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Evaluation of ACC deaminase producing *Pseudomonas fluorescens* strains for their effects on seed germination and early growth of wheat under salt stress

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Abstract

Plant growth-promoting rhizobacteria possessing ACC deaminase activity reduces the level of stress ethylene, conferring resistance and improving plant growth under different stress conditions. The present study aims at evaluating *Pseudomonas fluorescens* strains for their potential to produce ACC deaminase and quantifying their effects on seed germination and seedling growth of wheat under salinity stress. Bacteria were first evaluated for their ability to utilize ACC, an immediate precursor of stress ethylene, using DF minimal medium containing 3 mM ACC and it was revealed that they were all able to use ACC as sole nitrogen source under *in vitro* conditions. Thereafter, the influence of bacterial strains on seed germination and seedling growth of wheat varieties was evaluated under NaCl induced salt stress after seven days. The experimental design was completely randomized in 3×5×5 factorial scheme with three replications. Factors consisted of salinity (0, 3 and 6 dS/m), *P. fluorescens* bacteria (strains PGU2-79, WBO-3, WKZ1-93 and WB1-7 and sterile water as control), and wheat variety (Chamran, Kooh-Dasht, Dehdasht, Karim and Jam). Twenty wheat seeds were transferred to plates after being soaked for an hour in bacterial suspensions at concentration of 10^8 CFU/ml. Each plate contained a filter paper which was moistened with 10 ml of NaCl solution in different concentrations. Plates were incubated in a growth chamber with maximum and minimum temperatures maintained at 20°C and 15 °C, respectively, with 12 h day-night photoperiod and relative humidity of 80%. Results revealed that *P. fluorescens* strains had positive impacts on different growth parameters of wheat varieties including germination percentage and rate, seed vigor, length and dry weight of coleoptile and radicle and salinity tolerance index of plants under salinity stress as compared to control. This study reveals the efficacy of plant growth promoting *P. fluorescens* strains containing ACC deaminase for improving salt tolerance and consequently stimulating the growth of wheat seedlings under salinity stress.

Keywords*: Pseudomonas fluorescens,* salinity stress, siderophore, wheat.
Abbreviations: ACC _ aminocyclopropane-1-carboxylate; HCN _ hydrogen cyanide; IAA _ indole-3-acetic acid; PGPR _ plant growth-promoting *rhizobacteria*.

Introduction

Salinity is one of the main environmental constraints that negatively affects modern agriculture in several countries all over the world (Munns and Tester, 2008). Low precipitation, irrigation of crops with salty water, and poor cultural practices are among the major reasons for increasing soil salinity (Munns, 2005). As soil salinity increases, plant growth decreases either through osmotic inhibition of water absorption by plant roots or toxicity of specific ions. Salts around plant roots immediately influence cell growth and disturb associated metabolism (Munns and Tester, 2008). Abundance of specific ions, e.g., Na^+ and Cl^- can also lead to a reduction in accessibility and uptake of some elements including N, P, K, and Mg by the plant (Heidari and Jamshid 2010). It is also documented that salinity stress inhibits biosynthesis of phytohormones and maturation of plant cell walls (Xiong and Zhu, 2002).

When exposed to various stress factors, plants release ethylene, a phytohormone which is also known as a stress hormone, as a physiological response. Salt stress can enhance the rate of ethylene biosynthesis through increased levels of 1-aminocyclopropane-1-carboxylic acid (ACC), which results in physiological changes in plant tissues. It has been reported that bacteria with ACC deaminase activity are able to hydrolyze the endogenous ACC, an immediate precursor of ethylene, into α-ketobutyrate and ammonia resulting in reduced production of stress ethylene (Hontzeas et al., 2004).

Many plant growth-promoting rhizobacteria (PGPR) stimulate plant growth either directly by providing plants with fixed nitrogen, phytohormones, iron, and soluble phosphate (Lucy et al., 2004) or indirectly by preventing plant pathogens from inhibiting plant growth (Raaijmakers et al., 2009). Bacterial inoculants can increase germination, speed up plant growth, response stress conditions, and protect plants from several diseases (Lugtenberg et al., 2002). Following colonization of plant rhizosphere, PGPRs produce hydrogen cyanide (HCN), siderophores and phytohormones like indole-3-acetic acids (IAA) (Glick, 1995).

Several PGPRs can enhance plant resistance to various biotic and abiotic stress factors. Presence of ACC deaminase activity in rhizobacteria and regulation of ACC is one of the main mechanisms through which bacteria exert positive impacts on plants under abiotic stress conditions (Glick, 2005; Glick et al., 2007; Saleem et al., 2007). Earlier researches indicated that bacteria possessing ACC deaminase activity decrease the level of stress ethylene and lead to a better plant growth under various stress conditions such as salt stress, heavy metal stress, flooding stress, and pathogen attack (Glick et al., 2007). It is also proved that plants inoculated with PGPR possessing several PGP traits show more resistance to harmful effects of stress ethylene synthesized as a result of stress conditions (Zahir et al., 2008). Alleviation of salinity stress by PGPR inoculants has been observed in several crops including wheat, maize, rice, cucumber, tomato and lettuce (Bal et al., 2013; Egamberdiyeva, 2007; Egamberdiyeva, 2009; Han and Lee, 2005; Kang et al., 2014; Mayak et al., 2004; Nakbanponte et al., 2014).

Although salt stress can occur at all stages of plant growth, the unfavorable effects of salinity on seed germination and seedling stage can be even more harmful, since the seedling roots are in direct contact with soil and are affected by soil changes like salinity stress (Jamil et al., 2006). Many researches have proved that salinity inhibits seed germination in several crops including wheat (Egamberdieva, 2009). Furthermore, significant reduction has been observed in germination percentage and rate, and in seedling shoot and root lengths of various crops (Egamberdieva, 2011; Jamil et al., 2006). It is revealed that the impact of salinity on germination rate and percentage, and seedling root and shoot growth is a reliable test in tolerance assessment of many plant species (Munns, 2002).

