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Effect of Seed Inoculation with Growth-Promoting Bacteria (PGPR) on Yield and Yield Components of Wheat (*Triticum aestivum* L.) at Different Soil Salinity Levels

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Abstract

The aim of this study was to evaluate the effect of Pseudomonas fluorescence strains with ACC deaminase and IAA activity enhancing plant growth isolated from rhizosphere of wheat plants and to evaluate the effect of Rhizobacterium isolated (PGPR) on growth and yield of five wheat cultivars under salinity stress. Before evaluating the effectiveness of 5 wheat cultivars, we detected ACC deaminase activity, siderefore production, IAA and solubilization of phosphate strains under salinity stress. The best isolate results in ACC deaminase activity, produce IAA, Phosphate Solubization and Siderefore revealed that inoculation with bacterial strains had considerable positive impacts on several growth parameters and yield of wheat cultivars including plant height, peduncle length, spike length, weight and number, number of grains per spike, 1000-grain weight and grain yield under various levels of salinity (50, 100 and 150 mM NaCl), as compared to uninoculated control. Inoculation with ACC deaminase-producing strains reduced the negative effects of salinity stress and increased wheat growth and yield. In addition, with increasing salinity levels, growth and yield of wheat cultivars decreased, however, cultivars showed different responses to salinity stress. This study demonstrates the effectiveness of growth-promoting rhizobacteria containing ACC deaminase and IAA to increase salt tolerance and thus enhance the growth and yield of wheat cultivars under salinity stress. Current findings suggested that the used PGPR strains are potential candidates for improving crop growth in salt stressed agricultural systems. Based on this, we conclude that Pseudomonas

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fluorescence strains can be used as a strong PGPR inoculum to improve wheat yield under salinity stress.
Keywords: ACC Deaminase, IAA, Siderophore, Salt Stress, PGPR

Introduction

Wheat (*Triticum aestivum L.*) is a monocotyledonous, herbaceous and annual plant of the genus Gramineae and has many species and is one of the largest families of flowering plants and consists of 600 species in 450 genera. In recent years, due to the environmental pollution crisis, especially soil and water resources pollution, which have lead to the infection of food sources and threaten human health, wide efforts have been started. In sustainable agricultural systems, using bio-fertilizers especially in poor soils is of particular importance in enhancing the performance and maintaining high soil quality (Sharma, 2012). Salinity stress is one of the most important abiotic stresses that directly stops plant growth and ultimately leads to reduced crop production. More than 6% of total land (approx. 800 million hectares) that can potentially be used for agricultural purposes is severely affected by saline conditions in arid and semi-arid regions worldwide (Sarkar *et al*., 2018). Soils affected by salinity stress contain a large amount of soluble salt, which is present in the macro and micro spatial structure (Carmen and Roberto, 2011). Salinity/sodicity leads to nutritional disproportion with an increase in uptake that associated with Na+ or reduced uptake of Ca2+ as well as K+ and also reduces the mobility and transportation of the active growing plant parts which affect the quality of both the vegetative and reproductive organ (Guo *et al*., 2009). Soil salinity not only reduces plant growth and development, but also negatively affects the composition and activity of rhizosphere bacteria (Ofek *et al*., 2006). The yield of plants grown in saline and saline soils can be increased by genetic engineering methods or inoculation of Plants with Growth-Promoting bacteria (PGPR) (Upadhyay *et al*., 2012). Growth-Promoting Rhizobacteria (PGPR) can greatly increase the growth and yield of cereals and other crops (Santoyo *et al*., 2016). Rhizosphere bacteria that stimulate plant growth are a group of bacteria that can directly, including nitrogen fixation, the production of plant growth regulators, increase the uptake of various nutrients for plants and other plant growth stimulants, or indirectly through production. Antibiotics, competition with harmful species for root occupation, systemic resistance in plants, etc. increase plant growth (Valencia-Cantero *et al*., 2007). The PGPR are the cheap and easily available sources for the mitigation of different biotic and abiotic stresses. Their activities including enhanced nutrients.

Mobility, phytohormone production, controlling pathogens and stress alleviation can enhance plant growth (Kumar and Verma, 2018). PGPR are agriculturally important bacteria having specific symbiotic relationships with plants. The PGPR enhance plant growth and health by suppressing plant pathogens and making different nutrients available to plants (Babalola, 2010). Rhizobacteria are microorganisms found in the rhizosphere which are categorized into diverse groups on the basis of their activities. Some rhizobacteria are pathogenic to plants and other organisms while some owe strong beneficial potentials to other organisms. Some rhizhobacteria are antagonistic in their mode of action (Iqbal and Ashraf, 2017; Islam, 2018). The plants inoculated with PGPR having ACC- deaminase are relatively more tolerant to environmental stress (Naveed

