INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–0739/201x/00–0–000–000
DOI: 10.17957/IJAB/15.0979
http://www.fspublishers.org

Full Length Article

Auxin and 1-Aminocyclopropane-1-Carboxylate Deaminase Activity Exhibiting Rhizobacteria Enhanced Maize Quality and Productivity under Water Deficit Conditions

Muhammad Tahir[2*], Umaira Khalid[1], Muhammad Bismillah Khan[1], Muhammad Shahid[3], Iftikhar Ahmad[2], Muhammad Akram[2], Muhammad Ijaz[4], Mubashir Hussain[1], Abu Bakr Umer Farooq[2], Muhammad Asif Naeem[2] and Naveed Ahmad[2]
[1]*Department of Agronomy, Bahauddin Zakariya University Multan, Pakistan*
[2]*Department of Environmental Sciences, COMSATS University Islamabad, Vehari Campus, Pakistan*
[3]*Department of Bioinformatics and Biotechnology, Govt College University, Faisalabad, Pakistan*
[4]*Department of Agronomy, BZU, Bahadur Campus Layyah, Pakistan*
[*]For correspondence: muhammad_tahir@ciitvehari.edu.pk

Abstract

Drought, the major characteristic of arid region exerts drastic effects on the crop productivity. Rhizobacteria equipped with 1-aminocyclopropane-1-carboxylate (ACC) deaminase and indole-3-acetic acid producing activity (IAA) assist plants to manage the water stress. From the arid regions (Multan and Bahawalnagar) of Southern Punjab, a total of 95 bacterial morpho-types were isolated, out of which, 32 isolates showed acetylene reduction activity (ARA), 43 were positive for phosphate (P) solubilization activity and 30 bacterial isolates produced IAA in culture medium. Of the total 95 bacterial morpho-types, 34 showed catalase activity and 25 were positive for ACC deaminase activity. Two potent bacterial isolates MD-23 and BN-5 with highest ARA, phosphate solubilizing, IAA production and positive for ACC deaminase activity were selected and tested for exopolysachharide (EPS) secretion. Significantly higher amount of sugar (7163 μg g^{-1} and 7081 μg g^{-1}), protein (988 μg g^{-1} and 925 μg g^{-1}) and uronic acid (0.87 μg mg^{-1} and 0.82 μg mg^{-1}) were detected in EPS of BN-5 and MD-23, respectively. The results of field experiments revealed that both strains (BN-5, MD-23) reduced the excised leaf water loss, increased the relative leaf water contents, chlorophyll contents and grain yield in well-watered plants as well as in plants under restricted water supply over their respective non-inoculated control. The grain quality parameters like carbohydrate, protein and oil contents of bacterial inoculated plants were enhanced over their respective non-inoculated control plants under all the irrigation regimes. From these results, we concluded that PGPR strains equipped with ACC deaminase and IAA producing activity and EPS secretion improved the yield and quality of maize under all water regimes.

Keywords: Water stress; Exopolysaccharides; Rhizobacteria; Phytohormone; Arid region; Irrigation

Introduction

Water scarcity, the common characteristic of arid region prevails all over the world as well as in Pakistan. Arid region in Pakistan (consists of most districts of interior Sindh and Southern Punjab province) have great potential for agriculture productivity due to healthier and ample soil availability. The only limitation for better productivity is the prevalence of extremely high temperature, low rainfall and non-availability of irrigation water in these areas throughout the growth season. During limited supply of irrigation water, plants face osmotic stress that results in reduced vegetative growth and leaf expansion (Bartels and Sunkar, 2005; Tardieu, 2005), increased rate of senescence and abscission and in some cases, plants expand their root system in search of water (Gepstein and Glick, 2013). Reduction in antioxidant enzymes *viz.*, ascorbate, peroxidase, catalase and glutathione peroxidases are the consequence of prolonged water stress and cause decline in crop performance (Vardharajula *et al.*, 2011; Vurukonda *et al.*, 2016; Forni *et al.*, 2017). Scarcity of water in crops enhances the production of ethylene (a growth inhibiting hormone) and reactive oxygen species which result in reduced plant growth and productivity (Nautiyal *et al.*, 2013; Forni *et al.*, 2017).

Numerous strategies like use of advanced irrigation methods, sowing techniques and cultivation of less water requiring cultivars have been developed to cope with water stress in arid climate (Hussain *et al.*, 2013; Adak *et al.*, 2018; Gogoi *et al.*, 2018; Jia *et al.*, 2018). Isolation of rhizobacteria indigenous to arid region and their application as bio-inoculant is an emerging eco-friendly approach that enables plants to withstand under water stress conditions. These rhizobacteria offer diverse plant beneficial characteristics

To cite this paper: Tahir, M., U. Khalid, M.B. Khan, M. Shahid, I. Ahmad, M. Akram, M. Ijaz, M. Hussain, A.B.U. Farooq, M.A. Naeem and N. Ahmad, 201x. Auxin and 1-aminocyclopropane-1-carboxylate deaminase activity exhibiting rhizobacteria enhanced maize quality and productivity under water deficit conditions. *Intl. J. Agric. Biol.* 00: 000–000

(nitrogen fixation, phosphate solubilization, secretion of growth substances, provision of resistance against biotic and abiotic stress) in normal as well as in stress environment and these are termed as plant growth promoting rhizobacteria (PGPR) (Mehnaz *et al*., 2001; Vessey, 2003; Tahir *et al*., 2013; Tahir *et al*., 2015a, b). These PGPR produce growth hormone like cytokinin, gibrillins (Garcia de Salamone *et al*., 2001; Joo *et al*., 2005) and indole-3-acetic acid (IAA) that increased the root proliferation, water uptake, nutrient uptake and productivity (Vessey, 2003; Bianco and Defez, 2009; Tahir *et al*., 2013; Bhardwaj *et al*., 2014; Tahir *et al*., 2015a). Bacterial strains native to stress environment secrete enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase that catabolize the ACC (predecessor of ethylene produced in plants under water stress) and thereby increase the growth and overall performance of plant (Glick *et al*., 1998; Mayak *et al*., 2004; Glick *et al*., 2007). Exopolysaccharides (EPS), produced by soil bacteria, are actively found in the soil organic matter and protect the cell from water stress through helping in bacterial attraction and colonization, bio-film development and facilitate the interaction of plants with microbes (Naseem and Bano, 2014).

Maize (*Zea mays*. L) is one of the prominent cereals in the world. Maize contributes to more than 50% of global cereal production. This contribution must be increased up to 100% by the year 2050 to fulfill the demand of ever-increasing human population, livestock consumption and bio-fuel industry requirements (Ray *et al*., 2013). In Pakistan, maize is cultivated as seasonal as well as spring crop. During last 3-4 years, maize cultivation and production is decreased in Pakistan (Annonymous, 2016). The major constraint in reduction of maize yield is the limited precipitation and water shortage during entire growth period (Bartels and Sunkar, 2005; Ali *et al*., 2011; Vardharajula *et al*., 2011; Naveed *et al*., 2014). Reduced productivity of maize and other crops under drought stress conditions has been reported in number of earlier studies (Ali *et al*., 2011; Vardharajula *et al*., 2011; Naveed *et al*., 2014).

Water availability for base period of any arable crop always has been a problem throughout the world in general and particular in arid region. Additionally, water resources are diminishing day by day and their conservation for sustainable environment is the critical need of the day (Potter and Darmame, 2010; Fan *et al*., 2013; Santini and Rulli, 2015; Aprilea and Damiano, 2017). A very minute focus has been given to make effort of fresh water saving for future use. This can be made possible by reducing the crop water requirement without compromising the crop yield. Keeping all these problems in mind, we hypothesized that application of PGPR indigenous to arid region to maize grown under water deficit conditions may help the plants to cope with water stress and enhance its productivity. To test this hypothesis, the present study was designed to find the PGPR native to arid climate equipped with IAA and ACC deaminase producing potential. Further, the selected PGPR isolates were tested as bio-inoculant to mitigate effects of water stress on maize without compromising the crop performance.

Materials and Methods

Sample Collection and Isolation of Bacterial Cultures

The present study was started in the year 2013. Soil samples (top 15-30 cm layer) from agricultural soils of Multan (30°11′52″N 71°28′11″E) and Bahawalnagar (29°57′N 73°15′E) districts were collected in sterilized plastic sealed bags and immediately brought to laboratory of Microbiology, Hexon Chemicals Pvt. Ltd, Multan to store at 4°C for further processing. After homogenization of the collected soil, bacterial colonies were isolated using serial dilution plate count method (Tahir *et al.,* 2015a). Purification of the colonies was done by continuous streaking and the purified colonies were stored in glycerol (20%) at -20°C for further processing.

