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ROLE OF PGPR IN THE RECLAMATION AND REVEGETATION OF SALINE LAND

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Abstract

A field experiment was conducted to investigate the effect of PGPR inoculation on growth and yield of maize as well as on reclamation of saline sodic soil of Soil Salinity Research Institute Pindi Bhattian Pakistan, during 2015-16. Seed of maize genotype "Islamabad Gold" were soaked (2-3 h) prior to sowing in the broth culture of 4 bacterial strains i.e. *Pseudomonas putida* (accession no. KX580766), *Pseudomonas fluorescens* (accession no. KX644132), *Exiguobacterium aurantiacum* (accession no. KX580769), *Bacillus pumilus* (accession no. KX580768) and *Lysinibacillus sphaericus* (accession no. KX580767). *In vitro* analysis of bacteria confirmed that they metabolize ACC deaminase, solubilize insoluble phosphate and produce significant quantity of auxin in the presence of L-tryptophan. Inoculation of maize with bacteria along with application of 1L inocula / treatment in the field gave a significant (P = 0.05) increase in germination (76%), leaf chlorophyll (24%), proline (65%), anthocyanin (38%) and soluble sugar content (56%). *P. putida* inoculation resulted in maximum increase in plant height, leaf area, no of grains cob^{-1} (459.8), 1000 grain weight (330.9 g) and grain yield (3.25 tha^{-1}). *P. fluorescens* was least effective. The rhizosphere soil analysed after harvesting exhibited significant decrease in electrical conductivity (49%), sodium absorption ratio (98%), and cation exchange capacity (94%) concomitant with a significant increase in organic matter (52%), NO_3-N (37%), available P (48%) and K (31%). The highest efficiency of *P. putida* may be attributed to the maximum ACC deaminase activity, higher production of indole acetic acid and greater potential for Phosphate solubilization. The favorable effects of PGPR were more pronounced in the successive year 2016.

Key words: Saline sodic soil, Sodium absorption ratio, Electrical conductivity, Cation exchange capacity, Grain yield.

Introduction

Salinity is one of the major abiotic factor which adversely affects the arid and semi-arid region of the world and reduced the cultivated land area and crop productivity at all (Qadir *et al*., 2014; Hashem *et al*., 2018). Globally more than 831 m ha of land is affected either by salinity (397 m ha), and/or by sodicity (434 m ha) (Setia *et al.,* 2013). In Pakistan, salinity and sodicity has affected about 6.68 mha of land, 26% of irrigated land is saline and 56% is saline sodic. According to an estimate, 2.66 m ha are affected in the province of Punjab with varying degree of salinity and sodicity.

Maize (*Zea mays* L.) is the third economic agriculture commodities after wheat and rice. In Pakistan it is the fourth largest crop grown after wheat, cotton and rice. About 52% of human nutrition relies on maize (Anonymous., 2015). For optimum growth and yield of maize, an adequate amount of available nitrogen, phosphorous and potassium is required (Khan *et al.,* 2014). In saline sodic soil these nutrients are insoluble due to higher electrical conductivity and sodium absorption ratio. Maize is considered moderately sensitive to salt stress, can maintain growth in saline soil with an ECe 3-6 dS m^{-1} (Hasanuzzaman *et al.,* 2013). Deficiency of these nutrients hamper normal activity of photosynthetic pigments, carbohydrate production and yield in maize. Salinity also decline the endogenous levels of phytohormone that results into poor germination (Abd Allah *et al.,* 2017).

Biofertilizers contain living cells of PGPR that increase plant growth directly through N_2-fixation, production of phytohormones, lowering of ethylene concentration and solubilization of inorganic Phosphate (Ahmad & Kibert, 2014; Mishra *et al.,* 2018). These bacteria secrete an organic acid like gluconic acid which solubilize the phosphate complexes converting them into ortho-phosphate which is available for plant uptake and utilization (Otieno *et al.,* 2015). They also produce a unique enzyme, ACC deaminase, that hydrolyzes ACC into NH_3 and α-ketobutyrate (Ali & Kim, 2018). Thus, plants treated with PGPR containing ACC deaminase enzyme are dramatically more resistant to the deleterious effect of stress ethylene under salinity (Bal *et al.,* 2013; Han *et al.,* 2015). They secrete IAA which brings morphological changes in root which ultimately leads to improved growth of shoots and increase the yield (Asim *et al.,* 2013). Several strains promote plant growth by ACC deamise activity, IAA production and phosphate solubilization (Sarkar *et al.,* 2018). PGPR have been reported to increase chlorophyll content in maize plant under salinity stress (Hassan *et al*., 2018; Singh & Jha, 2017). Under salt stress, increased accumulation of leaf proline and total soluble sugar content has been reported in many PGPR treated plant species (Upadhyay *et al.,* 2015; Iqbal *et al.,* 2016).

The present study was conducted to evaluate the effect of rhizobacteria on pH, EC_e, SAR, CEC, OM (%), NO_3-N, available P and K of soil as well as their effect on growth, physiology and yield of *Zea mays* L. grown in saline sodic field in Soil Salinity Research Institute Pindi Bhattian, Pakistan, for two consecutive cropping years 2015-2016.