et al., 2008). Studies show that PGPRs provide nutrients to the plants and prevent the osmotic stress triggers by the addition of chemical fertilizers to saline land, while use of chemical fertilizers in the ecosystem, only damages the soil physical, chemical and biological structure, but also but also affects the quality of plant products (Kouchaki *et al*., 2008). Many PGPRs can also increase plant resistance to biotic and abiotic stress factors. Presence of 1-Aminocyclopropane-1-Carboxylate (ACC) deaminase activity in several rhizospheric bacteria and regulation of ACC, a precursor to plant ethylene levels, is one of the principal mechanisms by which bacteria exert beneficial effects on plants under abiotic stress (Glick, 2004; 2005; Saleem *et al*., 2007). Possibly by positively regulating the production of proline, antioxidant enzymes, siderophores, Hydrogen Cyanide (HCN) and IAA and the solubilization of phosphate, all resulting in increased chlorophyll content, plant growth and subsequent crop yield (Sarkar *et al*., 2018). According to (Creus *et al*., 2004), PGPR may alter plant–water relationships and show enhanced osmotic adjustment. The inoculation of seeds of various crop plants, such as tomato, pepper, canola, bean and lettuce, with PGPR can result in increased root and shoot growth, dry weight, fruit and seed yield and in enhanced tolerance of plants to salt stress (Glick *et al*., 1997; Mayak *et al*., 2004; Yildirim and Taylor, 2005; Barassi *et al*., 2006; Egamberdieva *et al*., 2013).

Taking into consideration the importance of salinity stress in Iran, the objectives of the present study were: (1) Evaluation of Pseudomonas fluorescence strains in terms of production of siderophore, hydrogen cyanide, solubility of mineral phosphate and production of indole acetic acid in vitro and determination of superior strains, (2) Investigation of the effect of different salinity levels on the growth of Pseudomonas fluorescent strains in laboratory conditions, (3) Study of the effect of selected strains on yield and yield components of wheat at different salinity levels in greenhouse conditions.

Material and Methods

Isolates and Culture Conditions

In this study, five isolates of Pseudomonas fluorescens (PGUF93, PGUF27, PGUF17 and PGUF3) were studied. The potential of these isolates as biofertilizers was evaluated in the bacteriology laboratory, Persian Gulf University, Bushehr, Iran. Bacteria were grown on Luria–Bertani broth (LB; Difco, Detroit, MI, USA) and 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) at 27°C. The purified strains were stocked with 20% glycerol and kept at -80°C. Seeds of wheat (*Triticum aestivum* L.) cvs. Chamran, Kuhdasht, Dehdasht, Karim and Bam obtained from Seed and Plant Improvement Institute (Karaj, Iran) and surface-disinfected in ethanol 95% (v/v), followed by 3 min in 10% (v/v) H_2O_2, rinsed thoroughly with sterile distilled water and germinated for four days at 25°C on 0.85% water agar under darkness conditions.

Evaluation of ACC Deaminase Activity

The potential of bacterial strains to utilize ACC was examined based on (Dell'Amico *et al*., 2005) with slight modifications. Bacterial strains were cultivated on Tryptic Soy Broth medium (TSB) (TSB, Merck) for 48 h. Then, 50 µL of bacterial suspension was transferred to 20 mL of DF minimal medium containing 3 mmol L^{-1} ACC (as the selective medium),

DF containing 2 gL^{-1} $(NH4)_2SO_4$ (as positive control) and DF minimal medium without any amendments (as negative control). Following incubation for 48 h at 27°C in shaker incubator with 120 rpm, an optical density at 405 nm was evaluated separately for each suspension.

Salt Tolerance Assay

Assessment of salt tolerance was performed based on the method of (Qin *et al*., 2014) with slight modifications. *Pseudomonas* strains were grown at 27°C on modified mineral-based nutrient agar (K_2HPO_4 0.2 g, $CaSO_4$ 0.1 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, peptone 1 g, agar 15 g in 1000 ml distilled water) amended with NaCl rising concentrations (0-10%, w/v) at intervals of 1%.

Evaluation of other Growth-Promoting Traits

Bacterial isolates were screened for solubilization of phosphate and production of siderophore and IAA based on (Islam *et al*., 2009). Siderophores detection was performed on Chrome Azural S (CAS) agar plates by the formation of orange halos around bacterial colonies after incubation for 24 h at 27°C. Potential of phosphate solubilization was evaluated on Pikovaskaya's agar medium containing 2.5% tricalcium phosphate. The formation of transparent halo zone around bacterial colonies was detected following incubation of bacterial isolates for 24-48 h at 27°C. IAA production ability was noticed by the colorimetric method of (Bric *et al*., 1991). The IAA secretion in the culture media was evaluated using a calibration curve of pure IAA (Sigma-Aldrich, St. Louis, MO) as a standard following the regression analysis. IAA production assay was performed in a completely randomized design with three replications. The experimental data were statistically analyzed using SAS software program and means were separated by Duncan's Multiple Range Test.

Plant Growth-Promotion Potential of Bacterial Strains under Salt Stress

The effects of *Pseudomonas* strains on wheat yield and growth factors were evaluated under greenhouse conditions. Bacteria were grown in LB broth medium for 16 h. After that, *Pseudomonas* cultures were washed with sterile NaCl solution (0.9%) and adjusted to an optical density at 600 nm of 0.125 corresponding to cell density of about 10^8 CFU mL^{-1}. Before performing greenhouse experiment, root tip colonization of bacterial strains on wheat cultivars was evaluated under gnotobiotic conditions under salinity levels (50, 100 and 150 mM NaCl) as described by (Egamberdieva, 2011), with some modifications. Results showed that all four strains were capable of colonizing the rhizosphere of wheat cultivars at the highest saline conditions (150 mM) (unpublished data).