Functional Characterization

Screening of bacterial morphotypes inhabiting phosphorous solubilizing activity: The 10 μL of the well-grown overnight bacterial culture in nutrient broth medium (50 mL) was inoculated on plates containing Pikovskaya (Pikovskaya, 1948) agar medium. These plates were incubated at various temperatures ranges *i.e.,* at 30°C, 35°C and 40°C for 72 h to observe P-solubilization potential of the morphotypes at various temperatures. After the said period, the colonies were observed for transparent halo-zone formation. The colonies showing halo-zone formation were counted and the morphotypes showing halo-zone formation at all the tested temperatures were selected and processed for quantification analysis. Samples for the quantification of bacterial solubilized P were prepared using the procedure adopted by Tahir *et al*. (2013). Phosphate solubilization activity of the bacteria was quantified colorimetrically (phosphomolybdate blue color method; Murphy and Riley, 1962) using double beam scanning spectrophotometer (PerkinElmer UV/VIS Spectrophotometer Lambda 25, USA) at 882 nm.

Screening of Bacterial Morphotypes with Potential to Produce Indole-3-Acetic Acid

To identify the bacterial colonies with IAA synthesis activity, all the colonies were grown in liquid combined carbon medium (CCM) supplemented with tryptophan separately for a week at 30±2°C. After a week, the samples were centrifuged at 6000 *g* for 10 min and pH of the supernatant was adjusted at 2.7 with HCl. Acidified supernatant was extracted with the same volume of ethyl acetate as that of the supernatant, suspended in ethanol after drying on lyophilizer (Tien *et al.,* 1979). These samples were analyzed on HPLC using the procedure described by Tahir *et al.* (2015a).

Screening of Morphotypes Positive for Acetylene Reduction Activity

The acetylene reduction activity (ARA) which represents biological nitrogen fixing potential of the bacteria was tested by growing the bacterial colonies in semi-solid nitrogen free medium (Okon *et al.,* 1977). Five mL of the semi-solid nitrogen free medium were poured in sterilized glass tubes (vials) of capacity 16 mL each. Purified individual bacterial colonies were inoculated to vials in triplicate. These inoculated tubes were incubated at 30±2°C for 16 h after closing the tubes with rubber stoppers. At visible bacterial growth in vials, 10% acetylene (C_2H_2) was added into the vials through injection and the vials were again incubated at 30±2°C for extra 16 h. After the said period, each vial was processed for ARA. Inoculated control (with bacteria but without C_2H_2) and non-inoculated control (without bacteria but with C_2H_2) were prepared and processed. All the samples were analyzed on a Gas Chromatograph (Gasukurokogyo model 370, Tokyo, Japan) using Porapak N column (Supelco Inc., Bellefonte, Pennsylvania) for the estimation of ethylene gas produced by bacterial cultures. Peak height of the samples was compared with that of standard (1% C_2H_4) to estimate the nitrogen fixing activity of the morphotypes.

Screening of Morphotypes with 1-Aminocyclopropane-1-Carboxylic Acid Deaminase and Catalase Activity

Potential of the isolates to utilize ACC as a sole source of nitrogen was measured in 5 mL DF minimal salt medium (Penrose and Glick, 2003) containing 3 μL of 0.5 M ACC. Cultures were grown at 30±2°C for 24 h in a shaker. ACC deaminase activity was determined by comparing the turbidity of the non-inoculated control sample with inoculated cultures. For catalase activity detection, single bacterial colony was inoculated on glass slide and placed single drop of 30% hydrogen peroxide (H_2O_2) on slide. Gas bubbles formation indicated the presence of CAT enzymes in the bacteria (Fadden, 1980).

Extraction and Analysis of Exopolysacharides (EPS) Produced by Bacterial Isolates

Exopolysaccharides produced by the two most efficient bacterial isolates were extracted and analyzed using the protocol described by (Naseem and Bano, 2014) with some kind of modifications. These bacterial colonies were grown in growth medium supplemented with different minerals salts with variable concentrations for ten days. Growth medium was prepared using K_2HPO_4 (0.126 g/L), KH_2PO_4 (0.182 g/L), NH_4NO_3 (0.1 g/L), $MgSO_4.7H_2O$ (0.01 g/L), $MnSO_4$ (0.006 g/L), $CaCl_2.2H_2O$ (0.01 g/L), $FeSO_4.2H_2O$ (0.0006 g/L), sodium molybdate (0.01 g/L), NaCl (0.15 g/L) and (0.002 g/L) glucose (Bramhachari and Dubey, 2006). Supernatant was obtained by centrifugation of the ten days grown bacterial cultures at 15,000 rpm for 20 min at 4°C. Extraction of the EPS was made by adding the double amount of cool 95% (v/v) ethanol. The extracted solutions were collected using the procedure described previously (Kumar *et al.,* 2011). Washing of the EPS was ensured by adding mixture of ethanol-water. The solutions were re-dissolved in distilled water and dialysis was performed for complete washing. The samples were dried on lyophilizer and stored at room temperature.

For the measurement of total sugars, protein and uronic acid in EPS, 2.0 gms of lyophilized samples were suspended in 10 mL distilled water. Total sugars were quantified using previously described phenol–sulfuric acid (PSA) method (DuBois *et al.,* 1956), while protein contents were measured by adopting already set procedure (Lowry *et al.,* 1951) using bovine serum albumin as standard. The absorbance was noted at 500 nm on spectrophotometer. Carbazole assay was used for the measurement of uronic acid (Taylor and Buchanan-Smith, 1992).

Soil Analysis

From the site of experiment, three soil samples were collected before the sowing of crop. Thereafter, samples were dried in air, ground to pass through a 2 mm sieve and analyzed for physico-chemical characteristics following analytical methods designated by Estefan *et al.* (2013).

Field Experiment

The two most efficient bacterial strains (BN-5, MD-23) with maximum level of P-solubilizing activity, IAA production, nitrogen fixing potential in terms of acetylene reduction assay (ARA), positive for ACC deaminase and catalase activity were selected and used for inoculation to maize under water stress in field experiments conducted during two consecutive years in spring, 2014 and 2015 at research farm of Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan. Both the experiments were laid out in Randomized Complete Block Design (RCBD) in split-plot arrangement with three replications by keeping water stress levels in main plots and bacterial inoculation in sub plots. Soil of experimental site was silt-loam with EC 2.4 dS m^{-1}, pH 7.8, organic matter 0.81%, phosphorous 8.81 ppm, nitrogen 0.083% and potassium 21 ppm.

Crop Husbandry

The experimental soil was applied with pre-soaking irrigation (*Rauni*) of 10 cm and seedbed was prepared by using tractor mounted cultivator twice each followed by planking when the field attained a moisture level feasible to cultivation. Seeds of the maize hybrid P1543 were inoculated with the bacterial inocula. Inoculated seeds were manually sown by dibbler on ridges (R×R=75 cm and P×P= 20 cm) in plots each of size 5 m × 3 m on 6th March, 2014 while in the year 2015, crop was sown on 10th of March using the same method.

The recommended chemical fertilizers (nitrogen, phosphorous and potassium, 200, 150 and 150 kg ha^{-1}, respectively) were applied to the crop in both the experiments using Urea ($(NH_2)_2CO$), diammonium phosphate ($(NH_4)_2PO_4$) and SOP as source of nitrogen, phosphorous and potassium respectively. Whole amount of phosphorous and potassium along with 1/3rd of nitrogen was applied at sowing time while the remaining nitrogen was applied in two equal doses. Maize plants were subjected to water stress at vegetative and tasseling stage (~50% FC), while ~75% FC water conditions were taken as control; and half of the soil saturation percentage was considered as 100% FC. The bacterial inoculation treatments (S_1, S_2 and S_3 mean isolate BN-5, MD-23 and non-inoculated control, respectively) were integrated with water stress levels. In case of water stress at vegetative stage, water application was stopped after 2nd irrigation and maintained at 50% FC level up to tasseling stage. While in case of water stress at tasseling stage, water application was restricted at tasseling stage to impose the water stress until the plots reached to 50% FC level and well-watered crop was irrigated at 75% FC level through-out the growing season. When soil attained workable moisture level after 1st irrigation, hoeing was done to remove the weeds. All the other cultural practices were uniformly maintained throughout the experimental set up to control the insects and diseases. The mature crop was harvested on 16th of June in 2014 while in 2015, the crop was harvested on 20th of June. The weather data of both the experimental years is given in Table 1.

Rhizosphere Bacterial Colonization

The rhizosphere associated bacterial population was estimated to observe the bacterial colonization. Rhizosphere soil samples (0.5 g) from each treatment were collected at harvest of the crop in sterilized plastic bags, the bags were sealed and stored at 4ºC for further processing. The bacterial population in terms of colony forming units (cfu g^{-1} soil) was estimated using serial dilution plate count method (Tahir *et al*., 2013).

