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Full Length Article

Auxin and 1-Aminocyclopropane-1-Carboxylate Deaminase Activity Exhibiting Rhizobacteria Improved Maize Quality and Productivity under Drought Conditions

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Abstract

Drought, the major characteristic of arid regions, exerts drastic effects on crop productivity. Rhizobacteria equipped with 1-aminocyclopropane-1-carboxylate (ACC) deaminase and indole-3-acetic acid producing activity (IAA) assist plants to cope with drought 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 sugars, (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 as compared to non-inoculated control. In two years field study, maize seeds inoculated with these bacterial isolates were grown in field under drought stress at vegetative and tasseling stages {(~50% field capacity (FC)} while well-watered conditions (~75% FC) throughout the season were taken as control. Results disclosed that both strains (BN-5, MD-23) reduced the excised leaf water loss, increased the relative leaf water contents, chlorophyll contents and grain yield both under well-watered and drought conditions 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 under well-watered and drought stress at both growth stages in both years of study. Moreover, grain yield and quality parameters were significantly lower in non-inoculated control compared with bacterial inoculation under drought stress imposed at vegetative and tasseling stages. In conclusion, the PGPR strains equipped with ACC deaminase and IAA producing activity and EPS secretion improved the yield and quality of maize under well-watered and drought stressed conditions.

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

Introduction

Drought stress, the common characteristic of arid region, prevails all over the world as well as in Pakistan (Hussain *et al*., 2018). 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. However, the only limitation is the prevalence of extremely high temperature, low rainfall and non-availability of irrigation water in these areas. Under drought stress, plants face osmotic stress that results in reduced vegetative growth and leaf expansion (Bartels and Sunkar, 2005; Hussain *et al*., 2018), 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 drought stress and cause decline in crop performance (Vardharajula *et al.*, 2011; 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

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reduced plant growth and productivity (Forni *et al*., 2017).

Maize (*Zea mays*. L) is one of the prominent cereals in the world and its production must be increased to fulfill the demand of ever-increasing human population, livestock/ poultry consumption and bio-fuel industry requirements (Ray *et al*., 2013). In Pakistan, maize is cultivated as autumn and spring crop. Drought stress occurred either at vegetative or reproductive stages of maize results in severe yield penalty (Hussain *et al*., 2013; Aslam, 2016). Substantial yield losses under drought stress are well reported in maize and other cereal crops (Khan *et al.,* 2001; Shaddad *et al.,* 2011; Nawaz *et al.,* 2013).

Numerous strategies like use of advanced sowing methods (Hussain *et al.,* 2013), variable planting density (Jia *et al.,* 2018) and cultivation of less water requiring cultivars (Adak *et al.,* 2018), have been developed to cope with drought stress in cereals in arid climates. Isolation of rhizobacteria indigenous to arid region and their application as bio-inoculant is an emerging eco-friendly approach that enables plants to withstand drought conditions. These rhizobacteria offer diverse plant beneficial characteristics (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) (Vessey, 2003; Tahir *et al*., 2013, 2015a). These PGPR produce growth hormone like cytokinin, gibberellins (Salamone *et al*., 2001; Joo *et al*., 2005) and indole-3-acetic acid (IAA) that increase the root proliferation, water uptake, nutrient uptake and crop productivity (Vessey, 2003; Tahir *et al*., 2013, 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).

This indicates the isolation of PGPRs native to arid lands and their application to field crops may ameliorate the negative effects of drought stress. For this study, we hypothesized that application of PGPRs indigenous to arid region will help the maize plants to ameliorate the damaging effects of drought and resultantly will enhance maize productivity. To test this hypothesis, the PGPRs native to arid climate equipped with IAA and ACC deaminase producing potential were isolated. Further, the selected PGPR isolates were tested as bio-inoculant to mitigate the negative effects of drought stress on maize productivity and its grain quality.

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 following 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 g 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). 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.

