



Full Length Article

Auxin Producing Plant Growth Promoting Rhizobacteria Improve Growth, Physiology and Yield of Maize under Saline Field Conditions

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Abstract

Soil salinity is one of the most widespread agricultural problems which reduce the field crop productivity. Salinity disturbs the hormonal balance in plants which results in poor growth. Use of plant growth promoting rhizobacteria (PGPR) is considered an economical and environment-friendly approach to combat salinity stress. This study was carried out to investigate the effect of auxin producing PGPR on the growth, antioxidant status and mineral content of maize (*Zea mays*) in salt affected soils. Rhizobacterial strains were isolated from the rhizosphere of maize growing under salt affected soil conditions. These strains were screened on the basis of auxin production and their ability to withstand salinity stress. Two stains (MA4 and MA11) which produced highest auxin and tolerated maximum salinity were selected for further evaluation in a field experiment. The results of field trial showed that soil salinity reduced the plant growth, mineral nutrient uptake and yield of maize while antioxidant activity and proline concentration was increased. However, rhizobacterial inoculation improved grain yield (31%), fresh biomass (56%) and phosphorous contents in grains (26%) while the proline concentration (41%), ascorbate peroxidase (59%) and SOD values (42%) in leaves were declined. The results of present study signify the role of auxin producing rhizobacteria under salt affected field conditions. © 2016 Friends Science Publishers

Keywords: Auxin; Physiology; Antioxidant activity; Salt stress; Maize

Introduction

Around the world, crop farming has to deal with varying biotic and abiotic stresses which limit the crop growth and productivity (Nemati *et al.*, 2011). Among these, soil salinization reduces the productivity of fertile lands (Lawlor, 2002). Salinity induces various physiological and biochemical alterations in plants which ultimately result in decreased growth and yield of crops (Krasensky and Jonak, 2012).

To surmount this problem, numerous approaches of defense against salt stress have been adopted. Some of these strategies include the development of salt tolerant plants via conventional and genetic engineering tools, reclamation and amelioration of salt affected soils (Mittler and Blumwald, 2010). Alternative to these techniques, plant growth promoting rhizobacteria are also being used to induce salinity tolerance in plants (Zahir *et al.*, 2010). Such beneficial microorganisms have been renowned in the maintenance of healthy root, facilitation of nutrient uptake and helping the plants to withstand environmental stresses and increase plant growth (Reed and Glick, 2004). Positive impacts of these PGPR include the production of phytohormones; auxin, cytokinins and gibberellins (Garcia de Salamone *et al.*, 2001), enhancing uptake of the nutrients (Nautiyal *et al.*,

2000) as well as preventing the deleterious effects of environmental stresses (Kloepper and Schroth, 1978).

Plants commonly face various types of biotic and abiotic stress by causing changes in their root morphology (Potters *et al.*, 2007). The plant hormones play a vital role in altering the root morphology. Patten and Glick (1996) reported that about 80% of the plant growth-promoting rhizobacteria possess the ability to produce indole acetic acid (IAA). Different plants inoculated with such bacteria showed an increase in root growth, lateral root formation, root hairs, which helped them to withstand abiotic stress. Activity of ACC-deaminase enzyme that degrades the ACC, the precursor of ethylene, is stimulated by the IAA produced by the bacteria (Glick, 2005). Under salt affected soil conditions, ACC-deaminase activity of bacteria can be helping for better growth and yield of crops. Positive effects of such phytohormone producing PGPR have been documented in different crops such as wheat (Egamberdieva, 2009) and rice (Mirza *et al.*, 2006).

Plant hormones play a vital role in regulating the protective response against different abiotic and biotic stresses (Ansari and Sharif-Zadeh, 2012). These phytohormones are involved in the expression of various genes via synergistic and/or antagonistic actions (Lucy *et*

al., 2004). Indole acetic acid is known to play a vital role in growth and developmental aspects of plants such as development of vascular tissues, lateral root formation, cell differentiation apical dominance as well as directive of gene expression (Wang *et al.*, 2001). Current proofs point out that auxin also responds to salinity in crop plants and its signaling is down-regulated under different stresses (Kazan and Manners, 2009). When plants faces various environments stresses, the internal levels of phytohormones are decreased which are mostly unsatisfactory for prime growth (De Salamone *et al.*, 2005). The negative effect of salinity on the germination of seed may be due to drop in the internal levels of phytohormones (Debez *et al.*, 2001). The growth inhibiting effect of salt stress have been found to be alleviated by the priming of wheat seed with various plant growth regulators by other researchers (Afzal *et al.*, 2005).

As plant responses to salt and drought stress are mostly mediated by plant hormones, it is of great importance to study IAA producing PGPR in saline conditions which could facilitate plant growth in such harsh environments. This study was planned to isolate, screen and evaluate the efficacy of auxin producing PGPR for growth promotion and yield of maize and furthermore to investigate the effect of these rhizobacteria on proline concentration and the activity of antioxidative enzymes under natural saline field conditions.

