



Full Length Article

Comparative Effectiveness of *Enterobacter aerogenes* and *Pseudomonas fluorescens* for Mitigating the Depressing Effect of Brackish Water on Maize

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ABSTRACT

Experiments were conducted under axenic and natural conditions to evaluate the comparative effectiveness of *Enterobacter aerogenes* and *Pseudomonas fluorescens* for improving maize growth and yield irrigated with synthetic brackish water [EC, 5 dS m⁻¹; SAR, 10 (mmol L⁻¹)^{1/2}]. In the first experiment under axenic conditions, normal water, brackish water and two PGPR strains (S₁₄, *E. aerogenes* & S₂₀, *P. fluorescens*) were tested. In the second experiment in pots, normal and brackish water irrigations at different stages of maize growth along with the same PGPR strains were tested. Brackish water significantly decreased the maize growth, whilst bacterial strains significantly reduced its adverse effects. Interestingly, inoculation was even more effective in case of brackish water as compared to normal water irrigation. In pot trial, PGPR inoculation reduced the adverse effects of salinity on maize growth and yield. The maximum improvement in growth and yield parameters was observed with S₂₀ inoculation under brackish water treatment. The chlorophyll content and K⁺/Na⁺ ratio of maize were also improved by PGPR strain S₂₀. It is concluded that *P. fluorescens* could be used as an effective tool to minimize the inhibitory effects of brackish water on the growth and yield of maize. © 2012 Friends Science Publishers

Key Words: Brackish water; PGPR; ACC-deaminase; Maize; Yield

INTRODUCTION

The limited supply of good quality canal water for irrigation has compelled the farmers to use water from alternate sources like sewage and ground water pumped by tube wells. This situation prevails in most of the countries situated in arid to semi arid regions of the world. Pakistan is no exception to this. According to an estimate, about 50-60% of the total water that is being pumped up is unfit for irrigation due to its high electrical conductivity (EC), sodium adsorption ratio (SAR) or residual sodium carbonate (RSC) (Ashfaq *et al.*, 2009). The continuous use of such water could result in an accumulation of soluble salts and/or exchangeable Na somewhere in the soil profile (Ahmed *et al.*, 2008). The presence of excessive soluble salts and/or exchangeable Na could result in severe yield losses by altering the soil physical or chemical properties (Nadeem *et al.*, 2006). The primary effect of high EC water on crop productivity is the development of physiological drought i.e. inability of the plants to compete with ions in the soil solution for water. Sodium in irrigation water can also cause toxicity problems for some crops (Bauder *et al.*, 2006). Water potential and osmotic potential of shoot became more

negative with an increase in brackish water salinity which is associated with an accumulation of ions in leaves (Gulzar *et al.*, 2003).

Moreover, salinity is one of the most detrimental stresses that affect the plant growth by influencing a number of physiological processes positively or negatively. It is a serious production problem for crops as saline conditions are known to suppress plant growth, particularly in arid and semiarid regions (Parida & Das, 2005). This implies that plants, growing in saline conditions come under stress and often suffer from more physiological disorders as compared to those growing in normal environment (Bernstein, 1975) and ultimately the yield is reduced.

Ethylene is one of the important growth hormones produced by almost all the plants, which mediates a wide range of plant responses (Arshad & Frankenberger Jr., 2002). The production of ethylene in most plant tissues is normally low; however, its accelerated production can be induced by various developmental and experimental cues, including seed germination, fruit ripening, leaf and fruit senescence and a number of other biotic and abiotic stresses (Theologis, 1992). It has been well documented that ethylene synthesis is accelerated in the presence of various

environmental stresses (Glick *et al.*, 1997) including salt stress and it has been reported to increase significantly in many plant species subjected to salt stress (Zapata *et al.*, 2004; Nadeem *et al.*, 2010). The production of ethylene may be inhibitory or stimulatory depending upon its concentration (Arshad & Frankenberger Jr., 2002), the nature of the physiological process (Johnson & Ecker, 1998) and the growth phase of the plant (Abeles *et al.*, 1992). Higher levels of ethylene ($> 25 \mu\text{g L}^{-1}$) can be damaging for plants, leading to epinasty, shorter roots and premature senescence (Holguin & Glick, 2001). Metabolizing ACC, the immediate precursor of ethylene, is an established approach to reduce ethylene level in plants. Some PGPR strains possess the enzyme ACC-deaminase, which can cleave the ACC and thereby lower the level of ethylene in a developing seedling or stressed plant (Mayak *et al.*, 2004). A decreased level of ACC results in a lower level of endogenous ethylene, which eliminates the inhibitory effect of high ethylene concentrations (Glick *et al.*, 1999).