Concerning greenhouse experiment, after soaking germinated wheat seeds in bacterial suspensions for 60 min, 25 seeds were transferred to plastic pots filled with 8 kg of sterilized soil. Soil was collected from the research field at College of Agriculture, University of Persian Gulf, Bushehr, Iran, air-dried, sieved (2-mm.10-mesh) and analyzed for physico-chemical traits. The soil used in greenhouse experiment was clay loam possessing organic matter 1.15%, total nitrogen 0.1%, total P 18 mg kg^{-1}, pH 6.2 and electrical conductivity 5.45 mSm^{-1}.

Plants were watered after emergence, as needed and no fertilizers were used. Salt

stress conditions were set up by adding 50, 100 and 150 mM of NaCl into the irrigation water (sterile distilled water) at four-leaf stage. Electrical Conductivity (EC) of saline solutions was 5, 10 and 15 ds/m for 50, 100 and 150 mM NaCl, respectively. Sterile distilled water without NaCl salt was considered as control. Wheat plants were grown in the glasshouse with a 16/8 day/night regime at 24-26°C. After 14 days, thinning was carried out to leave 15 uniform seedlings in every pot. At physiological maturity stage, morphological characteristics including spike weight, plant height, number of grains per spike, peduncle and spike length, 1000-grain weight and grain yield were evaluated.

Survival of Bacteria in the Rhizosphere of Wheat

The effects of salt stress on the survival of bacteria in the rhizosphere of wheat cultivars was estimated under greenhouse conditions as described above.

At harvest, plants were removed from the posts and shaken lightly to discard loosely adhering soil. Following that, roots were weighted with tightly adhering soil, put in 10 mL sterile saline solution (0.9%) and shaken for 30 min at 450 rpm. Number of bacterial cells in the resulting suspensions was examined as Colony Forming Units (CFU) by plating serial dilutions on KB agar Petri dishes.

Statistical Analysis

The greenhouse experiment was carried out in a split-split plot design based on randomized complete block with three replications (pots) per treatment. Main plots, subplots and sub-sub plots were consisted of three salinity levels (50, 100 and 150 mM), five wheat cultivars (Chamran, Kuhdasht, Dehdasht, Karim and Bam) and four bacterial strains (PGUF93, PGUF27, PGUF17 and PGUF3) respectively. In the greenhouse experiment, controls were consisted of wheat plants either not irrigated with NaCl solutions or not inoculated with bacteria. Every experimental unit was a pot including 15 plants. Data were analyzed using SAS Version 9.1 (SAS Institute Inc., Cary, NY, USA). Means were separated using Fisher's protected Least Significant Difference (LSD) method at ($P < 0.05$).

Results

Production of ACC Deaminase

All bacterial strains isolated from wheat rhizosphere were able to grow on DF minimal medium amended with ACC, revealing that they possess the ACC deaminase activities. Optical densities of bacterial suspensions in negative control (minimal medium without nitrogen source), positive control (amended with 2 g of $(NH_4)_2SO_4$ L^{-1}), or with ACC (3 mM L^{-1}) are displayed in (Table 1).

PGP Characteristics of Pseudomonas Bacteria and their Tolerance to NaCl

The *Pseudomonas* strains were screened for their plant growth promoting traits and the results are shown in (Table 2). In addition to solubilizing phosphate, all bacterial strains produced siderophore and IAA. Furthermore, results exhibited that all isolates could tolerate 8% of NaCl induced stress after which no growth was detected.

Influence of PGP Isolates on Wheat Growth and Yield under Salinity Stress

Analysis of variance indicated that the main effects of salinity, variety and *Pseudomonas*

bacteria and the interaction between them were significant on almost all measured traits ($p \leq 0.05$) (Table 3). Generally, as salinity increased, all measured traits decreased, nevertheless, inoculation of wheat seeds with *Pseudomonas* bacteria ameliorated salt stress and resulted in an increase in wheat growth and yield as compared to non-inoculated control plants.

Table 1.The effects of different media with salinity,on the growth and abilityof Pseudomonas fluorescens utilizing ACC as the sole N-source

	Optical density[a]		
Isolate	DF+$(NH4)_2SO_4$[b]	DF+ACC[c]	DF[d]
PGUF93	3.026±0.019	1.563±0.028	0.269±0.017
PGUF27	2.742±0.036	2.049±0.021	0.342±0.025
PGUF17	2.807±0.043	2.144±0.026	0.278±0.031
PGUF3	3.027±0.021	1.758±0.011	0.335±0.021

[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 and [d]DF minimal medium as a negative control (DF); values are means ± SE (Standard Error).