Materials and Methods

ACC-deaminase activity: ACC-deaminase activity was determined following the method of Li *et al.,* (2011). Fresh colony of bacterial strain was inoculated

into 20 mL liquid LB media, incubated at 37ºC for 24 hr on an orbital shaker (Excell E24, USA). 2 mL of culture was centrifuged at 8000 rpm for 5 min. Supernatant was discarded and the pellets were washed twice with 1 mL DF medium, then suspended into 2 mL DF medium (supplemented with 3 mM ACC) and incubated at 37ºC on shaker for 2 hr. After centrifugation, 100µL supernatant was diluted to 10X with DF medium. An aliquot 60 µL of diluted supernatant was mixed with 120 µL ninhydrin reagent in effendorf tube and heated on boiling water bath for 30 min. DF medium was used as a blank. After development of Ruhemann's purple color, absorbance was measured at 570 nm by using Elisa reader.

Estimation of phosphate solubilisation Index (SI): Phosphate solubilization index was checked on the Pikovskaya's agar medium (Pikovskaya, 1948). Fresh colony of strain was streaked in the center of plates containing Pikovskaya's agar medium (Ca_3PO_4 2.5 g, $C_6H_{12}O_6$ 13 g, $(NH_4)_2$ SO_4 0.5 g, NaCl 0.2 g, $MgSO_4.7H_2O$ 0.1 g, KCl 0.2 g, Yeast Extract 0.5 g, $MnSO_4$ trace, $FeSO_4.7H_2O$ trace, Agar 15 g in 1L dH_20 at pH 7.2), incubated at 28°C. After 7days, formation of halozone around colony was noted. SI was determined using the following formula:

SI = Colony diameter + Halozone diameter / Colony diameter

IAA production: Quantification of IAA was done using Salkowski reagent (Patten & Glick 2002). 50 µL of 24 hr old bacterial culture was transferred into test tube containing 10 mL liquid LB media, supplemented with 50µg/mL L-tryptophane or without L-tryptophan, kept on orbital shaker (Excell E24, USA) at 120 rpm for 5 days at 28°C. The bacterial culture was centrifuged at 10,000 rpm for 15 min at 4°C to separate supernatant. Afterward, 1 mL supernatant was transferred to a fresh tube, mixed vigorously with 100 µL of 10mM ortho H_3PO_4 and 2 mL of reagent (1 ml 0.5 M $FeCl_3$ in50 mL of 35% $HClO_4$). After 25 min, the absorbance of the developed pink color and was read at 530nm.

Field experiment

Geographical and physio-chemical characterization of experimental area: The study area falls within the semi-arid zone, located between latitude of 31°52′ N, longitude of 73°20′ E, and elevation of 195.6 m above sea level. The soil is characterized by alkalinity and sodicity, sandy clay loam in texture, pH 8.9, EC_e 2.6 S/dm, SAR 24.1, 0.43% OM, 16.48 mg/kg NO_3-N, 2.25 mg/kg available P and 63.7mg/kg available K, respectively. The climate of the area is dry and hot with summer temperature ranging from 27-50°C, winter temperature varies from 6-21°C and annual rainfall is 40 mm.

Experimental materials: *Zea Mays* L. (Islamabad Gold) was purchased from Crop Science Department, NARC Islamabad, Pakistan. Five PGPR used in this research work: *Pseudomonas putida* (accession no. KX580766), *Lysinibacillus sphaericus* (accession no. KX580767) and *Bacillus pumilus* (accession no. KX580768) isolated from roots of wheat seedling grown in Khewra salt range having pH 8.6, EC_e 3300 µS/dm. *Exiguobacterium aurantiacum* (accession no. KX580769) isolated from oily sludge in Chakwal having pH 7.9, ECe 630 µS/dm and *Pseudomonas fluorescens* (accession no. KX644132) isolated from rhizosphere of maize grown in Quaid-e-Azam University Islamabad having pH 7.2-7.4, EC_e 113 µS/dm.

Experimental design: Six treatments with five replicates were considered for experiment: T_1: un-inoculated treatment, T_2: inoculated with *P. fluorescens,* T_3: inoculated with *P. putida,* T_4: inoculated with *L. sphaericus,* T_5: inoculated with *B. pumilus,* T_6: inoculated with *E. aurantiacum.* The experimental design used was RCBD and different treatments were randomly allocated to the experimental plots. The size of plot/treatment was 4×5m with 50×100cm paths separating adjacent plots and blocks, respectively.

Inocula preparation, seed sterilization and inoculation: Surface sterilized maize seeds (0.024% NaClO for 2 min) were rinsed thoroughly with dH_2O (Gholami *et al.,* 2009). Five days old inocula (in 250 mL liquid LB media) was adjusted to OD ≈1 at 660 nm to obtain uniform population of bacteria (10^8-10^9CFU/ ml). 300 seeds for each treatment were soaked in respected inocula for atleast 2 hr. After sowing inocula of each bacterium (1L/bacterium) was mixed soil.

Germination (%), plant height and leaf area: At 13DAS, germination data was recorded using the following formula:

$$\text{Germination (\%)} = \frac{\text{No. of seed germinated}}{\text{Total no. of seeds}} \times 100$$

At 12WAS, five plants/replicate in the middle rows were randomly selected from each plot and tagged for the measurement of plant height (cm). Leaf area was determined using the following formula:

Leaf area = K × length × width, where k = 0.75 (Ruget *et al.,* 1996)

Measurement of yield traits: The maize was harvested at maturity (16 WAS) and data related to number of grain/cob and 1000 grain weight (g) was recorded. Grain yield (t/ha) was estimated as per the following relationship.

GYha = Yp x Pha

where, GYha = Grain yield per hectare, Yp = Average grain yield per plant, Pha = Plant population per hectare.

Soil pH and electrical conductivity (EC_e): Air dried soil sample (10g) was mixed in 10mL distilled water and stirred for 1 hr on magnetic stirrer for homogenous mixing (McClean, 1982). Filtered the suspension with Whatman No. 42 filter paper. The pH of filtrate was determined at 27.8°C with pH meter (Sartorius Professional meter PP-15) ECe of extracts was determined with conductivity meter (KL-138).