Leaf Relative Water Content (%) and Water Loss from Excised Leaves

From each plot young top leaves of three plants were removed, preserved in sealed plastic bags and immediately transferred to laboratory (Department of Agronomy, Bahauddin Zakariya University, Multan). Within 2-3 h of leaf removal, leaf fresh weight (FW) was recorded. To measure the leaf turgid weight (TW), leaves were placed in water at room temperature and after 16-18 h absorbing water these were dried with soft tissue and again weighed to get TW. Thereafter, these leaves were oven dried for three days at 65-70°C and weighed to obtain dry weight (DW). Relative water contents (RWC) of leaves were measured using the equation;

$$RWC\ (\%) = (FW - DW)/(TW - DW) \times 100$$

For the measurement of water loss from excised leaves, top third leaf were removed from three plants of each treatment and their fresh weight (FW) were noted. These samples were kept at room temperature for 6-8 h and then weighed for noting the reading of wilted weight (WW). After this, these leaves were oven-dried for one day at 65-70°C and weighed to record the dry weight (DW) of the samples. The values of FW, WW and DW were placed in the following formula to get water loss from the leaf.

Excised leaf water loss (ELWL) = (Weight of fresh leaves – weight of wilted leaves)/ oven dry weight.

Chlorophyll Contents, Yield and Yield Related Traits

Chlorophyll contents were measured at each 15 days interval started from 30 DAS up to 60 DAS using chlorophyll meter (SPAD 502, Spectrum Technologies, Inc, Aurora, IL). For the measurement of agronomic and yield parameters like number of grain rows per cob, number of grains per row, weight of 1000-grains, grain weight and stalk yield, crop was harvested at maturity *i.e*., after 100 days of sowing. Twenty plants from each treatment were chosen randomly and their cobs were separated. After separating the cobs from the selected plants, grain rows on each cob and the grains on each row of the cob were counted and averaged. From each treatment, after separating the cobs, both the cobs and the stalk were sun dried for 10 days and the cobs were threshed. All grains obtained after the threshing were weighed to get yield of grains. From the seed lot of each treatment, 1000-grains were randomly taken and weighed to record their weight (1000-grain weight). Similarly, dried stalks were weighed to get stalk weight. Biological yield was measured by adding grain yield and stalk yield while harvest index was calculated by using the following equation;

$$\text{Harvest index (HI)} = (\text{Grain yield/Biological yield}) \times 100$$

Quality of the Grains

Quality of the maize grains was measured by determining the protein, carbohydrates and oil contents in grains. Grains were grinded in a laboratory mill (Fritsch Pulverisitte 14, Germany) to pass through a sieve of 0.5 mm. Kjeldahl (Gerhardt, Germany) method (ICC, 1980) was used to determine the total protein contents. The oil analysis was done following the Soxhlet method. The total carbohydrate contents were determined using the Anthron method (Gerhardt *et al*., 1994) on spectrophotometer (PerkinElmer UV/VIS Spectrophotometer Lambda 25, USA).

Statistical Analysis

The obtained data was statistically analyzed in STATISTIX 8.1. When, the overall main effects were statistically significant, treatments were further compared using least significant difference (LSD) test at 5% probability level (Steel *et al*., 1997).

Results

Isolation and Functional Characterization of Bacteria

In this study, a total of 95 different bacterial colonies were isolated and purified from soil samples of Multan and Bahawalnagar collected from the depth of 15 cm. Among the total, 64 and 31 bacterial isolates were obtained from the Multan and Bahawalnagar region soil, respectively (data not shown). Catalase activity was detected in 34 bacterial isolates out of which 14 isolates were from Bahawalnagar district and 20 were from Multan district. Among the total bacterial isolates, twenty-five isolates were positive for ACC deaminase activity. From the ACC-deaminase positive isolates 10 were from Bahawalnagar and 15 were from Multan. The results showed that 32 bacterial isolates were positive for ARA, out of which 12 were from Bahawalnagar and 20 were from Multan region. Among all the isolates, the maximum ARA activity (855 n mole C_2H_4 h^{-1} mg^{-1} protein) was detected in isolate MD-23 from Multan region. The bacterial isolate BN-6 from Bahawalnagar region showed higher ARA activity (472 n mole C2H4 h^{-1} mg^{-1} protein) when compared with that of all other isolates from this region (Table 2).

Phosphate solubilization activity was detected in 43 bacterial isolates. Among these, 28 and 15 were from Multan and Bahawalnagar region, respectively. All these bacterial isolates were versatile in showing P-solubilizing activity ranging 4-375 μg mL^{-1}. The bacterial isolate BN-5 from Bahawalnagar soil showed maximum (375 μg mL^{-1}) P-solubilization activity (Table 2).

Characterization of all the obtained bacterial isolates showed that 30 individual cultures produced IAA in culture medium. Among these, 16 isolates were from Multan and 14 from Bahawalnagar. Maximum IAA synthesis was observed in BN-5 (643 μg mL^{-1}) obtained from Bahawalnagar and MD-23 (553 μg mL^{-1}) from Multan district (Table 2) Multan district.

Among all the obtained bacterial isolates in present study, ten isolates exhibited all the above-mentioned plant beneficial traits, while 26 isolates were those which did not show any PGPR activity.

After the functional characterization, two most efficient bacterial isolates MD-23 with maximum nitrogen fixing ability in terms of higher ARA activity and BN-5 inhabiting maximum IAA production as well as P-solubilizing potential were selected for further studies. Both these isolates were positive for ACC deaminase and catalase activities as well. Both these isolates were processed for exopolysaccharide secretion. Composition analysis of the EPS showed that both the isolates produced total sugar, protein and uronic acid in their EPS. However, significantly ($p \leq 0.05$) higher amount of sugars (7163 μg g^{-1}), protein (988 μg g^{-1}) and uronic acid (0.87 μg mg^{-1}) contents were observed in EPS of BN-5 bacterial isolate as compared to MD-23 and non-inoculated control (Table 3).

Table 1: Weather data during the course of experiment

Months	Mean temperature (°C)		Humidity % (Mean)		Rainfall (mm)	
	2014	2015	2014	2015	2014	2015
February	12.9	12.0	79.2	88.0	1.50	0.80
March	14.9	16.3	81.7	74.9	18.00	4.00
April	19.8	20.2	74.2	73.3	33.40	92.90
May	26.9	28.5	55.2	65.3	8.90	9.20
June	30.7	32.6	53.8	53.0	42.60	8.50

Table 2: Biochemical characterization of the bacteria isolated from Multan and Bahawalnagar

Isolate	ARA (nmole C_2H_4 h^{-1} mg^{-1} protein)	P-solubilization (mg L^{-1})	IAA (mg L^{-1})	Catalase	ACC deaminase activity
MD-10	75±2 d	41±2 d	210±1 c	+	+
MD-11	82±2 c	58±2 b	32±1 f	+	+
MD-23	855±3 a	15±1 f	553±3 b	+	+
MD-28	42±1 e	48±1 c	16±1 gh	+	+
MD-29	12±1 g	34±2 e	17±1 g	+	+
MD-30	18±2 f	27±1 e	19±2 g	+	+
BN-5	79±1 c	375±2 a	643±3 a	+	+
BN-6	472±3 b	43±2 d	45±2 e	+	+
BN-14	42±1.5 e	9±1 g	146±2 d	+	+
BN-23	19±1 f	14±2 f	17±2 g	+	+

BN and MD represent the bacterial isolates from Bahawalnagar and Multan district, respectively
ARA, P, IAA, ACC represent acetylene reduction activity, phosphate, indole-3-acetic acid and 1-aminocyclopropane-1-carboxylic acid, respectively
*Each value (mg L^{-1} and "n mole C_2H_4 h^{-1} mg^{-1} protein) in the table is the mean of three replicates and values with different letters a, b and c show significance level at 5% probability ($p \leq 0.05$)

Table 3: Characterization of exopolysaccharides produced by the selected bacterial isolates

Bacterial isolate	Sugar (μg g^{-1})	Protein (μg g^{-1})	Uronic acid (μg mg^{-1})
BN-5	7163±2 a	988±2 a	0.87±0.05 a
MD-23	7081±1 a	925±2 a	0.82±0.02 a
Non-inoculated	235±1 b	20±2 b	0.06±0.01 b
LSD at 5%	35	56	0.01

MD-23, BN-5 represent the inoculation with bacterial isolate MD-23 and BN-5 while Non-inoculated (control) means the growth media without any bacterial culture
*Each value (μg g^{-1} as well as μg mg^{-1}) are the mean of three replications. Values with different letters a, b and c show significance level at 5% probability ($p \leq 0.05$)

Field Experiment

Chlorophyll and relative water contents: The selected isolates were used as inoculants to maize in the field experiments conducted during two consecutive years in spring 2014 and 2015 under well water as well as water stress conditions. During the year 2014, chlorophyll contents were significantly higher in bacterial inoculated plants as compared to respective non-inoculated plants at 30 DAS under all water regimes (Fig. 1a). At 45 DAS, chlorophyll contents were significantly higher in inoculated plants as compared to respective non-inoculated control under well watering and water stress at vegetative as well as at tasseling stage. However, among the irrigation regimes, the treatments where water stress was imposed at vegetative stage produced lower chlorophyll contents as compared to that of other treatments.