Table 2. Biochemical properties of the isolated strains

Isolate	Phosphate solubilization	IAA (mg/L)	Siderophore	Salt tolerance range %
PGUF93	+	3.05b	+	0-8
PGUF27	+	2.82b	+	0-8
PGUF17	+	7.51a	+	0-8
PGUF3	+	2.65b	+	0-8

Regarding IAA production, means with the similar letters are not significantly different according to Fisher's protected least significant difference ($p \leq 0.05$).

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

Source changes	Df	Plant height	Peduncle length	Spike length	Spike number	Grains / spike	1000-grain weight	Spike weight	Grain yield	Rhizosphere colonization ($\times 10^7$ CFU g^{-1} rhizosphere)
Block	2	41.47**	10.32ns	1.90**	7.92**	50.68**	16.59**	0.673**	0.025ns	3.26**
Salinity	3	2293.37**	384.32**	114.85**	71.59**	672.96**	184.09**	1.127**	23.28**	1705.03**
Error 1	6	9.16	9.07	0.11	0.37	2.54	1.39	0.03	0.018	1.26
Variety	4	850.73**	229.10**	79.06**	28.75**	5170.72**	1406.26**	48.02**	0.141**	79.56**
Salinity × variety	12	37.73**	15.08^{ns}	1.61**	3.19**	7.03**	49.41**	0.077**	0.114**	2.45**
Error 2	32	2.3	39.13	0.29	2.06	3.07	0.58	0.056	0.0148	0.56
Bacteria	4	415.36**	39.31**	30.32**	32.64**	652.99**	438.19**	5.82**	2.08**	10.99**
Salinity × bacteria	12	21.01**	2.64**	0.65**	4.55**	11.42**	19.99**	0.051**	0.141**	3.04**
Variety × bacteria	16	50.72**	4.58**	3.83**	7.70**	145.25**	42.32**	2.43**	0.040**	5.19**
Salinity × variety × bacteria	48	14.99**	2.64**	1.18**	2.48**	4.66**	15.08**	0.071**	0.080**	0.78*
Error 3	160	3.06	1.52	0.16	0.93	2.16	0.25	0.036	0.0086	0.98
CV (%)		2.17	8.56	4.72	5.99	3.33	0.96	5.58	3.45	12.12

^{ns}, *, **, respectively significant and non-significant at 5 and 1%.

Table 4:Mean Comparison of the interactions of different levels of salinity and seed inoculation with growth-promoting bacteria on yield and yield components of wheat

Salinity levels (mM)	Bacteria	Plant height (cm)	Peduncle length (cm)	Spike length (cm)	Spike number	Grains / spike	1000-grain weight (g)	Spike weight (g)	Grain yield (g)	Rhizosphere colonization ($\times 10^7$ CFU g^{-1} rhizosphere)
0	Control	3.21b	3.08e	52.51d	42.20d	15.4e	8.68e	15.03d	80.50c	ND
	PGUF93	3.61a	3.70c	55.46a	50.66a	18b	10.10d	16.99c	88.48d	14.53c
	PGUF27	3.53a	3.71c	54.52b	47.80c	18.33a	10.27c	18.22a	88.94b	14.88c
	PGUF17	3.57a	3.85a	54.75b	48.66b	17.4d	10.44b	17.05b	89.81a	16.81a
	PGUF3	3.51a	3.52d	55.64a	48.60b	17.53c	10.97a	17.38b	88.37e	15.99b
50	Control	2.08d	2.88e	47.50g	40e	14.86d	7.80e	13.84f	77.73e	ND
	PGUF93	2.68c	3.39d	51.72e	49.40a	17.06a	9.18c	14.44e	83.14c	7.07e
	PGUF27	2.74c	3.64b	54.28b	46.20b	16.93b	9.37b	15.65d	84.31b	7.40de
	PGUF17	2.62c	3.74a	53.03c	45.53d	17.06a	8.90d	15.82d	85.08a	7.51de
	PGUF3	2.75c	3.45c	55.62a	45.60c	16.86c	9.90a	15.75d	82.40d	7.85d
100	Control	2.06d	2.84e	46.37h	38.20e	14.33d	6.90e	12.20g	74.34e	ND
	PGUF93	2.44c	3.29d	51.30e	47.66a	15.73c	8.28b	13.77f	78.88b	4.17gh
	PGUF27	2.52c	3.57b	53.68c	44.33b	15.73c	8.22c	14.10e	79.10a	4.67fg
	PGUF17	2.63c	3.68a	52.54d	43.13c	16.40a	8.02d	14.24e	78.5c	4.70fg
	PGUF3	2.59c	3.39c	54.82b	42.46d	15.86b	8.85a	14.12e	77.73d	4.89f
150	Control	2.05d	2.81e	45.50i	36.20e	14d	6.32e	11.06h	71.47e	ND
	PGUF93	2.21d	3.21d	50.49f	45.60a	15.43b	7.64b	11.84h	77.05a	3.60h
	PGUF27	2.27d	3.48b	53.11c	42.26b	16.20a	7.38c	11.93h	72.10d	4.10gh
	PGUF17	2.28d	3.60a	51.34e	39.60c	14.66c	7.10d	12.40g	76.34b	4.03gh
	PGUF3	2.29d	3.35c	54.14b	39.26d	14.66c	7.67a	12.02g	73.09c	4.05gh

Means, in each column and for each factor, followed with similar letter(s) are not significantly different at the 5% probability level- using LSD Test. ND: Not Detected.