In our previous research, it was revealed that the fluorescent *Pseudomonas* isolates PGU2-79, WKZ1-93, WBO-3 and WB1-7 had multiple PGP properties including production of indole-3-acetic acid, hydrogen cyanide and siderophore and solubilization of phosphate. The bacterial strains were also tolerant to NaCl induced salt stress (Safari et al., 2016). The objectives of the present study were: (1) to evaluate four promising plant growth promoting *P. fluorescens* strains for their potential to utilize ACC as sole nitrogen source under laboratory conditions and (2) to determine the potential of bacteria to increase root germination attributed traits and early seedling growth of five wheat varieties under different salinity levels.

Results

ACC deaminase activity

Four *Pseudomonas* strains were able to utilize ACC as the sole nitrogen source. Optical densities of bacteria grown in different media are shown in Table 1.

Influence of bacteria on seed germination and seedling growth under salt stress

Data analysis of variance showed that the main effects of salinity, variety and bacterial isolates and the interaction between them were significant on most measured traits (Table 2).

Under normal condition, isolate WB1-7 (with 81% increase), in 3 dS/m, PGU2-79 (with 63.7% increase) and in 6 dS/m, WBO-3 (with 72.7% increase) improved seed vigor comparing to control (Table 3). Wheat varieties showed variations in seed vigor during NaCl induced salinity stress. Under normal condition and 6 dS/m salt, Kouhdasht and Bam, and at 3 dS/m, Bam variety showed higher seed vigor comparing to other treatments (Table 4). Inoculation of wheat varieties with PGPRs increased SV as compared to non-inoculated plants (Table 5).

Results also showed that strains WB1-7, PGU2-79 and WBO-3 increased GP by 55.6, 23.9 and 34.6 %, under normal conditions and 3 and 6 dS/m salinity, respectively (Table 3). Wheat varieties represented different GP under normal and salt conditions, among which Koudasht and Bam varieties had a higher GP than the other varieties (Table 4). Inoculation of wheat varieties with PGPRs made a significant increase in GP comparing to non-inoculated plants. Application of WB1-7 resulted in a higher GP as compared to control and caused a significant increase in GP of Chamran, Dehdasht, Karim and Bam varieties, respectively. Besides, about 22% increase in GP was observed following treatment of Kouhdasht variety with PGU2-79 strain (Table 5).

WB1-7 increased GP by 56.2% under normal condition. Besides, GP was 5% and 34.4% higher following WBO-3 application at 3 and 6 dS/m, as compared to non-inoculated control (Table 3). As salinity increased, GR of all varieties decreased substantially. Bam variety had the highest GR under normal condition and salt stress (Table 4). Pre-treatment of wheat varieties with PGPRs caused an increase in GR comparing to non-inoculated control; however, varieties showed variations in GR after being inoculated with bacteria (Table 5). All bacteria caused a significant increase in GR of Kouhdasht and Bam varieties comparing to control, but no significant difference was observed between bacterial strains (Table 5).

Salt stress decreased radicle, coleoptile and seedling length of wheat seedlings, however, inoculation of seeds with PGPRs ameliorated salt stress and resulted in increase in these traits. Seed treatment with strain WB1-7 under normal conditions, with strain WBO-3 at 3 dS/m salinity and with strain WKZ1-93 at 6 dS/m promoted radicle, coleoptile and seedling length, respectively (Table 3). Under normal condition, Chamran and Kouhdasht had maximum radicle, coleoptile and seedling length comparing to other varieties. Bam and Kouhdasht had a longer radicle length in 3 and 6 dS/m, respectively. Chamran and Bam in 3dS/m and Dehdasht in 6 dS/m showed a longer coleoptile length comparing to other varieties (Table 3). Regarding seedling length, Koudasht, Bam and Dehdasht had the highest values at 0, 3 and 6 dS/m salinity, respectively (Table 4). Inoculation of wheat varieties with PGPRs increased radicle and coleoptile length. Inoculation of Chamran with Wkz1-93, Kouhdasht with WB1-7, Dehdasht with WB1-7, Karim with WBO-3 enhanced radicle, coleoptile and seedling length, respectively, comparing to uninoculated control (Table 5). In Bam variety, WB1-7 and WBO-3 strains, respectively, caused a 25.7% and 38.7% increase in radicle, coleoptile and seedling length (Table 5).

PGU2-79 and WB1-7 strains under normal condition, WB1-7 and WBO-3 at 3 dS/m and WB1-7 at 6 dS/m, had the

Table 1. ACC deaminase activity of bacterial strains.

Isolate	Optical density at 405 nm[a]		
	DF+$(NH4)_2SO_4$[b]	DF+ACC[c]	DF[d]
PGU2-79	3.045	1.735	0.289
WKZ1-93	2.948	1.834	0.347
WB1-7	3.031	1.721	0.293
WBO-3	3.044	1.818	0.338

[a]Optical density of bacterial strains grown in different media, [b]DF minimal medium amended with ammonium sulphate as a positive control, [c]DF minimal medium amended with ACC as a selective medium and [d]DF minimal medium as a negative control (DF).

Table 2. Mean squares resulted from analysis of variance for measured traits.

