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ORIGINAL ARTICLE

Mitigation of salinity-induced negative impact on the growth and yield of wheat by plant growth-promoting rhizobacteria in naturally saline conditions

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Abstract Salinity is one of the major environmental threats for successful crop production, hampering plant growth due to the osmotic effect and nutritional and hormonal imbalances. The application of naturally occurring plant growth-promoting rhizobacteria (PGPR) is an emerging technology aimed at ameliorating the negative impact of salinity. However, the results obtained in the laboratory can sometimes not be reproduced in the field. The aim of the study reported here was to evaluate the effect of PGPR inoculation on seed germination in a saline environment under axenic conditions and on enhancement of the growth and yield of wheat under natural salt-affected field conditions. Wheat seeds were inoculated with pre-isolated strains of *Pseudomonas putida*, *Enterobacter cloacae*, *Serratia ficaria*, and *Pseudomonas fluorescens* and sown at different salinity levels (1, 2, 3, 6, 9, 12, 15 dS m^{-1}). Inoculation with these strains was found to enhance the germination percentage, germination rate, and index of wheat seeds up to 43, 51, and 123 %, respectively, over the uninoculated control at the highest salinity level. The potential of these PGPR for improving the growth and yield of wheat was also evaluated at two natural salt-affected sites. Inoculation with PGPR resulted a significant increase in the growth and yield parameters of wheat at both sites. The inoculated plants also improved the nutrient status of the wheat plants. The inoculated plants had low sodium and high nitrogen, phosphorus, and potassium contents. Our results show that such rhizobacterial strains may be used as an effective tool for enhancing plant growth under salinity stress and for maximizing the utilization of salt-affected soils.

Keywords Plant growth-promoting rhizobacteria · Salt stress · Germination · Growth · Wheat

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Introduction

In arid and semi-arid regions with low rainfall and high temperature, salinity is one of the major environmental stresses which reduces plant growth. In these regions, groundwater continuously moves towards the cultivation layer (Li et al. 2003), and the concentration of soluble salts increases due to the recycling of poor quality drainage water for irrigation (Shakirova et al. 2003). A high salt concentration inhibits plant growth (Cuartero and Fernandez-Munoz 1999) and affects many aspects of plant metabolism, resulting in reduced growth and yield (Tank and Saraf 2010). Under salinity stress, Na^+ uptake increases, which may cause metabolic disturbance in those processes requiring low Na^+ and high K^+ and/or Ca^{+2} for normal function (Marschner 1995). Similarly, the uptake and accumulation of Cl^- may disrupt photosynthesis (Xu et al. 2000), and decreases in leaf production and leaf size reduces the expansion of leaf area, leading to the death of the plant (Suarez and Medina 2005).

On an overall basis, plant growth in a saline environment is affected by a complex interaction of osmotic effect, hormonal imbalance, specific ion toxicity, and mineral imbalance (Gorham et al. 1985; Ashraf 1994; Ruiz et al. 1995; Arbona et al. 2005). Among the various plant hormones, enhanced ethylene production has been associated with

increased salinity in different crops at different growth stages (Jones and El-Abd 1989; Arbona et al. 2005). Ethylene is considered to be a stress hormone, and its concentration increases under salinity stress via elevated levels of its precursor 1-aminocyclopropane-1-carboxylic acid (ACC), potentially leading to physiological changes in leaf tissue (Tank and Saraf 2010). Rapid seed germination is a critical factor for crop production, and early seedling growth is the developmental stage that is most sensitive to salinity stress (Ashraf and Foolad 2005). The significant decrease in wheat germination caused by salt stress (Ashraf and O'Leary 1997) might be associated to elevated levels of salinity reducing seed germination and causing poor root growth (Mattoo and Suttle 1991; Sarquis et al. 1991; Zapata et al. 2007). Studies by Heidstra et al. (1997) and Ma et al. (2003) have also shown that ethylene inhibits root growth and causes senescence in crop plants. To improve the germination and growth of plants in the presence of salts, it is necessary to limit ethylene production.

