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Variation in growth and ion uptake of maize due to inoculation with plant growth promoting rhizobacteria under salt stress

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Abstract

Soil salinity decreases plant growth and photosynthetic activity besides resulting in nutrient imbalance in plants. Plant growth promoting rhizobacteria (PGPR) can induce plant tolerance to salinity by producing various hormones and enhancing the availability of nutrients from soil matrix. A pot study was conducted to evaluate the effect of different PGPR strains on maize growth and ions uptake under salt stress conditions. Three salinity levels (4, 8 and 12 $dSm^{-1)}$ along with original EC were maintained in the pots using NaCl salt. Maize seeds inoculated with pre-selected strains (S5, S15 and S20) along with uninoculated control were sown in the pots. Recommended doses of NPK were applied. In general, maize growth was decreased with the increase in salinity. Results indicated that PGPR inoculation, even at higher EC (12 dS m^{-1}), significantly increased shoot/root fresh weight, shoot/root dry weight, chlorophyll a, b and cartenoid contents upto 64/114, 102/102, 154, 102 and 58%, respectively, compared with un-inoculated control. Similarly, inoculation restricted the uptake of Na^+/Cl^- ions and enhanced the accumulation of N, P and K in shoot compared to control. Among the three selected strains, S20 performed better at all EC levels. The growth promotion and increased ions uptake exhibited by strain S20 might be due to its high in vitro IAA production, chitinase activity, P-solubilition and more intensive root colonization, besides ACC-deaminase activity.

Key words: PGPR, growth, ions uptake, maize, salinity

Introduction

Plant growth promoting rhizobacteria are free living microorganisms having beneficial effects on plants by colonizing their roots. They include such effects as the production of phytoharmones; auxin, cytokinins and giberelins (Garcia de Salamone *et al*., 2001), enhancing release of the nutrients (Nautiyal *et al*., 2000; Idriss *et al*., 2002) as well as preventing the deleterious effects of environmental stress (Kloepper and Schroth, 1978).

Among various environmental stresses soil salinity limits plant growth and production in many parts of the world, particularly in arid and semi arid areas (Shannon, 1984). Under salinity, growth depression results from increased level of ethylene (Nukui *et al*., 2003), decreased photosynthetic capacity (Drew *et al*., 1990), water deficit and ion imbalance (Wyn Jones, 1981). Coping with salinity is a global issue to ensure sustainable crop production. Plant growth under salt stress is dependent on adaptation to re-establish ionic balance. Many attempts have been made to reduce the drastic effect mostly focusing on chemical amelioration and saline agriculture. Recently, a biological approach using microorganisms was attempted. Hasnain and Sabri (1996) reported that inoculation with *Pseudomonas* sp. stimulated plant growth by reduction of toxic ion up take and production of stress- specific proteins in plant under stress. Certain microorganisms contain an enzyme 1-aminocyclopropane-1-carboylic acid (ACC) deaminase that hydrolyses ACC, the immediate precursor of ethylene, thereby lowering the plant ethylene level (Glick *et al*., 1998) which eliminates the potential inhibitory effect of high ethylene concentration (Glick *et al*., 1999). PGPR strains can also produce exopolysaccharides (EPSs) to bind cations including sodium (Geddie and Sutherland, 1993), thus help alleviating salt stress in plants grown under saline environment (Ashraf *et al*., 2004).

So present study was carried out to elucidate the role of PGPR on growth and ion uptake of maize under salt stressed condition.

Materials and methods

Rhizosphere soil samples were collected from salt-affected field of maize. Several bacterial strains were isolated by dilution plate technique using DF minimal medium (Dworkin and Foster, 1958). Further streaking on fresh plates purified the collected rhizobacterial strains.

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Inoculum was prepared in flasks and each flask containing 60 mL broth was inoculated with selected strains of bacteria and incubated for 72 hours under shaking (100 rpm) conditions at 28 ± 1°C. An optical density of 0.5 measured at a wavelength of 535 nm was achieved by dilution to maintain uniform cell density (10^7–10^8 cfu mL^{-1}) prior to seed inoculation. The selected strains were characterized by measuring ACC-deaminase activity (Honma and Shimomura, 1978) by monitoring the amount of α-ketobutyrate produced when the enzyme ACC-deaminase cleaves ACC. *In vitro* auxin production was determined as indole-acetic acid (IAA) equivalents in the presence and absence of L-tryptophan by using protocol described by Khalid *et al*. (2004). Chitinase activity and phosphorus solubilizing activity was determined qualitatively as described by Chernin *et al*. (1998) and Mehta and Nautiyal (2001), respectively. Root Colonization ability of these strains was studied under axenic conditions as described by Simon *et al*. (1996).