Source of variations	df	SV	GP	GR	RL	CL	SL	RDW	CDW	SDW	STI
Salinity	2	6768943.94**	2346.25**	1.90**	192.54**	245.47**	863.27**	74**	110**	720**	2.70**
Variety	4	626355.25**	4568.17**	3.62**	0.78**	0.43*	0.85^{ns}	9.5**	6.9**	130**	0.68**
Bacteria	4	966815.03**	2205.49**	1.79**	7.43**	14.33**	42.32**	7.9**	43**	340**	1.74**
Salinity × variety	8	81766.70**	133.39**	0.10*	3.91**	2.18**	9.93**	2.4**	1.3**	34**	0.39**
Salinity × bacteria	8	319494.01**	741.55**	0.58**	1.41**	3.45**	7.56**	0.92**	5.7**	43**	0.45**
Bacteria × Variety	16	24138.99*	64.51^{ns}	0.05*	0.94**	1.30**	3.74**	1.8**	2.4**	98**	0.16**
Bacteria × Variety × Salinity	32	39636.09**	69.30^{ns}	0.06*	0.92**	1.03**	2.66**	1.4**	1.5**	86**	0.15**
Error	150	11838.11	39.75	0.03	0.10	0.18	0.38	0.15	0.21	0.39	0.03
Coefficient of variations		12.80	8.92	9.01	5.95	6.70	5.23	6.89	6.84	5.08	17.77

*, **, ^{ns}, respectively, significant at 5 and 1% and non-significant. SV: Seed vigor, GP: Germination percentage, GR: Germination rate, RL: Radicle length, CL: Coleoptile length, SL: Seedling length, RDW: Radicle dry weight, CDW: Coleoptile dry weight, SDW: Seedling dry weight, STI: Salinity tolerance index.

Table 3. Mean comparison of seed germination and seedling traits of wheat under salt stress with or without bacterial inoculations.

Salt concentrations (dS/m)	Treatments	SV	GP	GR	RL (mm)	CL (mm)	SL (mm)	RDW (µg)	CDW (µg)	SDW (µg)	STI
0	Control	850.60 ± 20.05ef	62.66 ± 0.025e	1.78 ± 0.331e	6.06 ± 0.193ed	7.26 ± 0.197cde	13.26 ± 0.861d	5400 ± 332d	7100 ± 201c	12500 ± 483c	1 ± 0g
	Wkz1-93	1127.15 ± 16.12b	75.46 ± 0.073b	2.15 ± 0.301b	6.96 ± 0.178b	8 ± 0.157b	14.96 ± 2.592b	6500 ± 140ab	7800 ± 185ab	14400 ± 240b	1.09 ± 0.029efg
	PGU2-79	1003.34 ± 29.90cd	71.13 ± 0.081bc	2.03 ± 0.335cb	6.50 ± 0.186c	7.51 ± 0.199cd	14.02 ± 2.832c	6800 ± 163a	7800 ± 193ab	14500 ± 265a	1.04 ± 0.039fg
	WB1-7	1540.22 ± 30.38a	97.53 ± 0.078a	2.78 ± 0.347a	7.28 ± 0.155a	8.56 ± 0.210a	15.78 ± 2.717a	6500 ± 267ab	8100 ± 157a	14600 ± 367a	1.09 ± 0.017efg
	WBO-3	1096.43 ± 34.49c	74.19 ± 0.098b	2.12 ± 0.413b	6.73 ± 0.235c	7.96 ± 0.196b	14.70 ± 3.342b	6400 ± 207b	8000 ± 206a	14400 ± 352b	1.09 ± 0.041efg
3	Control	636.38 ± 18.62g	61.80 ± 0.089e	1.76 ± 0.325e	5.04 ± 0.309g	5.33 ± 0.178f	11.14 ± 3.133f	5100 ± 98e	4100 ± 170f	9200 ± 149d	1 ± 0g
	Wkz1-93	823.91 ± 28.18f	70.53 ± 0.115bc	2.01 ± 0.358cb	5.64 ± 0.208f	7.09 ± 0.179e	12.47 ± 4.020e	6000 ± 341c	7400 ± 313c	13400 ± 599c	1.84 ± 0.129a
	PGU2-79	1041.85 ± 23.81cb	75.60 ± 0.080b	2.11 ± 0.390b	6.14 ± 0.167d	7.59 ± 0.211c	13.80 ± 2.819c	6300 ± 183b	7200 ± 169c	13600 ± 246b	1.48 ± 0.032c
	WB1-7	929.08 ± 36.56ed	71.40 ± 0.092bc	2.04 ± 0.354cb	5.86 ± 0.229ef	7.22 ± 0.190de	12.64 ± 3.236ed	6400 ± 350b	7500 ± 453bc	13900 ± 632a	1.20 ± 0.051def
	WBO-3	1058.43 ± 32.29cb	74.33 ± 0.068b	2.12 ± 0.369b	6.24 ± 0.207d	7.92 ± 0.183b	14.07 ± 2.657c	5900 ± 293c	8100 ± 141a	14000 ± 284a	1.67 ± 0.094b
6	Control	350.94 ± 15.01i	53 ± 0.098f	1.51 ± 0.220f	2.91 ± 0.164k	3.72 ± 0.141h	6.63 ± 3.424j	4100 ± 181g	3500 ± 181g	7600 ± 329c	1 ± 0g
	Wkz1-93	602.58 ± 20.22gh	67.60 ± 0.107cd	1.93 ± 0.372cd	4.10 ± 0.180h	4.74 ± 0.209g	8.84 ± 3.753g	4300 ± 96fg	5600 ± 154e	10000 ± 201b	1.65 ± 0.095b
	PGU2-79	531.76 ± 19.47h	67.66 ± 0.108cd	1.93 ± 0.226cd	3.31 ± 0.128j	4.50 ± 0.109g	7.82 ± 3.806i	4300 ± 219fg	5700 ± 185de	10100 ± 347b	1.17 ± 0.023defg
	WB1-7	550.92 ± 23.56 h	65.46 ± 0.104de	1.87 ± 0.316de	3.64 ± 0.144i	4.59 ± 0.202g	8.23 ± 3.653hi	4800 ± 147e	5800 ± 309de	10700 ± 430a	1.26 ± 0.042de
	WBO-3	606.16 ± 15.29gh	71.33 ± 0.101bc	2.03 ± 0.233bc	3.98 ± 0.145h	4.42 ± 0.111g	8.45 ± 3.711hg	4600 ± 260f	6000 ± 194d	10600 ± 368a	1.29 ± 0.043de

Note: Data with different letters in the same column are significantly different according to Duncan's multiple range test ($p \leq 0.05$). SV: Seed vigor, GP: Germination percentage, GR: Germination rate, RL: Radicle length, CL: Coleoptile length, SL: Seedling length, RDW: Radicle dry weight, CDW: Coleoptile dry weight, SDW: Seedling dry weight, STI: Salinity tolerance index.

