Pak. J. Bot., 51(5): 1839-1846, 2019. DOI: http://dx.doi.org/10.30848/PJB2019-5(24)

MULTI-STRAIN BACTERIAL INOCULATION OF *ENTEROBACTER CLOACAE*, *SERRATIA FICARIA* AND *BURKHOLDERIA PHYTOFIRMANS* WITH FERTILIZERS FOR ENHANCING RESISTANCE IN WHEAT AGAINST SALINITY STRESS

MUHAMMAD ZAFAR-UL-HYE[1*], NIAZ MUHAMMAD HUSSAIN[1], SUBHAN DANISH[1], UMAR ASLAM[1] AND ZAHIR AHMAD ZAHIR[2]

[1]Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University Multan, Pakistan
[2]Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan
[*]Corresponding author's email: zafarulhyegondal@yahoo.com

Abstract

Development of improved crop production technologies having less or no harmful effects on environment, has become centre to mitigate salinity stress. Soil salinity hampers crops productivity by elevation of ethylene level in plants which can be decreased by activity of ACC deaminase. Despite reduction of salinity stress produced ethylene by inoculation of single strain ACC deaminase producing PGPR, multistrains inoculation with inorganic fertilizer could be more effective. So far, single-strain seed inoculation with ACC deaminase producing PGPR to improve crop growth under stress conditions has been widely investigated. However, the current study was conducted to examine the efficacious role of multi-strain PGPR inoculation in the presence of recommended mineral fertilizer (RNPKF) on wheat growth under artificially induced salinity stress. The multi-strain PGPR, *Enterobacter cloacae* (W6), *Serratia ficaria* (W10) and *Burkholderia phytofirmans* (PsJN) with mineral fertilizers significantly increased root (40%) and spike length (54%), root (65%) and shoot (63%) dry weight in wheat as compared to control and even single-strain inoculation. A significant improvement in straw (0.96-fold), economic (1.69-fold), biological yields (1.20-fold), phosphorus concentration in grain (1.43-fold) and shoot (1.75-fold) as compared to control validated the use of multi strain inoculation with mineral fertilizers, as an effective and environmentally safe technique to improve resistance to wheat plants against salinity.

Key words: ACC-deaminase containing PGPR, Growth attributes, Nutrition, Salinity, Yield.

Introduction

In arid and semi-arid areas of the world, one of the major agricultural problems is an excessive accumulation of soluble salts in upper soil horizons. Salinity is an important abiotic stress (Debez *et al.,* 2006; Koyro, 2006). Brackish irrigation water (Shakirova *et al.,* 2003), high temperature, less rain fall (Ahmad *et al.,* 2003), poor soil drainage and improper methods of irrigation are major causes of soil salinity development. More than 20% arid and semi-arid zone soils have already become saline (Mühling & Läuchli, 2003).

Seed germination, seedling growth, root development (Neumann, 1995), leaf expansion, leaf number (El-Hendawy *et al.,* 2005), flowering, and fruit setting are adversely affected due to salinity. The reasons behind, may be the osmotic shock, ionic imbalance and interruption in metabolic processes (Arora *et al.,* 2008). Salinity not only damages the crop yield but also deteriorates the quality of the produce. Most of the crops cultivated under saline conditions produce and store less carbohydrates and proteins in their body (Parida *et al.,* 2002).

High demand for food due to increasing human population especially in developing countries has made the cultivation of saline soils, a necessity of time by adopting low cost and easily affordable technologies. In near past, the research showed that PGPR inoculation improved the germination, seedling growth and fresh biomass of plants cultivated in saline soils (Afrasayab *et al.,* 2010). The PGPR are present in the plant rhizosphere, make interaction with the plant roots, that is complex in nature (Sylvia *et al.,* 1999). Some of the PGPR increase the plant growth through the biosynthesis of auxins and gibberellins as well as control pest attack by the production of 2,4-diacetylphloroglucinol and phenazine (Burkhead *et al.,* 1994; Shanahan *et al.,* 1992).

It is documented that an abiotic stress increases ethylene in plants. Some of the PGPR also produce an enzyme ACC-deaminase that reduces the elevated ethylene in plants. The ACC-deaminase actually converts the 1-aminocyclopropane-1-carboxylic acid (ACC, ethylene precursor) into ammonia and α-ketobutyrate instead of producing ethylene in the rhizosphere and consequently in plant roots (Hall *et al.,* 1996). A variety of rhizobacteria have been found to produce ACC-deaminase, which help in reducing the abiotic stress (Zafar-ul-Hye *et al.,* 2015). The reduction in ethylene (as lower as 10 μg L^{-1}) would likely to improve the growth of roots in plants under salt affected soils (Jalili *et al.,* 2009; Zafar-ul-Hye *et al.,* 2007, 2014).

In most part of the world, wheat (*Triticum aestivum* L.) is cultivated as a staple food and considered as king of cereals. Wheat grain is a rich source of carbohydrates and proteins (approx. 75%) with a minor quantity of fats (1-3%). Moreover, wheat grain is a good source of vitamin B, thiamin, riboflavin, niacin and vitamin E (Brigid, 2004).

Therefore, the current study was conducted with the objective to explore the role of multi-strains PGPR producing ACC-deaminase under various soil salinity levels on wheat (*T. aestivum* L.) growth and yield. Although the impact of PGPR on crops growth under saline conditions has previously been studied but we have used multi-strain inoculation of PGPR producing ACC-deaminase for wheat productivity at different salinity levels for the first time. The experiment was planned with the hypothesis that the multi-strain inoculation would grant the wheat plants with more resistance against salinity and consequently promote growth and productivity as compared to single-strain inoculation and/or uninoculated condition.