Soil used in pots was analyzed for physicochemical characteristics (Sandy clay loam having pH, 7.9; ECe, 1.6 dSm^{-1}; organic matter, 0.72%; total nitrogen, 0.05%; Olsen phosphorus, 6.25 mg kg^{-1} and exchangeable potassium, 125 mg kg^{-1}). Surface-disinfected seeds of maize were inoculated with peat mixed with 10% sugar solution (inoculum to peat ratio 1:1 w/w). In the case of un-inoculated control, seeds were coated with sterilized (autoclaved) peat treated with sterilized broth. Five inoculated seeds of maize were sown in each pot containing 12 kg soil pot $^{-1}$ at four EC_e levels (Original, 4, 8 and 12 dS m^{-1}). There were four replications for each treatment. Pots were arranged in wire house under ambient light and temperature according to completely randomized design. Recommended doses of NPK fertilizers were applied in each pot as Urea, DAP and SOP, respectively. Phosphorus and potassium were applied as basal dose while Nitrogen was applied in two splits.

Leaf samples were collected after 75 days and stored in polypropylene centrifuge tubes at freezing temperature (Akhtar *et al*., 1998). Frozen leaf samples were thawed and crushed. The sap was collected in polypropylene tubes by Gilson pipette and centrifuged at 6500 rpm for 10 minutes. The supernatant sap was collected and sodium and potassium were determined using Sherwood 410 Flame Photometer (U.S. Salinity Lab. Staff, 1954). Chloride content was determined by chloride analyzer.

For chlorophyll pigments, 0.5 g of leaf samples from each treatment were homogenized with 80% acetone (v/v) and then the homogenate was filtered through filter paper. Absorbance of the resulting solution was read at 663, 645 and 480 nm for chlorophyll a, b and carotenoids, respectively (Arnon, 1949).

For N and P, dried leaf samples were digested with sulphuric acid and hydrogen peroxide according to the method of Wolf (1982). Nitrogen was determined by Kjeldhal method and P contents were determined by spectrophotometer after mixing the sample with Barton reagents. Data were collected and analyzed statistically using a completely randomized design (Steel and Torrie, 1980).

Results

The increase in fresh weight of shoot by inoculation with rhizobacterial strains at different salinity levels ranged from 2 to 64% over respective control (Table 1). At EC 8 and 12 dS m^{-1}, S20 was found to be more effective and increased weight significantly that was 40 and 64% higher than control, respectively, followed by S5. At original EC, rhizobacterial strains S5 and S20 increased the shoot fresh weight that was 2% higher than uninoculated control.

Data regarding the effect of inoculation on shoot dry weight of maize revealed that strain S20 caused maximum increase in the shoot dry weight i.e. 102% over uninoculated control at 12 dS m^{-1} (Table 1). At original EC level, treatments showed non significant results. At 8 dS m^{-1}, S20 strain increased the shoot dry weight by 65 % over control followed by S15 and S5 i.e. 47 and 45 % higher than control, respectively.

Inoculation with isolate S20 significantly increased the root fresh weight (up to 114%) at EC 12 dS m^{-1} while 48% increase was recorded at EC 8 dS m^{-1}over respective control, followed by S5, where as at 4 dS m^{-1}, S15 and S20 showed statistically same results. At original EC, strain S20 significantly increased root fresh weight (19%) compared to uninoculated control followed by S15.