Table 4. Mean comparison of seed germination and seedling traits of wheat varieties under different salinity levels.

Salt concentration (dS/m)	Varieties	SV	GP	GR	RL (mm)	CL (mm)	SL (mm)	RDW (µg)	CDW (µg)	SDW (µg)	STI
0	Chamran	1014.44 ± 37.16c	67.33 ± 0.114ef	1.91 ± 0.272fg	7.10 ± 0.207a	8.13 ± 0.119a	15.10 ± 3.94a	6100 ± 117cd	7700 ± 220ab	13700 ± 319cd	1.07 ± 0.038de
	Kouhdasht	1266.83 ± 26.22a	83.32 ± 0.046a	2.38 ± 0.319ab	7.05 ± 0.194ab	8.06 ± 0.142a	15.12 ± 1.63a	6400 ± 135b	7700 ± 188ab	14100 ± 259bc	1.06 ± 0.032de
	Dehdasht	1048.91 ± 58.44bc	72.53 ± 0.124cd	2.07 ± 0.590de	6.21 ± 0.313c	7.57 ± 0.289b	13.78 ± 4.35b	6600 ± 376ab	7900 ± 212a	14600 ± 539a	1.08 ± 0.033de
	Karim	1016.40 ± 34.96c	73.13 ± 0.109cd	2.08 ± 0.290de	6.36 ± 0.127c	7.48 ± 0.175b	13.84 ± 3.81b	6700 a ± 202a	7900 ± 172a	14700 ± 271a	1.04 ± 0.031e
	Bam	1271.16 ± 26.09a	85 ± 0.064a	2.42 ± 0.323a	6.82 ± 0.147b	8.05 ± 0.201a	14.88 ± 2.26a	5700 ± 239ef	7500 ± 140b	13200 ± 274ed	1.05 ± 0.021ed
3	Chamran	752.36 ± 19.03e	56.53 ± 0.059h	1.61 ± 0.333i	5.72 ± 0.174e	7.46 ± 0.190b	13.18 ± 1.99c	4900 ± 366h	6400 ± 418cd	11400 ± 440g	1.17 ± 0.049cde
	Kohdasht	892.14 ± 21.72d	78.73 ± 0.052b	2.24 ± 0.325bc	5.33 ± 0.245f	6.85 ± 0.164c	12.18 ± 1.81e	6400 ± 198bc	6700 ± 371c	13100 ± 471e	1.45 ± 0.100b
	Dehdasht	812.50 ± 22.28e	64 ± 0.070f	1.82 ± 0.173g	5.83 ± 0.248e	6.80 ± 0.138c	12.63 ± 2.43d	6000 ± 103de	7400 ± 586b	13400 ± 530e	1.91 ± 0.179a
	Karim	920.82 ± 38.89d	70.40 ± 0.051de	2.01 ± 0.664ef	6.13 ± 0.423e	6.74 ± 0.268c	12.94 ± 1.80c	6800 ± 139a	7500 ± 434b	14300 ± 545ab	1.43 ± 0.104 b
	Bam	1111.83 ± 41.51b	84 ± 0.048a	2.35 ± 0.552ab	5.92 ± 0.429de	7.31 ± 0.173b	13.10 ± 1.70c	5700 ± 174f	6200 ± 381d	12000 ± 445f	1.23 ± 0.101cd
6	Chamran	325.85 ± 10.52i	49.26 ± 0.063i	1.40 ± 0.299j	2.60 ± 0.115i	3.96 ± 0.335g	6.57 ± 2.24i	4100 ± 201j	4600 ± 194f	8000 ± 185i	1.16 ± 0.046cde
	Kouhdasht	651.43 ± 20.79f	76.33 ± 0.051bc	2.18 ± 0.257cd	3.95 ± 0.222g	4.39 ± 0.157ef	8.41 ± 1.81fg	4200 ± 233j	5400 ± 362e	9700 ± 292h	1.41 ± 0.110b
	Dehdasht	561.43 ± 17.20gh	63 ± 0.092fg	1.80 ± 0.256gh	4 ± 0.145g	4.83 ± 0.167d	8.84 ± 3.23f	5300 ± 185g	6100 ± 400d	11400 ± 424g	1.34 ± 0.102bc
	Karim	496.26 ± 14.14h	59.13 ± 0.074gh	1.68 ± 0.243hi	3.70 ± 0.117h	4.66 ± 0.219de	8.31 ± 2.69g	4500 ± 217i	5400 ± 201e	10000 ± 193h	1.17 ± 0.055cde
	Bam	607.76 ± 11.00fg	77.33 ± 36bc	2.21 ± 0.288cd	3.69 ± 0.118h	4.06 ± 0.232fg	7.84 ± 1.27h	4000 ± 140j	5100 ± 299e	9100 ± 263i	1.28 ± 0.082bc

Notes: Data with different letters in the same column are significantly different according to Duncan's multiple range test ($p \leq 0.05$). SV: Seed vigor, GP: Germination percentage, GR: Germination rate, RL: Radicle length, CL: Coleoptile length, SL: Seedling length, RDW: Radicle dry weight, CDW: Coleoptile dry weight, SDW: Seedling dry weight, STI: Salinity tolerance index.

Table 5. Mean comparison of seed germination and seedling traits of wheat varieties inoculated with different *P. fluorescens* strains.