Under normal condition and salinity levels of 50, 100 and 150 mM, all bacterial isolates elevated plant height (9.3-12.4, 23.6-31.7, 18.4-27.7 and 7.8-11.7%, respectively) in comparison with other treatments (Table 4). The results showed that treatment with PGUF3 strain significantly improved spike length and peduncle length by 25-30 and 19-17%, respectively, under normal conditions and salt stress (Table 4). Inoculation with PGUF93 strain increased the number of seeds per spike (with 20-26% increase) at all salinity levels. Under normal stress conditions and at a salinity level of 150 mm, inoculation with PGUF27 strain increased the number of spikes (19 and 10, respectively) significantly compared to the treatment without bacterial inoculation. However, at salinity levels of 50 and 100 mm, PGUF93 and PGUF17 strains perform better (Table 4). PGUF3 was the most effective isolate which enhanced 1000-grain weight up to about 28.3%. The maximum spike weight (21%) was obtained under normal condition following inoculation of seeds with PGUF27 strain comparing to other treatments. Seed inoculation with PGUF17 strain under normal conditions at salinity levels of 50 and 100 mM had the greatest effect on increasing grain yield (up to 46.6%). PGUF93 strain increases this property more than other treatments under salinity of 150 mm (Table 4).

Wheat varieties showed variations in plant height during NaCl induced salinity stress (Table 5). Under salinity levels of 50 na 100 mM, Chamran variety and under normal condition and 150 mM salinity Kuhdasht and Bam varieties showed the maximum plant height comparing to other varieties. Kuhdasht variety showed the maximum spike length under all salinity levels. Bam variety had the highest spike length, spike number and number of grains per spike at all salinity levels. Dehdasht variety had the most spike weight at all salinity levels comparing to other varieties. Under normal condition, Bam and Dehdasht varieties and under all salinity levels Bam variety had the most spike weight, in comparison to other varieties. Considering grain yield, under normal condition Kuhdasht and Bam varieties, under 50 mM salinity Chamran, Kuhdasht and Bam varieties, under 100 mM salinity Kuhdasht variety and under 150 mM Karim variety showed the maximum grain yield, comparing the other varieties (Table 5).

Inoculation of wheat varieties with tested strains caused an increase in different measured traits comparing to uninoculated control; however, varieties did not show identical response to inoculation with different strains (Table 6). Inoculation of Chamran and Kuhdasht varieties with PGUF93 and PGUF17 resulted in the maximum plant height as compared to other treatments. Seed inoculation of Dehdasht variety with PGUF17 and PGUF3 (with 23.8 and 23.3% increase, respectively) increased peduncle length more significantly than the other treatments. The maximum spike length was observed following the inoculation of Karim variety with PGUF3 (53.5%). Inoculation of Chamran variety with PGUF3 resulted in the most spike number as compared to other treatment. The most increase in the number of grain per spike (48.4%) was observed in Kuhdasht variety following seed inoculation with PGUF93 strain. Inoculation of Chamran variety with PGUF3 caused the maximum 1000-grain weight (21.9%). The maximum increase in spike weight was seen in Chamran variety after seed inoculation with PGUF27 strain.

Inoculation of Chamran with PGUF3, Karim and Bam with PGUF27, Kuhdasht with PGUF93 and PGUF3 and Dedasht with PGUF3 caused the most increase in grain yield of wheat cultivars as compared to other treatments (Table 6).