Table 1. Effect of inoculation with rhizobacteria containing ACC-deaminase on shoot /root fresh and shoot/root dry weight at different salinity levels

(Average of four replicates)

Strains	Shoot fresh weight (g)				Root fresh weight (g)			
	0 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}	0 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}
Control	207.8 abc*	206.2 abc	143.3 f	105.1 g	38.6 cde	45.5 bcd	27.2 g	15.8 h
S5	212.8 a	212.0 ab	189.2 d	171.8 e	43.1 abc	43.5 abc	37.3 de	32.0 fg
S15	206.9 abc	204.5 bc	184.9 d	166.3 e	45.9 ab	48.2 a	38.5 cde	28.4 g
S20	211.5 ab	210.5 ab	201.0 c	172.7 e	48.5 a	48.2 a	40.27 cd	33.9 ef
	LSD 5%= 6.72				LSD 5%= 5.02			
Strains	**Shoot dry weight (g)**				**Root dry weight (g)**			
	0 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}	0 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}
Control	90.3 a	83.9 b	55.4 e	38.7 f	28.2 abc	24.3 de	18.7 f	7.5 h
S5	94.0 a	94.2 a	80.4 b	74.0 cd	29.4 a	26.1 cd	23.2 e	15.3 g
S15	90.3 a	90.2 a	81.7 b	70.3 d	28.5 abc	24.0 de	22.0 e	15.1 g
S20	95.2 a	96.0 a	91.5 a	78.2 bc	28.7 ab	26.2 bcd	24.1 de	15.2 g
	LSD 5%= 5.76				LSD 5%= 2.37			

*Means sharing same letter (s) in a column do not differ significantly according to the Duncan s multiple range test (p=0.05)

At EC 8 and 12 dS m^{-1}, S5, S15 and S20 were statistically non significant with each other, but significantly different from control. However, maximum dry weight was observed by strain S20 at EC 8 and 12 dS m^{-1} (28 and 102%) followed by strains S5.

Results of pot study showed that on overall basis the increase in salinity level decreased the photosynthetic pigments (chlorophyll a, b and carotenoid contents) of the maize seedlings. However, inoculation with PGPR containing ACC-deaminase activity significantly increased the pigments under salinity stress compared to control.

Data regarding the effect of inoculation on chlorophyll a contents in maize plant revealed that the S20 strain caused maximum increase in chlorophyll a contents at original EC and at 8 dS m^{-1} (up to 13 and 76% increase over respective control, respectively) (Table 2) followed by S5. At EC 12 dS $m^{-}1$, strains S5 and S20 showed statistically same results and caused increase in chlorophyll a contents up to 154 and 150% over control, respectively.

Data in Table 2 depicted significant effect of strains inoculation on chlorophyll b contents. At EC original and 12 dS m^{-1}, S20 caused significant increase in chlorophyll b contents that was 6 and 102% higher than control. Next to it was S5 that increased chlorophyll b contents up to 84 % more than control at 12 dS m^{-1}. Data regarding carotenoids content indicates that treatments showed non-significant effect at original EC level. However, inoculation with rhizobacterial strain S20 significantly increased carotenoid contents at all the EC levels up to 58% as compared to control.

Ionic composition

Data in Table 2 and 3 depicted that N, P and K concentration significantly increased due to inoculation with PGPR. At original EC, treatments were statistically non significant. At 8 and 12 dS m^{-1}, maximum K concentration was recorded for S20 (51 and 119% higher over control, respectively) followed by S5.

Under salinity stressed conditions, up to 55% increase in nitrogen concentration occurred over control with PGPR inoculation. At original EC, treatments significantly increased the nitrogen concentration, however the effects among the strains were statistically non-significant. At EC 12 dS m^{-1}, S20 strain showed maximum N concentration (16 and 55%, respectively higher than control) while S5 and S15 were statistically at par with each other. Data regarding P concentration in leaf is indicated in Table 3. Results showed that treatment effect was significant over control. Similar to nitrogen, higher P concentration was also observed with S20 at original and EC 12 dS m^{-1} i.e. 28 and 56% higher than respective control followed by S5.