Chemical inhibitors of ethylene synthesis, such as cobalt ions (Co^{2+}) and aminoethoxyvinyle glycine (AVG), are often used to overcome the problems associated with salt stress. However, these chemicals are not only expensive, but they have harmful effects on the environment (Dodd and Belimov 2009). During recent years, a new biocontrol approach has been developed to mitigate the impact of higher ethylene concentrations on plant growth. This approach includes the use of plant growth-promoting rhizobacteria (PGPR) containing ACC-deaminase that can hydrolyze the ACC to reduce ethylene production (Mayak et al. 2004) and therefore facilitate plant growth under the salinity stress condition (Nadeem et al. 2010; Siddikee et al. 2010). These PGPR also facilitate plant growth by various direct and indirect mechanisms, such as nitrogen fixation, phosphorus solubilization, synthesis of siderophores, phytohormone production, and protection against pathogens (Kloepper et al. 1989; Glick et al. 1999; Saharan and Nehra 2011).

Although a number of studies conducted under laboratory and field conditions have shown that PGPR treatments can enhance plant growth (Abbaspoor et al. 2009; Zabihi et al. 2010; Siddikee et al. 2011), most of these studies were conducted under normal field conditions. Additionally, in certain cases the results obtained in the laboratory could not be reproduced in the field (Zhender et al. 1999; Smyth et al. 2011). Studies by Ahmad et al. (2011a, b) performed under salinity stress conditions demonstrated that the test strains performed differently under axenic and field conditions, possibly due to the low quality of the inoculums and/or the inability of the bacteria to compete with the indigenous population under adverse environmental conditions (Brockwell and Bottomley 1995; Catroux et al. 2001). Therefore, the selection of efficient strains of rhizobacteria with the ability to tolerate adverse field conditions could prove to be an efficient additive to biofertilizers. Only a few studies have evaluated the performance of PGPR in enhancing plant growth and development under natural salt-affected conditions. Thus, the aim of the study reported here was to evaluate the effectiveness of preselected rhizobacteria containing ACC-deaminase (Nadeem et al. 2010) for enhancing seed germination and improving the growth and yield of wheat cultivated on natural salt-affected soils. The efficient strains identified here can be considered as potential additives to biofertilizer for use in sustainable agriculture.

Materials and Methods

The strains used in this study had been screened for their growth-promoting activity in the presence of salinity in previous axenic and pot trials (Nadeem et al. 2010). The four strains showing the most prolific growth in these trials were identified as *Pseudomonas putida* (W2), *Enterobacter cloacae* (W6), *Serratia ficaria* (W10), and *Pseudomonas fluorescens* (W17) and selected for use in our field trials.

Preparation of inoculum

For inoculum preparation, DF medium without ACC was sterilized in 250-mL flasks at 121 °C for 20 min, following which a 3-mL aliquot of heat-labile, filter-sterilized (membrane pore size 0.2 mm) ACC was added to the cooled DF medium. After each flask was inoculated with one of the PGPR test strains, the broth was incubated at 28±1° C for 48 h in an orbital shaking incubator at 100 rpm. In order to obtain a uniform population of rhizobacteria (10^8–10^9 colony-forming units mL^{-1}), we adjusted the optical density of the broth to 0.5 at 535 nm using a turbidity meter (OD meter; model 21907; Biolog, Haywood, CA).

Germination assay

For evaluating the effect of PGPR stains on the germination of wheat seeds in the presence of different salinity levels, we first selected uniformly sized wheat seeds showing no signs of damage. The seeds were surface sterilized by a very short immersion in 95 % ethanol solution followed by a 3-min immersion in a 0.2 % $HgCl_2$ solution, and then washed thoroughly with sterilized distilled water to remove all remaining $HgCl_2$. The germination test was performed in sterilized petri dishes. Six salinity levels (1, 3, 6, 9, 12, and 15 dS m^{-1}) were obtained by dissolving a specified amount of NaCl in distilled water. The surface-sterilized seeds were first dipped in liquid broth (inoculum prepared in DF salt minimal medium with no agar) for 10 min and then sown in a petri dish (15 seeds per plate) containing cotton moistened with water containing the desired salt concentration (Rueda-Puente

et al. 2007). Seeds dipped in broth not containing a PGPR strain and then sown under the same conditions as the inoculated seeds served as the un-inoculated control. The petri dishes were placed in an incubator at 28±2 °C. Germination was observed daily according to the method described in by the Association of Official Seed Analysts (AOSA 2004). A seed was scored as germinated when the coleoptile and root reached lengths of 2–3 mm. The final germination percentage was determined after 7 days. The germination rate was calculated according to the formula described by Maguire (1962), and the germination index was calculated according to AOSA (1983).