Material and Methods

Site of experiment: The research was conducted in the experimental area of Department of Soil Science, Bahauddin Zakariya University Multan, Pakistan. The climatic condition of research area was sub-tropical to semi-arid.

Pots preparation and characteristics of soil: Plastic pots were used in the experiment with the capacity of carrying 10 kg soil. In each pot having no hole for drainage, 8 kg of soil was filled. The pH and original EC of soil used were 8.7 and 3.0 dSm^{-1} respectively. The soil was sandy clay loam in texture determined with a hydrometer and USDA textural triangle (Moodie *et al.,* 1959). In each pot having 8 kg soil, the RNPKF were applied as 1.04 g urea, 2.0 g diammonium phosphate and 0.40 g sulphate of potash (Table 1).

Table 1. Physio-chemical soil characteristics.

Parameters	Values	Unit
Physical analysis		
Sand	50	%
Silt	28	%
Clay	22	%
Textural class	Sandy clay loam	
Chemical analysis		
pH_s	8.70	-
EC_e	3.00	dSm^{-1}
Total nitrogen	0.80	%
Olsen's phosphorus	7.23	$\mu g\ g^{-1}$
Extractable potassium	97.0	$\mu g\ g^{-1}$

Soil salinity induction: The original EC of the soil was 3 dSm^{-1}. Further, to introduce artificial salinity 2 levels of EC were maintained as 6 dSm^{-1} (6.81 g / 8 kg soil) and 9 dSm^{-1} (13.6 g / 8 kg soil) by mixing Na_2SO_4 salt.

Bacterial strains and seed inoculation: Three strains W6 (*Enterobacter cloacae*), W10 (*Serratia ficaria*), and PsJN (*Burkholderia phytofirmans*) of PGPR producing ACC deaminase were obtained from Soil Microbiology and Biochemistry Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan.

The composition of DF salt minimal medium (Dworkin & Foster, 1958) was as follows:

KH_2PO_4 = 4 g/L, Na_2PHO_4 = 6 g/L, $MgSO_4.7H_2O$ = 0.25 g/L, $FeSO_4.7H_2O$ = 1 mg/L, H_3BO_3 = 10 µg/L, $MnSO_4$ = 10 µg/L, $ZnSO_4$ = 70 µg/L, $CuSO_4$ = SO µg/L, MoO_3 = 10 µg/L, glucose = 10 g/L, gluconic acid = 2 g/L, citric acid = 2 g/L, distilled water = 1 L and ACC = 5 mM/0.66 g/L

The seeds were inoculated according to protocol stated by Sharma *et al*., (2003). Broth cultures were kept at 28 ± 1°C with shaking at 100 rpm for 72 h. The paste used for seed coating included 30% sugar solution with the respective broth cultures and sterilized clay and peat.

Treatments plan: The PGPR strains, W6, W10 and PsJN were used in different combinations with recommended NPK fertilizer (RNPKF). There were eight treatments (T) with 3 replications including: control + RNPKF, W6 + RNPKF, W10 + RNPKF, PsJN + RNPKF, W6 × W10 + RNPKF, W10 × PsJN + RNPKF, W6 × PsJN + RNPKF and W6 × W10 × PsJN + RNPKF applied under 3 levels of salinity (3, 6 and 9 dS m^{-1} EC) following factorial complete randomized design (CRD).

Seeds sowing and harvesting: In each pot, inoculated 20 seeds of *T. aestivum* L. (FSD – 2008) were sown manually. After germination 5 plants in each pot were kept, and harvested after 110 days of sowing.

Data recording and chemical analyses: Data regarding plant height, root and spike length, root and shoot dry weights, number of tillers per plant, number of spikelets per spike, number of grains per spike, economic yield, straw yield and biological yield was noted after harvesting. The digestion of plant and grain samples for nitrogen, phosphorus and potassium determination was done by wet digestion methods (Wolf, 1982). The Kjeldahl's apparatus was used for the determination of nitrogen content (Van Schouwenberg & Walinge, 1973). Yellow color method was used for phosphorus determination on spectrophotometer at 420 nm wavelength (Chapman & Pratt, 1961).

Statistical analyses

Standard statistical procedure was followed to analyze the data (Steel *et al.*, 1997) on SPSS 18.0 statistical software (SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago). Two-way analysis of variance test was applied to calculate significance of treatment. The LSD test was applied for differentiation of means at $p \leq 0.05$.

Results

Main effects of treatments (T) and various levels of salinity (S) were significant for root length but their interaction (T × S) remained non-significant. Application of the W6×W10×PsJN + RNPKF performed significantly better as compared to other treatments for root length (Table 2). The treatments, W6×W10 + RNPKF, W10 + RNPKF and W6×PsJN + RNPKF were statistically alike to each but differed significantly as compared to control for root length. The performance of W10×PsJN + RNPKF was statistically similar to that of W10 + RNPKF and W6×PsJN + RNPKF but remained significantly better as compared to PsJN + RNPKF, W6 + RNPKF and control for root length. Application of PsJN + RNPKF and W6 + RNPKF also remained significantly better as compared to the control for root length. Maximum increase in root length (40%) was noted as compared to the control where W6×W10×PsJN + RNPKF was applied. For plant height, main effect of S was significant. Main effect of T and interaction of T × S did not differ significantly for plant height. It was noted that plant height was significantly better at 3 dSm^{-1} soil

EC as compared to 6 and 9 dSm^{-1} soil EC. Similarly, the plant height was significantly better at 6 dSm^{-1} as compared to 9 dSm^{-1} soil EC. Maximum increase of 21% was noted in plant height at 3 dSm^{-1} EC as compared to 9 dSm^{-1}. In case of spike length, main effect of T and S remained significantly different but T × S did not differ significantly. Application of W6×W10 + RNPKF, W10×PsJN + RNPKF and W6×W10×PsJN + RNPKF performed in a better way among all the treatments for spike length. The treatments W6 + RNPKF, W10 + RNPKF and PsJN + RNPKF were statistically alike but remained significantly better as compared to control for spike length. Maximum increase of 54% in the spike length was noted in W6×W10×PsJN + RNPKF as compared to the control.