Assay for organic matter (OM): OM was determined by Walkley-Black method (Walkey, 1947). 1g air dried soil sample was oxidized with 10 mL 1N $K_2Cr_2O_7$ solution and 20 mL conc. H_2SO_4, vigorously shaken for 1 mim. After 30 min, 200 mL DI water and 10 mL conc. H_3PO_4 was added, allowed to cool. 10-15 drops of $(C_6H_5)_2NH$ was added as an indicator. The samples were titrated against 0.5 M [(NH_4) $2SO_4.FeSO_4.6H_2O$] solution until the color changes from violet-blue to green. Organic matter was calculated by following formula.

Organic matter (%) = 1.724 × Total Organic Carbon (%)

Cation exchange capacity (CEC): 24g air dried soil was mixed 132 mL 1N $CH_3COONa.3H_2O$ solution, shook for 5 min and centrifuged at 3000 rpm. Samples were washed with 99 mL of 95% C_2H_6O, mixed will for 5 min and centrifuged at 3000 rpm. The supernatant liquid was discarded. Adsorbed Na was replaced from sample by extraction with 99 mL 1N NH_4OAc solution. Then supernant liquid was collected in 100 mL flask. Emission reading was taken by Flame photometer at 767-nm wavelength (Rhoades & Polemio, 1977).

Extractable potassium: Air dried soil was mixed with 1N NH4OAc in a ratio of 1:5 and shaked on a reciprocal shaker for at 200-300 rpm. The suspension was filtered using a Whatman No.1 filter paper and volume was raised to 50 ml with 1 N NH4OAc solution. Potassium in soil extracts was measured by taking emission reading on the flame photometer at 767-nm wavelength.

NO_3-N extraction: NO_3-N was determined using chromotropic acid method (Sims & Jackson, 1971)**.** Air dried soil (10g) was mixed with 50 mL 0.002 N $CuSO4.5H_2O$ solution, shook for 15 min and filtered through a double Whatmann No. 42 filter paper. After cooling, 3 mL filtrate was mixed with 1 mL 0.1% $C_{10}H_8O_8S_2$ solution drop by drop and allowed to cool. Then 6 mL conc. H_2SO_4 was added, swirled and left to cool. Yellow colour was developed after 45 min and OD of was recorded on the spectrophotometer at 430-nm wavelength.

Available phosphorous in soil: Available phosphorus in soil was determined using the $NaHCO_3$ method (Olsen & Sommers 1982). Air dried sample (5g) was mixed with 100 mL $NaHCO_3$ (0.5M) solution, shook for 30 min on shaker at 120 rpm. The suspension was filtered using Whatman No. 42 filter paper. Few drops of *p*-nitrophenol indicator and 1 mL 5N H_2SO_4 was mixed with 10 mL $NaHCO_3$ extract. Then 8 mL reagent B was added, and volume of the digested mixture was raised to 40 mL using dH_2O. After 10 min, the absorbance was recorded at 882-nm wavelength.

Sodium adsorption ratio (SAR): Concentrations of Na, Ca and Mg were determined using Ammonium Bicarbonate-DTPA (Diethylene triamine penta acetic acid) method. acetic acid) method (Soltanpour & Schwab, 1977). The extracts were then analyzed using atomic absorption spectrometry (Spectra AA-100). SAR was calculated through the following equation:

$$SAR = \frac{[Na+]}{\sqrt{\frac{1}{2}([Ca2+] + [Mg2+])}}$$

Statistical analysis

All data were collected for each treatment, mean values, and standard errors were calculated. Data were analyzed by analysis of variance (ANOVA) and pair wise comparison among treatment means was made by Barlett's test at p=0.05 using STATISTIX version 8.1. Pearson correlation was analyzed through XLSTAT.

Results

Plant growth promotion activities of PGPR: Amongst PGPR strains, a significant difference (p=0.05) in the solubilization index (SI) was recorded. Maximum SI was recorded in *B. pumilus*, followed by *P. putida*, *P. fluorescens* and *L. sphaericus*. Least SI was recorded in *E. aurantiacum* (Fig. 1). The addition of tryptophan in the culture medium augmented the production of IAA in *P. putida* by 91% of the control (-trp) and 80% of the control in *E. aurantiacum*. Trp has no significant effect on IAA content of *P. fluorescens* and *B. pumilus* whereas *L. sphaericus* showed decline in IAA content by 42% of control (Fig. 1). Similarly, maximum ACC-deaminase activity (186 nmol/h) was recorded for *P. putida*, while other strains showed non-significant difference in ACC activity (Fig. 1).

Effect of PGPR on soil health: In Ist year decrese in soil pH was too least, but in second year pH showed decline in inoculated soil. Lowest pH was due to *P. putida* (T_3). Inoculated soil with *P. fluorescens* (T_2) being least effective (Table 1). ECe and SAR were also decreased both in 1st and 2nd year in all the treatments. The % decrease was higher in T_3 (*P. putida*)>T_4 (*L. sphaericus*). *E. aurantiacum* (T_6) was slower in response and did not show any significant decline in ECe in 1st year. SAR was decreased by 62% of the inoculated over control in T_3 (*P. putida*), but in 2nd year SAR showed higher decline (111%) of the control. *L.sphaericus* (T_4) showed 75% decline in SAR in Ist year, in 2nd year the decrease was 85% of the SAR of Ist year (Table 1).