Plants inoculated with PGPR containing ACC-deaminase are more resistant to the deleterious effects of stress ethylene that is synthesized as a consequence of stressful conditions such as high salt concentrations (Mayak *et al.*, 2004; Nadeem *et al.*, 2010). Such inoculation was also reported to reduce the drastic effect of salinity stress in plants and increase the shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, number of leaves per plant, leaf relative water content, emergence percentage and chlorophyll content but decrease electrolyte leakages in the plants (Yildirim *et al.*, 2008). Inoculation of wheat with *P. putida* carrying ACC-deaminase activity under salinity stress resulted in increased plant height, root length, grain yield, 100-grain weight and straw yield up to 52, 60, 76, 19 and 67%, respectively over un-inoculated control at 15 dS m^{-1} (Zahir *et al.*, 2009). Similarly, chlorophyll content and K^+/Na^+ ratio of leaves were also increased by *P. fluorescens* over control when the maize plants were inoculated under salinity stress (Nadeem *et al.*, 2007). It is also reported that inoculation of maize under salinity stress with *P. fluorescens* carrying ACC-deaminase activity increased the nutrient uptake and plant yield efficiently over un-inoculated control by lowering the endogenous level of ethylene (Nadeem *et al.*, 2009). Inoculation with the same bacteria has also been reported to increase the wheat growth and yield (Nadeem *et al.*, 2010). *Pseudomonas fluorescens* increased the K^+/Na^+ ratio, relative water content and chlorophyll content in wheat (Nadeem *et al.*, 2010).

Plant growth promoting rhizobacteria containing ACC-deaminase are not only effective for reducing stress-induced ethylene, but are also helpful for the absorption of nutrients necessary for better growth. The *Klebsiella oxytoca* (Rs-5) containing ACC-deaminase mitigate the negative effects of salt stress and promote the plant growth. Inoculation also enhances the absorption of major nutrients such as N, P, K and Ca and decreases the uptake of the Na^+ (Yue *et al.*, 2007).

There is repeated evidence available in literature indicating that the microbial inoculation can play an important role to improve crop yields in salt affected soils. However, the prospects of using such PGPR in crops grown with brackish water have not been studied. Continued use of brackish water results in the development of secondary salinity in the long run. But it could also raise an immediate adverse effect on plant growth by disturbing the composition of soil solution. The unfavorable conditions in the soil solution through application of brackish water may upset the plant uptake of water and other essential nutrient elements. Therefore, it is imperative to explore all such possible ways, which may reduce the depressing effect of brackish water on plant growth. The present study was conducted to study the comparative effectiveness of *E. aerogenes* and *P. fluorescens* for mitigating/ eliminating the stress, induced by irrigating maize with brackish water.

MATERIALS AND METHODS

Basic experimental details: Two trials were conducted under axenic and natural conditions to test the comparative efficacy of two pre-isolated (Nadeem *et al.*, 2007) bacterial strains (S_{14} , *Enterobacter aerogenes*) and (S_{20} , *Pseudomonas fluorescens*). A mixture of salts NaCl, Na_2SO_4 , MgSO_4 and CaCl_2 in a specific ratio was used to produce brackish water containing almost all the major cations and anions found in ground water. Synthetic brackish water of EC, 5 dS m^{-1} ; SAR, $10 (\text{mmol L}^{-1})^{1/2}$ prepared with the aforementioned salts was used to grow crops in axenic and natural conditions.

Pouch trial: Maize seeds were surface-sterilized by momentarily dipping them in 95% ethanol solution and then in 0.2% HgCl_2 solution for 3 min. and were subsequently washed thoroughly with sterilized distilled water (Russell *et al.*, 1982). Petri dishes with filter paper sheets were autoclaved, surface-sterilized seeds were arranged in them and were placed in an incubator at 25°C for 4 days. Sterilized distilled water was used to maintain optimum moisture for germination. Germinating seeds were inoculated by dipping for 10 min. in culture media (10^7 – 10^8 cfu mL^{-1}) of strain S_{14} or S_{20} . Inoculated seedlings were transplanted in sterilized growth pouches. In case of un-inoculated control, sterilized broth was used for seed dipping.

Four treatments of synthetic brackish water treatments were applied: N_0 (normal water throughout the growth period), B_0 (brackish water throughout the growth period), C (alternate normal & brackish water) and D (2 normal water irrigations followed by 1 brackish water). In the growth room, the temperature was maintained at $25 \pm 1^\circ\text{C}$ and 14 h of light (intensity, $275 \mu\text{mol m}^{-2}\text{s}^{-1}$) alternated with 10 h darkness. The growth parameters were measured after 25 days of planting. Root length was measured by using Delta T-Scan (Win DIAS 3, England).