Main effects of T and S for root dry weight, shoot dry weight and 1000-grains weight were significantly different but T × S remained non-significant (Table 3). Application of W6×PsJN + RNPKF, W10×PsJN + RNPKF and W6×W10×PsJN + RNPKF remained significantly better as compared to the control for root dry weight. The treatments W6×W10 + RNPKF, PsJN + RNPKF, W10 + RNPKF and W6 + RNPKF were statistically alike but remained significantly better as compared to control for root dry weight. Maximum increase, 65% in root dry weight was noted where W6×W10×PsJN + RNPKF was applied. In case of shoot dry weight, application of W6×W10 + RNPKF, W10×PsJN + RNPKF and W6×W10×PsJN + RNPKF were significantly better as compared to control. It was noted that W6×PsJN + RNPKF, W6 + RNPKF and W10 + RNPKF also remained significantly better as compared to PsJN + RNPKF and the control for shoot dry weight. However, application of PsJN + RNPKF also differed significantly as compared to control for shoot dry weight. Maximum increase (63%) in shoot dry weight was noted with W6×W10×PsJN + RNPKF as compared to control. For 1000-grains weight, W6×W10 + RNPKF, W10×PsJN + RNPKF and W6×W10×PsJN + RNPKF remained statistically alike to each other but differed significantly as compared to control. It was noted that W10×PsJN + RNPKF, W6 + RNPKF and W6×PsJN + RNPKF were statistically alike to other but performed significantly better as compared to control for 1000-grain weight. No significant difference was noted among W10 + RNPKF and PsJN + RNPKF but the both differed significantly as compared to the control for 1000-grain weight. Maximum increase, 36% in 1000-grains weight was noted with W6×W10×PsJN + RNPKF as compared to the control.

Main effects of T and S were significantly different for straw, economic and biological yield while interactive effect of T and S was similar (Table 4). For straw, economic and biological yield, all the PGPR treatments differed significantly as compared to the control. Among all the treatments, application of W6×W10×PsJN + RNPKF remained significantly better for straw, economic and biological yield. In case of biological yield, W6×PsJN + RNPKF and W10×PsJN + RNPKF remained statistically similar to each but differed significantly as compared to the control. Maximum increase i.e. 0.96, 1.69 and 1.20-fold in straw, economic and biological yields was noted respectively where W6×W10×PsJN + RNPKF was applied as compared to the control.

Main effects of T and S remained significantly different for spikelets per spike and number of grains per spike while interaction of T and S remained non-significant. In case of number of tillers per plant both main and interactive effect of PGPR and S did not differ significantly (Table 5). The application of W6×W10×PsJN + RNPKF remained significantly better among all the treatments for spikelets per spike and number of grains per spike. The treatments W6×PsJN + RNPKF, W10×PsJN + RNPKF, W6×W10 + RNPKF, W10 + RNPKF and W6 + RNPKF remained statistically alike to each other but significantly different as compared to control for spikelets per spike. For number of grains per spike W6×PsJN + RNPKF and W10×PsJN + RNPKF were also statistically similar to each other but significantly better as compared to the control. Maximum increase, 56% and 58% in spikelets per spike and number of grains per spike was noted respectively as compared to control with W6×W10×PsJN + RNPKF.

Main effects of T and S were significantly different for nitrogen (grains) and phosphorus (grain and shoot) but interaction of T and S remained non-significant. For nitrogen in shoot, both main and interactive effects of PGPR and S remained non-significantly different (Table 6). All the PGPR treatments were statistically similar to each other but differed significantly as compared to the control for nitrogen concentration in grains. Maximum increase i.e., 67% in the grain nitrogen concentration was noted as compared to control where W6×W10×PsJN + RNPKF was applied. For phosphorus concentration in grains, W6×W10 + RNPKF, W6×PsJN + RNPKF, W10×PsJN + RNPKF and W6×W10×PsJN + RNPKF remained statistically alike to each other but remained significantly better as compared to the control. Application of W6 + RNPKF, W10 + RNPKF and PsJN + RNPKF also remained statistically alike and differed significantly as compared to the control. Among W6×W10 + RNPKF, W6×PsJN + RNPKF, W10×PsJN + RNPKF W10 + RNPKF and PsJN + RNPKF no significant difference was observed for phosphorus concentration in grains. However, W6×W10×PsJN + RNPKF and W6×W10 + RNPKF performed significantly better as compared to W6 + RNPKF. Maximum increase, 1.43-fold in grain phosphorus concentration was observed as compared to the control with W6×W10×PsJN + RNPKF. In case of phosphorus concentration in shoot, application of W6×PsJN + RNPKF, W10×PsJN + RNPKF and W6×W10×PsJN + RNPKF differ significantly as compared to control. The treatments PsJN + RNPKF, W6 + RNPKF, W6×W10 + RNPKF and W10 + RNPKF were statistically alike with each other but performed significantly better as compared to control for shoot phosphorus concentration. Maximum increase of 1.75-fold in shoot phosphorus concentration was noted as compared to the control where W6×W10×PsJN + RNPKF was applied.

Table 2. Effect of single and multi-strain PGPR inoculation with recommended NPK fertilizer (RNPKF) under various levels of salinity (S) on plant height, root length and spike length.