CEC was decreased in 2nd year by 39% of the 1st year. In both years, the maximum decrease in CEC was 94% due to T_3, while the minimum decrease was due to T_6 (11%). T_5 showed 69% decrease in CEC in the 2nd year while in the 1st year % decrease was 23% of the uninoculated control. The organic matter content of the rhizosphere soil was significantly (12-52%) higher following cultivation of inoculated plants. In both years, the maximum increase (50-52%) was due to T_3 respectively (Table 1).

The application of PGPR increased the fertility status of soil i.e. NO_3-N, available P and K availability. In both years, the concentration of NO_3-N, available P and K in rhizospheric soil significantly (p=0.05) increased from 9-37%, 7-48% and 11-31% over uninoculated soil (T_1). The maximum increase (37%) in NO3-N, available P (48%) and K (31%) was due to T_3 inoculated plants (Table 2).

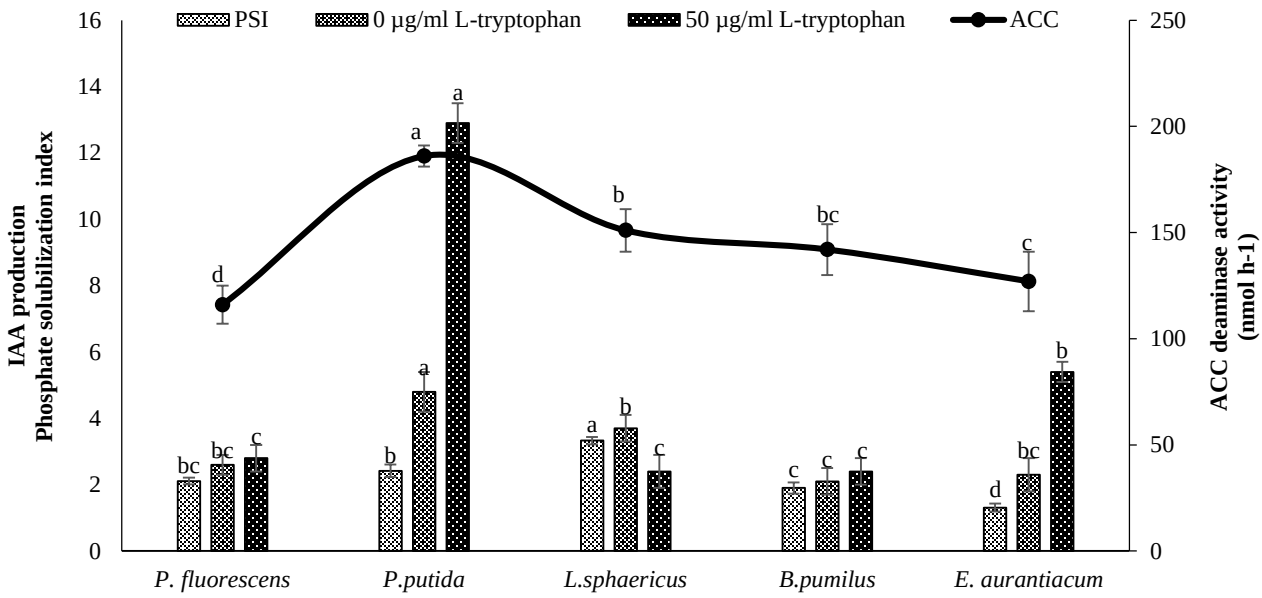

Fig. 1. Plant growth promoting activities of selected PGPR. Measurement for IAA (ug/ml) and ACC deaminase activity was made after 24 hr, for P-solubilization measurement was made after 7 days of growth on LB broth culture media. Values are mean of three replicates with ± standard deviation. Values followed by different letters in a column are significantly different (p=0.05).