Pot trial: A pot experiment was conducted under natural conditions to evaluate the comparative efficacy of two pre-selected strains (S_{14} & S_{20}) of PGPR to minimize the effect of salinity stress induced by brackish water on maize crop at vegetative and reproductive stages. For this purpose, synthetic brackish water of EC, 5 dS m^{-1} and SAR, 10 ($mmol\ L^{-1}$)^{1/2} was used. The soil was sandy loam with pH, 7.9; electrical conductivity (EC_e), 1.4 dS m^{-1} ; organic matter, 0.75 %; total N, 0.07 %; available (Olsen) P, 7.12 mg kg^{-1} and extractable K, 89 mg kg^{-1} . Surface-disinfected seeds were coated with PGPR strains S_{14} and S_{20} by using slurry prepared with sterilized peat, broth culture (10^7 – 10^8 cfu mL^{-1}) and sterilized sugar solution 10% in the ratio 5:4:1 (w/w). To the un-inoculated control, plain sterilized (autoclaved) broth was used in the slurry. Ten inoculated seeds of maize were sown in each pot containing 12 kg soil and one plant was maintained in each pot after germination. Four different treatments of brackish water were applied: N (all irrigations with normal water), B (all irrigations with brackish water), NB (normal water during vegetative growth & brackish water during reproductive growth) and BN (brackish water during vegetative growth & normal water during reproductive growth) and each treatment was repeated thrice. Pots were arranged in a wire house at ambient light and temperature using completely randomized design (CRD). Recommended dose of N, P, K fertilizers (220: 125: 125 kg ha^{-1}) were applied in each pot as urea, diammonium phosphate and sulphate of potash, respectively. Data regarding plant height, no. of leaves $plant^{-1}$, fresh shoot weight, dry shoot weight, root length, fresh root weight, dry root weight, cob length, fresh cob weight, dry cob weight, no. of rows cob^{-1} , no. of grains row^{-1} and 100-grain weight were recorded after harvesting.

The chlorophyll contents were measured in the leaf samples through chlorophyll meter. Leaf samples were collected prior to harvesting and stored in polypropylene centrifuge tubes at freezing temperature (Akhtar *et al.*, 1998). The sap was collected and analyzed for sodium (Na^+) and potassium (K^+) concentrations by flame photometer as described by US Salinity Laboratory Staff (1954).

Statistical analysis: The Analysis of variance (ANOVA) techniques (Steel *et al.*, 1980) was applied to analyze the data using complete randomized factorial design and means were compared by Duncan's Multiple Range Tests (Duncan, 1955).

RESULTS

Pouch trial: In growth pouch trial under axenic conditions, the application of brackish water always significantly reduced the maize growth but the alternate irrigation of normal and brackish water or two normal water irrigations followed by, one brackish water had a significantly less negative effect than application of brackish water throughout the growth. Furthermore, the bacterial strains significantly reduced the inhibitory effect of brackish water

on maize growth but *P. fluorescens* was more efficient.

The data regarding root length revealed that the brackish water significantly reduced the root length but the alternate irrigation of normal and brackish water (C) or two normal water irrigations followed by 1 brackish water (D) equally reduced this inhibitory effect though the root length was still significantly less than with normal water treatment (Table I). Moreover, the bacterial strains reduced the inhibitory effect of brackish water on root length, with the PGPR strain S_{20} being more efficient one in all the treatments. But the results were more pronounced in case of brackish water as compared to normal water treatment. The maximum increase in root length (124%) compared with the respective un-inoculated control was observed with the S_{20} inoculation in the treatment (B) where brackish water was applied throughout the growing period.

Data (Table I) showed that the PGPR inoculation significantly improved the root fresh weight of maize seedlings, which was otherwise reduced by brackish water application. The maximum increase in root fresh weight was observed with S_{14} inoculation in the brackish water treatment B, where increase in root fresh weight was about 5 fold over the respective un-inoculated control. As in the case of root fresh weight, the root dry weight of maize seedlings was also improved by both PGPR strains in all the treatments, and strain S_{20} gave the maximum increase in root dry weight in the treatment B over the respective un-inoculated control. But the increase was statistically non-significant in all the treatments.

It is evident from the data (Table II) that the shoot length of the maize seedlings was adversely affected by brackish water. However, it was less affected by alternate irrigation of normal and brackish water (C) or two normal water irrigations followed by, one brackish water (D). The inoculation of maize seeds with PGPR strains (S_{14} & S_{20}) on the other hand significantly reduced the inhibitory effect of brackish water. Both the bacterial strains increased the shoot length (7 to 50%) of maize seedlings. However, the bacterial isolate S_{20} was more efficient and it increased the shoot length up to 50% over the respective un-inoculated control in the treatment B where brackish water was applied.

Shoot fresh weight and shoot dry weight (Table II) were also significantly reduced by the application of brackish water and the minimum shoot fresh weight (0.993 g) and shoot dry weight (0.058 g) were observed in the treatment where brackish water was applied throughout the growth period. The bacterial inoculation significantly reduced the adverse effect of brackish water. The PGPR strain S_{14} increased the shoot fresh weight and shoot dry weight of maize seedlings grown with brackish water by 103 and 206%, respectively compared with the corresponding un-inoculated control.

