2^{de}	24.58±0.86^{c}
	B4-6	71.6±0.85^{jk}	13.37±0.23^{m}	7.65±0.15^{ab}	26±0.72^{b}
	B2-10	71.97±1.55^{ij}	14.95±0.73^{ij}	6.62±0.24^{g}	26.75±1.09^{ab}
	B2-11	71.45±1.42^{jk}	14.93±0.23^{ij}	6.13±0.26^{j}	25.75±0.55^{b}
Mid-day	Control	72.97±0.5^{h}	13.33±0.25^{m}	4.93±0.13^{n}	22.75±0.63^{e}
	B10	74.33±0.61^{g}	15.24±0.48^{gh}	5.25±0.22^{m}	25±0.78^{c}
	B4-6	75.47±0.83^{f}	14.63±0.37^{k}	5.75±0.16^{k}	23.75±0.63^{de}
	B2-10	78.24±1.22^{d}	16.91±0.79^{b}	6.44±0.19^{hi}	27±0.91^{a}
	B2-11	74.22±0.77^{g}	15.8±0.31^{de}	6.35±0.17^{hi}	24±0.56^{c}
South	Control	57.47±0.65^{q}	14.13±0.27^{l}	6.5±0.16^{gh}	20.08±0.62^{g}
	B10	60.76±1.31^{o}	16.08±0.52^{c}	6.99±0.18^{f}	21.67±0.78^{f}
	B4-6	59.72±0.65^{p}	17.95±0.22^{a}	7.53±0.16^{bc}	22.25±0.63^{e}
	B2-10	61.65±1.8^{o}	17.75±1^{ab}	7.76±0.23^{a}	23.92±0.92^{cd}
	B2-11	62.68±1.33^{n}	17.01±0.25^{b}	7.38±0.24^{cd}	23.83±0.61^{d}

Table 6. Continued.

Variety	Number of grains/spike	1000-grain weight (g)	Spike weight (g)	Grain yield (g)	Rhizosphere colonization ($\times 10^7$ CFU g^{-1} rhizosphere)
Karun	41.5±0.76^{l}	34.58±0.49^{fg}	2.31±0.06^{de}	2.01±0.04^{b}	ND
	43.08±0.86^{ij}	35.14±0.54^{ef}	2.54±0.06^{b}	2.08±0.04^{ab}	7.19±0.16^{e}
	45±0.72^{gh}	35.36±0.51^{e}	2.49±0.05^{c}	2.08±0.03^{ab}	6.78 ±0.18^{fg}
	49.25±1.51^{e}	36.94±0.58^{d}	2.58±0.08^{ab}	2.16±0.03^{a}	8.28±0.12^{bc}
	47±1.12^{f}	37.9±0.7^{c}	2.4±0.09^{c}	2.02±0.03^{b}	7.24 ±0.17^{e}
Zehak	40.91±0.78^{lm}	37.78±0.42^{c}	2.23±0.06^{e}	1.65±0.05^{i}	ND
	42.91±1.32^{jk}	39.89±0.55^{a}	2.4±0.07^{c}	1.8±0.06^{d}	4.86±0.13^{h}
	42±0.82^{kl}	38.84±0.37^{b}	2.36±0.06^{d}	1.91±0.04^{c}	5.1 ±0.17^{h}
	44.16±1.08^{hi}	30.96±0.81^{k}	2.37±0.08^{d}	2.03±0.02^{b}	5±0.23^{h}
	41.16±0.87^{l}	34.5±0.4^{fg}	2.31±0.08^{de}	2.02±0.03^{b}	4.99 ±0.13^{h}
Desert	41.75±0.84^{kl}	32.6±0.46^{i}	2.42±0.06^{c}	1.85±0.04^{d}	ND
	44.58±1.31^{h}	34.86±0.48^{fg}	2.5±0.08^{bc}	1.99±0.03^{bc}	6.56 ±0.26^{g}
	46±0.72^{fg}	33.76±0.39^{h}	2.55±0.06^{b}	1.99±0.03^{bc}	7.72±0.23^{d}
	46.75±1.18^{f}	37.72±0.81^{c}	2.51±0.08^{b}	2.04±0.02^{b}	7.91±0.24^{cd}
	44.08±1.34^{hi}	36.74±0.38^{d}	2.56±0.08^{b}	2.03±0.02^{b}	7.95 ±0.18^{cd}
Mid-day	56.25±0.95^{d}	30.89±0.34^{k}	2.38±0.06^{d}	1.94±0.02^{c}	ND
	60.25±1.06^{c}	35.21±0.52^{ef}	2.49±0.08^{c}	2.03±0.03^{b}	6.68±0.28^{fg}
	59.25±0.63^{c}	37.67±0.4^{c}	2.41±0.06^{c}	1.98±0.05^{c}	7.24±0.17^{e}
	61.83±0.81^{b}	38.56±1.01^{b}	2.61±0.07^{a}	2.05±0.04^{b}	7.81±0.23^{d}
	63.25±0.51^{a}	36.81±0.38^{d}	2.53±0.06^{b}	2.01±0.04^{b}	8.70±0.18^{a}
South	33.25±0.63^{p}	25.89±0.4^{l}	2.29±0.06^{e}	1.9±0.09^{cd}	ND
	34.66±0.86^{p}	31.77±0.49^{j}	2.39±0.08^{cd}	1.94±0.03^{c}	6.39±0.23^{g}
	36.25±0.82^{o}	32.76±0.43^{i}	2.44±0.06^{c}	1.97±0.03^{c}	7±0.17^{ef}
	38.5±0.97^{n}	34.19±0.85^{gh}	2.46±0.08^{c}	2±0.02^{b}	8±0.18^{bcd}
	39.91±0.99^{m}	30.74±0.4^{k}	2.51±0.09^{b}	2.03±0.09^{b}	8.39 ±0.18^{ab}