Treatments	Root length (cm)				Plant height (cm)				Spike length (cm)			
	Various levels of salinity (dS m^{-1})											
	*IE (T × S) (Means of 3 replicates)			$^{\alpha}$ME (T)	*IE (T × S) (Means of 3 replicates)			$^{\alpha}$ME (T)	*IE (T × S) (Means of 3 replicates)			$^{\alpha}$ME (T)
	3	6	9		3	6	9		3	6	9	
Control + RNPKF	8.86	7.97	7.18	8.00 ^{E}	59.2	54.8	49.2	54.4	6.06	6.00	5.41	5.82 ^{D}
W6 + RNPKF	10.1	8.77	8.07	9.04 ^{D}	72.3	63.1	59.1	64.8	8.80	7.86	7.06	7.90 ^{BC}
W10 + RNPKF	12.0	9.91	8.29	10.1 ^{BC}	68.9	64.3	59.1	64.1	8.26	7.73	6.73	7.57 ^{C}
PsJN + RNPKF	9.86	8.85	7.00	8.57 ^{D}	68.7	59.2	58.7	62.2	8.30	8.00	6.46	7.58 ^{C}
W6×W10 + RNPKF	12.0	10.0	9.31	10.4 ^{B}	67.9	63.7	57.7	63.1	10.6	8.53	7.60	8.91 ^{A}
W6×PsJN + RNPKF	11.0	10.0	9.33	10.1 ^{BC}	67.7	59.4	55.7	60.9	9.60	7.60	6.66	7.95 ^{B}
W10×PsJN + RNPKF	10.8	9.67	8.47	9.66 ^{C}	71.3	63.8	55.1	63.4	9.73	8.53	6.40	8.22 ^{AB}
W6×W10×PsJN + RNPKF	12.7	10.9	9.87	11.2 ^{A}	75.5	65.1	59.7	66.8	10.6	8.26	8.00	8.95 ^{A}
$^{\alpha}$ME (S)	10.9 ^{A}	9.52 ^{B}	8.44 ^{C}		68.9 ^{A}	61.7 ^{B}	56.8 ^{C}		8.99 ^{A}	7.81 ^{B}	6.80 ^{C}	

Means sharing different letters are significantly different ($p \leq 0.05$). Non-significant interactive effect (T × S) did not have any letter

$^{\alpha}$ ME indicates main effect; * IE indicates interactive effect; S indicates various levels of salinity; T indicates treatments

Table 3. Effect of single and multi-strain PGPR inoculation with recommended NPK fertilizer (RNPKF) under various levels of salinity (S) on shoot dry weight, root dry weight and 1000-grains weight.

Treatments	Root dry weight (g)				Shoot dry weight (g)				1000-grains weight (g)			
	Various levels of salinity (dS m^{-1})											
	*IE (T × S) (Means of 3 replicates)			$^{\alpha}$ME (T)	*IE (T × S) (Means of 3 replicates)			$^{\alpha}$ME (T)	*IE (T × S) (Means of 3 replicates)			$^{\alpha}$ME (T)
	3	6	9		3	6	9		3	6	9	
Control + RNPKF	0.69	0.35	0.25	0.43 ^{D}	0.61	0.58	0.54	0.57 ^{E}	27.6	23.0	21.0	23.9 ^{D}
W6 + RNPKF	0.91	0.44	0.35	0.56 ^{C}	0.96	0.81	0.63	0.80 ^{C}	30.3	25.3	23.3	26.3 ^{BC}
W10 + RNPKF	0.91	0.48	0.31	0.56 ^{C}	1.00	0.78	0.61	0.80 ^{C}	32.0	24.7	21.7	26.1 ^{C}
PsJN + RNPKF	0.98	0.49	0.34	0.60 ^{C}	0.95	0.84	0.58	0.79 ^{D}	32.0	28.3	24.3	28.2 ^{C}
W6×W10 + RNPKF	0.99	0.53	0.37	0.63 ^{BC}	1.07	0.90	0.71	0.89 ^{AB}	35.3	28.3	26.7	30.1 ^{A}
W6×PsJN + RNPKF	1.02	0.61	0.41	0.68 ^{AB}	1.06	0.85	0.64	0.85 ^{BC}	32.0	30.3	28.0	30.1 ^{B}
W10×PsJN + RNPKF	1.02	0.61	0.41	0.68 ^{AB}	1.05	0.88	0.69	0.87 ^{AB}	32.0	30.7	27.7	30.1 ^{AB}
W6×W10×PsJN + RNPKF	1.08	0.64	0.45	0.71 ^{A}	1.13	0.92	0.73	0.93 ^{A}	38.7	31.0	28.0	32.6 ^{A}
$^{\alpha}$ME (S)	0.95 ^{A}	0.51 ^{B}	0.36 ^{C}		0.98 ^{A}	0.82 ^{B}	0.64 ^{C}		32.5 ^{A}	27.7 ^{B}	25.1 ^{C}	

Means sharing different letters are significantly different ($p \leq 0.05$). Non-significant interactive effect (T × S) did not have any letter

$^{\alpha}$ ME indicates main effect; * IE indicates interactive effect; S indicates various levels of salinity; T indicates treatments

Table 4. Effect of single and multi-strain PGPR inoculation with recommended NPK fertilizer (RNPKF) under various levels of salinity (S) on straw, economic and biological yield.