Pot trial: The data from the pot trial revealed that brackish water significantly reduced the growth (Table III & IV) and yield (Table V) of maize crop, and decreased the chlorophyll content (Fig. 1) and K^+/Na^+ ratio (Fig. 4) in

Table I: Comaprative effect of bacterial strains on root growth of maize grown with brackish water under axenic conditions (Average of three replicates)

PGPR Strain	N ₀	B ₀	C	D
Root length (mm)				
Control	2450 ab	941 c	1351 bc	1446 bc
S ₁₄	2972 a	1612 bc	2967 a	1673 bc
S ₂₀	2908 a	2104 ab	2224 ab	2863 a
LSD value		967.5		
Root fresh weight (g)				
Control	0.448 e	0.137 f	0.382 e	0.174 f
S ₁₄	0.583 cd	0.667 ab	0.616 bc	0.584 cd
S ₂₀	0.734 a	0.529 d	0.587 cd	0.734 a
LSD value	0.0754			
Root dry weight (g)				
Control	0.095 ab	0.036 b	0.057 b	0.065 ab
S ₁₄	0.092 ab	0.065 ab	0.075 ab	0.066 ab
S ₂₀	0.120 a	0.069 ab	0.088 ab	0.079 ab
LSD value	0.0533			

Table II: Comaprative effect of bacterial strains on shoot growth of maize grown with brackish water under axenic conditions (Average of three replicates)

PGPR Strain	N ₀	B ₀	C	D
Shoot length (cm)				
Control	32.43 ef	24.83 g	30.67 f	31.33 f
S ₁₄	38.50 b	36.67 bc	34.58 cde	33.50 def
S ₂₀	42.33 a	37.33 bc	38.33 b	36.17 bcd
LSD value	2.819			
Shoot fresh weight (g)				
Control	2.685 ab	0.993 e	1.590 d	1.707 d
S ₁₄	2.655 ab	2.217 bc	1.868 cd	1.903 cd
S ₂₀	3.047 a	1.868 cd	2.232 bc	2.560 b
LSD value	0.4296			
Shoot dry weight (g)				
Control	0.198 bc	0.058 f	0.0780 ef	0.143 cd
S ₁₄	0.213 b	0.177 bcd	0.117 de	0.150 cd
S ₂₀	0.280 a	0.150 cd	0.186 bc	0.175 bcd
LSD value	0.0533			

Means sharing same letters are statistically at par at 5 % level of probability

S₁₄= *Enterobacter aerogenes*, S₂₀= *Pseudomonas fluorescens*

N₀ = Normal water throughout the growth period, B₀ = Brackish water throughout the growth period, C = Alternate normal and brackish water, D = 2 normal water irrigations followed by 1 brackish water

the maize leaves. However, the bacterial strains significantly reduced the inhibitory effect of brackish water on maize growth and yield, and increased the chlorophyll content and K⁺/Na⁺ ratio but with different degrees of efficacy. Moreover, brackish water application at the vegetative stage was more deleterious than at the reproductive stage.

The results (Table III) showed that the minimum plant height was found in the case of treatment B, where all irrigations were with brackish water. Where that was not the case, treatment NB, where brackish water was applied at the reproductive stage, inhibited significantly less the shoot growth than the treatment BN, where brackish water was applied at the vegetative stage. This inhibitory effect was again reduced by inoculation with both PGPR strains (S₁₄ & S₂₀). The bacterial strains increased the plant height up to 16% but the isolate S₂₀ was more efficient in the treatment B.

Table III: Comaprative effect of bacterial strains on shoot growth of maize grown with brackish water in pot trial (Average of three replicates)

PGPR Strain	N	B	NB	BN
Plant height (cm)				
Control	140.3 a	108.0 d	139.0 ab	113.7 cd
S ₁₄	142.7 a	114.5 cd	140.7 a	126.0 bc
S ₂₀	142.0 a	125.3 bc	136.7 ab	136.0 ab
LSD value		12.89		
Shoot fresh weight (g)				
Control	147.8 b	59.33 i	89.60 f	80.33 g
S ₁₄	147.4 b	73.67 h	116.4 d	96.67 e
S ₂₀	155.3 a	87.00 f	137.3 c	117.7 d
LSD value	5.041			
Shoot dry weight (g)				
Control	53.11 b	23.73 g	46.55 c	32.13 f
S ₁₄	56.52 b	26.13 g	35.84 de	38.67 d
S ₂₀	62.67 a	33.13 ef	54.93 b	47.07 c
LSD value	3.256			

Table IV: Comaprative effect of bacterial strains on root growth of maize grown with brackish water in pot trial (Average of three replicates)