Materials and Methods

Isolation of PGPR

Plant growth promoting rhizobacteria were isolated from the rhizosphere of maize growing in the salt affected soil by dilution plate technique using general purpose media (GPM). Fast growing colonies were selected and further purified. Isolated rhizobacteria were stored in refrigerator at 4°C for further experimentation (Khalid *et al.*, 2004).

Preparation of Inoculum

Sterilized general purpose media was inoculated with each rhizobacterial culture and incubated at 28 + 1°C for 3 days in shaking incubator at 100 rpm. Fresh inoculum were prepared for each experiment and diluted to get uniform population (10^8 – 10^9 CFU mL⁻¹) by measuring optical density (OD 0.5) using spectrophotometer at wavelength 535 nm.

Osmotic Adjustment Test

The isolated strains were tested according to the method as described by Zahir *et al.* (2010) for their ability to withstand salinity stress. For this purpose, strains were grown in test tubes on various salinity levels (original, 10, 20, 30 dS m⁻¹). Fifteen milliliters of broth was taken in test tubes and autoclaved. The tubes were inoculated with the selected

strains and incubated at 28 ± 1°C and the absorbance was measured at (λ) 540 nm by using spectrophotometer.

Measurement of Auxin Production

Auxin production measurement was carried out according to the method described by Sarwar *et al.* (1992). Twenty milliliters of sterilized GPM broth was taken in Erlenmeyer flasks of 100 mL. Five milliliters solution of 5% L-TRP after filter sterilization (0.2 μ m membrane filter, Whatman No. 2) was applied to the medium to prepare a media with final concentration of 1.0 g L⁻¹. Inoculation of the flask content was done with 0.1 mL of 3-day old broth culture {OD 0.5 (10^8 – 10^9 CFU mL⁻¹)}. After proper plugging, the flasks were incubated for 48 h using shaking incubator adjusted at 28 + 1°C and 100 rpm. A flask containing all the materials except inocula was kept as control for comparison. After incubation, the contents in the flask were filtered using Whatman No. 2 filter paper. For measuring IAA equivalents, 3 mL of filtrate was taken in test tubes and 2 mL of Salkowski reagent (2 mL 0.5 M FeCl₂ + 98 mL 35% HClO₄) was added to it. The mixture in the tubes was allowed to stand for 30 min for color development. Standard curve was used for the comparison to calculate auxin production by the selected isolates. The intensity of the color was measured at (λ) 535 nm by using a spectrophotometer. Rhizobacterial cultures giving higher auxin production and osmoadaptation were selected for field experiment.

Seed Inoculation

The inoculum for PGPR strain was prepared by culturing the strain on 250 mL of liquid medium in a 500 mL Erlenmeyer flask incubated for 72 h at 28°C and 100 rpm. Maize seeds (Monsanto-919) were surface sterilized with 70% ethanol for 2 min, treated with 2% sodium hypochlorite for 5 min, and followed by three washings with sterilized distilled water. For inoculation, PGPR inoculum of selected strains, having cell suspension of 10^8 – 10^9 CFU mL⁻¹, were injected into sterilized peat (150 mL kg⁻¹ of seed, seed to peat ratio 4:1 w/w). Maize seed dressing was done with inoculated peat mixed with 100 mL of 10% sterilized sugar (Sucrose) solution. In case of un-inoculated control, the seeds were coated with the sterilized peat treated with sterilized broth and 10% sterilized sugar solution but without bacterial inocula. While for co-inoculation (1:1 ratio) equal volume of both strains was used (Naveed *et al.*, 2014).

Field Experiment

Field experiment was conducted to further confirm the efficacy of selected strains to improve growth and yield of maize under salt affected field conditions. The surface soil was collected from the research area of the Institute of Soil and Environmental sciences, University of Agriculture

Faisalabad, air dried, thoroughly mixed, passed through 2 mm sieve and analyzed for various physico-chemical characteristics. The soil was sandy clay loam having pH 7.6; ECe 6.7 dS m⁻¹; organic matter, 0.63 %; total nitrogen, 0.05%; available phosphorus, 3.3 mg kg⁻¹ and extractable potassium, 84.2 mg kg⁻¹. Inoculated and non-inoculated (as described above) seeds were sown in the well-prepared field at 120 kg ha⁻¹ with a plot size of 20 m². Recommended doses of NPK fertilizers at 175-100-50 kg ha⁻¹ were applied as urea, single super phosphate and murate of potash, respectively. Data of growth and yield parameters were recorded at different stages of crop plants.

Plant Analyses

After 60 days, fresh leaf samples were collected and analyzed for proline, sodium, potassium and relative water content. At maturity, plants were harvested and data about growth and yield parameters were recorded. Grain samples were analyzed for nitrogen and phosphorous.

Chlorophyll Content (SPAD value)

Chlorophyll content (SPAD value) in leaf of maize plant was measured using chlorophyll meter SPAD-502 (Minolta, Minolta Co, Ltd, UK).

Relative Water Content

Relative water content (RWC) of leaves was determined using the following formula as described by Mayak *et al.* (2004).

$$\text{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fully turgid weight} - \text{Dry weight}}$$

The fully turgid weight of leaf was taken after putting it in 100% humidity in the dark at 4°C for 48 h.