Treatments	Straw yield (g pot^{-1})				Economic yield (g pot^{-1})				Biological yield (g pot^{-1})			
	Various levels of salinity (dS m^{-1})											
	*IE (T × S) (Means of 3 replicates)			$^{\alpha}$ME (T)	*IE (T × S) (Means of 3 replicates)			$^{\alpha}$ME (T)	*IE (T × S) (Means of 3 replicates)			$^{\alpha}$ME (T)
	3	6	9		3	6	9		3	6	9	
Control + RNPKF	31	27	23	27 ^{H}	17	13	9	13 ^{G}	48	40	32	40 ^{F}
W6 + RNPKF	41	37	33	37 ^{F}	24	20	16	20 ^{E}	65	57	49	57 ^{DE}
W10 + RNPKF	38	35	33	35 ^{G}	22	18	14	18 ^{F}	60	53	47	53 ^{E}
PsJN + RNPKF	43	39	35	39 ^{E}	26	23	19	22 ^{D}	69	62	54	62 ^{D}
W6×W10 + RNPKF	49	45	41	45 ^{D}	29	26	22	25 ^{C}	78	71	63	71 ^{C}
W6×PsJN + RNPKF	53	49	45	49 ^{B}	32	28	24	28 ^{B}	85	77	69	77 ^{B}
W10×PsJN + RNPKF	51	47	43	47 ^{C}	29	26	23	26 ^{C}	80	73	66	73 ^{BC}
W6×W10×PsJN + RNPKF	57	53	49	53 ^{A}	39	35	31	35 ^{A}	96	88	80	88 ^{A}
$^{\alpha}$ME (S)	45 ^{A}	41 ^{B}	37 ^{C}		27 ^{A}	24 ^{B}	20 ^{C}		73 ^{A}	65 ^{B}	58 ^{C}	

Means sharing different letters are significantly different ($P \leq 0.05$). Non-significant interactive effect (T × S) did not have any letter

$^{\alpha}$ ME indicates main effect; * IE indicates interactive effect; S indicates various levels of salinity; T indicates treatments

Table 5. Effect of single and multi-strain PGPR inoculation with recommended NPK fertilizer (RNPKF) under various levels of salinity (S) on number of tillers per plant, spikelets per spike and number of grains per spike.

Treatments	Number of tillers per plant				Spikelets per spike				Number of grains per spike			
	Various levels of salinity (dS m^{-1})											
	IE (T × S) (Means of 3 replicates)			$^{\alpha}$ME (T)	IE (T × S) (Means of 3 replicates)			$^{\alpha}$ME (T)	IE (T × S) (Means of 3 replicates)			$^{\alpha}$ME (T)
	3	6	9		3	6	9		3	6	9	
Control + RNPKF	3.67	3.33	3.00	3.33	9.73	8.33	7.33	8.46 ^{D}	14.3	14.7	12.3	13.7 ^{F}
W6 + RNPKF	4.33	4.33	4.33	4.33	12.6	11.9	10.4	11.6 ^{BC}	22.0	18.0	14.3	18.1 ^{CD}
W10 + RNPKF	4.67	4.33	4.33	4.44	12.9	12.2	9.89	11.7 ^{BC}	15.7	15.3	15.3	15.4 ^{E}
PsJN + RNPKF	4.33	4.33	4.00	4.22	12.3	11.0	10.7	11.3 ^{C}	18.7	15.0	14.3	16.0 ^{E}
W6×W10 + RNPKF	5.00	4.33	4.00	4.44	13.0	12.7	11.0	12.2 ^{B}	20.7	17.0	16.3	18.0 ^{D}
W6×PsJN + RNPKF	4.67	4.33	4.33	4.44	12.7	11.7	11.0	11.7 ^{BC}	22.7	19.0	18.3	19.2 ^{B}
W10×PsJN + RNPKF	4.33	4.33	4.00	4.22	13.0	11.7	11.0	11.9 ^{BC}	21.7	18.7	17.6	19.3 ^{BC}
W6×W10×PsJN + RNPKF	5.00	4.67	4.33	4.67	15.1	13.3	11.3	13.2 ^{A}	24.3	21.0	19.6	21.7 ^{A}
$^{\alpha}$ME (S)	4.50	4.25	4.04		12.7 ^{A}	11.6 ^{B}	10.3 ^{C}		19.2 ^{A}	17.3 ^{B}	16.0 ^{C}	

Means sharing different letters are significantly different ($p \leq 0.05$). Non-significant interactive effect (T × S) did not have any letter

$^{\alpha}$ ME indicates main effect; * IE indicates interactive effect; S indicates various levels of salinity; T indicates treatments

Table 6. Effect of single and multi-strain PGPR inoculation with recommended NPK fertilizer (RNPKF) under various levels of salinity (S) on nitrogen and phosphorus concentration (%) in grains and shoot.

Treatments	Nitrogen in grains (%)				Phosphorus in grains (%)			
	Various levels of salinity (dS m^{-1})							
	IE (T × S) (Means of 3 replicates)			αME (T)	IE (T × S) (Means of 3 replicates)			αME (T)
	3	6	9		3	6	9	
Control + RNPKF	1.70	1.54	1.36	1.53 ^{B}	0.33	0.28	0.22	0.28 ^{D}
W6 + RNPKF	2.66	2.30	1.94	2.30 ^{A}	0.70	0.55	0.44	0.56 ^{C}
W10 + RNPKF	2.60	2.44	1.80	2.28 ^{A}	0.74	0.60	0.40	0.58 ^{BC}
PsJN + RNPKF	2.62	2.60	1.90	2.37 ^{A}	0.72	0.57	0.42	0.57 ^{BC}
W6×W10 + RNPKF	2.76	2.50	2.16	2.47 ^{A}	0.83	0.64	0.49	0.65 ^{AB}
W6×PsJN + RNPKF	2.81	2.53	2.09	2.48 ^{A}	0.76	0.66	0.46	0.63 $^{A-C}$
W10×PsJN + RNPKF	2.72	2.48	2.18	2.46 ^{A}	0.79	0.62	0.47	0.63 $^{A-C}$
W6×W10×PsJN + RNPKF	2.84	2.61	2.24	2.56 ^{A}	0.85	0.67	0.52	0.68 ^{A}
αME (S)	2.59 ^{A}	2.38 ^{A}	1.96 ^{B}		0.72 ^{A}	0.57 ^{B}	0.43 ^{C}	
	Nitrogen in shoot (%)				Phosphorus in shoot (%)			
Control + RNPKF	1.32	1.21	1.16	1.23	0.19	0.16	0.13	0.16 ^{E}
W6 + RNPKF	1.75	1.61	1.35	1.57	0.46	0.36	0.21	0.34 ^{D}
W10 + RNPKF	1.80	1.58	1.38	1.59	0.48	0.32	0.24	0.35 ^{D}
PsJN + RNPKF	1.84	1.56	1.40	1.60	0.50	0.35	0.23	0.36 ^{CD}
W6×W10 + RNPKF	1.92	1.70	1.48	1.70	0.53	0.38	0.25	0.39 $^{B-D}$
W6×PsJN + RNPKF	1.94	1.62	1.49	1.68	0.54	0.41	0.28	0.41 ^{AB}
W10×PsJN + RNPKF	1.90	1.66	1.50	1.69	0.52	0.42	0.27	0.40 $^{A-C}$
W6×W10×PsJN + RNPKF	1.99	1.74	1.52	1.75	0.56	0.45	0.31	0.44 ^{A}
αME (S)	1.81	1.59	1.41		0.47 ^{A}	0.36 ^{B}	0.24 ^{C}	