PGPR Strain	N	B	NB	BN
Root length (cm)				
Control	61.33 ab	53.33 b	59.00 ab	58.67 ab
S ₁₄	62.50 ab	57.00 ab	68.33 a	61.67 ab
S ₂₀	65.67 ab	59.33 ab	69.67 a	61.33 ab
LSD value	10.87			
Root fresh weight (g)				
Control	32.24 ab	10.80 f	13.50 ef	12.74 f
S ₁₄	31.56 abc	11.78 f	14.24 ef	18.98 def
S ₂₀	38.11 a	19.58 def	24.99 bcd	22.82 cde
LSD value	8.572			
Root dry weight (g)				
Control	7.217 abc	2.660 d	5.973 bcd	5.070 bcd
S ₁₄	7.430 abc	3.963 cd	6.567 abc	6.867 abc
S ₂₀	10.20 a	5.107 bcd	7.377 abc	8.310 ab
LSD value	3.352			

Means sharing same letters are statistically at par at 5 % level of probability

S₁₄= *Enterobacter aerogenes*, S₂₀= *Pseudomonas fluorescens*

N = All irrigations with normal water, B = All irrigations with brackish water, NB = Normal water during vegetative growth and brackish water during reproductive growth, BN = Brackish water during vegetative growth and normal water during reproductive growth

Shoot fresh weight (Table III) of maize was improved by inoculation with PGPR strains. Both the PGPR strains reduced the inhibitory effect of brackish water at vegetative as well as reproductive stages. These strains increased the shoot fresh weight up to 24 to 47%, in the treatment B; where brackish water was applied throughout the growth period, as compared with the respective un-inoculated control. However, the maximum increase in shoot fresh weight was observed with the strain S₂₀, which was 47% more than the respective un-inoculated control. As in the case of shoot fresh weight, the bacterial inoculation was also equally efficient in increasing the shoot dry weight of maize plants and the results were similar to those of shoot fresh weight in case of S₂₀ but S₁₄ was non-significant in case of brackish water treatment. A 6 to 40% increase in shoot dry weight was observed in case of inoculated plants compared

Table IV: Comaprative effect of bacterial strains on root growth of maize grown with brackish water in pot trial (Average of three replicates)

PGPR Strain	N	B	NB	BN
Root length (cm)				
Control	61.33 ab	53.33 b	59.00 ab	58.67 ab
S ₁₄	62.50 ab	57.00 ab	68.33 a	61.67 ab
S ₂₀	65.67 ab	59.33 ab	69.67 a	61.33 ab
LSD value	10.87			
Root fresh weight (g)				
Control	32.24 ab	10.80 f	13.50 ef	12.74 f
S ₁₄	31.56 abc	11.78 f	14.24 ef	18.98 def
S ₂₀	38.11 a	19.58 def	24.99 bcd	22.82 cde
LSD value	8.572			
Root dry weight (g)				
Control	7.217 abc	2.660 d	5.973 bcd	5.070 bcd
S ₁₄	7.430 abc	3.963 cd	6.567 abc	6.867 abc
S ₂₀	10.20 a	5.107 bcd	7.377 abc	8.310 ab
LSD value	3.352			

Table V: Comaprative effect of bacterial strains on yield parameters of maize grown with brackish water in pot trial (Average of three replicates)

PGPR Strain	N	B	NB	BN
Cob length (cm)				
Control	13.00 b	6.333 f	9.000 de	7.667 ef
S ₁₄	13.50 b	8.000 e	12.67 b	11.00 c
S ₂₀	15.80 a	9.667 cd	14.00 b	10.83 c
LSD value		1.442		
No. of grains row⁻¹				
Control	12.83 ab	8.00 d	11.00 abcd	8.333 cd
S ₁₄	12.82 ab	8.887 bcd	12.17 abc	10.48 abcd
S ₂₀	13.61 a	9.220 bcd	12.56 ab	10.61 abcd
LSD value	3.537			
100-grain weight (g)				
Control	22.98 ab	12.41 e	19.96 abcd	17.98 bcde
S ₁₄	22.71 ab	14.76 de	21.76 abc	20.27 abcd
S ₂₀	24.97 a	16.01 cde	24.58 a	22.44 ab
LSD value	5.722			
Grain yield plant⁻¹ (g)				
Control	29.22 cde	19.15 h	24.17 g	20.08 h
S ₁₄	31.24 bcd	24.95 fg	31.63 bc	23.74 g
S ₂₀	34.92 a	27.70 ef	34.09 ab	28.21 de
LSD value	2.93			

Means sharing same letters are statistically at par at 5 % level of probability

S₁₄= *Enterobacter aerogenes*, S₂₀= *Pseudomonas fluorescens*

N = All irrigations with normal water, B = All irrigations with brackish water, NB = Normal water during vegetative growth and brackish water during reproductive growth, BN = Brackish water during vegetative growth and normal water during reproductive growth

with the respective un-inoculated control in all the treatments but the maximum increase in shoot dry weight (40%) over the respective un-inoculated control was observed in the case of S₂₀ inoculation in treatment B, where all irrigations were applied with brackish water.