Electrolyte Leakage

Electrolyte leakage (EL) was measured following the protocol described by Jambunathan (2010). For this purpose, leaf discs were transferred in 5 mL de-ionized water and electrical conductivity (EC) (R1) was recorded with EC meter (Jenway Conductivity Meter Model 2052) after 4 h incubation at 28 ± 1°C and 100 rpm in orbital shaking incubator. The same samples were autoclaved at 121°C for 20 min to determine EC (R2).

$$\text{EL (\%)} = (\text{EC before autoclaving R1} / \text{EC after autoclaving R2}) \times 100$$

Analysis of Stress Related Metabolites

Total soluble sugars were measured by anthrone reagent (0.2%) following Sadasivam and Manickam (1992). A reaction mixture (10 mL) consisting of 200 µL leaf extract (1 g leaf homogenized in de-ionized water), 1,800 µL DI water and 8 mL anthrone reagent was heated for 10 min in boiling water and cooled in ice bath to stop the

reaction. Absorbance was measured at 630 nm and total soluble sugar concentration (µg mL⁻¹) was calculated using glucose standard curve. Determination of total phenolics was carried out by the method as devised by the Singleton *et al.* (1999). For this purpose 2 mL of reaction mixture containing 20 µL leaf extract, 300 µL Na₂CO₃ (1 N), 1,580 µL DI water and 100 µL Folin Ciocalteu's reagent (0.25 N) was incubated in the dark at room temperature for 2 h. Then absorbance was recorded at 760 nm and concentration in µg mL⁻¹ was calculated following gallic acid standard curve.

Proline Content

Proline content was measured following Bates *et al.* (1973). A reaction mixture consisting of leaf extract (1 g leaf homogenized with 3% sulphosalicylic acid), ninhydrin and glacial acetic acid were heated at 100°C for 1 h in water bath. The reaction was stopped by cooling in ice bath and absorbance was recorded at 520 nm after mixture extraction with toluene. Proline content (µmol g⁻¹) was calculated following standard curve of L-proline.

Enzymatic/non Enzymatic Antioxidant Activity Assays

Ascorbate peroxidase (APX) activity was determined by tracking the ascorbate reduction through H₂O₂ with decrease in spectrophotometer absorbance at 290 nm (Nakano and Asada, 1981). Two milliliters reaction mixture consisting of 20 µL crude leaf extract, 660 µL ascorbic acid solution, 660 µL potassium phosphate buffer (pH 7.0, 50 mM) and 660 µL H₂O₂ was used to measure APX activity. Decrease in absorbance was monitored for 3 min just after the addition of H₂O₂. Enzyme activity was calculated in the form of µmol ascorbate min⁻¹ g⁻¹ leaf fresh weight (Elavarthi and Martin, 2010). SOD activity was measured by monitoring the inhibition of nitroblue tetrazolium (NBT) reduction at 560 nm as described by Van Loon (1971).

Statistical Analysis

Statistical procedures were applied to analyze the data (Steel *et al.*, 1996) using completely randomized and randomized complete block designs and means were compared by Duncan's Multiple Range Tests (Duncan, 1955). Selection of efficient rhizobacterial strains was done by using the principal component scoring method using MIITAB software.

Results

Osmoadaptation Assay

The ability to withstand salinity of isolated rhizobacterial

strains was tested by carrying out the osmoadaptation assay (Table 1). It was found from the results that all the strains have variable ability to withstand salinity stress. Most of the strains were capable of facing the salinity stress and did not showed significant decrease in growth with increasing salinity. Maximum optical density at highest salinity level was observed by the strain MA4 followed by MA11.

Auxin Production Assay

The data in Table 2 shows auxin production ability of the rhizobacterial strains. It is evident from the data that all rhizobacterial strains were able to produce auxin (IAA equivalents) to varying degrees, both in the presence and absence of L-TRP. However, the auxin production was enhanced by applying the precursor L-TRP.

Rhizobacterial strain MA-11 produced maximum IAA equivalents when supplemented with L-TRP. The minimum auxin production was recorded by strain MA-21. The data also suggested that these strains were not over auxin producing, but produced moderate amounts of IAA equivalents.

Field Trial

Two most efficient strains (MA4 and MA11) which tolerated the salinity stress and produced auxin were selected as PGPR and further evaluated by conducting a field trial under salt affected soil conditions.