Variety	Bacteria	SV	GP	GR	RL (mm)	CL (mm)	SL (mm)	RDW (µg)	CDW (µg)	SDW (µg)	STI
Chamran	Control	491.55 ± 92.89l	46.33 ± 0.203j	1.32 ± 0.874j	4.54 ± 0.744i	5.87 ± 0.247h	10.31 ± 7.10h	4400 ± 174i	4900 ± 545j	9200 ± 540k	1 ± 0g
	Wkz1-93	684.08 ± 43.93k	54.77 ± 0.084i	1.56 ± 1.357i	5.41 0.653de	6.93 ± 0.724a	12.34 ± 2.92bcd	4400 ± 401i	6000 ± 387h	10400 ± 763j	1.26 ± 0.075cdef
	PGU2-79	713.87 ± 47.36jk	59 ± 0.078hi	1.68 ± 1.197hi	5.32 ± 0.628ef	6.46 ± 0.587defg	11.78 ± 2.75def	5800 ± 482cd	6800 ± 648fg	12400 ± 1065gh	1.13 ± 0.042fg
	WB1-7	811.68 ± 41.47ghij	65.55 ± 0.085fg	1.87 ± 1.064fgh	5.04 ± 0.504fg	6.37 ± 0.584gf	11.31 ± 3.03efg	5800 ± 387cd	6900 ± 616efg	12800 ± 856fgh	1.15 ± 0.043efg
	WBO-3	786.55 ± 49.27hijk	62.33 ± 0.063gh	1.78 ± 1.485gh	5.40 ± 0.751de	6.94 ± 0.736abc	12.34 ± 2.08dcd	4700 ± 375hi	6900 ± 362efg	11600 ± 596i	1.11 ± 0.72fg
Kouhdasht	Control	694.88 ± 93.92jk	67.77 ± 0.139efg	1.94 ± 1.371defg	4.67 ± 0.915hi	5.42 ± 0.501i	10.10 ± 3.99h	4800 ± 195hg	4800 ± 658j	9600 ± 832k	1 ± 0g
	Wkz1-93	901.30 ± 31.32efgh	83.33 ± 0.053ab	2.38 ± 0.625ab	5.37 ± 0.335de	6.77 ± 0.336bcdef	12.15 ± 1.88cd	5700 ± 437de	7100 ± 304def	12800 ± 725fgh	1.63 ± 0.164ab
	PGU2-79	1001.08 ± 44.85bcde	82.88 ± 0.058ab	2.36 ± 0.904ab	5.45 ± 0.457cde	6.54 ± 0.454cdefg	12 ± 2.04d	5400 ± 478ef	6800 ± 360fg	12200 ± 819ghi	1.41 ± 0.033cd
	WB1-7	1099.16 ± 41.55abc	83.33 ± 0.034ab	2.38 ± 0.940ab	6.02 ± 0.480a	6.98 ± 0.471abc	13.01 ± 1.17a	6100 ± 270abcd	6600 ± 151fg	12800 ± 318fgh	1.21 ± 0.046defg
	WBO-3	987.66 ± 52.82cde	79.98 ± 0.078bc	2.28 ± 1.196bc	5.70 ± 0.612abcd	6.45 ± 0.614efg	12.26 ± 2.76bcd	6300 ± 606ab	7800 ± 353bc	14100 ± 888bcd	1.28 ± 0.035cdef
Dehdasht	Control	567.34 ± 112.23l	53.44 l ± 0.202i	1.52 ± 1.289i	4.54 ± 0.839i	5.37 l ± 0.528i	9.92 ± 7.04h	5200 ± 323g	5200 ± 969j	9800 ± 1259k	1 ± 0g
	Wkz1-93	899.14 ± 38.00efgh	69.77 ± 0.086def	1.99 ± 0.671def	5.88 ± 0.365ab	6.91 ± 0.319abcde	12.80 ± 3.03abc	5800 ± 457de	7300 ± 352cde	13100 ± 758ef	1.83 ± 0.280a
	PGU2-79	727.02 ± 44.22jk	65.44 ± 0.105fg	1.87 ± 0.715fgh	4.82 ± 0.348hig	6.26 ± 0.372gh	11.08 ± 3.67g	6000 ± 207bcd	7300 ± 290cde	13400 ± 411ef	1.40 ± 0.019cde
	WB1-7	1038.11 ± 27.03bcd	74.44 ± 0.037cd	2.12 ± 0.656cd	6.03 ± 0.436a	7.18 ± 0.240ab	13.22 ± 1.30a	6500 ± 260a	8000 ± 322a	15100 ± 537a	1.18 ± 0.038defg
	WBO-3	805.83 ± 29.71ghij	69.44 ± 0.101def	1.98 ± 0.817def	5.46 ± 0.384cde	6.26 ± 0.435gh	11.73 ± 3.53efd	6400 ± 302a	7900 ± 333b	14300 ± 561b	1.82 ± 0.055a
Karim	Control	571.35 ± 88.20l	54.88 ± 0.212i	1.56 ± 0.756i	4.88 ± 0.581gh	5.24 ± 0.161i	10.13 ± 7.41h	5100 ± 173g	5400 ± 566i	10600 ± 577j	1 ± 0g
	Wkz1-93	778.10 ± 42.95hij	65.33 ± 0.088fg	1.86 ± 0.961fgh	5.52 ± 0.439cde	6.18 ± 0.525gh	11.71 ± 3.11defg	6400 ± 544ab	7800 ± 479bc	14200 ± 990bc	1.40 ± 0.114bc
	PGU2-79	863.27 ± 50.01fghi	67.22 ± 0.059efg	1.91 ± 1.260efg	5.68 ± 0.591abcd	6.85 ± 0.632abcde	12.65 ± 2.06abc	6400 ± 451ab	7100 ± 294def	13600 ± 724de	1.07 ± 0.035fg
	WB1-7	932.20 ± 27.41def	80.11 ± 0.065bc	2.29 ± 0.821bc	4.96 ± 0.399hg	6.22 ± 0.432gh	11.18 ± 2.27fg	6200 ± 446abc	7400 ± 568bcd	13700 ± 990cde	1.19 ± 0.055defg