Means sharing different letters are significantly different ($p \leq 0.05$). Non-significant interactive effect (T × S) did not have any letter
α ME indicates main effect; * IE indicates interactive effect; S indicates various levels of salinity; T indicates treatments

Discussion

Suppression of plant growth due to abiotic stresses, is a major hurdle in achieving the goal of improvement in crops yield (Glick *et al.,* 2007; Zafar *et al.,* 2018). Soil salinity is one of such abiotic stresses that elevates endogenous ethylene concentration in higher plants (Cuartero & Fernandez-Munoz, 1999; White & Broadley, 2001; Tester & Davenport, 2003; Munas & Tester, 2008). The elevated ethylene level in plant roots decreases root elongation (Penrose & Glick, 2001; Mayak *et al.,* 2004). Glick *et al.,* (1998), stated that the ACC deaminase cleaved the rhizosphere ethylene into ammonia (NH_3) and α-ketobutyrate and resultantly lowers the endogenous ACC concentration and then ethylene level (Glick *et al.,* 1998). In present study, the improvement in root and spike length of wheat under various levels of salinity (S) might be due to the reduction in ethylene accumulation by multi-strain PGPR inoculation. Significant improvement in growth and yield via single and multi-strains PGPR inoculation under abiotic stresses were frequently reported (Belimov *et al.,* 2002; Nadeem *et al.,* 2009; Naz *et al.,* 2013; Zafar-ul-Hye *et al.,* 2014; Kiani *et al.,* 2015). Higher concentration of salts, reduces the uptake of water and nutrients by disturbing ion transport and osmosis, which cause leaf discoloration and growth inhibition (Tester & Davenport, 2003). Mantelin & Touraine (2004) suggested PGPR symbiosis as an important factor that enhances root surface area and promote the growth of root tips due to which bioavailability of water and nutrients is improved. Contesto *et al.,* (2008) had documented 3% increase in the elongation of root hair in *Arabidopsis,* where consortia of PGPR were applied. According to Liu *et al.,* (2009) the growth and yield of crops might be enhanced due to uptake of essential nutrients. Under salinity stress, poor phytoavailibility of essential nutrients especially P is considered one of the growth limiting factors in plants (Borch *et al.,* 1999). Secrections of phosphatase enzyme by PGPR significantly enhanced P and essential nutrients mobilization and their availability to plants (Naveed *et al.,* 2008). Similarly, Panjebashi *et al.,* (2012) suggested the secretions of PGPR phytohormones as an allied factor that might improve N uptake. In connection with the above argument, a significant improvement in grain N and P concentration under salinity stress proved the efficacious role of multi-strain PGPR (W6 × W10 × PsJN) inoculation in the presence of RNPKF. Remans *et al.,* (2008) and Guiñazú *et al.,* (2010) documented a significant improvement in the uptake of N as a result of PGPR inoculation. Rokhzadi *et al.,* (2008) observed a significant increase in grain yield and biomass of chickpea where multi-strains of PGPR (*Mesorhizobium, Azotobacter, Pseudomonas and Azospirillum*) were applied as an inoculum. In agreement with above cited finding we observed a significant improvement in 1000-grain weight, spikelets per spike, number of grains per spike, straw, economical and biological yields of wheat where W6 × W10 × PsJN + RNPKF was applied under salinity stress.

Conclusion

The improvement in root length, plant height, spike length, straw, economic, biological yields, N and P concentration in grains and P concentration in shoot of wheat might be due to reduction in ethylene by ACC deaminase under salinity stress. Although, single inoculation is effective too when applied but multi-stains (W6×W10×PsJN) inoculation with RNPKF is suggested as more efficacious and effective approach to enhance the resistance in wheat plants against salinity.

References

Afrasayab, S., M. Faisal and S. Hasnain. 2010. Comparative study of wild and transformed salt tolerant bacterial strains on *Triticum aestivum* growth under salt stress. *Brazilian J. Microbiol.,* 41(4): 946-955.

Ahmad, R., S. Qadir, N. Ahmad and K.H. Shah. 2003. Yield potential and stability of nine wheat varieties under water stress conditions. *Int. J. Agri. Biol.,* 5(1): 7-9.

Arora, N., R. Bhardwaj, P. Sharma and H.K. Arora. 2008. Effects of 28-homobrassinolide on growth, lipid peroxidation and antioxidative enzyme activities in seedlings of *Zea mays* L. under salinity stress. *Acta Physiol. Plant.,* 30(6): 833-839.