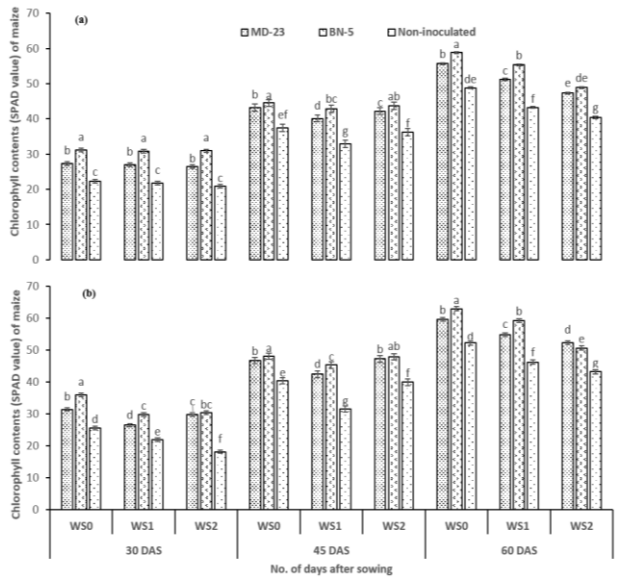

Fig. 1: Chlorophyll contents (SPAD value) of maize recorded at 30, 45 and 60 days of sowing influenced by irrigation levels and bacterial isolates inoculation

MD-23, BN-5 represent the plants inoculated with bacterial isolate MD-23 and BN-5 while Non-inoculated means the plants without any bacterial culture inoculation

* WS0, WS1, WS2 represent plants under no water stress, water stress at vegetative stage (V7) and water stress at reproductive (tasseling) stage, respectively

*Bars with different letters like a, b, c etc. show significance level at 5% probability value ($p \leq 0.05$) and standard errors bars are given on each bar on the basis of standard error values

*(a) and (b) represents the chlorophyll contents of maize in experiments conducted during the year 2014 and 2015, respectively

At 60 DAS, again chlorophyll contents were observed higher in bacterial inoculated plants as compared to respective non-inoculated control plants under all water regimes. In plots, where water stress was imposed (water stress at tasseling stage), chlorophyll contents were lower as compared to other water regimes. Among the bacterial isolates, BN-5 inoculation gave higher chlorophyll contents as compared to MD-23 inoculated plants at 30, 45 and 60 DAS under all water regimes. A similar trend was observed in the experiment conducted during the year 2015 (Fig. 1b). The number of days after sowing showed significant effects on SPAD chlorophyll contents of maize leaves regardless to inoculation and water levels.

Application of the bacterial isolates under different water regimes reduced the water loss from excised leaves and increased the leaf relative water contents (LRWC) of maize grown during both the years (Table 4). Significantly ($p \leq 0.05$) higher values of excised leaf water loss and lower LRWC were observed in well-watered non-inoculated control plants in both the experiments. Excised leaf water loss was significantly lower in inoculated plants while in these plants LRWC were significantly ($p \leq 0.05$) higher as compared to non-inoculated control at all water regimes (Table 4). Among the bacterial isolates, the isolate BN-5 produced significantly ($p \leq 0.05$) higher values of LRWC and lower values of excised leaf water loss as compared to respective non-inoculated control in the experiments conducted in two consecutive years (Table 4).

Yield Attributes, Grain Yield and Harvest Index of Maize

The yield parameters of maize like number of rows per cob, number of grains per row, 1000–grains weight, grain yield, stalk yield, biological yield and harvest index were increased significantly due to bacterial inoculation under well watering and water stress conditions over respective non-inoculated control in consecutive two year's field trials. The maximum numbers of rows per cob and number of grains per row were produced in plants with no water stress when compared with those observed in plants faced water stress at vegetative as well as reproductive (tasseling) stage (Table 4) in both the experimental years. The number of rows per cob were not significantly different when comparison was made between the plants under water stress at vegetative and reproductive (tasseling) stage but this was not the same in case of number of grains per row. Among the bacterial treatments, inoculation with isolate BN-5 gave higher number of rows per cob and number of grains per row as compared to that of MD-23 at all water regimes in both the experiments (Table 4).

Inoculation of isolates MD-23 and BN-5 gave higher 1000-grain weight, grain yield, stalk yield, biological yield and harvest index as compared to non-inoculated control under all water regimes in both the experiments conductive during two consecutive years. Among the bacterial isolates, inoculation of BN-5 produced significantly higher 1000-grain weight of 319, 296 and 280 g in plants grown under no water stress, water stress at vegetative stage and water stress at tasseling stage, respectively during the year, 2014 (Table 4). Similarly, inoculation of this bacterial isolate gave the significantly higher values of 1000-grain weight as compared to that of MD-23 and non-inoculated control plants under all water regimes during the experimental year 2015. Inoculation of the bacterial isolate BN-5 produced significantly higher grain yield (6.8, 3.7 and 3.3 t ha^{-1} in plants under no water stress, water stress at vegetative and water stress at tasseling stage, respectively) as compared to that of non-inoculated control plants in the experiment during the year, 2014. A similar trend was observed in field trial conducted during 2015. In this experiment, inoculation with BN-5 produced a 8.1, 4.5 and 4.0 t ha^{-1} grain yield in plants with no water stress, water stress at vegetative stage and water stress at reproductive stage, respectively. Inoculation of bacterial isolate MD-23 increased the grain yield over respective non-inoculated control but not more than that was obtained from BN-5 inoculation under all water regimes.

Quality of Maize Grain

Inoculation of both the bacterial isolates to maize increased the grain carbohydrate contents over non-inoculated control under no water stress, water stress at vegetative and water stress at tasseling stage (Table 4).

Table 4: Effect of PGPR inoculation on physiological, yield and grain quality of maize under normal and water stress conditions

Treatments	Excised leaf water loss (%)						Leaf relative water contents (%)					
	2014			2015			2014			2015		
	WS_0	WS_1	WS_2	WS_0	WS_1	WS_2	WS_0	WS_1	WS_2	WS_0	WS_1	WS_2
MD-23	1.14 d	0.86 f	0.92 e	1.26cd	0.93ef	1.0 e	65.8 b	59.8 d	56.2 f	59.9 b	54.4 d	52.6 e
BN-5	1.15 d	0.82 f	0.84 f	1.23 d	0.90 f	0.93ef	70.3 a	62.5 c	59.4 d	63.9 a	56.9 c	54.0 d
Non-inoculated	1.76 a	1.36 b	1.23 c	1.90 a	1.46 b	1.33 c	57.8 e	53.2g	52.1 h	51.2 f	48.4 g	47.4 h
LSD 5%		0.05			0.08			0.59			0.60	
	Number of rows per cob						Number of grains per row					
MD-23	12.7bc	11.3 c	11.0 c	13.3bc	11.9 c	11.6 c	29.3 b	26.0 c	22.0 d	31.9 b	28.3 c	24.0 d
BN-5	16.3 a	13.3 b	13.3b	17.2 a	14.0 b	14.1 b	33.3 a	29.3 b	27.3bc	36.4 a	31.9 b	29.8bc
Non-inoculated	12.3bc	8.7 d	8.3 d	12.9bc	9.1 d	8.8 d	28.3bc	19.0 e	17.7 e	30.9bc	20.7 e	19.2 e
LSD 5%		1.82			1.47			2.48			1.4	
	1000 grain weight (g)						Grain yield (t ha^{-1})					
MD-23	294b	259d	234 e	318 b	280d	253e	4.2 b	2.5 d	1.8 e	5.0 b	2.97 d	2.17 e
BN-5	319 a	296 b	280 c	345 a	320 b	302 c	6.8 a	3.7bc	3.3 c	8.1 a	4.5 bc	4.0 c
Non-inoculated	224 e	205 f	185g	242 e	221 f	200 g	2.5 d	0.9 f	0.7 f	2.97 d	1.13 e	0.9 f
LSD 5%		9.9			7.81			0.66			0.59	
	Stalk yield (t ha^{-1})						Biological yield (t ha^{-1})					
MD-23	12.1 c	10.8 d	9.2 f	13.1 c	11.7 d	9.9 f	16.3b	13.3 c	11 d	18.1 b	14.7cd	12.1de
BN-5	14.1 a	12.6 b	10.3 e	15.1 a	13.6 b	11.1e	20.9 a	16.3 b	13.6c	23.2 a	18.1b	15.1 c
Non-inoculated	9.4 f	8.2 g	6.5 h	10.1 f	8.9 g	7.0 h	11.9 d	9.1 e	7.2 f	13.1 d	10.0 e	7.9 f
LSD 5%		0.37			0.42			0.68			0.65	
	Harvest index (%)						Carbohydrate contents in grains (%)					
MD-23	25.8b	18.8de	16.4 e	27.6 b	20.2 d	18de	66.9 a	61.1 c	59.3 d	72.7a	66.4 c	64.5 d
BN-5	32.5 a	22.7bc	24.3bc	34.9 a	24.9bc	26.5b	67.5 a	64.8 b	60.3cd	73.3a	70.5 b	65.5 cd
Non-inoculated	21 cd	9.9 f	9.7 f	22.7 c	11.3 e	11.4 e	47.1 e	41.8 g	43.8 f	51.2e	45.4 g	47.7 f
LSD 5%		3.84			3.80			2.1			1.84	
	Protein contents in grains (%)						Oil contents in grains (%)					
MD-23	9.98 b	9.4cd	8.96 d	10.5 b	9.9 cd	9.4 d	4.91 a	3.66bc	3.13 d	5.17a	3.85bc	3.29 d
BN-5	10.6 a	9.8bc	9.0 d	11.7 a	10.2 bc	9.5 d	5.14 a	3.96 b	3.49cd	5.41a	4.17 b	3.67cd
Non-inoculated	8.14 e	6.9 f	6.27 g	8.56 e	7.27 f	6.60 g	3.88 b	1.32 e	1.27 e	4.1 b	1.39 e	1.33 e
LSD 5%		0.48			0.50			0.37			0.39	