	WBO-3	910.88 ± 49.51efg	70.22 ± 0.072def	2 ± 1.031def	5.92 ± 0.514ab	6.88 ± 0.528abcde	12.81 ± 3.19abc	5900 ± 440cd	7000 ± 418defg	12900 ± 826g	1.27 ± 0.036cdef
	Control	738.05 ± 83.42jk	73.33 ± 0.118de	2.09 ± 1.137de	4.71 ± 0.691hi	5.27 ± 0.450i	9.98 ± 4.14h	4700 ± 243hi	5200 ± 698j	9600 ± 696k	1 ± 0g
	Wkz1-93	993.55 ± 61.08cde	82.77 ± 0.079ab	2.36 ± 1.112ab	5.65 ± 0.628bcde	6.24 ± 0.492gh	11.90 ± 2.77de	5900 ± 450cd	6500 ± 379g	12400 ± 772gh	1.40 ± 0.116cde
Bam	PGU2-79	989.67 ± 58.79cde	82.77 ± 0.071ab	2.28 ± 1.143bc	5.32 ± 0.622ef	6.55 ± 0.524cdefg	11.87 ± 2.51de	5400 ± 385ef	6700 ± 172fg	12100 ± 525hi	1.07 ± 0.038fg
	WB1-7	1152.55 ± 56.08a	87.22 ± 0.053a	2.49 ± 0.933a	5.92 ± 0.580ab	7.18 ± 0.414ab	12.90 ± 1.86ab	4900 ± 202hi	5700 ± 338h	10900 ± 472j	1.19 ± 0.046defg
	WBO-3	1110.77 ± 73.58ab	84.44 ± 0.028ab	2.41 ± 1.518ab	5.78 ± 821abc	7.31 ± 0.700a	13.05 ± 1.00a	4800 ± 365hi	7300 ± 440cde	12200 ± 787ghi	1.28 ± 0.125cdef

Notes: Data with different letters in the same column are significantly different according to Duncan's multiple range test ($p \leq 0.05$). SV: Seed vigor, GP: Germination percentage, GR: Germination rate, RL: Radicle length, CL: Coleoptile length, SL: Seedling length, RDW: Radicle dry weight, CDW: Coleoptile dry weight, SDW: Seedling dry weight, STI: Salinity tolerance index.

Table 6. Correlation analysis of different traits of seed germination under salt inoculated with PGPR bacteria.

Characteristics	SV	GR	GP	RL	CL	SL	RDW	CDW	SDW	STI
SV	1									
GR	0.73^{**}	1								
GP	0.74^{**}	0.99^{**}	1							
RL	0.84^{**}	0.35^{**}	0.36^{**}	1						
ShL	0.86^{**}	0.34^{**}	0.34^{**}	0.95^{**}	1					
SL	0.74^{**}	0.34^{**}	0.35^{**}	0.94^{**}	0.81^{**}	1				
RDW	0.43^{**}	0.13^{*}	0.14^{**}	0.58^{**}	0.51^{**}	0.60^{**}	1			
ShDW	0.55^{**}	0.21^{**}	0.21^{**}	0.58^{**}	0.65^{**}	0.44^{**}	0.43^{**}	1		
SDW	0.59^{**}	0.21^{**}	0.21^{**}	0.68^{**}	0.69^{**}	0.60^{**}	0.79^{**}	0.88^{**}	1	
STI	0.008^{ns}	0.06^{ns}	0.05^{ns}	-0.03^{ns}	-0.01^{ns}	-0.05^{ns}	-0.03^{ns}	0.26^{**}	0.16^{*}	1

*, **, ^{ns}, respectively, significant at $p \leq 0.05$ and $p \leq 0.01$ and non-significant. SV: Seed vigor, GP: Germination percentage, GR: Germination rate, RL: Radicle length, CL: Coleoptile length, SL: Seedling length, RDW: Radicle dry weight, CDW: Coleoptile dry weight, SDW: Seedling dry weight, STI: Salinity tolerance index.

maximum impact on radicle and coleoptile weight, respectively (Table 3). The maximum increase in seedling dry weight under normal condition and 6dS/m (16.8 and 40.8%, respectively), was observed following inoculation with WB1-7; WBO-3 was the most effective strain in 3 dS/m (with 52.1% increase over control) (Table 3). Decrease in radicle and coleoptile dry weight was observed in varieties following increase in salinity levels; however, slight, non-significant increase was seen in Karim variety, as salinity increased from 0 to 3 dS/m (Table 4). In normal condition, Dehdasht and Karim, in 3dS/m, Karim and in 6 dS/m Dehdasht had the maximum radicle and coleoptile dry weight in comparison to other varieties. Besides, Karim (in 0 and 3 dS/m) and Dehdasht (in 6 dS/m) had the maximum seedling dry weight (Table 4). Inoculation of wheat varieties with PGPRs improved radicle and coleoptile dry weight. Treatment of Koudasht with WBO-3 and Dehdasht with WB1-7 caused maximum increase in radicle and coleoptile dry weight, respectively, comparing to uninoculated control (Table 5). Inoculation of Chamran variety with PGU2-79 and WB1-7, Karim with WKZ1-93 and PGU2-79 and Bam with KZ1-93 promoted radicle dry weight more significantly as compared to other treatments. Furthermore, inoculation of Chamran with WB1-7 and WBO-3, Karim with WKZ1-93 and Bam with WBO-3 increased coleoptile dry weight more effectively than the other treatments. Inoculation of Chamran and Dehdasht with WB1-7 and WBO-3, Kouhdasht with WBO-3, Karim with WKZ1-93 and PGU2-79 and Bam with PGU2-79 gave maximum seedling dry weight over untreated control (Table 5).