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. Maize seeds were inoculated with both above cited bacterial strains while un-inoculated seeds were taken as control. Inoculated and un-inoculated maize seeds were grown during two consecutive years in spring, 2014 and 2015 at research farm of Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan (30°11′52″N 71°28′11″E) and subjected to drought stress at vegetative and tasseling stage {(~50% field capacity (FC)}, while ~75% FC water conditions were taken as control. The FC was based on soil moisture contents and was maintained by collecting soil samples from 15 cm soil depth on week basis following Farooq et al. (2015). Soil moisture contents were 50.24% at saturation percentage; half of which was labeled as 100% field capacity, and half of 100% FC was designed as 50% FC. Moreover FC of soil was maintained by giving measured volume of water when the soil moisture level was dropped below the above cited levels. Experiment was laid out in Randomized Complete Block Design (RCBD) in split-plot

arrangement by keeping drought stress levels in main plots and bacterial inoculations in sub plots. In both years experiment was replicated three times with net plot size of 5 m × 3 m

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 and un-inoculated maize seeds were manually sown by dibbler on ridges (R×R = 75 cm and P×P = 20 cm) on 6th March, 2014 while in the year 2015, crop was sown on 10th of March using the same method. Crop was fertilized with NPK at 200, 150 and 150 kg ha^{-1}, respectively in both years using urea, diammonium phosphate and SOP as source of N, P and K, respectively. Whole amount of P and K along with 1/3rd of N was applied at sowing time while the remaining N was applied in two equal doses. Maize crop was irrigated according to treatments. 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 as a ratio of grain yield to biological yield in percentage.

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 were 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, 25 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 functional characterization, two most efficient bacterial isolates MD-23 with maximum N 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 isolates were positive for ACC deaminase and catalase activities as well. Both isolates were processed for exopolysaccharide secretion. Composition analysis of EPS showed that both isolates produced total sugar, protein and uronic acid in their EPS. However, significantly ($p \leq 0.05$) higher amounts 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 compared to MD-23 and non-inoculated control (Table 3).

Table 1: Weather data during the course of experiment

Months	Mean temperature (°C)		Mean humidity (%)		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 and 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: During the year 2014, chlorophyll contents were significantly higher in bacterial inoculated plants as compared to respective non-inoculated plants at 30 days after sowing (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 drought stress at vegetative and tasseling stage. However, the plants subjected to drought stress at vegetative stage had lower chlorophyll contents as compared to that of other treatments. 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 drought stress was imposed, drought stress at tasseling stage in particular,

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
WS_0, WS_1, WS_2 represent plants under well-watered, drought stress at vegetative stage (V7) and drought 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 ($p \leq 0.05$)

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 reduced the water loss from excised leaves and increased the 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 drought conditions over respective non-inoculated control in both years. 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 drought stress at vegetative as well as reproductive (tasseling) stage (Table 4) in both the experimental years. The number of rows per cob was not significantly different when comparison was made between the plants under drought 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

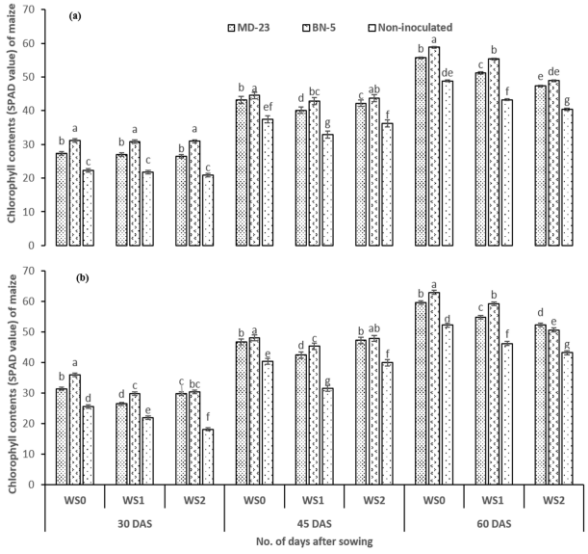

Fig. 1: Chlorophyll contents (SPAD value) of maize recorded at 30, 45 and 60 days of sowing influenced by irrigation levels and bacterial isolates inoculation