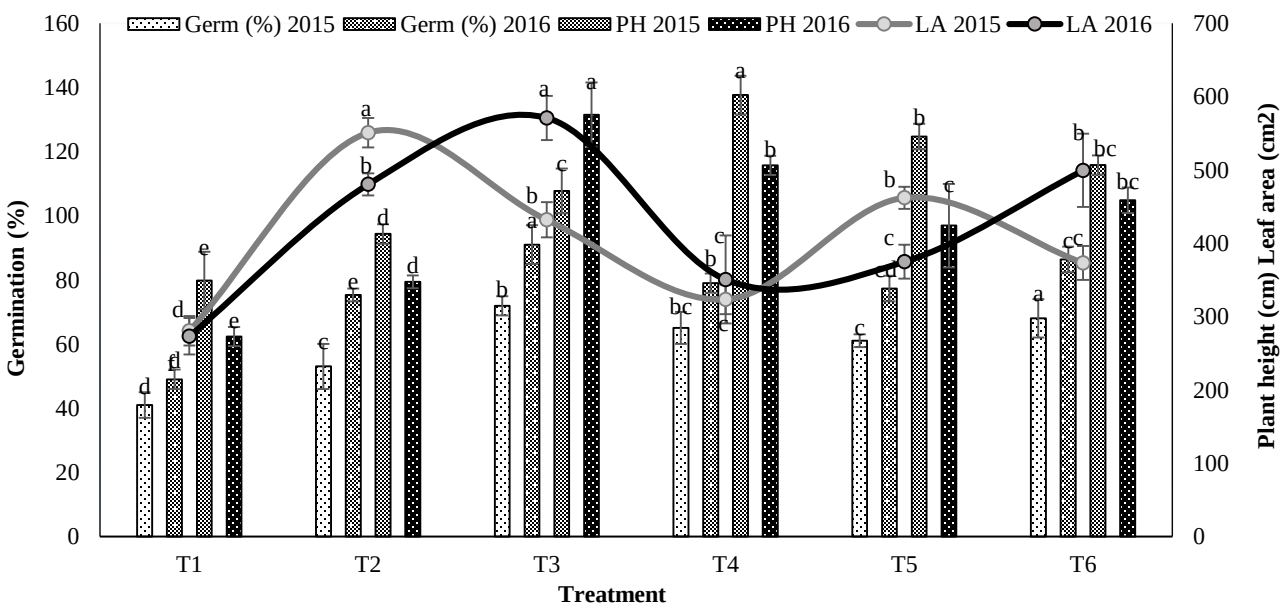

Fig. 2. Effects of rhizobacteria on the seed germination, plant height an leaf area of maize grown in the saline sodic field of Soil Salinity Research Institute, Pindi Bhattian.
Germ: Germination, PH: Plant height, LA: Leaf area, T_1: *Control*, T_2: *P.fluorescens*, T_3: *P.putida*, T_4: *L.sphaericus*, T_5: *B.pumilus*, T_6: *E. aurantiacum.* Germination data was taken after 13 DAS while plant height and leaf area was measured 12WAS. Values are mean of five replicates with ± standard deviation. Values followed by different letters in a column are significantly different (p=0.05).

Effect of PGPR on germination, plant physiology and yield: At 13DAS, germination data was recorded for two consecutive years. In 1st year, the inoculation effect of PGPR had significantly (p=0.05) increased germination by 28-55% over uninoculated plants (Fig. 2). The highest germination was recorded in T_6 (55%), least germination was recorded in T_5 =T_2 =T_3= T_4. In 2nd year, germination was improved by 26 to 76% in inoculated plants over un-inoculated plants. The highest germination was recorded in T_3 (76%), least germination was recorded in T_2 (26%) respectively.

The physiochemical analysis carried out at 8WAS, showed that inoculated plants exhibited maximum chlorophyll content (11-24%), leaf proline content (2-3 fold), leaf soluble sugar content (18-56%), and anthocyanin content (13-38%) over uninoculated plants in both years (Table 3). A significant (p=0.05) increase in chlorophyll content was recorded in T_3 (24%) followed by T_5 =T_6 (16%) inoculated plants. T_3 inoculated plants showed increase in proline content (58-65%) followed by T_5 (27-42%) respectively. High soluble sugar content was recorded in T_3 (56%), followed by T_6 (45%). T_6 and T_3

inoculated plants expressed significant amount of anthocyanin content (38%, 25%) respectively.

Vegetative parameters evaluated at 12WAS, indicated that plant height and leaf area increased significantly (p=0.05) with PGPR inoculation (Fig. 2). In 1st year, T_4 showed a significant (p =0.05) increase in plant height (53%) followed by T_5 (43%). The minimum plant height was recorded in T_2 (17%). In the 2nd year, a significant (p=0.05) increase in plant height was recorded in T_3 (71%) followed by T_4 (69%), T_6 (51%) and T_5 (44%). T_2 showed least increase in plant height upto 24%. Similarly, a significant increase (p=0.05) in leaf area was also recorded. In 1st year maximum leaf area was recorded in T_2 (551 cm^2) > T_5 (462 cm^2). In 2nd year maximum leaf area was recorded in T_3 (571cm^2) >T_6 (480cm^2) >T_2 (490 cm^2) respectively (Fig. 2).

Data presented in Fig. 3 showed that inoculation of PGPR had significant effect on grains and grain related parameters. In 1st year maximum number of grain/cob were recorded in T_5 and T_3. In 2nd year maximum number of grains/cob were recorded in T_3 > T_6. Similarly, T_3 and T_5 exhibited maximum 1000 grain weight and grain yield (t/ha) in both years. However, T_2, T_6 and T_4 showed least significant (p=0.05) increase in 1000 grain weight and grain yield over un-inoculated T_1 plants, respectively.

Correlation of grain yield with SAR, OM, available NO3-N and P, and IAA: Pearson correlation showed that a strong positive relationship (r=1) between organic matter and available phosphorous in soil. A significant correlation was obtained between IAA production in presence of tryptophan by PGPR (r=0.95) and presence of tryptophan precursor and available nitrates in soil (r=0.94) at p=0.05 (Table 4). This showed strong association of available NO_3-N with grain yield (r=0.93). Similarly, the available P was strongly correlated with IAA content produced by using tryptophan as precursor (r=0.92) available NO_3-N (0.86) and grain yield (r=0.93) at p=0.05