The root length of maize plants (Table IV) was inhibited by the application of brackish water and the minimum root length was observed in the un-inoculated plants with treatment B, where all irrigations were applied with brackish water. This was followed by BN, where brackish water was applied at the vegetative stage. The maximum increase in root length was observed in case of

S₂₀ inoculation in NB treatment, where brackish water was applied at reproductive stage; it increased the root length up to 18% over respective un-inoculated control but the increase was statistically non significant in all the treatments.

Root fresh and dry weight of maize plants was significantly reduced by brackish water application both at vegetative as well as reproductive stages but the effect was more pronounced at vegetative stage. However, PGPR inoculation reduced the inhibitory effect of brackish water on the root fresh and dry weight but the difference with the respective un-inoculated control was statistically non-significant. The strain S₂₀ was more efficient in increasing the root fresh as well as dry weight of maize plants under normal and brackish water application at vegetative as well as reproductive stages (Table IV).

The results (Table V) showed that bacterial inoculation reduced the inhibitory effect of brackish water on the cob length of maize both at vegetative as well as reproductive stages. Brackish water reduced the cob length 51% over the normal water treatment. However, PGPR inoculation increased the cob length (4 to 53 %) over the respective un-inoculated control. The maximum increase (53%) in cob length over the respective un-inoculated control was observed with S₂₀ inoculation in B, where throughout brackish water was applied. The number of grains per row (Table V) was also improved by PGPR inoculation with normal as well as brackish water applications at vegetative and reproductive stages. But the results were statistically non-significant compared with the respective un-inoculated controls.

Brackish water significantly reduced 100-grain weight (Table V) but a lesser reduction in 100-grain weight was observed in the treatments NB and BN, where brackish water was applied at reproductive and vegetative stage, respectively. The PGPR inoculation improved the 100-grain weight in all the treatments but the increase was statistically non-significant compared with the respective un-inoculated control. PGPR inoculation also improved the grain yield of maize, which otherwise was drastically reduced by the brackish water application (Table V). Again the strain S₂₀ was the more efficient one in all the brackish water treatments. The maximum increase in grain yield (45%) over the respective un-inoculated control by S₂₀ was observed in treatment B, where all irrigations were applied with brackish water. The PGPR inoculation also significantly improved the grain yield in treatment NB and BN, where brackish water was applied at vegetative and reproductive stage, respectively. However, non significant results were observed by S₁₄ inoculation in N; where throughout normal water was applied.

It is evident from the data (Fig. 1) that the PGPR inoculation increased the chlorophyll contents of maize plant, which otherwise were decreased by brackish water application and the increase was more in the treatment B, where all irrigations were applied with brackish water.

Fig. 1: Comparative effect of bacterial strains on Chlorophyll content of maize grown with brackish water in pot trial (Average of three replicates)

Values sharing same letters are statistically at par at 5% level of probability

S₁₄= *Enterobacter aerogenes*, S₂₀= *Pseudomonas fluorescens*

N = All irrigations with normal water, B = All irrigations with brackish water, NB = Normal water during vegetative growth and brackish water during reproductive growth, BN = Brackish water during vegetative growth and normal water during reproductive growth

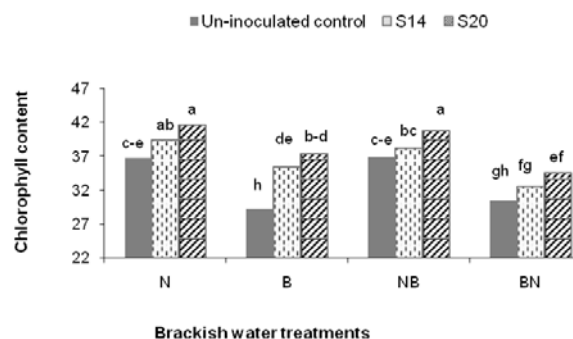
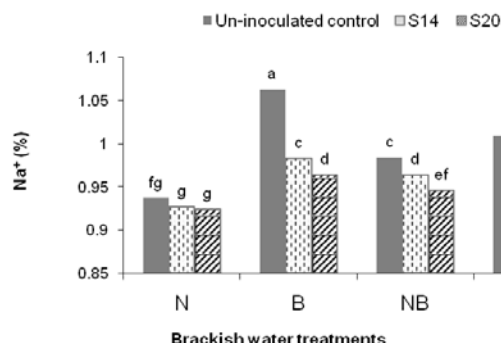


Fig. 2: Comparative effect of bacterial strains on Na⁺ concentration (%) in maize leaf grown with brackish water in pot trial (Average of three replicates)

Values sharing same letters are statistically at par at 5% level of probability

S₁₄= *Enterobacter aerogenes*, S₂₀= *Pseudomonas fluorescens*

N = All irrigations with normal water, B = All irrigations with brackish water, NB = Normal water during vegetative growth and brackish water during reproductive growth, BN = Brackish water during vegetative growth and normal water during reproductive growth



The PGPR strain S₂₀ was more efficient in increasing the chlorophyll contents and the maximum increase (28%) over the respective control was observed in case of B.