Field trials

The performance of PGPR stains containing ACC-deaminase activity were evaluated under natural salt-affected conditions during field experiments conducted at the Postgraduate Agriculture Research Station (PARS), University of Agriculture, Faisalabad, Pakistan. The experiments were conducted at two different locations with different levels of soil salinity. Soil samples were collected, air dried, mixed thoroughly, sieved (pore size 2.0 mm), and analyzed for physico-chemical characteristics (Table 1).

For inoculation, the inoculum (prepared as described earlier) was mixed with sterilized peat. Wheat seed dressing was done using inoculated peat mixed with a 10 % sterilized sugar (sucrose) solution. In the case of the un-inoculated control, seeds were coated with sterilized peat treated with sterilized broth only and the 10 % sterilized sugar solution. The experiment was laid out according to randomized complete block design (RCBD) with a plot size of 5 × 2 m and three replications of each condition.

Table 1 Physico-chemical characteristics of soils analyzed in the field trials

Parameter	Unit	Site I	Site II
Textural class		Sandy clay, loam	Sandy clay, loam
Saturation percentage	%	31	34
ECe	dS m^{-1}	11.8	14.2
Na^+	m mol_c L^{-1}	82.4	98.2
K^+	m mol_c L^{-1}	3.0	3.6
$Ca^{2+} + Mg^{2+}$	m mol_c L^{-1}	29.6	45.2
CO_3^{2-}	m mol_c L^{-1}	-	2.0
HCO_3^-	m mol_c L^{-1}	10.5	13.3
Cl^-	m mol_c L^{-1}	48	58.5
SO_4^{2-}	m mol_c L^{-1}	60	69.0
SAR	(m mol $L^{-1})^{½}$	16.6	20.6
Organic matter	%	0.81	0.75
Cation exchange capacity	$Cmol_c$ kg^{-1}	4.65	5.01

ECe, Electrical conductivity of the saturation extract; SAR, sodium absorption rate

Nutrients were supplied through the application of the recommended dose of NPK fertilizers (120:90:60 kg ha^{-1}) in the form of urea, diammonium phosphate, and murate of potash, respectively. Phosphorus and potassium were applied as a basal dose, while nitrogen was applied in splits, i.e., at the tillering, booting stage, and grain filling stages.

Plants were irrigated with good quality canal water that meting the irrigation quality criteria for crops, i.e., an electrical conductivity of 0.05 dS m^{-1}, a sodium adsorption ratio of 0.1 [(mmol $L^{-1})^{½}$], and a residual sodium carbonate content of 0.02 meq L^{-1} (Ayers and Westcot 1985). At plant maturity, data on growth and yield-contributing parameters were recorded following standard procedures. Data were collected from a 1-m^2 area randomly selected in each plot. Total nitrogen, phosphorus, and potassium uptake was calculated after measuring their contents in plant tissues according to the methods described by Ryan et al. (2001). Briefly, the plant samples were first digested with concentrated H_2SO_4 and H_2O_2 according to the method of Wolf (1982). A 5-mL aliquot of the digested plant tissue was placed in a Kjeldhal flask, and the nitrogen content was determined by the Kjeldhal method. For the phosphorus determination, a 5-mL aliquot of the digested plant tissue was mixed in 10 mL of Barton reagents, and samples were allowed to stand for 30 min, following which phosphorus was determined by spectrophotometer using a standard curve.

Potassium and sodium were determined using a flame photometer according to the method described by the U.S. Salinity Laboratory staff (Richards 1954). The values of potassium and sodium determined from the flame photometer were compared with a standard curve and total quantities were computed

The data collected on germination were analyzed statistically using MSTATC software by applying a completely randomized design (CRD). The data on the effect of inoculation on the various growth and yield parameters of wheat were analyzed by applying a CRBD (Steel et al. 1997). Means were compared using the DMR test (p=0.05) to detect significant differences among treatments (Duncan 1955).