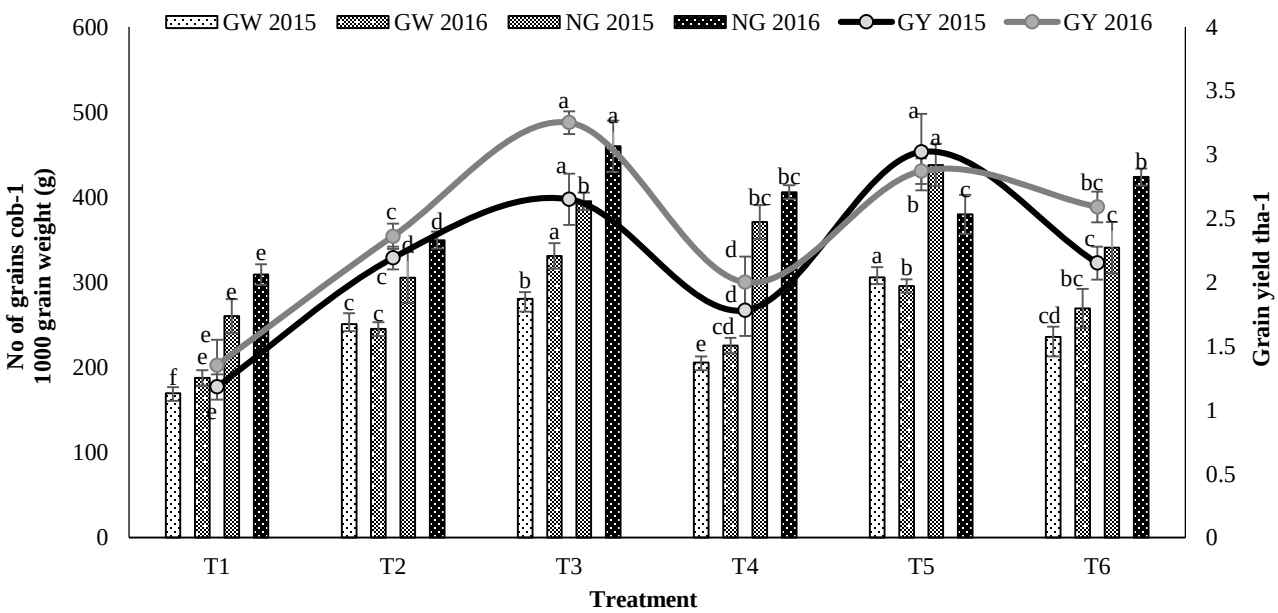

Fig. 3. Effects of rhizobacteria on the seed germination and physiology of maize grown in the saline sodic field of Soil Salinity Research Institute,Pindi Bhattian. The maize was harvested at 16 WAS and number of grain/cob, 1000 grain weight (g) and grain yield(t/ha) were recorded. Treatment details as in Fig. 2. GW: Grain weight, NG: Number of grains, GY: Grain yield. Values are mean of five replicates with ± standard deviation. Values followed by different letters in a column are significantly different (p=0.05).

Table 1. Physiochemical characteristics of the rhizosphere soil as affected by growing maize inoculated with PGPR. The rhizosphere soil was collected after harvesting (16WAS) and analysis were made. Means with same letters are non significant at P=0.05.

Treatment	pH		ECe (S/dm)		SAR (m mol/L)$^{1/2}$		CEC (mmeq/L)		OM (%)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
T1	8.9±0.76^{c}	8.4±0.45^{c}	2.6±0.34^{c}	2.3±0.47^{c}	24.1±0.23^{a}	15.8±0.22^{a}	260±0.81^{a}	175±0.84^{a}	0.43±0.27^{e}	0.47±0.62^{f}
T2	8.3±0.53^{ab}	7.7±0.61^{b}	2.0±0.71^{b}	1.9±0.27^{b}	17.6±0.49^{b}	8.7±0.19^{b}	149±0.57^{d}	121±0.63^{bc}	0.58±0.67^{d}	0.63±0.28^{d}
T3	8.1±0.91^{a}	7.4±0.82^{a}	1.7±0.83^{a}	1.4±0.51^{a}	12.7±0.18^{bc}	5.4±0.31^{d}	134±0.37^{e}	63±0.38^{d}	0.73±0.33^{a}	0.78±0.51^{a}
T4	8.2±0.27^{a}	7.6±0.38^{b}	1.9±0.76^{ab}	1.5±0.17^{a}	10.9±0.22^{c}	6.4±0.56^{c}	210±0.62^{bc}	100±0.15^{c}	0.59±0.91^{c}	0.64±0.38^{b}
T5	8.2±0.73^{a}	7.7±0.62^{b}	1.8±0.84^{ab}	2.2±0.52^{bc}	15.1±0.43^{b}	7.6±0.82^{bc}	201±0.47^{bc}	85±0.29	0.68±0.68^{b}	0.62±0.73^{c}
T6	8.3±0.58^{ab}	7.6±0.42^{b}	2.04±0.52^{b}	1.6±0.37^{a}	14.3±0.36^{b}	9.2±0.76^{b}	232±0.19^{b}	157±0.53^{b}	0.51±0.39^{d}	0.53±0.65^{e}

T1: *Control*, T2: *P.fluorescens*, T3: *P.putida*, T4: *L.sphaericus*, T5: *B.pumilus*, T6: *E. aurantiacum*. ECe: electrical conductivity, SAR: sodium absorption ratio, CEC: cation exchange capacity, OM: organic matter. Values are mean of three replicates with ± standard deviation. Values followed by different letters in a column are significantly different (p=0.05). Means with same letters are non significant at p=0.05

Table 2. Effect of PGPR on nutrient content of rhizospher soil during 2015-15 cropping year. The rhizosphere soil was collected after harvesting (16WAS) and analysis related to nitrate, available P and extractable K were made. Values are mean of three replicates with ± standard deviation. Values followed by different letters in a column are significantly different (p=0.05). Means with same letters are non significant at p=0.05. Treatment details as in Table 1.