Table 2. Effect of inoculation with rhizobacteria containing ACC-deamianse on Chlorophyll a, b, carotenoid and nitrogen contents at different salinity levels

(Average of four replicates)

Strains	Chlorophyll a (mg g^{-1})				Chlorophyll b (mg g^{-1})			
	0 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}	0 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}
Control	2.91 abc*	2.62 d	1.66 ef	0.74 g	1.46 b	1.38 bc	1.05 e	0.45 h
S5	2.93 abc	2.75 abcd	2.66 cd	1.89 e	1.45 b	1.39 bc	1.32 cd	0.83 g
S15	2.96 ab	2.94 ab	2.70 bcd	1.45 f	1.44 b	1.46 b	1.25 d	0.79 g
S20	3.02 a	2.98 ab	2.91 abc	1.85 e	1.56 a	1.56 a	1.32 cd	0.91 f
	LSD 5%= 0.241				LSD 5%= 0.074			
Strains	Carotenoid (mg g^{-1})				N (%)			
	0 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}	0 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 d Sm^{-1}
Control	0.41 a	0.38 ab	0.26 d	0.11 f	1.57 cd	1.51 d	1.25 f	0.89 g
S5	0.40 a	0.39 a	0.33 bc	0.18 e	1.81 a	1.74 ab	1.60 c	1.23 f
S15	0.40 a	0.40 a	0.31 c	0.17 e	1.80 a	1.68 b	1.39 e	1.23 f
S20	0.42 a	0.42 a	0.38 ab	0.19 e	1.82 a	1.69 b	1.49 d	1.38 e
	LSD 5%= 0.053				LSD 5% =0.0.74			

*Means sharing same letter (s) in a column do not differ significantly according to the Duncan s multiple range test (p=0.05)

Table 3. Effect of inoculation with rhizobacteria containing ACC-demainase on P, K, Na and Cl concentration at different salinity levels

(Average of four replicates)

Strains	P (%)				K (%)			
	0 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}	0 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}
Control	0.23 de*	0.24 cde	0.22 ef	0.17 f	1.93 a	1.85 c	1.71 f	1.64 g
S5	0.30 abc	0.28 abcd	0.26 cde	0.21 ef	1.94 a	1.92 a	1.84 cd	1.79 de
S15	0.27 bcde	0.28 abcd	0.25 cde	0.22 ef	1.93 a	1.90 ab	1.83 cd	1.77 e
S20	0.33 a	0.32 ab	0.26 bcde	0.25 cde	1.96 a	1.94 a	1.85 bc	1.83 cd
	LSD 5% = 0.052				LSD5%= 0.052			
Strains	Na (%)				Cl (%)			
	0 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}	0 dS m^{-1}	4 dS m^{-1}	8 dS m^{-1}	12 dS m^{-1}
Control	0.96 fg	0.94 fg	1.15 b	1.26 a	0.19 gh	0.24 fg	0.38 b	0.56 a
S5	0.94 fg	0.91 gh	1.05 de	1.09 cd	0.21 g	0.27 def	0.35 bc	0.38 b
S15	0.97 f	0.93 fgh	1.06 de	1.13 bc	0.15 hi	0.21 g	0.31 cd	0.39 b
S20	0.93 fgh	0.88 h	1.02 e	1.05 de	0.13 i	0.19 gh	0.25 efg	0.30 cde
	LSD5%= 0.052				LSD5% = 0.052			

*Means sharing same letter (s) in a column do not differ significantly according to the Duncan s multiple range test (p=0.05)

Data regarding Na^+ and Cl^- concentration in leaf sap are summarized in Table 3. Na^+ and Cl^- concentration significantly increased with the increasing salinity particularly in control treatment. At EC 8 and 12 dS m^{-1}, minimum Na^+ concentration was observed in incase of inoculation with S20 that was 11 and 16% lower than control, respectively, followed by S5. However, at original EC, S15 had slightly high concentration of Na^+ than control. Similar to Na^+, Cl^- concentration also increased with salinity. Minimum Cl^- concentration was recorded with S20 i.e. 36 and 40 % lower than control at 8 and 12 dS m^{-1}, respectively. At original EC, S20 had minimum Cl^- concentration (30% lower than control) and S15 had high concentration of Cl^- (10% higher than control).

Discussion

In this study, three strains of PGPR were selected for their growth promoting activity under

different salinity levels (Original, 4, 8 and 12 dS m^{-1}) by conducting pot experiment on maize. It was observed that inoculation with these rhizobacterial strains significantly improved shoot/root fresh weight and shoot/root dry weight at all salinity levels. In general, growth was reduced with increase in salinity. However, inoculation was effective even in the presence of higher salinity levels (12 dS m^{-1}).