Growth and Yield Parameters

Soil salinity adversely affects the growth and yield of plants. The data in Table 3 demonstrate that plant height was improved due to bacterial inoculation in the absence and presence of L-TRP. The maximum increase in plant height (45%) was found as a result of co-inoculation with MA4 and MA11 in the presence of L-TRP over respective control. Sole inoculation with MA4 and MA11 along with L-TRP significantly improved plant height up to 34 and

32%, respectively, over uninoculated control. Sole and co-inoculation significantly improved the fresh biomass both in the presence and absence of L-TRP application (Table 3). Maximum increase (56%) in fresh biomass was recorded by co-inoculation with MA4 x MA11 when it was tested in the presence of L-TRP. Sole inoculation with MA4 and MA11 also increased the fresh biomass both in the presence and absence of L-TRP. Sole inoculation with MA4 and MA11 in the absence of L-TRP significantly enhanced the straw yield up to 47 and 46%, respectively (Table 3). Along with L-TRP application, the maximum increase (51%) in straw yield was observed by single inoculation with MA4 over its respective control. Without L-TRP application increase in cob length due to sole inoculation/co-inoculation varied from 27 to 40% (Table 3). Along with L-TRP application the improvement in cob length ranged from 32 to 49% over control. Results regarding the effect of rhizobacterial inoculation on cob weight are demonstrated in Table 3. Co-inoculation (MA4xMA11) in the absence and presence L-TRP showed maximum increase (53 and 63%) in cob weight over respective un-inoculated control, respectively. Strain MA4 along with L-TRP application resulted an increase of up to 47% in cob weight as compared with uninoculated control. Inoculation/co-inoculation with and without L-TRP significantly improved the 1000-grain weight (Table 4). Maximum increase in 1000-grain weight (28%) over respective control was seen in case of MA4xMA11, when it was done along with L-TRP. Sole inoculation with MA4 and MA11 also significantly enhanced the 1000-grain weight both in the presence and absence of L-TRP. Along with L-TRP application the inoculation with both strains (i.e. MA4 and MA11) showed up to 22 and 23% increase in 1000-grain weight, respectively, over control. Similarly, inoculation/co-inoculation also caused improvement in grain yield (Table 4). Co-inoculation (MA4 x MA11) showed maximum increase in grain yield when it was tested in the presence (31%) and absence (28%) of L-TRP over control. Single inoculation with strains MA4 and MA11 along with L-TRP improved grain yield up to 23 and 22%, respectively, over control.

Table 1: Growth of rhizobacterial strains in broth culture after 3 days under salt stress conditions

Strain	Original	10 dS m ⁻¹	20 dS m ⁻¹	30 dS m ⁻¹	Strain	Original	10 dS m ⁻¹	20 dS m ⁻¹	30 dS m ⁻¹
MA-1	1.21 ± 0.10	0.89 ± 0.04	0.97 ± 0.02	0.97 ± 0.23	MA-15	1.04 ± 0.09	0.50 ± 0.03	1.02 ± 0.14	0.80 ± 0.01
MA-2	1.26 ± 0.20	1.05 ± 0.06	1.00 ± 0.12	0.75 ± 0.12	MA-16	0.99 ± 0.07	0.20 ± 0.01	1.07 ± 0.09	0.83 ± 0.11
MA-3	1.08 ± .01	1.11 ± 0.01	0.98 ± 0.12	1.00 ± 0.08	MA-17	0.04 ± 0.04	0.07 ± 0.05	0.09 ± 0.14	0.08 ± 0.14
MA-4	1.00 ± 0.08	1.11 ± 0.36	1.08 ± 0.02	0.98 ± 0.04	MA-18	1.02 ± 0.01	0.78 ± 0.10	0.94 ± 0.05	0.65 ± 0.07
MA-5	0.70 ± 0.03	1.40 ± 0.12	0.70 ± 0.05	0.83 ± 0.03	MA-19	0.68 ± 0.01	0.84 ± 0.03	0.80 ± 0.20	0.91 ± 0.05
MA-6	0.81 ± 0.03	0.94 ± 0.05	0.89 ± 0.04	0.79 ± 0.07	MA-20	1.30 ± 0.01	1.22 ± 0.08	1.06 ± 0.06	1.10 ± 0.12
MA-7	0.97 ± 0.02	0.85 ± 0.02	0.84 ± 0.17	0.77 ± 0.26	MA-21	1.08 ± 0.03	1.26 ± 0.17	1.09 ± 0.16	1.03 ± 0.01
MA-8	0.63 ± 0.01	1.14 ± 0.06	0.93 ± 0.11	0.78 ± 0.17	MA-22	0.80 ± 0.01	0.78 ± 0.11	0.67 ± 0.08	0.59 ± 0.06
MA-9	0.52 ± 0.01	0.64 ± 0.12	0.40 ± 0.15	0.63 ± 0.21	MA-23	0.86 ± 0.01	0.78 ± 0.02	0.82 ± 0.02	0.81 ± 0.08
MA-10	0.87 ± 0.07	0.51 ± 0.03	0.50 ± 0.03	0.68 ± 0.02	MA-24	0.92 ± 0.02	0.87 ± 0.06	0.77 ± 0.01	0.85 ± 0.23
MA-11	1.05 ± 0.04	0.62 ± 0.21	0.69 ± 0.02	0.89 ± 0.15	MA-25	1.05 ± 0.02	1.01 ± 0.07	1.21 ± 0.12	0.76 ± 0.11
MA-12	0.95 ± 0.03	0.60 ± 0.20	0.64 ± 0.03	0.75 ± 0.01	MA-26	0.63 ± 0.02	0.66 ± 0.04	0.62 ± 0.11	0.67 ± 0.06
MA-13	1.48 ± 0.04	1.38 ± 0.06	1.04 ± 0.04	0.78 ± 0.23	MA-27	0.81 ± 0.01	0.80 ± 0.15	0.77 ± 0.03	0.78 ± 0.05
MA-14	1.43 ± 0.06	0.77 ± 0.08	1.50 ± 0.23	0.65 ± 0.08	MA-28	0.58 ± 0.01	0.54 ± 0.13	0.58 ± 0.05	0.57 ± 0.12
MA-29	0.75 ± 0.02	0.82 ± 0.02	0.79 ± 0.07	0.63 ± 0.16					

