Pak. J. Bot., 53(3): 893-903, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-3(28)

INTERACTION OF ACC DEAMINASE AND ANTIOXIDANT ENZYMES TO INDUCE DROUGHT TOLERANCE IN *ENTEROBACTER CLOACAE* 2WC2 INOCULATED MAIZE GENOTYPES

SAMAN MAQBOOL[1], AMNA[1], AMMARAH MAQBOOL[1,2], SHEHZAD MEHMOOD[1,3], MUHAMMAD SUHAIB[4], TARIQ SULTAN[4], MUHAMMAD FAROOQ HUSSAIN MUNIS[1], SHAFIQ-UR-REHMAN[5] AND HASSAN JAVED CHAUDHARY[1*]

[1]*Department of Plant Sciences, Quaid-I-Azam University, Islamabad 45320, Pakistan*
[2]*Department of Botany, Govt. Sadiq College Women University, Bahawalpur 63100, Pakistan*
[3]*Department of Environmental Sciences, COMSATS University, Islamabad, Vehari Campus, 61100, Pakistan*
[4]*Land Resources Research Institute, National Agricultural Research Center Islamabad, Pakistan*
[5]*Department of Botany, University of Okara, Okara 56300, Pakistan*
[*]*Corresponding author's email: hassaan@qau.edu.pk*

Abstract

Beneficial endophytic bacteria are well known for plant growth enhancement, induced plant defense responses and antioxidant activities that confer resistance against various biotic and abiotic stresses by utilization of 1-aminocyclopropane-1-carboxylate deaminase activity. A plant growth promoting endophytic (PGPE) bacteria *Enterobacter cloacae* 2WC2 strain was investigated for its role in drought stress amelioration and plant growth promotion via ACC deaminase activity and enhanced levels of antioxidant enzymes in stressed plants. *Invitro* screening revealed drought tolerance of *E. cloacae* at various stress levels (10-40 % PEG 6000) and showed resistance against 15 different antibiotics. Furthermore, its positive results for indole acetic acid (IAA), ammonia production, catalase, and phosphate solubilization revealed its growth promoting attributes. Exopolysaccharide production and ACC deaminase activity (0.95 µM/mg protein/h exposed its potential as competing candidate in drought stressed conditions. In a pot experiment, two maize genotypes TP 30 and TPSSWD either inoculated with or without *E. cloacae* were grown to investigate its effectiveness under drought stress. Experiment was conducted in a completely randomized design under factorial arrangement with three water regimes 100% FC (control), 75% FC (mild stress) and 40% FC (severe stress). Drought stress significantly reduce maize growth, however inoculation with *E. cloacae* 2WC2 elevated the morphological variables, relative water content and antioxidant activity, at all stress levels. Results showed that maize variety TP 30 was found to be more drought tolerant as compared to variety TPSSWD. Current findings suggest inoculation of plants by ACC deaminase producing endophytic bacteria, could be harnessed as an effective approach for sustainable crop production in drought stressed conditions.

Key words: ACC deaminase, PGPE, Water stress, Exopolysaccharide, Antioxidants.

Introduction

Drought stress confers about 50% or more annual crop yield losses (Wang *et al.*, 2003) which is a key obstacle to fulfill increasing food demands. Over time, increasing intensity of drought stress threatened the world's food security among other abiotic stresses (Vurukonda *et al.*, 2016). Moreover, it has been reported that due to global climate change 64 % of the land is affected by drought and it might get more severe, longer, and frequent in future (Meena *et al.*, 2017) as it relates to a severe food crisis, hunger, and sustained poverty (Bryan *et al.*, 2013). Furthermore, to meet world food requirements, agricultural movements are going to be expanded with time to overcome water scarcity and practical implementation of alternative strategies (Foley *et al.*, 2011).