Belimov, A.A., V.I. Safronova and T. Mimura. 2002. Response of spring rape (*Brassica napus* var. *oleifera* L.) to inoculation with plant growth promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase depends on nutrient status of the plant. *Can. J. Microbiol.,* 48(3): 189-199.

Borch, K., T.J. Bouma, J.P. Lynch and K.M. Brown. 1999. Ethylene: A regulator of root architectural responses to soil phosphorus availability. *Plant, Cell Environ.,* 22(4): 425-431.

Brigid, F. 2004. The Hungarian Status Law: Nation Building and/or Minority Protection. In: (Eds.): Kántor, Z., B. Majtényi, O. Ieda, B. Vizi and I. Halász. Hokkaido University, Japan.

Burd, G.I., D.G. Dixon and B.R. Glick. 1998. A plant growth-promoting bacterium that decreases nickel toxicity in seedlings. *Appl. Environ. Microbiol.,* 64(10): 3663-3668.

Burkhead, K.D., D.A. Schisler and P.J. Slininger. 1994. Pyrrolnitrin production by biological control agent *Pseudomonas cepacia* B37w in culture and in colonized wounds of potatoes. *Appl. Environ. Microbiol.,* 60(6): 2031-2039.

Chapman, H.D. and P.F. Pratt. 1961. Methods of analysis for soils, plants and water. Berkeley, CA, USA.

Contesto, C., G. Desbrosses, C. Lefoulon, G. Béna, F. Borel, M. Galland, L. Gamet, F. Varoquaux and B. Touraine. 2008. Effects of rhizobacterial ACC deaminase activity on Arabidopsis indicate that ethylene mediates local root responses to plant growth-promoting rhizobacteria. *Plant Sci.,* 175(1-2): 178-189.

Cuartero, J. and R. Fernandez-Munoz. 1999. Tomato and salinity. *Sci. Hort., (Amsterdam)*. 78(1-4): 83-125.

Debez, A., D. Saadaoui, B. Ramani, Z. Ouerghi, H.W. Koyro, B. Huchzermeyer and C. Abdelly. 2006. Leaf H+-ATPase activity and photosynthetic capacity of Cakile maritima under increasing salinity. *Environ. Exp. Bot.,* 57(3): 285-295.

Dworkin, M. and J.W. Foster. 1958. Experiments with some microorganisms which utilize ethane and hydrogen. *J. Bacteriol.,* 75(5): 592-603.

El-Hendawy, S.E., Y. Hu, G.M. Yakout, A.M. Awad, S.E. Hafiz and U. Schmidhalter. 2005. Evaluating salt tolerance of wheat genotypes using multiple parameters. *Eur. J. Agron.,* 22(3): 243-253.

Glick, B., D. Penrose and J. Li. 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *J. Theor. Biol.,* 190(1): 63-68.

Glick, B.R., Z. Cheng, J. Czarny and J. Duan. 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. In: *New Perspectives and Approaches in Plant Growth-Promoting Rhizobacteria Research*, pp. 329-339.

Guiñazú, L.B., J.A. Andrés, M.F.D. Papa, M. Pistorio and S.B. Rosas. 2010. Response of alfalfa (*Medicago sativa* L.) to single and mixed inoculation with phosphate-solubilizing bacteria and *Sinorhizobium meliloti*. *Biol. Fertil. Soils,* 46(2): 185-190.

Hall, J.A., D. Peirson, S. Ghosh and B.R. Glick. 1996. Root elongation in various agronomic crops by the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. *Isr. J. Plant Sci.,* 44(1): 37-42.

Jalili, F., K. Khavazi, E. Pazira, A. Nejati, H.A. Rahmani, H.R. Sadaghiani and M. Miransari. 2009. Isolation and characterization of ACC deaminase-producing fluorescent pseudomonads, to alleviate salinity stress on canola (*Brassica napus* L.) growth. *J. Plant Physiol.,* 166(6): 667-674.

Kiani, M.Z., A. Ali, T. Sultan, R. Ahmad and S.I. Hydar. 2015. Plant growth promoting rhizobacteria having 1-aminocyclopropane-1-carboxylic acid deaminase to induce salt tolerance in sunflower (*Helianthus annus* L.). *Nat. Resour.,* 15(6): 391-397.

Koyro, H.W. 2006. Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.). *Environ. Exp. Bot.,* 56(2): 136-146.

Liu, W., L. Du and Q. Yang. 2009. Biogas slurry added amino acids decreased nitrate concentrations of lettuce in sand culture. *Acta Agric. Scand. Sect. B. Soil Plant Sci.,* 59(3): 260-264.

Mantelin, S. and B. Touraine. 2004. Plant growth-promoting bacteria and nitrate availability: Impacts on root development and nitrate uptake. *J. Exp. Bot.,* 55(394): 27-34.

Mayak, S., T. Tirosh and B.R. Glick. 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem.,* 42(6): 565-572.

Moodie, C.D., H.W. Smith and R.A.M. Creery. 1959. Laboratory Manual for Soil Fertility. Dept. of Agron. State college of Washington Pullman, Washington, USA.

Mühling, K.H. and A. Läuchli. 2003. Interaction of NaCl and Cd stress on compartmentation pattern of cations, antioxidant enzymes and proteins in leaves of two wheat genotypes differing in salt tolerance. *Plant Soil,* 253(1): 219-231.

Munas, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.,* 59: 651-681.

Nadeem, S.M., Z.A. Zahir, M. Naveed and M. Arshad. 2009. Rhizobacteria containing ACC-deaminase confer salt tolerance in maize grown on salt-affected fields. *Can. J. Microbiol.,* 55(11): 1302-1309.