Values are means of three replicates ± Standard error (SE)

Table 2: Amount of IAA equivalents ($\mu\text{g mL}^{-1}$) produced by the selected rhizobacterial strains

Strains	L-TRP(+)	L-TRP(-)	Strains	L-TRP(+)	L-TRP(-)
MA-1	9.23 d	6.11 gh	MA-14	5.56 a	3.09 op
MA-2	7.25 b	5.61 lm	MA-15	4.61 c	0.53 q
MA-3	10.19 g	7.07 jk	MA-18	2.25 j	2.02 k
MA-4	13.26 g	7.19 g	MA-19	5.12 b	2.08 jk
MA-5	6.76 e	5.68 i	MA-20	4.61 c	1.63 l
MA-6	2.53 i	2.48 i	MA-21	1.18 no	0.91 p
MA-7	8.13 d	4.53 f	MA-23	2.95 h	1.40 mn
MA-8	3.07 gh	0.98 op	MA-24	2.06 jk	1.11 op
MA-11	19.19 g	12.08 jk	MA-25	3.25 g	1.45 lm
MA-13	6.12 b	3.52 q			

Values are means of three replicates. *Means sharing similar letter(s) do not differ significantly

and presence of L-TRP application (Table 5). Co-inoculation with MA4xMA11 showed maximum improvement in total chlorophyll content (29 and 25%) over its respective un-inoculated control in the presence and absence of L-TRP application, respectively. Data regarding proline contents (Table 5) showed that inoculation with rhizobacterial strains both in the presence and absence of L-TRP significantly decreased the proline contents in maize plants grown under salt stressed soil conditions. Along with L-TRP application, reduction in proline contents ranged from 34 to 41% over respective un-inoculated controls. Minimum proline contents were recorded by the co-inoculation with MA4xMA11 both in the absence and presence of L-TRP application. Similarly, inoculation/co-inoculation significantly reduced the electrolyte leakage

Table 3: Effect of auxin producing bacterial inoculants on growth parameters of maize plants under saline field conditions

Bacterial inoculants	Plant height (cm)		Fresh biomass (t ha ⁻¹)		Straw yield (t ha ⁻¹)		Cob length (cm)		Cob weight (g)	
	L-TRP		L-TRP		L-TRP		L-TRP		L-TRP	
	without	with	without	with	without	with	without	with	without	with
Control	159.3fg*	169.0ef	6.7k	7.9i	3.91f	4.75e	12.5gh	14.4d-g	107.8f	119.8e
MA4	208.7b	214.7b	9.9de	10.1bc	5.59bc	6.16b	15.9b-e	16.5b-d	153.6cd	158.5bc
MA11	205.3b	211.7b	9.8de	10.0cd	6.02bc	6.12b	15.6b-e	16.7bc	149.7cd	156.8b-d
MA4 x MA11	228.0a	232.3a	10.3b	10.83a	6.13b	6.45a	17.6ab	18.7a	165.3cd	176.4a

Values are means of three replicates. *Values sharing the same letter(s) in a column do not differ significantly at $p < 0.05$ according to Duncan's multiple range test

Table 4: Effect of auxin producing bacterial inoculants on yield and nutritional parameters of maize plants under saline field conditions

Bacterial inoculants	Grain yield (t ha ⁻¹)		1000-grain weight (g)		K: Na ratio		Phosphorus content in grains (%)		Potassium content in grains (%)	
	L-TRP		L-TRP		L-TRP		L-TRP		L-TRP	
	without	with	without	with	without	with	without	with	without	with
Control	2.95i*	3.23h	191.2fg	197.0f	1.19g	1.25ef	0.57gh	0.58c-f	0.26fg	0.27f
MA4	3.55cd	3.65c	229.3cd	234.2bc	1.39f	1.54bc	0.66b-e	0.68a-d	0.31de	0.33bc
MA11	3.45de	3.60c	227.9cd	235.0bc	1.36d-f	1.48bc	0.63ef	0.65bc	0.35bc	0.31de
MA4 x MA11	3.78b	3.88a	239.6ab	245.0a	1.73de	1.85a	0.69a-c	0.72a	0.33bc	0.39a

Values are means of three replicates. * Values sharing the same letter(s) in a column do not differ significantly at $p < 0.05$ according to Duncan's multiple range test

Table 5: Effect of auxin producing bacterial inoculants on physiological parameters of maize plants under saline field conditions