Results

Germination assay

The germination of both inoculated and un-inoculated seeds was significantly affected at higher salinity levels. Increasing levels of salinity depressed seed germination, but this effect was decreased by inoculation with PGPR, and inoculated seeds showed an improved germination compared to the

un-inoculated control. At low salinity levels, the effect of inoculation with the PGPR strains was statistically similar (Table 2). At 9 dS m^{-1}, seeds inoculated with strain W2 showed the maximum germination percentage, which differed non-significantly from that of seeds inoculated with W17. At 12 dS m^{-1}, seeds inoculated with strain W17 showed the maximum germination percentage, which was 22 % higher than that of un-inoculated control, followed by seeds inoculated with strains W10 and W2, whose seed germination was statistically similar. At the highest salinity level (15 dS m^{-1}), seeds inoculated with W17 showed the maximum germination percentage (43 % higher than the control); however, the germination of these seeds was statistically similar to that of seeds inoculated with the other strains.

The data presented in Table 2 show that at the highest salinity level, inoculation with PGPR significantly increased the germination rate compared to un-inoculated control. The maximum germination rate was obtained with W17 at 9 and 12 dS m^{-1} and was up to 34 % higher than that of un-inoculated control. This germination rate was statistically similar with that of seeds inoculated with W2 at 9 dS m^{-1} and with those inoculated with the other strains at 12 dS m^{-1}. At the highest salinity level, i.e., 15 dS m^{-1}, the maximum germination rate (82 % more than that of the control) was obtained in seeds inoculated with strain W2, followed those inoculated with strain W17. The germination rates of seeds inoculated with strains W6 and W10 were statistically similar and were up to 50 % higher than that of the un-inoculated control.

Table 2 Effect of inoculation on germination percentage, age, germination rate, and germination index of wheat seeds under different salinity levels

Strain	Salinity (dS m^{-1})					
	1	3	6	9	12	15
	Germination (%)					
Control	98.0 ab	97.0 ab	95.0 bc	81.6 gh	74.0 j	54.6 k
W2	98.6 ab	99.3 a	97.0 ab	93.0 cd	85.6 f	75.0 ij
W6	97.3 ab	96.0 abc	95.0 bc	87.7 ef	80.6 h	75.6 ij
W10	96.3 abc	96.0 abc	97.0 ab	88.0 ef	86.0 f	75.6 ij
W17	97.6 ab	98.3 ab	96.0 abc	90.3 de	90.0 de	78.0 hi
	Germination rate					
Control	5.0 ab	4.8 abcd	4.5 defg	3.8 j	3.2 k	2.2 l
W2	5.2 a	5.0 abc	4.7 bcde	4.5 defg	4.1 hij	4.0 hij
W6	5.1 a	4.8 abcd	4.2 ghi	4.0 ij	4.0 hij	3.3 k
W10	5.0 ab	4.9 abc	4.4 efgh	4.2 ghi	4.0 hij	3.0 k
W17	5.1 a	5.0 ab	4.8 abcd	4.6 cdef	4.3 fghi	3.8 j
	Germination index					
Control	77.1 abc	73.3 cd	65.1 h	61.2 i	56.0 j	53.0 k
W2	74.5 abc	74.8 abc	73.6 bcd	70.1 ef	70.0 ef	66.4 gh
W6	75.7 ab	74.2 abcd	70.8 ef	69.2 f	68.5 fg	65.2 h
W10	74.8 abc	73.7 abcd	73.8 abcd	69.5 f	69.2 f	64.1 h
W17	75.9 a	74.5 abc	74.3 abc	72.0 de	69.8 ef	69.0 f

W2, *Pseudomonas putida*; W6, *Enterobacter cloacae*; W10, *Serratia ficaria*; W17, *Pseudomonas fluorescens*

Data are presented as the mean of three replicates. Means followed by the same letter(s) do not differ significantly at $p<0.05$ according to Duncan's multiple range test