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

Salinity (mM)	Variety	Plant height (cm)	Peduncle length (cm)	Spike length (cm)	Spike number	Grains/ spike	1000-grain weight (g)	Spike weight (g)	Grain yield (g)	Rhizosphere colonization ($\times 10^7$ CFU g^{-1} rhizosphere)
0	Chamran	89.96b	15.77e	10.05c	16.26e	49.13b	53.36g	3.71c	3.44b	16.26ab
	Kuhdasht	90.14a	19.14a	11.3b	16.66d	44.33d	52.51h	2.56d	3.59ab	12.39c
	Dehdasht	88.54d	19.50a	8.8e	17.20c	48.53c	64.90a	4.04ab	3.46b	16.21b
	Karim	78.80e	16.56d	8.47d	17.80b	34.14e	53.97g	2.65d	3.32c	16.85a
	Bam	88.64c	13.70g	11.48a	18.73a	61.60a	48.14l	4.77a	3.63a	16.05b
50	Chamran	86.14a	13.42g	9.13c	16.20c	48.92	48.92	3.70c	2.59d	7.75e
	Kuhdasht	84.02c	18.47b	9.45b	16.6d	42.33c	51.28i	2.50e	2.61d	4.89fghi
	Dehdasht	84.86b	16.46d	8.29d	16.20c	46.40b	58.4b	3.60c	2.58d	7.94de
	Karim	75.34e	14.57f	7.81e	16.66b	32.53d	56.10d	2.62d	2.53e	8.16de
	Bam	82.29d	12.58h	10.48a	17.66a	59.06a	47.40l	4.65a	2.55e	8.56d
100	Chamran	81.38a	13.46g	8.44c	15.40d	44.13b	47.20l	3.69c	2.42f	5.04fgh
	Kuhdasht	79.53c	17.01c	8.68b	14.53c	41.06c	50.90j	2.48e	2.62d	2.87j
	Dehdasht	75.88d	13.42g	7.26d	15.13e	44.13b	57.64c	4ab	2.37g	5.10fg
	Karim	72.16e	12.84h	6.58e	15.73b	30.53d	55.77e	2.60d	2.40f	5.15f
	Bam	79.60b	11.69i	9.32a	16.86a	55.93a	47.21m	4.89a	2.42f	4.87fghi
150	Chamran	76.44b	11.13i	7.74b	15.03d	40.53c	47.16m	3.67c	2.12k	4.38hi
	Kuhdasht	76.10c	13.86g	7.42c	15.40b	39d	49.82k	2.49e	2.23i	2.24j
	Dehdasht	73.04d	11.52i	6.17e	15.06c	41.20b	56.48d	3.99b	2.24i	4.41ghi
	Karim	69.88e	13.12g	6.20d	14.33e	28.8e	54.59f	2.63d	2.34h	4.47fghi
	Bam	77.59a	9.64j	8.59a	16.13a	53.40a	46.53n	4.8a	2.17j	4.23i

Means, in each column and for each factor, followed with similar letter(s) are not significantly different at the 5% probability level- using LSD Test.

Table 6. Mean comparison of yield and yield components of wheat varieties inoculated with different *P. fluorescens* strains

Variety	Bacteria	Plant height (cm)	Peduncle length (cm)	Spike length (cm)	Spike number	Grains/ spike	1000-grain weight (g)	Spike weight (g)	Grain yield (g)	Rhizosphere colonization ($\times 10^7$ CFU g^{-1} rhizosphere)
Chamran	Control	79.09e	11.87g	8.29e	12.58e	35.41e	44.56n	2.43e	2.24f	ND
	PGUF93	87.56a	13.48e	9.04b	16.20c	46.66c	49.14i	3.11d	2.71c	8.18de
	PGUF27	84.50c	14.17d	8.96c	16.16d	49.66a	48.07j	4.80a	2.72c	7.80ef
	PGUF17	85.35b	14.23d	8.82d	16.58b	47.41b	49.70i	4.35b	2.75bc	9.29ab
	PGUF3	80.91d	13.47e	9.09a	17.08a	46.08d	54.34f	3.35c	2.79b	8.17de
Kuhdasht	Control	77.76e	15.36c	8.02e	15.33c	35.83e	48.51j	2.24e	2.40d	ND
	PGUF93	82.60c	17.09a	9.08d	16.33a	53.16a	50.75h	2.72a	2.85ab	5.96g
	PGUF27	82.34d	17.63a	9.58c	16.08d	42.16b	52.55g	2.43d	2.80b	6.05g
	PGUF17	86.11a	17.87a	9.7a	14.83e	38.66c	50.58h	2.45c	2.85ab	5.39gh
	PGUF3	83.43b	17.65a	9.67b	15.75b	38.58d	53.23f	2.62b	2.90a	4.99h
Dehdasht	Control	76.07e	13.21d	6.10e	14.91d	42.33e	52.95g	3.41d	2.33e	ND
	PGUF93	83.08a	14.73d	8.18a	16.25c	52.33a	59.04c	3.77c	2.64bd	7.58ef
	PGUF27	81.61b	16.36b	8.16b	16.25c	43.58c	63.55a	3.25e	2.71d	8.71bcd
	PGUF17	81.08c	15.53c	7.92c	16.58b	43.33d	60.31b	4.34a	2.80b	8.67bcd
	PGUF3	81.06d	16.29b	7.78d	15.50a	43.75b	60.97b	3.82b	2.82ab	8.70bcd
Karim	Control	71.52e	12.67f	6.17e	14.75e	26.83e	48.67j	2.47e	2.31e	ND
	PGUF93	74.86b	13.85e	7.07b	16.16d	32.08d	56.14e	2.48d	2.72bc	7.68ef
	PGUF27	73.30d	15.22c	6.95c	17.33a	32.66c	58.87c	2.66b	2.79b	8.26cde
	PGUF17	74.61c	14.67d	6.67d	16.50c	33.41a	54.67f	2.56c	2.73bc	8.98abc
	PGUF3	76.19a	14.95d	9.47a	15.91b	32.75b	57.19d	2.71a	2.69c	9.71a
Bam	Control	75.87e	11.49 g	8.55e	16.91c	55.33e	45.15l	3.97e	2.47d	ND
	PGUF93	81.33c	12.16f	10.64b	17.83b	57.41d	46.15k	4.90a	2.76bc	7.31f
	PGUF27	87.56a	12.04f	10.40c	18.16a	57.66c	46.43k	4.86c	2.80b	8.01def
	PGUF17	85b	12.08f	9.95d	16.91c	58.33b	49.31i	4.88b	2.73bc	8.99ab
	PGUF3	80.38d	11.71g	10.73a	16.91c	58.75a	49.55i	4.61d	2.72bc	9.39ab

Means, in each column and for each factor, followed with similar letter(s) are not significantly different at the %5 probability level- using LSD Test. ND: Not Detected.