Means followed by the same letters in columns are not significantly different according to DMRT ($p \leq 0.05$). Values are means ± SE (n = 12). ND: not detected.

and phosphorous. IAA production is an important PGPR trait, since this phytohormone allows plant to develop extremely organized root system by which nutrient uptake becomes more efficient.

The variation in the ability of bacterial strains to produce IAA found in the current study was also reported by Qin *et al*. (2014) and Majeed *et al*. (2015). IAA production by bacteria isolated from rhizosphere of different plants such as wheat and rice had already been reported by Cakmakci *et al*. (2007) and Majeed *et al*. (2015).

In the present study, *Pseudomonas* strains revealed as efficient phosphate solubilizers. The beneficial influence of PGPR in maintaining sufficient levels of nutrients mainly the phosphorus in plant productivity was documented earlier by Majeed *et al*. (2015). The phosphate solubilizing bacteria increase the availability of phosphorus to plants through mineralization of organic phosphate and conversion of inorganic phosphate into more accessible forms (Bar-Yosef *et al*., 1999). Solubilization of phosphate is mainly related to the production of microbial metabolites like organic acids which reduces the pH of the culture media (Shahid *et al*., 2012).

Present study revealed that seed treatment with PGPR strains improved barley growth and yield over the non-inoculated seeds (Table 6). Similarly, improvement in growth indices and yields-of different crop plants like rice, maize and wheat in response to inoculation with PGPR were reported earlier by Khalid *et al*. (2004), Gholami *et al*. (2009) and Bal *et al*. (2013).

In pot experiment, it was observed that inoculation of barley seeds with *P. fluorescens* strains significantly increased plant height, peduncle length, spike length, spike number, number of grains per spike, MGW, spike weight and grain yield under different salinity levels.

The results of our current study confirmed the findings of earlier studies performed by other researchers who demonstrated the increased resistance to various stresses in plants treated with ACC deaminase containing PGPR (Mayak *et al*., 2004; Qin *et al*., 2014).

Inoculation of barley seeds with *P. fluorescens* strains under different salinity levels resulted in increase in plant height, yield and yield components in comparison to control plants. Using rhizobacteria with multiple PGP traits is believed to help improve crop productivity on a sustainable basis. All the four ACC deaminase producing strains were tested positive for several PGP traits such as production of IAA and siderophore and solubilization of phosphate. Furthermore, inoculation of plants with ACC deaminase and siderophore producing PGPR helps plants to conquer the effects induced by the environmental stresses as observed in the present study (Dimkpa *et al*., 2009; Bal *et al*., 2013).

In the current study, the tested bacterial isolates had the ability to produce both IAA and ACC deaminase. It is probable that ACC deaminase and IAA stimulate root growth in a coordinated manner (Glick *et al*., 2007b). The collection of specific PGP traits of studied strains suggests that these rhizobacteria are able to improve plant growth by more than one mechanism.