Salt tolerance index (STI) in plants

Analysis of variance showed that the main effects of salinity, variety and bacteria and their interactions were significant on salt tolerance index in plants (Table 2). Under normal condition, although all isolates increased salt tolerance index as compared to non-inoculated control, their effects were not significantly different. In 3 and 6 dS/m, WKZ1-93 had the greatest impact on STI and increased this index by 84% and 65%, respectively (Table 3). Under normal condition, no significant difference was observed between varieties. However, in 3 dS/m and 6dS/m, Dehdasht and Koudasht had the maximum STI, respectively (Table 4). Pretreatment of Chamran, Kouhdasht, Karim and Bam with WKZ1-93 resulted in a 26%, 63%, 40% and 40% increase in STI, respectively. Regarding Dehdasht variety, inoculation with Wkz1-93 and WBO-3 caused 83% increase in STI over uninoculated control (Table 5).

Simple correlation coefficients between traits

Results indicated that germination rate had positive and highly significant ($p \leq 0.01$) relationship with germination percentage ($r=0.99$) and seed vigor index ($r=0.73$). Correlation coefficients between germination percentage and seed vigor ($r=0.74$) was highly significant as well (Table 6).

Discussion

This study reveals the efficacy of ACC deaminase-producing *Pseudomonas fluorescens* strains in inducing salt tolerance, and hence improving germination attributed traits and early seedling growth of wheat varieties under salt stress.

Four *P. fluorescens* strains (PGU2-79, WBO-3, WB1-7 and WKZ1-93) with plant growth promoting (PGP) traits (Safari et al., 2016) were evaluated for ACC deaminase activity. It was revealed that they were all able to utilize ACC as sole nitrogen (N) source under *in vitro* conditions. Inoculation of various crops with rhizobacteria possessing ACC deaminase activity under salt stress causes a better root system, which consequently results in better shoot growth of developing seedlings (Glick et al., 1998). PGPRs with ACC deaminase activity assist plants to confront biotic or abiotic stresses by making decrease in stress ethylene level (Mayak et al., 2004). Initial stimulatory effects of PGPRs on plants at germination stage and following growth play a significant role in attaining a strong crop stand. In the current study, seeds inoculated with PGPR strains exhibited a substantial improvement in germination rate and percentage and seedling vigor index under salt stress ($p \leq 0.05$) over non-inoculated control. These results may be due to the enhanced biosynthesis of phytohormones in particular IAA and gibberellins (GA), which are involved in seed germination. It is suggested that auxin might assist in GA biosynthesis and thereby, regulate amylase activity which encourages early germination (Kim et al., 2006). Besides, during germination process, the enlargement of cortical cells of growing axis e.g., radicle, is related to GA biosynthesis (Feurtado and Kermode, 2007). In the current study, it is feasible that an increase in seed vigor is associated to high levels of auxin (IAA) synthesized by *Pseudomonas* strains which have triggered emergence of growing axis. It is documented that the significant increase in seed vigor is occurred by superior biosynthesis of growth hormones (Bharathi et al., 2004). Furthermore, it seems that the production of ACC deaminase by *Pseudomonas* strains in this study helps wheat seedlings to tolerate salinity conditions and improves germination attributed traits. It is documented that *P. fluorescens* TDK1 showing ACC deaminase activity significantly elevated groundnut vigor index and yield under salinity (Saravanakumar and Samiyappan, 2007).

Seed treatment with PGPR strains enhanced fresh and dry weight of radicle and coleoptile of wheat seedlings. It has been presumed that greater dry weight would mean longer and more powerful roots and shoots, and hence plants that would be able to combat salinity stress (Mayak et al., 2004). Application of bacterial strains with diverse PGP characteristics is believed to assist plants to have higher productivity. Increase in weight of the growing embryonic axis in the current study could be related to different embryo development resulted from bacterial growth regulators, which together with water, penetrate the seed coat speeding up root growth (Cassán et al., 2009). Phytohormone biosynthesis is the main cause of PGPR effects during very early growth stages that can change subsequent growth and metabolism of plants and increase water and mineral uptake (Bashan et al., 2004).

As seen in the current study, seed inoculation with ACC deaminase producing PGPRs assist plants to deal with many obstacles generated by environmental stresses (Bal et al., 2013). Similar to other studies (Bal et al., 2013; Mayak et al., 2004) inoculation with PGPR strains of *P. fluorescens* enhanced radicle and coleoptile length of wheat cultivars varieties to non-inoculated controls. It is revealed that there

is a significant positive relationship between the ability of bacteria to produce ACC deaminase and root elongation under salt stress (Bal et al., 2013; Glick et al., 1998). The impact of PGPRs at early growth stages of plant development is believed to be associated with production of critical growth substances (Bashan et al., 2004). It has also been proposed that ACC deaminase and IAA can coordinately stimulate growth of plant roots (Li and Glick, 2001).

The difference in plant growth promotion activity of bacterial strains used in this study may be attributed to their individual root colonization potential and utilization of ACC produced in roots. Furthermore, it seems that the difference between the compositions of root exudates of various wheat cultivars is involved in bacterial colonization levels. Host specificity of the bacterial strains has been proved even at cultivar level (Jamali et al., 2009). The host specialization is related to use of different combinations of carbon and nitrogen in root exudates and their impact on bacterial growth and population structure (Lemanceau et al., 1995).

Salt stress prevents plant growth through oxidative stress and an enhanced biosynthesis of ethylene, as well as homeostasis disruption in water status and ionic distribution (Shannon and Grieve, 2003; Tester and Davenport, 2003). In the present study, we observed higher STI in inoculated seedlings comparing to non-inoculated control under salt stress. Similar effects of PGPRs on salinity tolerance of plants have been reported by other researchers (Kang et al., 2014). High ethylene levels in plants grown under salt stress might be alleviated by ACC deaminase producing rhizobacteria (Mayak et al., 2004). The ameliorative influence of *Pseudomonas* strains under salt stress in this study might be due to their ability to produce ACC deaminase. In this context, growth promoting rhizobacteria can play a crucial role in increasing germination rate, and thus cause a significant impact on seedling strength under saline conditions.