The data regarding Na⁺ content (Fig. 2) showed that the Na⁺ content increased in all the treatments with brackish water application. However, inoculation with PGPR strains significantly decreased the Na⁺ content as compared to un-inoculated control. It was also observed that the effect of strains was more pronounced in the treatments, where brackish water was applied than with the normal water treatment where the results were non-significant. The PGPR inoculation when brackish water was used decreased the

Fig. 3: Comparative effect of bacterial strains on K⁺ concentration (%) in maize leaf grown with brackish water in pot trial (Average of three replicates)

Values sharing same letters are statistically at par at 5% level of probability

S₁₄= *Enterobacter aerogenes*, S₂₀= *Pseudomonas fluorescens*

N = All irrigations with normal water, B = All irrigations with brackish water, NB = Normal water during vegetative growth and brackish water during reproductive growth, BN = Brackish water during vegetative growth and normal water during reproductive growth

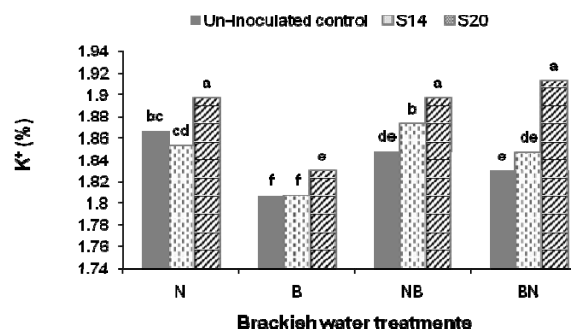
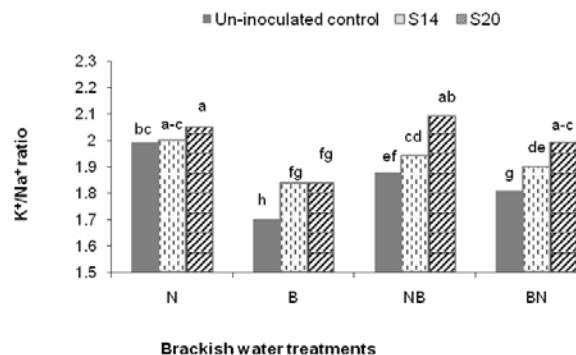


Fig. 4: Comparative effect of bacterial strains on K⁺/Na⁺ ratio of maize grown with brackish water in pot trial (Average of three replicates)

Values sharing same letters are statistically at par at 5% level of probability

S₁₄= *Enterobacter aerogenes*, S₂₀= *Pseudomonas fluorescens*

N = All irrigations with normal water, B = All irrigations with brackish water, NB = Normal water during vegetative growth and brackish water during reproductive growth, BN = Brackish water during vegetative growth and normal water during reproductive growth



Na⁺ contents from 2 to 10% and the maximum decrease (10%) was observed with the S₂₀ strain in treatment B, where all irrigations were with brackish water.

In contrast to Na⁺, K⁺ contents of maize plant decreased under salinity stress. However, plants treated with PGPR strains showed a higher K⁺ content compared with un-inoculated control at the respective brackish water treatments (Fig. 3). The PGPR strain S₂₀ was more efficient and it increased the K⁺ content up to 5% in the treatment BN, where brackish water was applied at vegetative stage. Under salinity stress due to brackish water, Na⁺ concentration in the leaf sap increased in all the treatments

and K^+ concentration decreased, however inoculation with PGPR strains decreased the Na^+ content and enhanced the K^+ uptake thus improving the K^+/Na^+ ratio in maize. The maximum increase (15%) in K^+/Na^+ ratio (Fig. 4) was observed in NB with the bacterial strain S_{20} over the respective un-inoculated control, which otherwise was reduced up to 17% by the brackish water application. After harvesting, the EC of the soil in the treatments N, B, NB and BN was 1.88, 17.06, 8.01 and 6.67 dS m^{-1} , respectively.

DISCUSSION

This study conducted under axenic and natural conditions, demonstrates the potential of plant growth promoting rhizobacteria (PGPR) containing ACC-deaminase along with some other plant growth promoting characteristics (Nadeem *et al.*, 2007) for improving growth and yield of maize irrigated with synthetic brackish water. It was observed that PGPR strains (*E. aerogenes* & *P. fluorescens*) reduced the depressing effect of brackish water on the growth and yield of maize but *P. fluorescens* was more efficient. The application of brackish water significantly reduced the maize growth and yield, which might be attributed to increased ethylene production. It is well documented that ethylene synthesis is accelerated in response to various environmental stresses, including salinity (Mayak *et al.*, 2004) and its high concentration inhibits the plant growth. It is well established that brackish water results in the buildup of secondary salinity which induces osmotic stress by limiting absorption of water from the soil (Mayak *et al.*, 2004) and ionic stress resulting from high concentrations of potentially toxic ions within the plant cells, which may reduce plant growth. It also affects the crop yield as it has pronounced adverse effects on reproductive growth, and the number and fresh weight of pods $plant^{-1}$ in mung bean (*Vigna radiata* L.) decreased with increasing salinity (Elahi *et al.*, 2004).