Barley cultivars showed variations in their response to saline conditions; however, salinity reduced growth parameters and yield irrespective of the cultivar. The changes in growth factors were evident at the lowest salinity level and became more pronounced at the highest level of 150 mM salinity. The better performance of a genotype under salt stress conditions appear to be determined mainly by the tolerance of the host plant.

In this study, barley varieties responded differently to inoculation with different rhizobacterial strains. The observed differences in response to inoculation with bacteria might be due to the differences in the amount and/or composition of compounds present in root exudates, which may result in a different level of rhizosphere colonization by introduced strains. Many studies have demonstrated the effect of host plant genotype and root exudates, not only on the production of biologically active substances, but also on rhizosphere colonization of root-associated bacteria (Dazzo *et al*., 2000; Jamali *et al*., 2009). It has been reported that the benefits of bacterization to plant growth can vary with plant genotype and cultural conditions as well as PGPR strains (Nowak, 1998; Khalid *et al*., 2004). In addition, application of PGPR appears to be a useful biological tool in agriculture for alleviation of the negative effects of salinity and improvement of the salt tolerance of crops.

In our study, four *Pseudomonas* strains stimulated growth of barley plants, but the activity of some strains was reduced under salinity conditions. It has been shown that the root colonization and plant growth promoting traits of PGPRs is affected by several biotic and abiotic factors including indigenous soil type, drought, salinity and temperature (Compant *et al*., 2010). Salt tolerant and root colonizing bacteria which are adapted to abiotic stress can survive in such severe conditions and assist plants to tolerate salinity stress (Egamberdieva *et al*., 2016). Egamberdieva *et al*. (2017) reported that *Pseudomonas extremorientalis*, a salt tolerant and root colonizing bacteria could increase growth of tomato plants under saline soil conditions. Salt stress has negative effects on the rhizosphere colonization of introduced bacteria; however, salt tolerant bacteria can survive in the rhizosphere of plants through their persistence and proliferation in semi-arid soils (Paul & Nair, 2008). The bacterial strains used in this study were salt tolerant (up to 8% NaCl) and showed potential root colonizing ability for barley cultivars (unpublished data). In our current study the colonization potential of bacterial strains was not inhibited by salt stress since they were able to colonize barley rhizosphere under salinity conditions. The colonization potential of introduced bacteria is mentioned as a crucial mechanism in the positive effects of introduced bacteria (Lugtenberg *et al*., 2001) and this potential of bacterial strains was not inhibited by salt stress in our study.

Application of bacterial strains with multiple PGP traits is expected to help increase crop productivity on a sustainable basis. The present study concluded that inoculation with the four ACC deaminase producing PGPR caused significant alleviation of salinity stress and thus enhanced the growth of barley cultivars under salinity stress conditions. The presence of various PGP traits in the strains may be the possible reason to protect the plant from the suppressive effects of salinity and consequently induce salinity tolerance in barley plants. However, further study is essential to examine the efficacy of these bacterial strains under field conditions to use them for minimizing salinity stress to the growing plants.

References

Akhgar AR, Arzanlou M, Bakker PAHM, Hamidpour M, 2014. Characterization of 1-aminocyclopropane-1-carboxylate (ACC) deaminase-containing *Pseudomonas* spp. in the rhizosphere of salt-stressed canola. Pedosphere 24: 461-468. https://doi.org/10.1016/S1002-0160(14)60032-1

Bal HB, Nayak L, Das S, Adhya TK, 2013. Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under

salt stress. Plant Soil 366: 93-105. https://doi.org/10.1007/s11104-012-1402-5

Bar-Yosef B, Rogers RD, Wolfram JH, Richman E, 1999. *Pseudomonas cepacia*-mediated rock phosphate solubilization in kaolinite and montmorillonite suspensions. Soil Sci Soc Am J 63: 1703-1708. https://doi.org/10.2136/sssaj1999.6361703x

Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S, Glick BR, 2005. Cadmium-tolerant plant growth-promoting rhizobacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern). Soil Biol Biochem 37: 241-250. https://doi.org/10.1016/j.soilbio.2004.07.033

Bric JM, Bostock RM, Silverstone SE, 1991. Rapid *in situ* assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl Environ Microbiol 57: 535-538.