Treatment	NO3-N (mg Kg^{-1} soil)		Available P (mg kg^{-1} soil)		Extractable K (mg kg^{-1} soil)	
	2015	2016	2015	2016	2015	2016
T1	13.48 ± 1.28^{c}	15.72 ± 1.16^{c}	2.25 ± 1.11^{c}	2.37 ± 0.91^{c}	57.3 ± 1.83^{c}	54.6 ± 1.42^{c}
T2	17.07 ± 1.32^{ab}	18.12 ± 1.13^{b}	3.02 ± 0.82^{a}	2.54 ± 1.41^{bc}	65.5 ± 1.59^{b}	68.3 ± 1.93^{b}
T3	20.89 ± 1.41^{a}	22.99 ± 1.37^{a}	3.22 ± 1.04^{a}	3.86 ± 1.31^{a}	71.4 ± 1.62^{a}	74.5 ± 1.63^{a}
T4	15.11 ± 1.53^{b}	20.42 ± 1.29^{ab}	2.81 ± 1.39^{b}	3.14 ± 1.91^{ab}	72.6 ± 1.22^{a}	69.6 ± 1.39^{b}
T5	15.11 ± 1.19^{b}	17.34 ± 1.05^{b}	3.19 ± 1.46^{a}	2.83 ± 1.73^{b}	68.6 ± 0.93^{ab}	71.3 ± 1.25^{ab}
T6	15.98 ± 1.09^{b}	19.33 ± 1.32^{b}	2.67 ± 1.21^{b}	2.99 ± 1.62^{b}	69.3 ± 1.46^{ab}	70.7 ± 1.04^{ab}

Table 3. Effect of PGPR on clorophyll, anthocyanin, proline and sugar content of maize leaves. Maize leaf samples were collected 12WAS and chlorophyll, anthocyanin, proline and sugar contents were determined in three replicates. Values are mean of three replicates with ± standard deviation. Values followed by different letters in a column are significantly different (p = 0.05). Means with same letters are non significant at p=0.05. Treatment details as in Table 1.

Treatment	Chlorophyll content (SPAD)		Anthocyanin		Proline (µmol g^{-1} FW)		Sugar (mg g^{-1} FW)	
	2015	2016	2015	2016			2015	2016
T1	36.4±01.42^{c}	38.3±1.17^{d}	1.18±0.97^{d}	1.21±1.18^{e}	23.4±0.28^{d}	28.7±0.49^{c}	19.05±0.86^{d}	16.8±0.92^{d}
T2	43.2±0.81^{b}	42.9±0.85^{bc}	1.38±1.09^{b}	1.43±0.86^{c}	27.6±0.41^{c}	31.5±0.79^{bc}	22.9±0.47^{c}	23.9±0.69^{c}
T3	46.3±0.73^{a}	47.7±1.32^{a}	1.40±1.14^{b}	1.56±0.96^{b}	42.7±0.58^{a}	56.4±0.82^{a}	28.55±0.99^{a}	29.8±0.57a
T4	42.2±1.49^{b}	43.4±0.88^{b}	1.39±0.88^{b}	1.63±1.31^{b}	29.8±0.35^{c}	32.8±0.51^{b}	23.9±0.73^{b}	23.8±0.39^{c}
T5	44.6±0.93^{ab}	42.6±0.82^{bc}	1.34±1.23^{c}	1.29±1.18^{d}	36.2±0.29^{b}	37.7±0.59^{b}	26.9±0.38^{ab}	23.9±0.54^{c}
T6	43.3±1.46^{b}	44.9±1.38^{b}	1.65±0.93^{a}	1.78±1.01^{a}	34.3±0.52^{b}	36.1±0.63^{b}	24.45±0.44^{b}	26.7±0.66^{b}

Table 4. Pearson correlation analysis between grain yield, available NO_3-N, P, sodium absorption ratio, organic matter and IAA production by PGPR. P-15: available phosphorous in soil in year 2015, P-16: available phosphorous in soil in year 2016, SAR-15: sodium absorption ratio in soil 2015; SAR-16: sodium absorption ratio in soil 2016; OM-15: organic matter in soil in year 2015; OM-16: organic matter in soil in year 2016. Letters in bold show significant level at alpha = 0.05.

	IAA(-trp)	IAA(+trp)	NO3-N15	NO3-N16	P-15	P-16	SAR15	SAR16	OM15	OM16	GY15	GY16
IAA(-trp)	**1**											
IAA(+trp)	0.78	**1**										
NO3-N15	**0.81**	**0.94**	**1**									
NO3-N16	**0.95**	**0.87**	**0.83**	**1**								
P-15	0.58	0.79	**0.84**	0.54	**1**							
P-16	**0.92**	0.78	**0.86**	**0.83**	0.78	**1**						
SAR15	**-0.87**	-0.53	-0.48	-0.80	-0.32	-0.68	**1**					
SAR16	**-0.93**	-0.61	-0.66	-0.80	-0.56	**-0.85**	**0.93**	**1**				
OM15	0.79	0.66	0.72	0.65	**0.83**	**0.91**	-0.67	**-0.85**	**1**			
OM16	**0.91**	0.77	**0.86**	**0.83**	0.78	**1.00**	-0.68	**-0.85**	**0.91**	**1**		
GY15	0.45	0.51	0.47	0.38	0.67	0.42	-0.57	-0.63	0.64	0.42	**1**	
GY16	**0.82**	**0.93**	**0.93**	**0.84**	0.81	0.78	-0.65	-0.75	0.71	0.78	0.71	**1**

Discussion

The PGPR stains used produced Indole Acetic Acid (IAA) and have the potential to convert tryptophan to IAA. It is a growth promoting hormone secreted by many rhizospheric bacteria including *P. putida* (Barucha *et al.,* 2013). IAA helps in seed germination, production of longer roots with extensive root hair which indirectly helps in nutrient uptake and possess great potential for large scale production of cereal crops (Hassan & Bano, 2015; Khalid *et al.,* 2013; Kang *et al.,* 2014). The ability of the strains to have ACC deaminase activity is also another strategy to cope with salt stress**.** Under saline sodic condition ethylene is produced which inhibits seed germination and plant growth. However, colonization of ACC deaminase producing bacteria to the roots of seedlings hydrolyze ACC into ammonia and a-ketobutyrate, thereby diverting the pathway for ethylene production hence the adverse effect of ethylene is overcome and germination is enhanced leading to improved plant growth under salinity stress as reported (Carlos *et al.,* 2016; Sarkar *et al.,* 2018; Kumari *et al.,*