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

* WS_0, WS_1 and WS_2 represent well-watered, drought stress at vegetative (V7) stage and drought 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

under all water regimes in both 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 well-watered conditions, drought stress at vegetative stage and 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 well-watered, drought stress at vegetative and drought 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 8.1, 4.5 and 4.0 t ha^{-1} grain yields in plants with no water stress, drought stress at vegetative stage and drought 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 bacterial isolates increased the grain carbohydrate contents over non-inoculated control under well-watered and drought conditions (Table 4). 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 drought 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 drought 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=0.77–0.94) exist between EPS concentration (sugars) and 1000-grain weight under well-watered conditions, drought stress at vegetative and tasseling stage (Table 5). The grain yield was positively and linearly correlated with the concentration of EPS (sugars) with R^2 values 0.65, 0.83 and 0.68 under well-watered and drought conditions during 2014. A similar trend was observed during 2015 (Table 5). Strong positive correlation existed (Table 5) between EPS (sugar) and LRWC (R^2= 0.88, 0.93 and 0.82 under well-watering, drought stress at vegetative and reproductive stage, respectively) in experiment during the spring 2014. Similarly, EPS (sugar) and LRWC were positively correlated under well-watered conditions (R^2=0.91), drought stress at vegetative (R^2=0.93) and reproductive stage (R^2=0.96) 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, 0.99, and 0.97), under well-watered, drought stress at vegetative and reproductive stage, respectively, during 2014 (Table 5). Similarly, a strong negative correlation was observed between bacterial produced sugar and ELWL (R^2=1.0, 0.99 and 0.98 under well-watering, drought stress at vegetative and reproductive (tasseling) stage, respectively in 2015 (Table 5). The grain carbohydrate contents were positively correlated with bacterial EPS *i.e*., sugars (R^2=1.0, 0.98 and 0.99 under no water stress, drought at vegetative and reproductive stage, respectively in 2014 (Table 5). A similar

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	0.94^{**}	0.85^{**}	0.7728^{**}	0.9405^{**}	0.85^{**}	0.77^{**}
Grainyieldandsugars	0.65^{*}	0.83^{**}	0.68^{*}	0.65^{*}	0.80^{**}	0.67^{*}
LRWC and sugars	0.88^{**}	0.93^{**}	0.82^{**}	0.91^{**}	0.92^{**}	0.96^{**}
ELWL and sugars	1.00^{**}	0.99^{**}	0.97^{**}	1.00^{**}	0.99^{**}	0.98^{**}
Grain carbohydrate and sugars	0.99^{**}	0.98^{**}	1.00^{**}	1.00^{**}	0.98^{**}	0.99^{**}

WS_0, WS_1 and WS_2 represent well-watered, drought stress at vegetative (V7) stage and drought stress at reproductive stage (tasseling; VT) stage, respectively. LRWC and ELWL represent leaf relative water content and excised leaf water loss, respectively

$^{*}p \leq 0.05$; $^{**}p \leq 0.01$

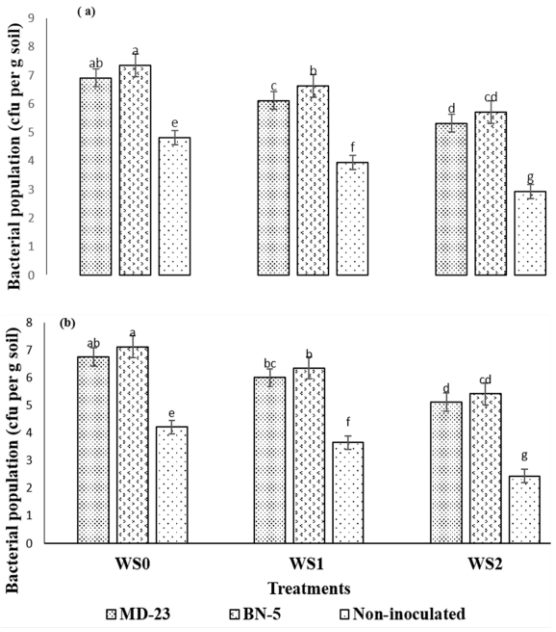

Fig. 2: Effect of PGPR inoculation on rhizosphere bacterial population (log cfu g^{-1} soil) in maize under no water stress, water stress at vegetative stage and water stress at tasseling stage during the year, 2014 (**a**) and the year, 2015 (**b**)

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 represent well-watered, drought stress at vegetative (V7) stage and drought 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

trend was observed in the experiment during the year 2015 between EPS (sugar) and LRWC (R^2=1.00, 0.98 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). Drought 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). Drought 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 N 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 (Mayak *et al*., 2004; Glick *et al*., 2007; Naseem and Bano, 2014). Presence of rhizobacteria with ACC deaminase and IAA producing activity in arid region is due to the reason that the bacteria having these characters can survive better under drought conditions. Bacterial strains with ACC deaminase activity have been isolated from rhizosphere of canola (Penrose and Glick, 2003), tomato (Mayak *et al*., 2004) and coastal soil (Siddikee *et al*., 2010). 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).