Cakmakci R, Erat M, Erdogan U, Dönmez MF, 2007. The influence of plant growth-promoting rhizobateria on growth and enzyme activities in wheat and spinach plants. J Plant Nutr Soil Sci 170: 288-295. https://doi.org/10.1002/jpln.200625105

Compant S, Clément C, Sessitsch A, 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42: 669-678. https://doi.org/10.1016/j.soilbio.2009.11.024

Dazzo FB, Yanni YG, Rizk R, de Bruijn FJ, Rademaker J, Squartini A, Corich V, Mateos P, Martínez-Molina E, 2000. Progress in multinational collaborative studies on the beneficial association between *Rhizobium leguminosarum* bv. *trifolii* and rice. In: The quest for nitrogen fixation in rice; Ladha JK, Reddy PM (eds.). pp: 167-189. IRRI, Los Baños, Philippines.

Deepa CK, Dastager SG, Pandey A, 2010. Isolation and characterization of plant growth promoting bacteria from non-rhizospheric soil and their effect on cowpea (*Vigna unguiculata* (L.) Walp.) seedling growth. World J Microbiol Biotechnol 26: 1233-1240. https://doi.org/10.1007/s11274-009-0293-y

Dell'Amico E, Cavalca L, Andreoni V, 2005. Analysis of rhizobacterial communities in perennial Graminaceae from polluted water meadow soil, and screening of metal-resistant, potentially plant growth-promoting bacteria. FEMS Microbiol Ecol 52: 153-162. https://doi.org/10.1016/j.femsec.2004.11.005

Dimkpa C, Weinan T, Asch F, 2009. Plant-rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell Environ 32: 1682-1694. https://doi.org/10.1111/j.1365-3040.2009.02028.x

Dworkin F, Foster J, 1958. Experiments with some microorganisms which utilize ethane and hydrogen. J Bacteriol 7: 592-601.

Egamberdieva D, 2007. The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Appl Soil Ecol 36: 184-189. https://doi.org/10.1016/j.apsoil.2007.02.005

Egamberdieva D, 2009. Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. Acta Physiol Plant 31: 861-864. https://doi.org/10.1007/s11738-009-0297-0

Egamberdieva D, 2011. Survival of *Pseudomonas extremorientalis* TSAU20 and *P. chlororaphis* TSAU13 in the rhizosphere of common bean (*Phaseolus vulgaris*) under saline conditions. Plant Soil Environ 57: 122-127. https://doi.org/10.17221/316/2010-PSE

Egamberdieva D, Li L, Lindström K, Räsänen L, 2016. A synergistic interaction between salt tolerant *Pseudomonas* and *Mezorhizobium* strains improves growth and symbiotic performance of liquorice (*Glycyrrhiza uralensis* Fish.) under salt stress. Appl Microbiol Biotechnol 100: 2829-2841. https://doi.org/10.1007/s00253-015-7147-3

Egamberdieva D, Davranov K, Wirth S, Hashem A, Abd Allah EF, 2017. impact of soil salinity on the plant-growth-promoting and biological control abilities of root associated bacteria. Saudi J Biol Sci 24: 1601-1608. https://doi.org/10.1016/j.sjbs.2017.07.004

Gholami A, Shahsavand S, Nezarat S, 2009. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. World Acad Sci Eng Technol 49: 19-24.

Glick BR, 1995. The enhancement of plant growth by free living bacteria. Can J Microbiol 41: 109-117. https://doi.org/10.1139/m95-015

Glick BR, Penrose DM, Li J, 1998. A model for the lowering of plant ethylene concentration by plant growth-promoting bacteria. J Theor Biol 190: 63-68. https://doi.org/10.1006/jtbi.1997.0532

Glick BR, Cheng Z, Czarny J, Duan J, 2007a. Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur J Plant Pathol 119: 329-339. https://doi.org/10.1007/s10658-007-9162-4

Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B, 2007b. Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26: 227-242. https://doi.org/10.1080/07352680701572966

Gray EJ, Smith DL, 2005. Intracellular and extracellular PGRP: Commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem 37: 395-412. https://doi.org/10.1016/j.soilbio.2004.08.030

Grönemeyer JL, Burbano CS, Hurek T, Reinhold-Hurek B, 2012. Isolation and characterization of root-associated bacteria from agricultural crops in the Kavango region of Namibia. Plant Soil 356: 67-82. https://doi.org/10.1007/s11104-011-0798-7

Hu YC, Schmidhalter U, 2005. Drought and salinity: a comparison of their effects on the mineral nutrition of plants. J Plant Nut Soil Sci 168: 541-549. https://doi.org/10.1002/jpln.200420516