MD-23, BN-5 represent the plants inoculated with bacterial isolate MD-23 and BN-5 while Non-inoculated means the plants without any bacterial culture inoculation
WS0, WS1, WS2 represent plants under no water stress, water stress at vegetative stage (V7) and water stress at reproductive (tasseling; VT) stage, respectively
Each value (%, g as well as t ha^{-1}) in the table are the mean of three replications. Values with different letters a, b, and c show significance level at 5% probability ($p \leq 0.05$)

The maximum carbohydrate contents of 67% and 68% were determined in grains obtained from well-watered plants inoculated with bacterial isolate MD-23 and BN-5, respectively during the year 2014. However, the inoculation with BN-5 to plants under water stress at vegetative and tasseling stage produced the higher carbohydrate contents of 65% and 60% respectively, as compared to MD-23 inoculation. A similar trend was observed in experiment conducted during the year, 2015. The maximum protein (10.6%) and oil (5.14%) contents were determined in BN-5 inoculated well-watered plants during the year 2014 (Table 4). In experiment during the year 2015, BN-5 inoculation again produced maximum protein (11.7%) and oil (5.41%) contents in plants with no water stress. Under water stress either at vegetative or tasseling stage, oil and protein contents was increased due to BN-5 inoculation significantly over respective non-inoculated control in both the experiments (Table 4). Inoculation with MD-23 also increased the protein and oil contents over non-inoculated control plants but not more than BN-5 inoculation under all water regimes in both the experiments.

Regression Analysis

Regression analysis showed that strong positive linear correlation (R^2=0.7723-0.9405) exist between EPS concentration (sugars) and 1000-grain weight under no water stress, water stress at vegetative and water stress at tasseling stage (Table 5). The grain yield was positively and linearly correlated with the concentration of EPS (sugars) with R^2 values 0.6495, 0.8255 and 0.6794 under well water, water stress at vegetative and water stress at tasseling stage, respectively in the experiment of spring, 2014. A similar trend (R^2 values 0.6499, 0.8027 and 0.665 respectively) in plant under no water stress, water stress at vegetative stage and water stress at tasseling stage was observed in the experiment conducted during spring, 2015 (Table 5). Strong positive correlation existed (Table 5) between EPS (sugar) and LRWC (R^2= 0.8805, 0.9259 and 0.8169 under well-watering, water stress at vegetative and water stress at reproductive stage, respectively) in experiment during the spring 2014. Similarly, EPS (sugar) and LRWC were positively correlated under well-watered conditions (R^2=0.9111), water stress at vegetative stage (R^2=0.9237) and water stress at reproductive stage (R^2=0.9634) in the experiment during the year, 2015. The regression equation drawn between *in vitro* sugar concentration of bacterial produced EPS and ELWL, showed that relationship was linearly negative (R^2=1.0, 0.9968, and 0.9661), under well-watered, water stress at vegetative stage and water stress at reproductive stage, respectively, during 2014 (Table 5).

Table 5: Relationship of various physiological and productivity attributes of maize grown in field experiments under different irrigation regimes with the bacterial produced exopolysaccharides (sugars) *in vitro*

Parameters	Year 2014			Year 2015		
	WS_0	WS_1	WS_2	WS_0	WS_1	WS_2
1000-grain weight and sugars	0.9405^{**}	0.8469^{**}	0.7728^{**}	0.9405^{**}	0.8472^{**}	0.7723^{**}
Grainyieldandsugars	0.6495^{*}	0.8255^{**}	0.6794^{*}	0.6499^{*}	0.8027^{**}	0.6650^{*}
LRWC and sugars	0.8805^{**}	0.9259^{**}	0.8169^{**}	0.9111^{**}	0.9237^{**}	0.9634^{**}
ELWL and sugars	1.00^{**}	0.9968^{**}	0.9661^{**}	0.9991^{**}	0.9986^{**}	0.9764^{**}
Grain carbohydrate and sugars	0.9998^{**}	0.9805^{**}	0.9981^{**}	0.9998^{**}	0.9798^{**}	0.9984^{**}

WS_0, WS_1 and WS_2 represent the no water stress, water stress at vegetative (V7) stage and water stress at reproductive stage (tasseling; VT) stage, respectively. LRWC and ELWL represent leaf relative water content and excised leaf water loss, respectively
$^{*}p\leq0.05$; $^{**}p\leq0.01$

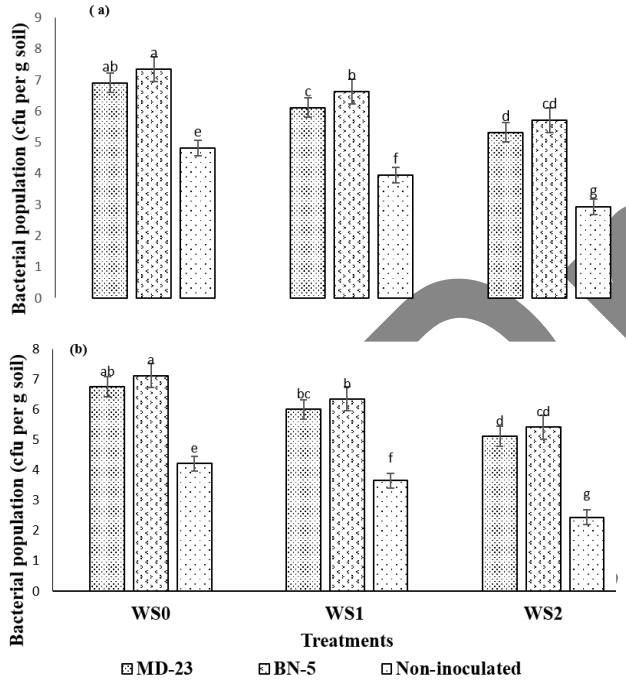

Fig. 2: Effect of PGPR inoculation on rhizosphere bacterial population (log cfu g^{-1} soil) in maize under no water stress, water stress at vegetative stage and water stress at tasseling stage during the year, 2014 (a) and the year, 2015 (b)

Bacterial population was estimated in rhizosphere soil samples (0.5 g) using serial dilution plate count method. Colony forming units (cfu/g of dry soil) were calculated by counting the colonies and their log values were calculated

WS0, WS1 and WS2 represents no water stress, water stress at vegetative stage and water stress at reproductive stage, respectively

Bars with different letters like a, b, c etc. show significance level at 5% probability value ($p\leq0.05$) and standard errors bars are given on each bar on the basis of standard error values

Similarly, a strong negative correlation was observed between bacterial produced sugar and ELWL (R^2=0.9991, 0.9986 and 0.9764 under well-watering, water stress at vegetative stage and water stress at reproductive (tasseling) stage, respectively) in the experiment during year 2015 (Table 5). The grain carbohydrate contents were positively correlated with bacterial EPS *i.e*., sugars (R^2=0.9998, 0.9805 and 0.9981 under no water stress, water stress at vegetative and water stress at reproductive stage, respectively) in the experiment during 2014 (Table 5). A similar trend was observed in the experiment during the year 2015 between EPS (sugar) and LRWC (R^2=0.9998, 0.9798 and 0.9984 under no water stress, water stress at vegetative and water stress at reproductive stage (Table 5).