Under salinity stress, ethylene is produced at higher concentration and this higher concentration of ethylene is inhibitory to plant growth. It influences various phases of vegetative growth in plants resulting in overall reduced growth (Smalle and Van der Straeten, 1997). In many instances, removing or blocking the effect of stress ethylene results in alleviation of the stress effect. It is very likely that the PGPR strains promoted root growth by lowering the endogenous inhibitory levels of ethylene in roots because of their ACC-metabolizing ability (Table 4). This may imply that the inoculation with rhizobacteria could result in the development of much better root system, which subsequently affects shoot growth positively. Very recently, Shaharoona *et al*. (2006) reported a significantly positive correlation between ACC-deaminase activity and root elongation in maize due to inoculation with rhizobacteria containing ACC-deaminase activity under axenic conditions. The data in this study revealed that inoculation with rhizobacterial strain S20 proved to be the most effective at all salinity levels followed by S5, which increased root growth and other parameters even at high salt stress i.e. 12 dS m^{-1}. This may be attributed to its intensive root colonization ability and ACC-deaminase activity (Table 4) compared to other strains which made it more competitive under stress conditions. Similar findings were also obtained by Shaharoona *et al*. (2006) where strain having good root colonization ability showed more promising results than others.

It was also observed that inoculation with strains also increased the chlorophyll pigments (a, b and carotenoids contents) of maize. This may be the result of increased photosynthetic leaf area of plant even at high salt stress by PGPR inoculation compared to control where leaf area reduced due to stress (Marcelis and Van Hooijdonk, 1999). Similar results were also reported by Han and Lee (2005b) that inoculation increased the chlorophyll content in lettuce.

The uptake of Na^+ and Cl^- varied between inoculated and uninoculated treatments. The Na^+ and Cl^- concentration increased with salinity and the increase was much greater in control treatment than PGPR inoculation treatments. However, in case of S15, Na^+ and Cl^- concentration increased compared to control. It means that inoculation with PGPR strains S5 and S20 retarded the absorption and consequently accumulation of these ions. These results are in accordance with Hamdia *et al*. (2004) who observed decrease in Na^+ content in maize under salinity due to inoculation with *Azospirillum brasilence.* This may be due to the reason that PGPR alleviate the salinity stress due to their ACC-deaminase activity and this may also be due to the expolysaccharides (EPS) activity of bacteria. The PGPR strains can produce bacterial exopoloysaccahrides which bind cations including Na^+ (Geddie and Sutherland, 1993) and decrease the content of Na available for plant uptake, thus helping alleviate salt stress in plants (Ashraf *et al.,* 2004). Thus PGPR strains markedly increased the tolerance of maize plant by lowering the Na concentration and consequently Na^+/K^+ ratio. This is also confirmed from the findings of Han and Lee (2005a). They observed that Na^+ content of soybean grown under saline conditions decreased due to inoculation of EPS producing strains.

Inoculation not only reduced the Na^+ and Cl^- concentration in maize but also induced a marked and progressive increase in N, P and K

Table 4. Characteristics of selected strains of plant growth promoting rhizobacteria

PGPR strains	ACC-deaminase activity (α-ketobutarate nmol g^{-1} biomass h^{-1})	Chitinase activity (qualitative)	Phosphate solublization (qualitative)	IAA production (mg L^{-1})		Root Colonization ($cfug^{-1}$)
				Without L-TRP	With L-TRP	
S5	440	-	+	0	18.2	4.50 x 10^5
S15	405	-	+	0	15.0	5.12 x 10^4
S20	442	+	+	0	19.0	7.80 x 10^5

concentration under salinity stress. This means that PGPR strains could alleviate the effects of salinity stress in maize. Han and Lee (2005a) also observed increase in N, P and K concentration under salinity stress due to inoculation with PGPR. Similarly, Vivas *et al*. (2003) reported that N, P and K concentration in lettuce inoculated by *Bacillus sp*. under stress conditions were increased by about 5, 70 and 50 %, respectively, over control.

The results of this study suggested that inoculation of salt-stressed plants with PGPR strains containing ACC-deaminase could alleviate salinity stress. These PGPR strains can induce salinity tolerance, enhance nutrient uptake and growth promotion in maize under salinity stress. However, further work is needed to explore the effectiveness of strains under field conditions.

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