Survival of Bacterial Strains in the Wheat Rhizosphere

The survival of *Pseudomonas* bacteria in the rhizosphere of five wheat cultivars grown under different salinity levels was evaluated under greenhouse conditions. Analysis of variance showed that the main effects of salinity, variety and bacterial isolates and the interaction between them were significant on wheat rhizosphere colonization ($p \leq 0.05$) (Table 3). All bacterial strains were able to colonize and survive in the rhizosphere of wheat cultivars. Despite that their colonization level was partly prevented under salinity conditions. Under normal condition isolate PGUF17, at 50 and 100 mM, isolates PGUF27, PGUF17, PGUF3 and at 150 mM salinity all bacterial strains had higher rhizosphere colonization in comparison to other treatments (Table 4). Wheat varieties exhibited variations in rhizosphere colonization by bacterial strains during salt stress (Table 5). Under normal condition, Karim and Chamran varieties, at 50 and 100 mM, Bam, Karim and Dehdasht varieties and at 50 mM, all wheat varieties except for Kuhdasht variety presented higher rhizosphere colonization. Wheat genotype at the cultivar level had notable influence on rhizosphere colonization by Pseudomonas strains under greenhouse conditions (Table 5). In Chamran variety the rhizosphere colonization by PGUF17 was higher than other strains. In Kuhdasht and Dehdasht varieties, PGUF93, PGUF27 and PGUF17 strains performed better than PGUF3 regarding rhizosphere colonization. In Karim and Bam varie ties, PGUF3 and PGUF17 strains colonized the rhizosphere better than the others.

Discussion

This study reveals the effectiveness of PGPR strains of P. fluorescens possessing ACC deaminase activity for induction of salt tolerance and consequently enhancement of wheat growth and yield under salinity conditions. Rhizospheric bacteria belonging to different species of Pseudomonas and the genera Alcaligenes, Bacillus, Rhodococcus and Variovorax more widely display ACC deaminase activity (Bal *et al*., 2013; Akhgar *et al*., 2014). 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 wheat isolates for IAA production. The ability of Pseudomonas strains to produce IAA indicates their potential to use as growth hormones or growth regulators. These results suggest that the selected strains can be beneficial in enhancing growth of wheat and other host plants by providing nutrients like iron 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; Majeed *et al*., 2015). IAA production by bacteria isolated from rhizosphere of different plants such as wheat and rice had already been reported by (Çakmakçı *et al*., 2007; Majeed *et al*., 2015). Inoculation plant with Growth Promoting Rhizobacteria (GPR) can help plant to grow in such stressful conditions

and increased the productivity of crops (Parida and Das, 2005). The underlying mechanism of PGPR for promoting plant growth involves in the increased nutrient cycling, reduced pathogens either producing siderophores and antibiotics or plant hormones such as ethylene. The concentration of ethylene, a key stress hormone, is increased in response to salinity stress by elevated levels of its precursor 1-Aminocyclopropane-1-Carboxylic Acid (ACC), resulting in physiological alterations in plant tissues (Tank and Saraf, 2010). Sergeeva *et al*. (2006) reported that PGPR under salinity stress might promote the ACC-deaminase activity and were able to metabolize ACC, a precursor of ethylene which produced in response to stress. The PGPR might reduce the ethylene content and consequently eliminate damage caused by high concentration of ethylene due to salt stress. In the present experiment, the increased wheat production was primarily because of the PGPR inoculation that contains ACC-deaminase which could reduce salt stress by regulating the endogenous levels of ethylene in plants through their ACC-deamine activity (Sergeeva *et al*., 2006). The ACC exuded from seeds under salt stress is taken up by the surrounding microbes, hydrolyzed to ammonia and a-ketobutyrate that maintain the equilibrium between the internal and external ACC. Decrease in ACC directly restricts the biosynthesis of stress induced ethylene in host plants and stimulate the seedling growth (Din *et al*., 2019). That's why difference in growth attributes was observed in bacterial inoculated wheat plants as compared to non-inoculated ones in the present study. Bacterial inoculation may diminished the inhibitory effect of salt stress on the roots and aid in the promote of more effective root systems, which could help plants absorb relatively more water from deeper soil under stress conditions (Marulanda *et al*., 2010; Kalayu, 2019). Vivas *et al*. (2003) suggested that there are synergistic effects on plant growth when PGPR are inoculated, particularly under growth limited conditions. Arough *et al*. (2016) showed that salinity stress negatively affected growth, yield, antioxidant enzymes and ions accumulation in barley plants. The PGPR strains used in this study may, therefore, protect the plant from the harmful effect of ethylene by decreasing its concentration, resulting in better root growth. Many researchers have also shown that PGPR strains do improve crop growth under salinity stress conditions (Saravanakumar and Samiyappan, 2007; Nadeem *et al*., 2009; Zahir *et al*., 2009; Tank and Saraf, 2010). Our results also reveal that the four PGPR strains tested had different potentials for improving plant growth under salt stressed conditions. The better performance was observed following inoculation with Pseudomons spp. The better performance of Pseudomonas spp. was also observed by other researchers in both laboratory and field studies (Saravanakumar and Samiyappan, 2007; Zahir *et al*., 2009; Abbaspoor *et al*., 2009; Ahmad *et al*., 2011; 2012). In a study, Pseudomonas stutzeri, Pseudomonas fluorescens and Pseudomonas aeruginosa were isolated from the rhizospheric zone of tomato growing under salinity stress caused by NaCl; these isolates showed plant growthepromoting activities. These strains help plants to get over from the stress condition by producing various plants hormones including ACC deaminase activity