Rhizosphere Bacterial Colonization

A significantly higher bacterial population was recorded in rhizosphere of the plants under no water stress in both the experiments (Fig. 2a and b). Water stress at vegetative stage affected the bacterial colonization, and due to which the bacterial population was decreased from 6.91 to 6.12 cfu g^{-1} soil in rhizosphere of plants inoculated with the isolate MD-23, 7.35 to 6.63 cfu g^{-1} soil in rhizosphere of plants inoculated with bacterial isolate BN-5 and 4.81-3.95 cfu g^{-1} soil in rhizosphere of non-inoculated plants during the year 2014 (Fig. 2a). Water stress at tasseling stage severely affected the bacterial colonization and further decreased the values to 5.72-2.94 cfu g^{-1} soil. The minimum values of cfu g^{-1} (2.94 cfu g^{-1} soil) were recorded in the samples collected from the rhizosphere of non-inoculated control plants in the experiment conducted during 2014. A similar trend of bacterial colonization was observed in the experiment conducted in growing season, 2015 (Fig. 2b).

Discussion

In present study, a total of 95 different rhizospheric bacterial isolates obtained from two different districts (located in arid region) of Punjab were characterized for plant beneficial traits like EPS secretion, P-solubilization, IAA production, ARA and ACC deaminase activity. The results indicated that among all the 95 isolates, 23% isolates showed ARA activity, 31% isolates were positive for P-solubilization potential, 21% isolates produced IAA and 18% were positive for ACC deaminase activity. Among all the bacterial isolates, 7% isolates exhibited all the plant beneficial traits. From these, two most efficient isolates MD-23 (highest nitrogen fixation and IAA production potential) and BN-5 (highest P-solubilization and IAA production) were tested for EPS (sugars, protein and uronic acid) secretion in culture medium. This was observed that the isolate BN-5 produced maximum amount of sugars (7163 μg g^{-1}), protein (988 μg g^{-1}) and uronic acid (0.87 μg mg^{-1}) in culture medium. Rhizosphere is dominated by microorganisms inhabiting diversity of functions and play vital role in soil-plant-microbe interaction (Tahir *et al*., 2015a; Adu *et al*., 2017; Dimitrov *et al*., 2017). Further, presence of bacteria in arid region with characters (like ACC deaminase activity, IAA and EPS production) that enable them to survive in stress environment is well documented (Glick *et al*., 1998; Mayak *et al*., 2004; Glick *et al*., 2007; Naseem and Bano, 2014).

Bacterial strains with ACC deaminase activity have been isolated from rhizosphere of wheat, maize, canola, tomato and coastal soil (Penrose and Glick, 2003; Siddikee *et al*., 2010; Ahmad *et al*., 2014, 2016; Vurukonda *et al*., 2016). Isolation of bacteria with nitrogen fixation, P-solubilization and IAA production potential from the rhizosphere of various crops grown in Punjab province have been reported in glut of studies (Tahir *et al*., 2013; Tahir *et al*., 2015a; Hussain *et al*., 2016).

On the basis of plant valuable traits, two most potent bacterial isolates MD-23 (Multan region) and BN-5 (Bahawalnagar region) were selected and tested as bioinoculant to maize crop sown under well-watered as well as water limiting conditions in field experiments in consecutive two years. In present experiments, significantly ($p \leq 0.05$) higher chlorophyll contents, number of grain rows per cob, number of grains per row, 1000-grain weight, stalk yield, grain yield, grain carbohydrate, protein and oil contents were observed in well-watered plants as compared to that were obtained in plants under water stress conditions. These results indicated that well-watered plants obtained optimum amount of water for root growth, nutrient uptake and metabolic functioning which resulted in better growth, productivity and quality of maize. Similar results have been reported in previous studies (Paredes *et al*., 2014; Kresovic *et al*., 2016). In our study, water stress was imposed at vegetative and tasseling stage. Limited water supply exerts osmotic stress (Forni *et al*., 2017) which impose membrane damage, reduced photosynthesis (Tardieu, 2005; Forni *et al*., 2017) and production of stress ethylene *i.e*., a well-known growth inhibiting phytohormone in high concentration (Forni *et al*., 2017) in plants. All these effects lead to reduced growth and grain development of maize in our study and is well reported in plethora of previous studies (Cakir, 2004; Bartels and Sunkar, 2005; Vardharajula *et al*., 2011; Naveed *et al*., 2014). In both the experiments, water stress at tasseling stage resulted in lower relative leaf water contents, number of rows per cob, number of grains per row, 1000-grain weight, grain and stalk yield as compared to that of water stress at vegetative stage. This indicated that flowering stage is more critical to water stress as compared to vegetative stage. Similar results were reported by Cakir (2004) that water stress at reproductive stage reduced the corn yield by 66-93% in a three-years field experiment.

Inoculation of the plant growth promoting rhizobacteria (BN-5 and MD-23) to maize resulted in lower excised leaf water loss, higher values of leaf relative water contents, chlorophyll contents, number of rows per cob, number of grains per row, 1000-grain weight, grain yield and quality attributes as compared to respective non-inoculated control under well watering as well as under water stress conditions in both the experiments. The higher values of growth, productivity and quality attributes in inoculated plants under water stress condition indicated that inoculated bacteria produced ACC deaminase which hydrolyzed the ACC (the immediate biosynthetic precursor of ethylene; Glick *et al*., 1998) produced by the plants under stress, reduced the stress ethylene level, enables the plant to escape from deleterious effects of water stress and resulted in increased growth, productivity and quality of maize. This mechanism has been well explained by Cakir (2004), Glick *et al.* (2007) and Forni *et al.* (2017). The inoculation of ACC deaminase producing bacteria resulted in reduced stress ethylene level in plants and improved the growth and productivity of crops in previous studies (Mayak *et al*., 2004; Cheng *et al*., 2007; Ali *et al*., 2014; Belimov *et al*., 2015; Han *et al*., 2015). In addition to ACC deaminase activity, inoculated bacteria also paraded IAA producing activity in *in vitro* culturing (Table 2). Indole-3-acetic acid production is a famous mechanism which plant and bacteria adopt under drought stress conditions (Forni *et al*., 2017). Indole-3-acetc acid has vital role in improving the root system of plants under water stress to absorb more water from the depth. In our study, the increased growth, productivity and quality of maize in bacterial inoculated treatments may also be attributed to IAA production by these bacteria under stress conditions in 2 years consecutive field experiments. Improvement in growth and productivity of crops due to inoculation with IAA producing bacteria particularly in drought stress conditions has been reported previously (Egamberdieva, 2009; Sandhya *et al*., 2010; Arzanesh *et al*., 2011).

As mentioned above, the inoculated bacterial isolates also secreted EPS (sugar, protein and uronic acid) in culture medium (*in-vitro*). This may be another mechanism that the inoculated bacteria adopted to protect the plants under water stress from membrane damage and to improve growth, productivity and quality of maize. In our study, the value of excised leaf water loss was negatively correlated with bacterial produced sugars *in vitro* under all water regimes. Leaf relative water contents and bacterial EPS (sugar) were positively correlated incase of plants under no water stress, water stress at vegetative stage and water stress at tasseling stage. In our study, bacterial EPS (sugar) contributed positively in grain carbohydrate accumulation which was confirmed through positive correlation between EPS sugars and grain carbohydrate contents. This indicated that bacterial produced EPS (sugars) has a vital role in protecting plants under water stress and improving growth, productivity and quality. Earlier, Naseem and Bano (2014) represented the growth improvement of maize due to inoculation of EPS producing rhizosphere bacteria. Both the bacterial isolates used in our study were also positive for catalase activity. The growth and productivity improvement of maize under water stress condition (50% FC level) due to bacterial inoculation might also be attributed to catalase activity of the inoculated isolates in our study. Previously, this was elaborated that catalase enzyme play role as scavenger of reactive oxygen species produced in drought stress conditions (Timmusk *et al*., 2014) and helped the plants to cope with water stress.

The performance of the inoculated isolates in improving chlorophyll contents, number of rows per cob,

number of grains per row, 1000-grain weight, grain yield, grain carbohydrate, protein and oil contents was significantly better as compared to non-inoculated control even under no water stress conditions (75% FC level). This was due to the fact that the tested bacterial isolates possessed plant beneficial traits like nitrogen fixation, P-solubilization, IAA production and EPS production which helped the plants in getting more nutrients and water, and provided the growth hormone and increased the growth, productivity and quality of maize over non-inoculated control. Several other studies have reported the effect of PGPR inoculation in improving growth and productivity of maize in particular (Biari *et al*., 2008; Ahmad *et al*., 2016; Iqbal *et al*., 2016; Mosimann *et al*., 2016) as well as in other crops in general (Tahir *et al*., 2013; Tahir *et al*., 2015a, b; Hussain *et al*., 2016; Rubin *et al*., 2017). Our results showed that grain protein and oil contents were increased due to bacterial inoculation in both the experiments (Table 4). Our results are similar with the previous studies (Akbari *et al*., 2011; Tahir and Shehzadi, 2017). Moreover, positive correlation between grain protein and oil contents of maize has been reported previously (Alda *et al*., 2011).