Materials and Methods

Plant materials

Wheat (*Triticum aestivum* L.) seeds, varieties Chamran, Kooh-Dasht, Dehdasht, Karim and Jam were kindly obtained from Falat Agricultural Company, Iran.

Bacterial strains

Four *P. fluorescens* strains including PGU2-79, WBO-3, WKZ1-93 and WB1-7 with tolerance to NaCl induced stress (concentrations of 200-400 mM) and PGP traits including production of HCN, IAA and siderophore and the ability to solubilize mineral phosphate (Safari et al., 2016) were used in this study.

ACC deaminase activity assay

The bacterial potential to utilize ACC as N source is a consequence of ACC deaminase activity. The test was performed based on Dell'Amico et al. (2005) with slight modifications. Bacteria were grown on tryptic soy broth medium (TSB, Difco) for 48 hours. Thereafter, 50 µl of bacterial suspension was transferred to 20 ml of DF minimal medium and DF medium containing 3 mM ACC (Sigma-Aldrich, USA) instead of $(NH4)_2SO_4$ as N source. ACC solution (0.5 M) (Sigma-Aldrich) was filter-sterilized (0.2 µm), and frozen at -20 °C. The ACC solution was thawed and added to sterile DF medium before inoculation with strains. Cultures were incubated at 27 ºC on a rotary shaker at 120 rpm following inoculation with bacteria and after 48 h an optical density at 405 nm was determined separately for each suspension. The potential of a strain to utilize ACC was confirmed by inoculating the strain in tubes containing DF medium without any N source (as negative control), and incubating the tubes in the mentioned conditions for a week. The lack of growth verified the utilization of ACC as N source.

Influence of bacteria on seed germination and growth of wheat seedlings

Bacterial inoculum preparation

Wheat seeds were surface disinfected by 1% (v/v) sodium hypochlorite, following by 70% (v/v) ethanol for 10 min. Seeds were washed thoroughly with sterile distilled water to remove residual bleach and ethanol. *Pseudomonas* strains were cultivated in Luria Bertani-Broth (LB; Difco, Detroit, MI, USA) for 16 hours. Bacterial cells were collected by centrifugation at 10000g, washed with sterile saline solution (0.9%) and re-suspended with sterile double-distilled water to obtain 10^8 CFU/ml (A600nm = 0.125).

Treatment application and experimental design

Germination test under salt stress was carried out with 20 wheat seeds per petri plate (90 mm). Seeds were transferred to petri plates after being soaked for 1 hour in bacterial suspensions at room temperature with constant agitation, to let bacteria bind to the seed coat and for seed imbibitions (Patten and Glick, 2002). Each plate contained one autoclaved Whatman No.1 filter paper moistened with 10 ml of NaCl solution of 0% (distilled water as control), 0.18% and 0.36% to obtain an electrical conductivity (EC) at 0, 3, 6 dS/m. Three petri dishes were used as replications. Plates were partially sealed with parafilm to prevent water loss through evaporation. Petri plates were incubated in a growth chamber (Binder, USA) with maximum and minimum temperatures maintained at 20°C and 15 °C, respectively, with 12 h day-night photoperiod and relative humidity of 80%.

The experimental design was completely randomized in 3×5×5 factorial scheme with three replications. Each experimental unit was a petri plate consisted of 20 seeds. Factors consisted of seed inoculation with bacteria (strains PGU2-79, WBO-3, WKZ1-93 and WB1-7 and sterile water as control), salinity (0, 3 and 6 dS/m) and wheat varieties (Chamran, Kooh-Dasht, Dehdasht, Karim and Jam).

Estimation of seed germination attributed traits and seedling growth factors

Wheat germination was investigated for seven days by measuring radicle and coleoptile length, dry weight and fresh weight. Other characteristics such as germination rate and percentage and seed vigor index were evaluated as

following. Germination percentage was estimated using the formula (Ikić et al., 2012):

GP= (number of germinated seeds/total number of seeds) ×100

Germination rate (GR) is also calculated as follows:

$GR=\Sigma(G_t/D_t)$

where G_t is the number of germinated seed on day t and D_t is the number of days after seed treatment when the germinated seeds were counted (Kalsa and Abebie, 2012). Additionally, vigor index was calculated using formula:

$SV = (HL+RL) \cdot GP$

where SV is seed vigor index, RL is radicle length, HL is coleoptile length and GP is germination percentage (Abdul Baki and Anderson, 1973). Salinity tolerance index (STI) of plant was calculated using the formula:

STI=TWSs/TWSc

where TWSs and TWSc are coleoptile dry weight of seedlings under stress and coleoptile dry weight of control seedlings (Sopha et al., 1991).

Statistical analysis

Statistical analysis was performed using SAS software version 9.1 and MSTAT-C. The normality of different variables was tested using Shapiro-Wilk test. Pearson correlation test (95%) was used to assess the relations between traits. Mean of the treatments were separated using Dunkan's multiple range test at $p \leq 0.05$. The data are presented in the tables as the means and their standard error.

Conclusion

Regarding the present study, it is concluded that treatment of wheat seeds with ACC deaminase producing strains of *P. fluorescens* resulted in remarkable mitigation of NaCl induced stress and hence, increasing seed germination attributed traits and seedling growth of wheat varieties. The potential to produce ACC deaminase and presence of various PGP characteristics in these strains can be the possible reason to keep plants safe from the inhibitory influence of salt on growth and consequently induce tolerance to salt levels in wheat varieties. This can be helpful in many arid areas where salinity is the main obstacle. However, further study is demanded to examine the influence of these strains on amelioration of salt stress and improving plant growth under natural soil conditions.

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