In our study, inoculation with PGPR strains, showing ACC-deaminase activity significantly enhanced the root, shoot and other growth and yield contributing parameters of maize grown with brackish water under axenic and pot conditions. It was observed that inoculation was even more effective in the treatment B, where brackish water was applied throughout the growth period. It is very likely that PGPR strains promoted root growth by lowering the endogenous ethylene levels due to their ACC-deaminase activity which metabolizes ACC, the immediate precursor of ethylene thus lowering the ethylene level in plants and eliminating the inhibitory effect of stress-induced high ethylene concentrations (Glick *et al.*, 1999; Zhenyu *et al.*, 2007). Very recently, Nadeem *et al.* (2010) have also reported a positive correlation between ACC deaminase activity and root elongation in maize seedlings grown under salt-stressed axenic conditions. The better root growth due to inoculation with PGPR containing ACC-deaminase activity was positively correlated with shoot growth. Our

results are also supported by the findings of Principe *et al.* (2007) who reported that plants inoculated under saline conditions produced more shoot dry weight as compared to un-inoculated control (Nadeem *et al.*, 2009; Ahmad *et al.*, 2011) have confirmed the ability of PGPR containing ACC-deaminase to reduce the salinity-induced classical triple response in maize, a typical assay for the confirmation of ethylene production.

In the present study that inoculation with PGPR strains increased the chlorophyll contents of maize. Increased stress-induced ethylene causes senescence (Arshad & Frankenberger Jr., 2002) thus decreasing chlorophyll content. So this may be due to the inhibition of ethylene synthesis by PGPR, thus decreasing the chlorophyll decay. The increased chlorophyll content may also be due to increased leaf area index due to decreased inhibitory effect of ethylene. These results are supported by the work of Nadeem *et al.* (2010) who reported significantly increased chlorophyll contents in maize plants inoculated with PGPR strains grown under salinity stress.

It was observed in the present study that the brackish water treatment changed the Na^+ and K^+ concentration and thus induced nutritional imbalance in plants which may be one of the main damages caused by the salt stress (Greenway & Munns, 1980). It was reported that an increase in Na^+ content in the rooting medium caused an increase in Na^+ uptake and a decrease in K^+ content of plant (Pervaiz *et al.*, 2002). Na^+ inhibits the uptake of K^+ and results in toxic accumulation of Na^+ (Saqib *et al.*, 2000). In our study, inoculation markedly altered the selectivity of Na^+ and K^+ uptake by plants. Inoculation restricted the Na^+ uptake and enhanced the uptake of K^+ , consequently increasing K^+/Na^+ ratio. The lower uptake of Na^+ by inoculated roots might be due to the decreased apoplasmic flow of Na^+ into the vascular tissues, caused by a higher proportion of root zone being covered with soil sheath due to inoculation. Nadeem *et al.* (2010) also reported that maize plants treated with PGPR having ACC-deaminase activity showed low Na^+ contents and high K^+ than un-inoculated plants. This decrease in Na^+ concentration may also be due to the production of exopolysaccharides (EPSs) by these bacteria (Nadeem *et al.*, 2007). Such bacterial strains have the ability to bind cations including Na^+ (Geddie & Sutherland, 1993). These EPS-producing bacteria under salt-stressed conditions have been found to restrict Na^+ uptake by roots (Ashraf *et al.*, 2004).

It was also observed in our studies that the application of brackish water at the vegetative stage was more deleterious than at reproductive stage and it is in line with the findings of Maas *et al.* (1983) who reported that maize was more sensitive during the vegetative growth stage than during the germination and reproductive stages. They also concluded that brackish water could be used during and after tasseling without reducing the yield.

The variable efficacy of strains was observed in reducing the effect of brackish water and thus improving

plant growth and yield. This variation in growth promotion might be due to variation in their efficacy to colonize the germinating roots and hydrolyze ACC along with some other mechanisms which have already been established in our previous study (Nadeem *et al.*, 2007).

In conclusion, adverse effects associated with the application of brackish water could effectively be reduced through bacterial inoculation and the strain *P. fluorescens* was more effective in this regard, which may be tested further to develop a biofertilizer. Moreover, application of brackish water at reproductive stage had less inhibitory effect on maize growth and yield, thus the salt tolerance by maize crop during the later stages of growth was much higher than during the seedling stage. So if normal water is available, it may be applied at vegetative stage followed by brackish water at reproductive stage with less yield loss. The approach has very good prospects for using brackish water for sustainable maize production particularly in the scenario deteriorating/decreasing water resources.

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