In field 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 plants subjected to drought stress. 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 (Ahmad *et al.,* 2018; Paredes *et al*., 2014; Kresovic *et al*., 2016). In our study, water stress was imposed at vegetative and tasseling stage. Restricted water application at these stages may have induced osmotic stress to plants that caused damage to membrane and photosynthesis reduction which reduced the growth, yield and quality of maize. Previously, this has been reported that limited water supply exerts osmotic stress (Forni *et al*., 2017) which impose membrane damage, reduced photosynthesis (Forni *et al*., 2017; Hussain *et al*., 2018) 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 years, drought stress at tasseling stage resulted in lower number of rows per cob, number of grains per row, 1000-grain weight, grain and stalk yield as compared to that of drought stress at vegetative stage. This indicated that flowering stage is more critical to drought stress as compared to vegetative stage. Similar results were reported by Cakir (2004) that drought 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 drought conditions indicated that inoculated bacteria BN-5 and MD-23 (positive for ACC deaminase; Table 2) might had 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 drought and resulted in increased growth, productivity and quality of maize in both years. 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; Belimov *et al*., 2015). In addition to ACC deaminase activity, inoculated bacteria (BN-5 and MD-23) 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 (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*; Table 3). Exopolysaccharides (EPS), produced by soil bacteria, are actively found in the soil organic matter and protect the cell from drought stress through helping in bio-film development, bacterial attraction and colonization, and facilitate the interaction of plants with microbes. In present study, 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 (Table 5). Leaf relative water contents and bacterial EPS (sugar) were positively correlated in case of plants under no water stress, water stress at vegetative stage and water stress at tasseling stage (Table 5). In our study, bacterial EPS (sugars) contributed positively in grain carbohydrate accumulation which was confirmed through positive correlation between EPS sugars and grain carbohydrate contents (Table 5). This indicated that bacterial produced EPS (sugars) have 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 *in-vitro* (Table 2). The inoculated bacteria might have produced catalase enzyme which scavenged the ROS produced in drought stressed plants and helped the plants to cope with deleterious effects of drought stress. The growth and productivity improvement of maize under drought due to bacterial inoculation might be attributed to catalase activity of the inoculated isolates (BN-5 and MD-23) 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 well-watered conditions (75% FC level). This was due to the fact that the tested bacterial isolates possessed plant beneficial traits like N fixation, P-solubilization, IAA production and EPS production *in-vitro* culturing (Table 2–3). Due to having the plant growth promoting traits the inoculated PGPR (BN-5 and MD-23) 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 PGPRs inoculation in improving growth and productivity of maize in particular (Biari *et al*., 2008; Naveed *et al*., 2014; Iqbal *et al*., 2016) as well as in other crops in general (Tahir *et al*., 2013, 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). This was due to the fact that PGPR (BN-5 and MD-23) on their inoculation to maize resulted in better photosynthate accumulation in grain and increased the protein and oil contents of the grain. 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 were similar to those recorded in BN-5 inoculated plants under water stress conditions. 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 under water stress 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 normal and drought conditions compared with isolate MD-23. The bacterial isolate BN-5 was more efficient in P solubilization (375±2 mg L^{-1}) and IAA production (643±3 mg L^{-1}) as compared to the bacterial isolate MD-23 in culture medium (Table 2). Moreover, this may be due to the better adoptability conditions specific for each bacterial isolate in two growing seasons. The overall grain yield of the present experiments was low as compared to the actual yield in field. This was due to the reason that the experimental soil was less fertile and was under the process of fertility improvement through green manuring. A minute rainfall during the growth season was observed but this was not at the stages of restricted water applications. 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 as well as water limiting conditions.

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 N fixers. Drought 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 N fixing activities. Application of the selected bacterial isolates to maize grown in the arid environment enabled the maize to perform better under drought stress without compromising its performance in terms of growth, productivity and quality.

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