The data on the germination index are also presented in Table 2. At low salinity levels (1 and 3 dS m^{-1}), the inoculation effect was non-significant; however, at high salinity levels, seeds inoculated with PGPR stains showed a higher germination index than untreated seeds. At 6 and 9 dS m^{-1}, seeds inoculated with strain W17 had the highest germination index which was statistically similar with those of seeds inoculated with strains W2 and W10 at 6 dS m^{-1} and that of seeds inoculated with strain W2 at 9 dS m^{-1}. At 12 dS m^{-1}, seeds inoculated with PGPR showed up to a 87 % increase in the germination index; however, the treatments were statistically similar. At the highest salinity level, i.e., 15 dS m^{-1}, seeds inoculated with strain W17 had the maximum germination index (1.63-fold higher than the inoculated control) followed by those inoculated with strains W2, W6, and W10.

Field trials

In two salt-affected fields [site I, electrical conductivity (EC) 11.8 dS m^{-1}; site II, EC 14.2 dS m^{-1}], inoculation with PGPR was found to significantly affect the growth and yield parameters of wheat compared to the un-inoculated control. However, the strains differed significantly in their performance for improving growth of wheat under salt-stressed conditions.

The data collected on plant height at site I showed that the effect of inoculation was non-significant. At site II, however, strains W2 and W17 significantly increased plant height (up to a 29.6 % increase) compared to the un-inoculated control (Table 3). The effect of strains W6 and W10 was statistically similar, and inoculation with either strain also increased plant height compared to the control, but the effect was non-significant.

Like plant height, PGPR strains differed non-significantly from each other in the number of tillers produced at both sites (Table 3); however, inoculation resulted in a significant increase in tiller number over the un-inoculated control at site I, with an up to 21 % increase in tiller number relative to the control. At site II, inoculation resulted in a 24 % increase in tiller number compared to the control, but the effect of inoculation was at par with the un-inoculated control. Inoculation with strain W17 caused the maximum increase in number of tillers at both sites, followed by W2.

Table 3 Effect of inoculation with plant growth-promoting rhizobacteria on wheat in salt-affected fields

Strain	Plant height (cm)		Tillers (no. m^{-2})		Number of grains $spike^{-1}$	
	Site I	Site II	Site I	Site II	Site I	Site II
Control	57.3 a	54.0 b	293.0 b	280.3 a	24.3 c	25.7 c
W2	69.0 a	70.0 a	340.7 a	341.7 a	43.3 a	38.7 a
W6	66.3 a	63.3 ab	328.3 a	335.0 a	31.7 b	34.3 b
W10	67.7 a	59.3 ab	336.3 a	332.0 a	35.7 b	36.3 ab
W17	73.7 a	70.0 a	355.0 a	349.3 a	41.0 a	40.0 a
	Grain yield (t ha^{-1})		1000 Grain weight (g)		Straw yield (t $^{-1}$ha)	
	Site I	Site II	Site I	Site II	Site I	Site II
Control	1.95 c	1.80 c	21.0 c	18.0 c	3.58 c	2.71 c
W2	2.39 a	2.29 ab	32.0 ab	30.7 ab	5.04 a	4.42 a
W6	2.24 b	2.15 b	28.3 b	28.0 b	4.40 b	4.10 ab
W10	2.29 ab	2.11 b	28.0 b	30.7 ab	4.42 b	3.52 b
W17	2.41 a	2.35 a	36.3 a	33.7 a	5.25 a	4.70 a

Data are presented as the average of three replicates. Values followed by the same lower-case letter(s) do not differ significantly at $P<0.05$ according to Duncan's multiple range test

Data on the number of grains per spike showed that inoculation significantly increased the number of grains per spike at both sites (Table 3). At site I, the number of grains per spike with strains W2 and W17 was statistically similar, with 68 % more grains per spike produced than the control. At site II, inoculation with strain W17 resulted in the maximum increase in number of grains per spike (56 % higher than the un-inoculated control), followed by W2 and W10.