Bacterial inoculants	Total chlorophyll content (SPAD values)		Relative water content		Proline content ($\mu\text{g g}^{-1}\text{FW}$)		Electrolyte leakage (dS m ⁻¹)		Crude protein	
	L-TRP		L-TRP		L-TRP		L-TRP		L-TRP	
	without	with	without	with	without	with	without	with	without	with
Control	39.8fg	41.9ef	67.7e	71.1fg	2.94b	2.64c	1.38a	1.19b	9.9f	10.9de
MA4	44.6c-e	47.8bc	74.2bc	75.0bc	2.25ef	2.04h	1.00cd	0.94c-e	11.7bc	12.0b
MA11	45.2c-e	47.3bc	74.5bc	76.9b	2.36fd	2.17fg	1.01cd	0.96c-e	11.6bc	11.9bc
MA4 x MA11	49.9ab	51.7a	72.1fg	78.3a	2.08gh	1.72i	0.95c-e	0.89e	12.1b	12.6a

Values are means of three replicates. * Values sharing the same letter(s) in a column do not differ significantly at $p < 0.05$ according to Duncan's multiple range test

Physiological Parameters

Salt stress causes severe physiological disorders within the plants, which are depicted in the Table 5. Significant improvement in total chlorophyll contents of maize was recorded by inoculation/co-inoculation both in the absence

both in the presence and absence of L-TRP (Table 5). Maximum reduction (35%) in electrolyte leakage was recorded by MA4xMA11 when it was carried out in the presence of L-TRP. Sole inoculation with MA4 and MA11 in the presence of L-TRP also reduced the electrolyte leakage up to 31 and 30%, respectively, as compared to

uninoculated control. Data in Table 5 revealed that co-inoculation in the presence of L-TRP caused an increase of 15% in relative water contents as compared with control. While such improvement in relative water content with sole inoculation ranged from 10 to 13%. The results regarding protein contents (Table 5) indicated that rhizobacterial inoculation significantly improved the protein contents in the presence and absence of L-TRP application. Co-inoculation (MA4xMA11) showed maximum results (27%) regarding improvement in protein contents of maize as compared to uninoculated control.

Nutritional Parameters

Under salt stress uptake of essential mineral nutrients is decreased. A significant improvement in mineral nutrient content was observed due to inoculation/co-inoculation. In the absence of L-TRP application, sole inoculation with MA4 and MA11 caused up to 16 and 11% increase in phosphorous concentration over respective control, respectively (Table 4). Along with L-TRP application, these strains caused up to 19 and 11% increase in P contents over their respective control, respectively. Maximum increase in P contents, in the absence (21%) and presence of L-TRP (26%) as compared with uninoculated control were recorded by combined inoculation.

Due to ionic competition during salinity stress uptake of Na^+ is greater than K^+ and salt tolerance of plants can be accessed by K:Na. Data in Table 4 highlights that inoculation/co-inoculation significantly improved K:Na in leaves of maize plants. The maximum increase (55%) in K:Na in the presence of L-TRP was recorded by MA4xMA11 over uninoculated control. Strains MA4 and MA11 in single inoculation along with L-TRP gave 29 and 24% increase over respective controls. Inoculation/co-inoculation in the absence of L-TRP also significantly improved the K/Na. Potassium content of grains also significantly improved (up to 50%) due to rhizobacterial inoculation both in the presence and absence of L-TRP.

Stress Related Metabolites and Antioxidant Enzyme Activity

Data shows that phenolic contents improved by inoculation/co-inoculation (Fig. 1). The improvement in total phenolic contents in presence of L-TRP ranged from 59 to 73% as compared with uninoculated control. In the absence of L-TRP, maximum improvement in total phenolic contents was observed with combined inoculation. Sole inoculation with MA4 and MA11 along with L-TRP showed up to 45 and 41% increase in total soluble sugars as compared to their respective uninoculated controls (Fig. 2). Maximum increase in total soluble sugars was recorded by MA4xMA11 in the absence (46%) and presence (63%) of L-TRP, respectively. When plants are subjected to any kind of stress such as salinity,

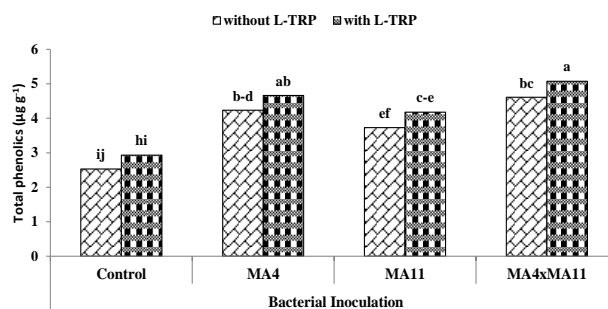


Fig. 1: Effect of rhizobacteria inoculation on the phenolic contents of maize under salt affected field conditions. In this and other figures, values are means of three replicates. * Bars sharing the same letter(s) do not differ significantly at $p < 0.05$ according to Duncan's multiple range tests