Islam MR, Madhaiyan M, Deka Boruah HP, Yim W, Lee G, Saravanan VS, Fu Q, Hu H, Sa T, 2009. Characterization of plant growth-promoting traits of three-living diazotrophic bacteria and their inoculation effects on growth and nitrogen uptake of crop plants. J Microbiol Biotech 19: 1213-1222. https://doi.org/10.4014/jmb.0903.3028

Jamali F, Sharifi-Tehrani A, Lutz MP, Maurhofer M, 2009. Influence of host plant genotype, presence of a pathogen, and coinoculation with *Pseudomonas fluorescens* strains on the rhizosphere expression of hydrogen cyanide-and 2, 4-diacetylphloroglucinol biosynthetic genes in *Pseudomonas fluorescens* biocontrol strain CHA0. Microb Ecol 57: 267-275. https://doi.org/10.1007/s00248-008-9471-y

Khalid A, Arshad M, Zahir ZA, 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J Appl Microbiol 96: 473-480. https://doi.org/10.1046/j.1365-2672.2003.02161.x

King EO, Ward MK, Raney DE, 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J Lab Clin Med 44: 301-307.

Lucy M, Reed E, Glick BR, 2004. Applications of free living plant growth-promoting rhizobacteria. Antonie van Leeuwenhoek 86: 1-25. https://doi.org/10.1023/B:ANTO.0000024903.10757.6e

Lugtenberg BJJ, Dekkers L, Bloemberg G V, 2001. Molecular determinants of rhizosphere colonization by *Pseudomonas*. Annu Rev Phytopathol 39: 461-490. https://doi.org/10.1146/annurev.phyto.39.1.461

Majeed A, Kaleem Abbasi M, Hameed S, Imran A, Rahim N, 2015. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Front Microbiol 6: 1-11. https://doi.org/10.3389/fmicb.2015.00198

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. https://doi.org/10.1016/j.plaphy.2004.05.009

Munns R, Tester M, 2008. Mechanisms of salinity tolerance. Ann Rev Plant Biol 59: 651-681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Niu X, Bressan RA, Hasegawa PM, Pardo JM, 1995. Ion homeostasis in NaCl stress environments. Plant Physiol 109: 735-742. https://doi.org/10.1104/pp.109.3.735

Nowak J, 1998. Review benefits of in vitro bacterization of plant tissue cultures with microbial inoculants. In vitro Cell Dev Biol Plant 34: 122-130. https://doi.org/10.1007/BF02822776

Paul D, Nair S, 2008. Stress adaptations in a plant growth promoting rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. J Basic Microbiol 48: 378-384. https://doi.org/10.1002/jobm.200700365

Qin S, Xing K, Jiang JH, Xu LH, Li WJ, 2011. Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. Appl Microbiol Biotechnol 89: 457-473. https://doi.org/10.1007/s00253-010-2923-6

Qin S, Zhang YJ, Yuan B, Xu PY, Xing K, Wang J, Jiang JH, 2014. Isolation of ACC deaminase-producing habitat-adapted symbiotic bacteria associated with halophyte *Limonium sinense* (Girard) Kuntze and evaluating their plant growth-promoting activity under salt stress. Plant Soil 374: 753-766. https://doi.org/10.1007/s11104-013-1918-3

Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moënne-Loccoz T, 2009. The rhizosphere: a playground and battlefield for soil-borne pathogens and beneficial microorganisms. Plant Soil 321: 341-361. https://doi.org/10.1007/s11104-008-9568-6

Shahid M, Hameed S, Imran A, Ali S, van Elsas JD, 2012. Root colonization and growth promotion of sunflower (*Helianthusannuus* L.) by phosphate solubilizing *Enterobacter* sp. Fs-11. World J Microbiol Biotechnol 28: 2749-2758. https://doi.org/10.1007/s11274-012-1086-2

Tank N, Saraf M, 2010. Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. J Plant Interact 5: 51-58. https://doi.org/10.1080/17429140903125848

Yao L, Wu Z, Zheng Y, Kaleem I, Li C, 2010. Growth promotion and protection against salt stress by *Pseudomonas putida* Rs-198 on cotton. Eur J Soil Biol 46: 49-54. https://doi.org/10.1016/j.ejsobi.2009.11.002

Yildirim E, Taylor AG, Spittler TD, 2006. Ameliorative effects of biological treatments on growth of squash plants under salt stress. Scientia Hort 111: 1-6. https://doi.org/10.1016/j.scienta.2006.08.003