Inoculation with all the strains significantly increased the grain yield of wheat grown under salinity stress (Table 3). Similar to number of grains per spike, at site I inoculation with strains W2 and W17 caused the maximum increase (up to 24 %) in grain yield compared to the un-inoculated control. Also, at site II, inoculation with strain W17 produced the maximum grain yield (up to 31 % more grain yield compared to un-inoculated control) and this effect was statistically similar to that of strain W2. The effects of strains W6 and W10 on grain yield were also statistically similar and caused up to a 20 % increase in grain yield compared to the control.

At site I, inoculation with W17 resulted in the maximum 1,000 grain weight, which was 72 % more than that of the un-inoculated control, followed inoculation with W2 (Table 3); however, the 1,000 grain weight of both strains was statistically similar. At site II, strain W17 again resulted in the maximum 1,000 grain weight (69 % more than un-inoculated control), but the value was statistically similar to that of strains W2 and W10. Strain W6 caused a minimum increase in 1,000 grain weight compared to other strains; however, its increase was significantly higher (40 %) than the 1,000 grain weight of the un-inoculated control.

Inoculation with the PGPR strains significantly increased the straw yield (Table 3). At both site I and II, strain W17 showed the maximum increase in straw yield (up to 73 %) compared to the un-inoculated control, and the increase was statistically similar with that of W2 at both site I and W2 and with that of W6 at site II. Overall, strains W2 and W17 were the most effective strains in terms of improving the growth and yield parameters of wheat at both sites under salt-stressed conditions.

Our data revealed that PGPR inoculation had variable effects on the uptake of major nutrients, i.e., nitrogen, phosphorus, and potassium (Table 4). Inoculation with strain W17 caused up to a 22 % increase in nitrogen uptake over

Table 4 Effect of inoculation with plant growth-promoting rhizobacteria on total nitrogen, phosphorus, and potassium uptake and K^+/Na^+ ratio of wheat under salt-stress conditions

Strain	Nitrogen uptake (kg/ha)		Phosphorus uptake (kg/ha)		Potassium uptake (kg/ha)		K^+/Na^+	
	Site I	Site II	Site I	Site II	Site I	Site II	Site I	Site II
Control	103 c	99 cd	18 d	14 c	124 c	121 c	1.25 c	1.19 d
W2	121 ab	109 b	26 c	27 a	142 a	130 b	1.70 a	1.45 b
W6	119 ab	104 c	26 c	17 bc	127 bc	125 bc	1.56 b	1.24 c
W10	107 c	106 bc	28 b	20 b	129 b	129 b	1.60 b	1.26 c
W17	126 a	115 a	29 a	21 b	147 a	141 a	1.64 ab	1.56 a

Data are presented as the average of three replicates. Values followed by the same lower-case letter(s) do not differ significantly at $P<0.05$ according to Duncans' multiple range test

the control at both sites, and this increase was statistically similar with that of W2 and W6 at site I. The increase in nitrogen uptake following inoculation with strains W2 and W10 was statistically similar at site II, while inoculation with strain W10 resulted in a non-significant difference from the control at both sites. Inoculation significantly increased the uptake of phosphorus at both sites, with strain W17 causing the maximum increase in phosphorus content at site I (61 % more than control) and W2 (92 % more than control) at site II. Phosphorus uptake caused by strains W6, W10, and W17 were statistically similar at site II. The data on potassium uptake also showed that inoculation significantly enhanced the uptake of potassium. Maximum potassium uptake was observed with W17 at both sites and was up to 17 % higher than that of the un-inoculated control, followed by W2, which caused up to a 15 % increase in potassium uptake compared to the control.

We also observed that inoculated plants showed low Na^+ contents as compared to the un-inoculated control, which resulted in a significant increase in the K^+/Na^+ ratio in the inoculated treatments. The maximum K^+/Na^+ ratio was noted with W2 at site I, followed by W17. At site II, strain W17 performed better and a caused maximum increase in the K^+/Na^+ ratio, followed by W2. Strains W6 and W10 also caused a significant increase in the K^+/Na^+ ratio compared to un-inoculated control; however, these ratios were statistically similar at both sites.