Naveed, M., Z.A. Zahir, M. Khalid, H.N. Asghar, M.J. Akhtar and M. Arshad. 2008. Rhizobacteria containing ACC-deaminase for improving growth and yield of wheat under fertilized conditions. *Pak. J. Bot.,* 40(3): 1231-1241.

Naz, I., A. Rehim, M. Zafar-ul-Hye, Z.A. Zahir, M. Abid, M.A. Ali and M. Hussain. 2013. Effectiveness of ACC-deaminase containing *Pseudomonas* strains to induce salinity tolerance in maize under fertilized and unfertilized field conditions. *Soil Environ.,* 32(2): 167-172.

Neumann, P.M. 1995. The role of cell wall adjustments in plant resistance to water deficits. *Crop Sci.,* 35(5): 1258-1266.

Panjebashi, M., M.R.H.S. Hadi and M.T. Darzi. 2012. Effects of the Rhizobium and PGPRs bacterium on seed yield and yield components in chickpea (*Cicer arietinum*). *Int. J. Agron. Plant Prod*., 3: 651-655.

Parida, A., A.B. Das and P. Das. 2002. NaCl stress causes changes in photosynthetic pigments, proteins, and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. *J. Plant Biol.,* 45(1): 28-36.

Penrose, D.M. and B.R. Glick. 2001. Levels of ACC and related compounds in exudate and extracts of canola seeds treated with ACC deaminase-containing plant growth-promoting bacteria. *Can. J. Microbiol.,* 47(4): 368-372.

Remans, R., L. Ramaekers, S. Schelkens, G. Hernandez, A. Garcia, J.L. Reyes, N. Mendez, V. Toscano, M. Mulling, L. Galvez and J. Vanderleyden. 2008. Effect of Rhizobium-Azospirillum coinoculation on nitrogen fixation and yield of two contrasting *Phaseolus vulgaris* L. genotypes cultivated across different environments in Cuba. *Plant Soil,* 312(1-2): 25-37.

Rokhzadi, A., A. Asgharzadeh, F. Darvish, G. Nour-Mohammadi and E. Majidi. 2008. Influence of plant growth-promoting rhizobacteria on dry matter accumulation and yield of chickpea (*Cicer arietinum* L.) under field conditions. *Am. J. Agric. Environ. Sci.,* 3(2): 253-257.

Shakirova, F.M., A.R. Sakhabutdinova, M. V. Bezrukova, R.A. Fatkhutdinova and D.R. Fatkhutdinova. 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci.,* 164(3): 317-322.

Shanahan, P., D.J. O'Sullivan, P. Simpson, J.D. Glennon and F. O'Gara. 1992. Isolation of 2,4-diacetylphloroglucinol from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. *Appl. Environ. Microbiol.,* 58(1): 353-358.

Sharma, A., B.N. Johri, A.K. Sharma and B.R. Glick. 2003. Plant growth-promoting bacterium *Pseudomonas* sp. strain GRP3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck). *Soil Biol. Biochem*., 35(7): 887-894.

Steel, R.G., J.H.Torrie and D.A. Dickey. 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd ed. McGraw Hill Book International Co., Singapore.

Sylvia, D.M., J.J. Fuhrmann, P.G. Hartel and D.A. Zuberer. 1999. Principles and applications of soil microbiology. Prentice Hall Inc, Upper Saddle River.

Tester, M. and R. Davenport. 2003. Na+ tolerance and Na+ transport in higher plants. *Ann. Bot.,* 91(5): 503-527.

Van Schouwenberg, J.C. and I. Walinge. 1973. Methods of Analysis for Plant Material. Agricultural University Wageningen, Wageningen, The Netherlands.

White, P.J. and M.R. Broadley. 2001. Chloride in soils and its uptake and movement within the plant: A review. *Ann. Bot.,* 88(6): 967-988.

Wolf, B. 1982. A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. *Commun. Soil Sci. Plant Anal.,* 13(12): 1035-1059.

Zafar-ul-Hye, M., A. Shahjahan, S. Danish, M. Abid and M.F. Qayyum. 2015. Mitigation of cadmium toxicity induced stress in wheat by ACC-deaminase containing PGPR isolated from cadmium polluted wheat rhizosphere. *Pak. J. Bot.,* 50(5): 1727-1734.

Zafar-ul-Hye, M., A. Shahjahan, S. Danish, M. Abid and M.F. Qayyum. 2018. Mitigation of cadmium toxicity induced stress in wheat by ACC-deaminase containing PGPR isolated from cadmium polluted wheat rhizosphere. *Pak. J. Bot.,* 50(5): 1727-1734.

Zafar-ul-Hye, M., H.M. Farooq, Z.A. Zahir, M. Hussain and A. Hussain. 2014. Application of ACC-deaminase containing rhizobacteria with fertilizer improves maize production under drought and salinity stress. *Int. J. Agri. Biol.* 16(3): 591-596.

Zafar-ul-Hye, M., M.H. Farooq and M. Hussain. 2015. Bacteria in combination with fertilizers promote root and shoot growth of maize in saline-sodic soil. *Brazilian J. Microbiol.,* 46(1): 97-102.

Zafar-ul-Hye, M., Z.A. Zahir, S.M. Shahzad, M. Naveed, M. Arshad and M. Khalid. 2007. Preliminary screening of rhizobacteria containing ACC-deaminase for promoting growth of lentil seedlings under axenic condition. *Pak. J. Bot.,* 39(5): 1725-1738.

Zhu, J.K., P.M. Hasegawa, R.A. Bressan and H.J. Bohnert. 1997. Molecular aspects of osmotic stress in plants. CRC. *Crit. Rev. Plant Sci*., 16(3): 253-277.

(Received for publication 16 April 2018)