In addition to all these results, there were some results with exception like in the experiment conducted during 2015, in which the number of grains per row produced in non-inoculated well-watered plants (75% FC level) were similar to those recorded in BN-5 inoculated plants under water stress conditions (50% FC level). Logically, this make sense that under the stress conditions the isolate BN-5 (positive for nitrogen fixation, P-solubilization, IAA production, ACC deaminase activity, catalase activity and EPS production) when inoculated to plants utilized all these mechanisms to boost up the plant tolerance against drought stress, stimulated water absorption and nutrient up take and enable the plant to perform better even underwater stress conditions (50% FC level) which was at par to that of plants growing under optimum water conditions.

As for as the bacterial isolates are concerned, this was observed that the isolate BN-5 performed better in both the experiments and produced the higher number of rows per cob, number of grains per row, 1000-grain weight, grain yield and grain quality under no water stress (75% FC level), water stress (50% FC level) at vegetative stage and water stress (50% FC level) at tasseling stage as compared to the isolate MD-23. This may be due to the better adoptability conditions specific for each bacterial isolate in two different growing periods. The reason for conducting experiments in two consecutive years was to test and confirm the potential of the bacterial isolates under varying seasons. The performance of the two isolates was consistent in both the experiments over non-inoculated control in case of well-water (75% FC level) as well as water limiting conditions (50% FC level).

Conclusion

Rhizobacteria with ability to produce indole-3-acetic acid, 1-aminocyclopropane-1-carboxylate deaminase and exopolysaccharides were dominant in rhizosphere of arid zone crops. Most of the bacterial isolates were efficient phosphate solubilizers and nitrogen fixers. The water stress either at vegetative or reproductive (tasseling) stage posed deleterious effects and reduced the growth, productivity and quality of maize over well-watered treatment. However, these deleterious effects were overwhelmed by the inoculation of ACC-deaminase, IAA, catalase and EPS producing bacteria equipped with P-solubilizing and nitrogen fixing activities. Application of the selected bacterial isolates to maize grown in the arid environment enabled the maize to withstand under water stress (50% FC level) without compromising its performance in terms of growth, productivity and quality. Moreover, these isolates enhanced the growth, productivity and quality of well-watered plants due to P-solubilization, nitrogen fixation and IAA production over non-inoculated plants.

Acknowledgement

I am thankful to Department of Agronomy, BZU, Multan and Laboratory of Environmental Sciences Department, COMSATS University Islamabad, Vehari for providing me the research facilities. Technical assistance of Mr. Ali Ahmad (Lab Assistant) in performing lab and field work at BZU, Multan is thankfully acknowledged. I am grateful to Hexon Group of Companies, Multan for providing Biofertilizer lab facilities to conduct microbiological research.

References

Adak, N., H. Gubbuk and N. Tetik, 2018. Yield, quality and biochemical properties of various strawberry cultivars under water stress. *J. Sci. Food Agric.*, 98: 304–311

Adu, M.O., P.A. Asare, D.O. Yawson, F.K. Ackah, K.K. Amoah, M. Nyarko and D.A. Andoh, 2017. Quantifying variations in rhizosheath and root system phenotypes of landraces and improved varieties of juvenile maize. *Rhizosphere*, 3: 29–39

Ahmad, I., H.N. Akhtar, U. Asghar, M. Ghafoor and M. Shahid, 2016. Differential effects of plant growth promoting rhizobacteria on maize growth and cadmium uptake. *J. Plant Growth Regul.*, 35: 303–315

Ahmad, I., M.J. Akhtar, Z.A. Zahir, M. Naveed, B. Mitter and A. Sessitsch, 2014. Cadmium tolerant bacteria induce metal stress tolerance in cereals. *Environ. Sci. Pollut. Res*., 21: 11054–11065

Akbari, P., A. Ghalavand, A. Sanavy, M. Agha Alikhani and S. Kalkhoran, 2011. Comparison of different nutritional levels and the effect of plant growth promoting rhizobacteria (PGPR) on the grain yield and quality of sunflower. *Aust. J. Crop Sci.*, 5: 1570–1576

Alda, S., A. Lazureanu, G. Carciu, L. Nita, L. Alda, T. Cristea and M. Danci, 2011. Quality evaluation of some maize hybrids cultivated under pedoclimatic conditions of Banat area (West of Romania). *J. Hortic. For. Biotechnol.*, 15: 207–211

Ali, S., T.C. Charles and B.R. Glick, 2014. Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. *Plant Physiol. Biochem.*, 80: 160–167

Ali, S.Z., V. Sandhya, M. Grover, V.R. Linga and V. Bandi, 2011. Effect of inoculation with a thermotolerant plant growth promoting *Pseudomonas putida* strain AKMP7 on growth of wheat (*Triticum* spp.) under heat stress. *J. Plant Interact.*, 6: 239–246

Annonymous, 2016. *Economic Survey of Pakistan*. Ministry of Food and Agriculture, Islamabad, Pakistan

Aprilea, M.C. and F. Damiano, 2017. Water conservation behavior and environmental concerns: Evidence from a representative sample of Italian individuals. *J. Clean Prod.*, 159: 119–129

Arzanesh, M.H., H. Alikhani, K. Khavazi, H. Rahimian and M. Miransari, 2011. Wheat (*Triticum aestivum* L.) growth enhancement by *Azospirillum* sp. under drought stress. *World J. Microb. Biotech*., 27: 197–205

Bartels, D. and R. Sunkar, 2005. Drought and salt tolerance in plants. *Crit. Rev. Plant Sci*., 24: 23–58

Belimov, A., I. Dodd, V. Safronova, A. Shaposhnikov, T. Azarova, N. Makarova and W. Tikhonovich, 2015. Rhizobacteria that produce auxins and contain 1-amino-cyclopropane-1-carboxylic acid deaminase decrease amino acid concentrations in the rhizosphere and improve growth and yield of well-watered and water-limited potato (*Solanum tuberosum*). *Ann. Appl. Biol*., 167: 11–25

Bhardwaj, D., M.W. Ansari, R.K. Sahoo and N. Tuteja, 2014. Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microb. Cell Fac*., 13: 66–76

Bianco, C. and R. Defez, 2009. Medicago truncatula improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing *Sinorhizobium meliloti* strain. *J. Exp. Bot*., 60: 3097–3107

Biari, A., A. Gholami and H. Rahmani, 2008. Growth promotion and enhanced nutrient uptake of maize (*Zea mays* L.) by application of plant growth promoting rhizobacteria in arid region of Iran. *J. Biol. Sci*., 8: 1015–1020

Bramhachari, P.V. and S. Dubey, 2006. Isolation and characterization of exopolysaccharide produced by *Vibrio harveyi* strain VB23. *Lett. Appl. Microbiol*., 43: 571–577

Cakir, R., 2004. Effect of water stress at different development stages on vegetative and reproductive growth of corn. *Field Crop Res*., 89: 1–16

Cheng, Z., E. Park and B.R. Glick, 2007. 1-Aminocyclopropane-1-carboxylate deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. *Can J. Microbiol*., 53: 912–918

Dimitrov, M.R., A.J. Veraart, M. de Hollander, H. Smidt, J.A. van Veen and E.E. Kuramae, 2017. Successive DNA extractions improve characterization of soil microbial communities. *Peer J*., 5: e2915

DuBois, M., K.A. Gilles, J.K. Hamilton, P.T. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem*., 28: 350–356

Egamberdieva, D., 2009. Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. *Acta Physiol. Plant.*, 31: 861–864

Estefan, G., R. Sommer and J. Ryan, 2013. *Methods of Soil, Plant and Water Analysis: A Manual for the West Asia and North Africa Region*, 3rd edition. ICARDA, Beirut, Lebanon

Fadden, M., 1980. *Biochemical Tests for Identification of Medical Bacteria*, pp: 51–54. Williams and Wilkins, Baltimore, USA

Fan, L., G. Liu, F. Wang, V. Geissen, C.J. Ritsema and Y. Tong, 2013. Water use patterns and conservation in households of Wei River Basin, China. *Resour. Conserv. Recy.*, 74: 45–53

Forni, C., D. Duca and B.R. Glick, 2017. Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. *Plant Soil.*, 410: 335–356

Garcia de Salamone, I.E., R.K. Hynes and L.M. Nelson, 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. *Can. J. Microbiol*., 47: 404–411

Gepstein, S. and B.R. Glick, 2013. Strategies to ameliorate abiotic stress-induced plant senescence. *Plant Mol. Biol.*, 82: 623–633

Gerhardt, P., R.G.E. Murray, W.A. Wood and N.R. Krieg, 1994. *Methods for General and Molecular Bacteriology*, pp: 55518. ASM Washington DC., ISBN 1-55581-55048-55589 Glick, B.R., 2012. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*, 2012. http://dx.doi.org/10.6064/2012/963401

Glick, B.R., Z. Cheng, J. Czarny and J. Duan, 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. *Eur. J. Plant Pathol.*, 119: 329–339