Discussion

Salinity is one of the most important constraints which hamper agricultural productions in most of the arid and semi-arid regions of the world. Rapid seed germination is a critical factor in crop production, and in many crop species, seed germination and early seedling growth are the most sensitive developmental stages to salinity stress (Ashraf and Foolad 2005). A high concentration of salts has a negative impact on seed germination and plant growth by affecting various metabolic processes (Sidari et al. 2008). In plants, certain physiological processes are regulated by endogenous ethylene (Mattoo and Suttle 1991; Frankenberger and Arshad 1995), and although a burst of ethylene is required to break seed dormancy (Esashi 1991), the high concentration of ethylene produced under salinity stress (Arbona et al. 2005; Zapata et al. 2007) has a negative impact on seed germination and root growth (Bernardo et al. 2000; Mattoo and Suttle 1991; Sarquis et al. 1991).

As seed germination and early seedling growth are generally the most sensitive stages affected by salinity (Foolad 2004), mitigation of the effect of salts at these early stages will improve the chance of establishing a successful crop under salt stress (Sallam 1999; Foolad 2000; Ashraf et al. 2003). In our study, the inoculation of wheat seeds with PGPR not only enhanced seed germination but also improved the growth and yield of wheat under naturally salt-affected conditions. The improved germination might be due to their ability to degrade ACC produced during stress and, therefore, reduce the elevated ethylene level in the immediate vicinity of seeds. It is also evident from the results of our previous study that the intensity of the classical triple response imposed by ethylene can be diluted through treating the seeds with these PGRP strains (Nadeem et al. 2010).

Inoculation also enhanced the germination rate and germination index under salinity stress. Other researchers have also reported enhanced germination percentages and germination rates under saline conditions through inoculation with PGPR (Nelson 2004; Barassi et al. 2006; Mishra et al. 2010). Although, no mention was made about the role of ACC-deaminase in their respective studies, these authors did correlate the growth promotion with a number of specific particular activities of PGPR. Rueda-Puente et al. (2007) suggested the possible role of plant growth promoting substances for growth enhancement under saline conditions.

In addition to enhanced germination, the better performance of inoculated wheat seedlings at two salt-affected sites further demonstrates their effectiveness in salinity tolerance. It is evident from the literature that under salinity stress, plant growth is affected by physiological disorders, such as enhanced ethylene production and nutritional and hormonal imbalances (Ashraf 1994; Marschner 1995; Glick et al. 1997; Sairam and Tyagi 2004). Elevated levels of ethylene concentration inhibit root growth, which ultimately affects overall plant growth (Mattoo and Suttle 1991; Sobeih et al. 2004). Any check on this enhanced synthesis of ethylene is essential for improving plant growth (Glick et al. 1997; Mayak et al. 2004; Saleem et al. 2007). 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).

Nutritional imbalance is another major impact of salinity on crop production. A balance in ion accumulation is necessary for good plant growth (Ashraf 2004; Foolad 2004). Salt tolerance in plants has been related to a restricted and controlled uptake of Na^+ and continued uptake of K^+ (Jeschke and Wolf 1988), and a high K^+/Na^+ ratio is a good indicator of salt tolerance (Ashraf and O'Leary 1996; Hamdia et al. 2004). In our study, PGPR strains also played an important role in regulating nutrient uptake and maintaining a nutritional balance in wheat plants. We found that the restricted uptake of Na^+ resulted in an increased K^+ concentration, which in turn

resulted in a high K^+/Na^+ ratio. Although the exact mechanism by which these strains regulate nutrient uptake is not fully understood, it may be due to a better root growth that increases the surface area for the uptake of nutrients from large volumes of soil. It may also be due to the exopolysaccharide activity of bacteria that have the ability to bind sodium and decrease the availability of sodium ions for plant uptake (Ashraf et al. 2004; Kohler et al. 2006; Nadeem et al. 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. (W2 and W17) and might be due to the higher ACC-deaminase activity and better root colonization ability of these strains, as indicated in our previous study (Nadeem et al. 2010). 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. 2011a, b).

In summary, the data presented in this manuscript are quite encouraging in terms of promoting the use of PGPR as a means for improving plant growth under field conditions and maximizing the utilization of salt-affected soils for increasing agricultural production from these soils. The more efficient PGPR strains should be further evaluated as inoculums in biofertilizers.

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