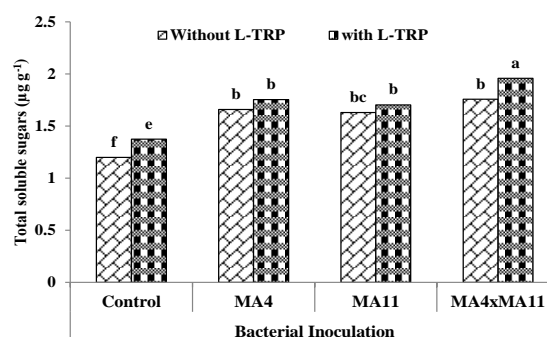


Fig. 2: Effect of rhizobacteria inoculation on the total soluble sugar contents of maize under salt affected field conditions

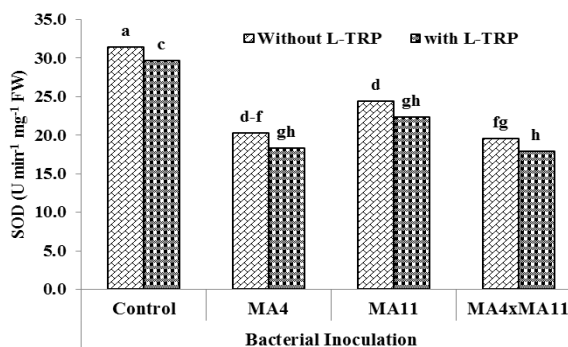


Fig. 3: Effect of rhizobacteria inoculation on SOD values of maize under salt affected field conditions

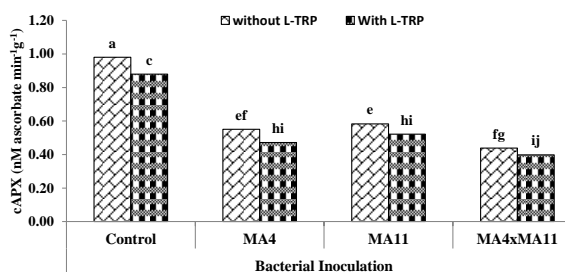


Fig. 4: Effect of rhizobacteria inoculation on cAPX values of maize under salt affected field conditions

the activity of antioxidant enzymes is enhanced. It is clear from the results that the rhizobacterial inoculation/co-inoculation significantly reduced the SOD values both in the presence and absence of L-TRP. Co-inoculation caused a maximum reduction in SOD values up to 38 and 42% in the absence and presence of L-TRP, respectively, as compared with respective uninoculated control (Fig. 3). Single inoculation with MA4 and MA11 also caused significant reduction in SOD values in the presence and absence of L-TRP. Similarly, significant reduction (up to 59%) in ascorbate peroxidase (cAPX) were also noted by the rhizobacterial inoculation both the presence and absence of L-TRP (Fig. 4).

Identification of Rhizobacterial Strains

The selected strains were identified by BIOLOG[®] identification system as *Pseudomonas fluorescens* (M4) and *Serratia proteamaculans* (M7).

Discussion

Soil salinity negatively affects the plant growth by causing osmotic and ionic stress (Azooz, 2004). Rhizobacterial inoculation improves the growth and development of plants through various mechanisms mainly by producing plant growth regulators. Auxin is an important plant hormone and its application can alleviate the adverse effects of soil salinity on plant growth (Kaya *et al.*, 2013). This study highlight the importance of using auxin producing growth promoting rhizobacteria for inducing salt tolerance in maize. In our laboratory study we observed that salinity adversely affected the growth of rhizobacterial strains. At normal conditions, the growth of strains was optimum but the growth of rhizobacterial strains varied under salinity stress. The growth of some strains was improved even at higher salinity levels. As we isolated the strains from the salt affected soil this might be the reason to tolerate saline conditions. The ability of these bacterial strains to tolerate higher levels of salinity may be due to changes in morphology and metabolism, which makes them able to cope and adopt saline conditions (Sgroy *et al.*, 2009).

When subjected to stress plants produce excess amount of auxin as a strategy to counter stress situation by limiting their life cycle (Yuerkli *et al.*, 2004). Plants use this strategy as an adaptive mechanism to cope stressful conditions. But along with normal metabolic activities, this auxin production by the plants requires extra metabolic energy. This extra energy consumed in the production of auxins results in the decrease of plant growth. So auxin produced by the PGPR can reduce the energy requirements of plant (Zahir *et al.*, 2010). In our field study we observed that salinity reduced the growth and yield of maize. This reduction in growth and yield caused by salinity may be due to hormonal imbalance. In previous studies, it is reported that salinity causes progressively decline in plant hormones (Nilsen and Orcutt, 1996). In our study we observed that

inoculation with auxin producing rhizobacteria caused positive increase in growth of maize under salt stress. The improvements in growth parameters by rhizobacterial strains may be due to the correction of this hormonal balance as they were able to produce auxin. Some years earlier, Egamberdieva, (2009). Exogenous application of hormones has also been reported to improve plant growth in salt affected conditions (Sastry and Shekhawa, 2001; Afzal *et al.*, 2005).