Glick, B.R., D.M. Penrose and J. Li, 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *J. Theor. Biol.*, 190: 63–68

Gogoi, B., R.K. Thakuria and N. Deka, 2018. Impact of Water Management on Growth and Yield of Ahu Rice Varieties of Assam, India. *Int. J. Curr. Microbiol. App. Sci.*, 7: 936–941

Han, Y., R. Wang, Z. Yang, Y. Zhan, Y. Ma, S. Ping, L. Zhang, M. Lin and Y. Yan, 2015. 1-aminocyclopropane-1-carboxylate deaminase from *Pseudomonas stutzeri* A1501 facilitates the growth of rice in the presence of salt or heavy metals. *J. Microbiol. Biotechnol*., 25: 1119–1128

Hussain, M., W. Bashir, S. Farooq and A. Rehim, 2013. Root development, allometry and productivity of maize hybrids under terminal drought sown by varying method. *Intl. J. Agric. Biol.*, 15: 1243–1250

Hussain, M., Z. Asgher, M. Tahir, M. Ijaz, M. Shahid, H. Ali and A. Sattar, 2016. Bacteria in combination with fertilizers improve growth, productivity and net returns of wheat (*Triticum aestivum* L.). *Pak. J. Agric. Sci*., 53: 633–645

ICC, 1980. ICC Standard No: 105/1. *Method for the Determination of Crude Protein in Cereals and Cereal Products for Food and for Feed. Standard Methods of the International Association for Cereal Chemistry* (ICC). Verlag Moritz Schafer, Detmold

Iqbal, M.A., M. Khalid, Z.A. Zahir and R. Ahmad, 2016. Auxin producing plant growth promoting rhizobacteria improve growth, physiology and yield of maize under saline field conditions. *Intl. J. Agric. Biol*., 18: 37–45

Jia, Q., L. Sun, J. Wang, J. Li, S. Ali, T. Liu, P. Zhang, Y. Lian, R. Ding and X. Ren, 2018. Limited irrigation and planting densities for enhanced water productivity and economic returns under the ridge-furrow system in semi-arid regions of China. *Field Crop Res.*, 221: 207–218

Joo, G.J., Y.M. Kim, J.T. Kim, I.K. Rhee, J.H. Kim and I.J. Lee, 2005. Gibberellins-producing rhizobacteria increase endogenous gibberellins content and promote growth of red peppers. *J. Microbiol*., 43: 510–515

Kresovic, B., A. Tapanarova, Z. Tomic, L. Zivotic, D. Vujovic, Z. Sredojevic and B. Gajic, 2016. Grain yield and water use efficiency of maize as influenced by different irrigation regimes through sprinkler irrigation under temperate climate. *Agric. Water Manage.*, 169: 34–43

Kumar, M.A., K.T.K. Anandapandian and K. Parthiban, 2011. Production and characterization of exopolysaccharides (EPS) from biofilm forming marine bacterium. *Braz. Arch. Biol. Techn*., 54: 259–265

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193: 265–275

Mayak, S., T. Tirosh and B.R. Glick, 2004. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Sci*., 166: 525–530

Mehnaz, S., M.S. Mirza, J. Haurat, R. Bally, P. Normand, A. Bano and K.A. Malik, 2001. Isolation and 16S rRNA sequence analysis of the beneficial bacteria from the rhizosphere of rice. *Can. J. Microbiol*., 47: 110–117

Mosimann, C., T. Oberhansli, D. Ziegler, D. Nassal, E. Kandeler, T. Boller, P. Mader and C. Thonar, 2016. Tracing of two *Pseudomonas* strains in the root and rhizoplane of maize, as related to their plant growth-promoting effect in contrasting soils. *Front. Microbiol*., 7: 2150–2164

Murphy, J. and J.P. Riley, 1962. Modified solution method for determination of phosphate in natural water. *Anal. Chim. Acta*., 27: 31–36

Naseem, H. and A. Bano, 2014. Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize. *J. Plant Interact.*, 9: 689–701

Nautiyal, C.S., S. Srivastava, P.S. Chauhan, K. Seem, A. Mishra and S.K. Sopory, 2013. Plant growth-promoting bacteria *Bacillus amyloliquefaciens* NBRISN13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. *Plant Physiol. Biochem*., 66: 1–9

Naveed, M., B. Mitter, T.G. Reichenauer, K. Wieczorek and A. Sessitsch, 2014. Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* spp. FD17. *Environ. Exp. Bot.*, 97: 30–39

Okon, Y., S.L. Albercht and R.H. Burris, 1977. Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. *Appl. Environ. Microbiol*., 33: 85–88

Paredes, P., G. Rodrigues, I. Alves and L. Pereira, 2014. Partitioning evapotranspiration, yield prediction and economic returns of maize under various irrigation management strategies. *Agric. Water Manage.*, 135: 27–39

Penrose, D.M. and B.R. Glick, 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol. Plant.*, 118: 10–15

Pikovskaya, R., 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya*, 17: 362–370

Potter, R.B. and K. Darmame, 2010. Contemporary social variations in household water use, management strategies and awareness under conditions of 'water stress': the case of Greater Amman, Jordan. *Habitat Intl.*, 34: 115–124

Ray, D.K., N.D. Mueller, P.C. West and J.A. Foley, 2013. Yield trends are insufficient to double global crop production by 2050. *PloS One*, 8: e66428

Rubin, R.L., K.J. van Groenigen and B.A. Hungate, 2017. Plant growth promoting rhizobacteria are more effective under drought: a meta-analysis. *Plant Soil*, 416: 309–323

Siddikee, M.A., P.S. Chauhan, R. Anandham, H. Gwang-Hyun Han and S. Tongmin, 2010. Isolation, characterization, and use for plant growth promotion under salt stress, of acc deaminase-producing halotolerant bacteria derived from coastal soil. *J. Microbiol. Biotechnol*., 20: 1577–1584

Sandhya, V., S.Z. Ali, M. Grover, G. Reddy and B. Venkateswarlu, 2010. Effect of plant growth promoting Pseudomonas spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. *Plant Growth Reg*., 62: 21–30

Santini, M. and M.C. Rulli, 2015. Water resources in Italy: the present situation and future trends. *In: The Water We Eat*, pp: 139–143. Antonelli, M. and F. Greco (eds.). Springer Water. Springer, Cham

Steel, R.G.D., J.H. Torrie and D.A. Deekey, 1997. *Principles and Procedures of Statistics: A Biometrical Approach*, 3rd edition, pp: 400–428. McGraw Hill Book, New York, USA

Tahir, M., M.S. Mirza, S. Hameed, M.R. Dimitrov and H. Smidt, 2015a. Cultivation-based and molecular assessment of bacterial diversity in the rhizosheath of wheat under different crop rotations. *PloS One*, 10: e0130030

Tahir, M., A. Nadeem, H. Nazim, M. Ijaz, M. Shahid, M. Bismillah Khan and H. Ali, 2015b. Plant growth promotingrhizobacteria improve growth of nursery transplanted riceunder agro ecological conditions of Southern Punjab. *Environ Plant Syst.*, 1: 22–27

Tahir, M., M.S. Mirza, A. Zaheer, M.R. Dimitrov, H. Smidt and S. Hameed, 2013. Isolation and identification of phosphate solubilizer*Azospirillum*, *Bacillus* and *Enterobacter* strains by 16SrRNA sequence analysis and their effect on growth of wheat (*'Triticum aestivum* L.). *Aust. J. Crop Sci.*, 7: 1284–1292

Tahir, M. and M. Shahzadi, 2017. Response of Spring and Autumn sunflower to chemical and bio-ertilizer for yield and quality traits. *Pak. J. Agric. Res.*, 30: 122–128

Tardieu, F., 2005. Plant tolerance to water deficit: physical limits and possibilities for progress. *Comptes Rendus Geosci.*, 337: 57–67

Taylor, K.A. and J.G. Buchanan-Smith, 1992. A colorimetric method for the quantitation of uronic acids and a specific assay for galacturonic acid. *Anal. Biochem.*, 201: 190–196

Tien, T., M. Gaskins and D. Hubbell, 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Appl. Environ. Microb*., 37: 1016–1024

Timmusk, S., I.A.A. El-Daim, L. Copolovici, T. Tanilas, A. Kannaste, L. Behers, E. Nevo, G. Seisenbaeva, E. Stenstrom and U. Niinemets, 2014. Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. *PloS One*, 9: e96086

Vardharajula, S., A.S. Zulfikar, M. Grover, G. Reddy and V. Bandi, 2011. Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. *J. Plant Interact*., 6: 1–14

Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil*, 255: 571–586

Vurukonda, S.S.K.P., S. Vardharajula, M. Shrivastava and A. SkZ, 2016. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiol. Res*., 184: 13–24

(Received 24 May 2018; Accepted 22 November 2018)