(Tank and Saraf, 2010; Bal *et al*., 2013). Salinity treatments caused a statistically significant decrease in all growth parameters compared to control. Similar reduction in growth performance were found in some plants under saline conditions (Ates and Tekeli, 2007; Azooz, 2009; Ekmekçi and Karaman, 2012; Kaya *et al*., 2013). These findings may be due to the increased synthesis of phytohormones like IAA, which would have triggered the activity of enzymes like α-amylase that promoted early germination by bringing an increase in availability of soluble sugars from starch decomposition (Kim *et al*., 2006). Therefore, significant increase in wheat growth and yield might be associated with improved plant attributes, which was likely accomplished by alleviation of salt stress to seed germination through PGPR inoculation (Lee and Bae, 2011). The experimental results reported here show the likelihood of use of PGPR for growth and yield improvement of wheat which is the most important staple crop of world. PGPR have been reported to have potential to promote growth in many crops like barley, sorghum, tomato, (Baldani *et al*., 1986) cotton (Hafeez *et al*., 2004; Yasmin *et al*., 2013), maize (Naureen *et al*., 2005) and rice (Mehnaz *et al*., 2001). Previous studies have revealed that inoculation of PGPR promoted plant growth attributes including plant height, root and shoot dry weight, number of tillers and yields of various crops (Khalid *et al*., 2004; Tank and Saraf, 2010). Plant Growth-Promoting Rhizobacteria (PGPR) inhabit plant roots and thereby enhance the plant growth (Upadhyay *et al*., 2012). In pot experiment, it was observed that inoculation of wheat 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 (Zahir *et al*., 2009; Ahmad *et al*., 2011). Inoculation of wheat 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). Shaharoona *et al*. (2006) reported that bacterial inoculation showed improvement in spike length and plant height at different salinity levels as compared to the control. PGPR strains containing ACC-deaminase activity have the ability to hydrolyze the ACC; therefore, they eliminated the negative effect of high ethylene concentration on plant growth (Príncipe *et al*., 2007). The inoculation of PGPR also increased nutrient contents in wheat. Results of increased nutrients in wheat by PGPR inoculation are supported by previous studies. Wu *et al*., (2012) reported that PGPR presence showed positive effect on nitrogen and phosphorus

concentration. It appeared that PGPR inoculation substantially increased nutrients in wheat plant which led to increased plant growth of wheat. The PGPR were inoculated on seeds prior sowing which could efficiently promote germination. Rapid and healthy seed germination is primary stage of obtaining vigorous crop production. Early seedling growth is the critical developmental stage that has been identified the most sensitive to soil salinity (Ashraf and Foolad, 2005). Therefore, significant increase in wheat growth and yield might be associated with improved plant attributes, which was likely accomplished by alleviation of salt stress to seed germination through PGPR inoculation (Lee and Bae, 2011). The inoculation of wheat seeds with selected strains of PGPR substantially improved growth and yield of wheat. Results of the present study are in accord with the findings of (Dobbelaere *et al*., 2001). That plant treated with PGPR having ACC-deaminase activity showed low Na contents and more K than uninoculated plants. It is concluded that inoculation with Pseudomonas or strains containing ACC-deaminase could be an alleviating the stressinduced ethylene and consequently improving the growth and yield of wheat even in the presence of high salinity stress.

Conclusion

This study showed that rhizosphere engineering is a good option for producing environmentally friendly stress tolerance in crops. Our results showed that inoculation of different wheat cultivars with Pseudomonas fluorescence increased vegetative parameters, improved yield, yield components and nutrient uptake, as well as reduced salt stress. In general, the results showed that the isolated strains PGUF93, PGUF27 PGUF17 and PGUF3 have a high potential to improve wheat growth under saline soils. It can be concluded that all four strains helped to withstand salinity stress by reducing the toxicity of sodium ions and masking the effect of salt on the plant. Of all Pseudomonas isolates tested, PGUF27 and PGUF3 isolates showed the highest PGPR growth and positive traits. The present study concluded that inoculation with four ACC deaminases containing PGPR significantly reduced stress-induced ethylene production and thus improved wheat growth under high salinity stress. The results shown in this study show a promising agricultural solution for crop growth in semi-arid regions.

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