Plants accumulate different compatible solutes such as proline under different stresses (Saravanakumar *et al.*, 2011). Plants synthesize proline in response to diverse abiotic or biotic stresses at high energy cost. High concentration of proline in plants is also considered as an indicator of stress (Rai *et al.*, 2003). We observed that the proline concentration in plant leaves increased under salinity stress but rhizobacterial inoculation decreased the proline concentration in salt stressed plants. The decrease in proline concentration in plants may be due to the fact PGPR lessened the effect of salinity stress on plants. This is confirmed by the work of Nadeem *et al.* (2007) who reported a decrease in proline contents during salt stress owing to rhizobacterial inoculation.

Salt tolerance of plants can be indicated by K^+/Na^+ (Hamida-Sayari *et al.*, 2005). It was found in our study that salinity stress caused the ionic imbalance in plants. It resulted in higher intake of Na^+ than K^+ . This increased uptake of Na^+ results in reduction of K^+/Na^+ (Nadeem *et al.*, 2010). Uptake of higher concentration of K^+ as compared to Na^+ is vital to maintain higher K^+/Na^+ in cells (Kavitha *et al.*, 2012). In our study, we found that rhizobacterial inoculation /co-inoculation significantly improved the K^+/Na^+ . This might be due to lowered uptake of Na^+ than K^+ due to longer roots caused by inoculation/co-inoculation with rhizobacterial strains as reported previously (Yue *et al.*, 2007; Ahmad *et al.*, 2012). These strain produced the exopolysaccharides which restrict the entry of Na^+ into the plant roots and caused ionic balance which may be one of the reasons of better growth and yield of maize under salt stress.

Membrane permeability (MP) is a good indicator of salt stress and is judged by measuring electrolyte leakage (Farkhondeh *et al.*, 2012). In our study, we observed marked increase in electrolyte leakage in plant subjected to salinity stress rhizobacterial inoculation caused decline in electrolyte leakage. This might be due to the reduction in the negative effects of salinity on membrane disintegration by these rhizobacterial strains. We also observed that a strong relationship existed between chlorophyll contents and electrolyte leakage. This type of relationship has been documented in many studies (Kaya *et al.*, 2001). It is likely that membrane integrity somehow is dependent upon chlorophyll contents; because decrease in chlorophyll contents as caused by salinity stress induces senescence and membrane become more permeable (De Araujo *et al.*,

2006). In our study we also observed improvement in relative water contents of inoculated plants which might be due to development of longer roots that were able to extract more water from soil.

Changes in nutrient uptake is the most common phenomenon which plants undergo in different stresses such as salinity stress (Greenway and Munns, 1980). In our field experiment, we observed that salinity stress caused marked decrease in phosphorous and potassium contents of maize grains however; rhizobacterial inoculation significantly improved the mineral nutrient contents. This might be due increase in the root area and provision of growth hormones such as auxins would have helped the plant in taking more nutrients from the soil. In a previous study, it is reported that auxin enhances the uptake of mineral nutrients (Haschke and Lutjge, 1973). Nadeem *et al.* (2006) and Hameeda *et al.* (2008) also reported increase in nutrient uptake by microbial inoculation under stressful conditions. These strains also possessed phosphate solubilization ability which may have helped the plants to get extra nutrients.

Under salt stress situations, plants accumulate higher levels of antioxidant enzymes such as SOD, CAT, POD and APX as a defense mechanism to counter oxidative stress caused by salinity (Munns and Tester, 2008; Kaya *et al.*, 2001). Auxin (IAA) and reactive oxygen species (ROS) play a critical role in salt stress tolerance. In our studies, we also found marked increase in antioxidant enzymes under salinity stress however; inoculation/co-inoculation caused reduction in antioxidant enzymes. This reduction in antioxidant enzymes highlights the fact that these rhizobacterial strains relieved the plants from the deleterious effects of salinity. Baniaghil *et al.* (2013) also reported that bacterial inoculation lowered the activity of antioxidant enzymes under salt stress. Contrary to our results Naveed *et al.* (2014) reported increase in antioxidant enzymes activity under abiotic stress in wheat by bacterial inoculation. Along with production of antioxidant enzymes, plants also accumulate some other compounds such as phenolics and sugars which not only act as osmoregulators but also scavenge reactive oxygen species (ROS) (Fernandez *et al.*, 2012; Theocharis *et al.*, 2012). In our study, we found increase in content of sugars and phenolics under salt stressed conditions.

It is highly likely that along with auxin production ability of these strains other characters of these strains might have helped the plants to tolerate the adverse effects of salinity. We observed that both the selected strains varied in their growth promotion ability, which might be due to presence of different other growth promoting characters such as root colonization, phosphate solubilization and chitinase activity. Chitinase activity of these strains might have helped the plants against their pathogens thus resulting in better growth. Nadeem *et al.* (2007) also observed that rhizobacterial strains varied in their ability to improve the plant growth. These differences may also be due to other growth promoting characteristics than auxin production of these strains.

Conclusion

These findings highlight the role of auxin producing rhizobacteria for improving the growth and yield of maize under salt affected conditions. Further studies using these bacteria on diverse field conditions is necessary before putting them into technology.

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