2018; Marag *et al.,* 2018). This was evident from the present research that the higher yield due to *P. putida* inoculation may be attributed to the greater potential of conversion of tryptophan to IAA and the increased area of leaf for photoassimilation. Under salt stress Phosphate precipitate out and become unavailable for plant growth. PGPR induced P solubilization to make available P is another mechanism of PGPR to alleviate salt stress (Sharma 2013a; Chen *et al.,* 2014; Paul & Sinha, 2017). Many authors have reported that PGPR also secrete phosphate enzyme which solubilize inorganic phosphate which is easily taken up by plant roots and elicit a strong growth promoting effect on plants grown under saline condition (Sharma, 2013b; Kadmiri *et al.,* 2018).

One of the strategy PGPR utilized to mitigate the adverse effects of salinity and sodicity in the field is to enhance organic matter production concomitant with the decrease in EC and SAR of the rhizosphere soil. Introduction of 1L liquid inocula treatment^{-1} (OD_{660}≈1) into the rhizospheric soil added more population of selected PGPR which had competitive advantage over indigenous microflora in saline sodic field. It has a positive effect on OM in soil (Mehdi *et al.,* 2007), NO_3-N, available P and K while decreasing soil pH, EC_e, CEC and SAR over un-inoculated soil from first year to second year. The observed higher decrease in SAR of the rhizospheric soil in the successive year demonstrates the persistence of these PGPR applied in the first year as bioinoculants on maize. However, the efficiency of each PGPR bioinoculants differed and is measured by the decrease in Ec, SAR and pH as well as increase in organic matter production. *P. putida* and *B. pumilus* were more efficient to enhance organic matter content of soil and to reduce the EC and SAR. The highest efficiency of *P. putida* may be attributed to the maximum ACC deaminase activity, higher production of IAA and greater potential for phosphate solubilization.

PGPRs have been known to promote germination in a wide range of cereal crops. In current study, it was observed that inoculation of maize with liquid formulation of PGPR resulted higher germination (76%) compared to uninoculated plants. *P. putida* treated plants showed the highest germination %. This improvement in seed germination by application of PGPR have been reported previously in many cereal crops (Laloo *et al.,* 2017; Hossain *et al.,* 2016) and appears to be the enhanced activity of ACC deaminase by the PGPR resulting in inhibition of ethylene production- a germination inhibitor as discussed earlier.

Salinity stress negatively impact photosynthetic activity in maize (Sali *et al.,* 2015). Therefore, the use of PGPR as bioinoculant to enhance chlorophyll content in maize under saline condition is promising to mitigate salt stress (Ullah & Bano 2105). Presently inoculation of *P. putida* significantly stimulated the chlorophyll content of leaves and augmented the leaf area. Kang *et al.,* (2014) reported higher chlorophyll content under saline condition in plants inoculated with PGPR compared to untreated plants.

Compatible solutes have been demonstrated to play positive role in drought tolerance of plants (Liu *et al.,* 2015). Significant positive correlation was established between Proline and sucrose content and salt tolerance in tomato (Almeida *et al.,* 2014). During the present investigation augmented production of proline and soluble sugar was recorded in *P. putida* inoculated plants over uninoculated plants. It is an adaptive response to salinity stress, that protect plants from salt stress through osmotic adjustment, detoxification of ROS, enzymes and protein stabilization (Abdel Latef & Chaoxing, 2014; Fukami *et al.,* 2018).

Application of PGPR inocula to maize exhibited different pattern of plant height under saline sodic condition. Inoculation with *L. sphaericus* in first year and *P. putida* in the second year significantly (p=0.05) improved plant height. Similar increase in plant height was observed in maize inoculated with *P. putida,* and other rhizobacteria (Abd El-Ghany *et al.,* 2015; Matsumura *et al.,* 2015).

Both grain number and grain weight were higher in *P. putida* >*B. pumilus* inoculated plants. this may be attributed to the higher leaf area for photo assimilate production, greater CEC and production of IAA, a growth promoter. The osmoregulant production and availability of the higher NO_3 –N in PGPR inoculated plants may be attributed for higher field. Inoculation of such PGPR exhibited positive effect on grain weight and yield in maize (Abo-kora, 2016; Ferreira *et al.,* 2013; Noumavo *et al.,* 2013). Zafarulhye *et al.,* (2014) and Iqbal *et al.,* (2016) reported increased growth and grain yield in maize inoculated with PGPR.

The correlation analyses made during the present investigation depicted significant positive correlation between IAA, NO_3-N, available P content and grain yield. The r value was much higher and significant in the second year demonstrating that PGPR induced reclamation of saline sodic soil was progressive in the second year. This was better achieved by adding extra inocula with irrigation water which resulted in the enhancement of the competency of the PGPR against indigenous microflora.

Conclusion

The revegetation of saline land depends on ECe, SAR and CEC of the soil which is modulated by the PGPR used as bioinoculant. Their efficacy in the field depend on their persistence. The PGPR also increases organic matter content, NO_3-N, available P and K in the soil which mitigates salt stress. The efficiency of PGPR varies *P. putida* being more effective. The highest efficiency of *P. putida* may be attributed to the maximum ACC deaminase activity, higher production of IAA and greater potential for P solubilization.

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