Plants show multiple and interconnected responses towards water scarcity. Impairment of various physiological and metabolic processes under drought stress leads to stunted growth, variation in cell water content, variation in biomass and photosynthetic pigments (Jaleel *et al.*, 2009). Previously, stunted plant growth under prevailing drought stress has been studied both at biochemical and molecular levels (Koh *et al.*, 2015). Plant growth retardation under this stress is well documented in maize (Cairns *et al.*, 2012). Maize, the third most important crop used as food, provides energy, protein to human nutrition and used as fodder. During its life span, maize requires an average of 500-800mm water; however the drought imposition during the growth phase may hamper its water use efficiency and nitrogen content that leads to a significant reduction in yield (Anjum *et al.*,2017; Lobell *et al.*, 2014). Annual 15% annual yield loss has been reported in maize due to drought stress (Schittenhelm, 2010). Crop productivity can be enhanced by interrupting prevailed drought conditions by various physical and biological means.

Utilization of microbes to ameliorate abiotic stresses as well as to enhance plant growth promotion is highlighted as a cost-effective and sustainable approach gaining more attention nowadays (Nadeem *et al.*, 2014; Timmusk *et al.*, 2014) to address the problem. Some isolated bacterial strains have been noticed to colonize plants internal tissues as endophytes, which are reported to enhance plant growth and defense mechanisms (plant growth promoting endophytes; PGPE) (Compant *et al.*, 2010; Khan *et al.*, 2016). These bacteria being able to survive in adverse environmental conditions can also protect associated plants from drought stress in semi-arid regions (Kavamura *et al.*, 2013; Kasim *et al.*, 2013). Previously, many rhizobacteria have been reported to induce drought tolerance in various crops like sugarcane, sunflower, maize, and chickpea (Vardharajula *et al.*, 2011; Saikia *et al.*, 2018; Kasim *et al.*, 2013). Under a biotic stress, production of free radicals directly interferes with cellular machinery and plant cell membranes. However, the drastic effects of reactive

oxygen species (ROS) can be counteracted by the enhanced activity of various antioxidant enzymes such as catalase, peroxidase, and superoxide dismutase by drought tolerant PGPE under water deficit conditions (Saikia *et al.,* 2018) as previously reported in *Lactuca sativa* L. (Kohler *et al.,* 2008); *Phaseolus vulgaris* L. (Figueiredo *et al.,* 2008); *Vigna radiate* L. (Sarma & Saikia, 2014). Moreover, exopolysaccharide producing ability of growth promoting bacteria has also been reported to enhance ROS scavenging enzymatic activities of host plants along with the root colonization and biofilm formation (Saikia *et al.,* 2018).

Endophytic bacteria have competing benefits over rhizospheric microbes due to their intimate contact with plants. These strains develop a mutualistic association with plants predominantly by root colonization or sometimes even get associated with fruit, seeds, or flowers (Sorensen & Sessitsch, 2015). This aid in efficient uptake of iron, phosphorous, nitrogen, modulation of plant hormones (auxin, gibberellin, cytokinin, ethylene) and growth-enhancing molecules (phosphatase, hydrogen cyanide, siderophore, nitrogen) thus, aid in the survival of plants under abiotic stresses (Kour *et al.,* 2019; Santoyo, 2016). ACC deaminase producing root-associated bacteria facilitate plant growth (Glick, 2014) under drought stress (Mayak *et al.,* 2004) by degrading ACC, either directly or indirectly (Glick *et al.,* 2014; Rashid *et al.,* 2012; Khan *et al.,* 2016). The underlying mechanism is the consumption of ACC by bacteria before its oxidation by ACC oxidase, an enzyme produced by plants. Thus, based on highly induced ACC deaminase activities, plant growth-promoting endophytes might be excellent growth promotors and stress ameliorators, reducing ethylene levels (Santoyo *et al.,* 2016). The current study was an attempt to evaluate the potential of an endophytic bacterial strain, *Enterobacter cloacae* 2WC2 in terms of plant growth promotion and also as an adaptive mechanism to suppress the drastic effects of drought stress on maize plants. Thus, the synergistic use of plant and ACC deaminase producing PGPE under water deficient conditions has been suggested as an effective remedy to enhance drought tolerance.

Materials and Methods

Source of bacterial strain: Formerly isolated *E. cloacae* 2WC2 (KR076430) from a medicinal plant *Withania coagulans* was provided by Plant-Microbe Interactions lab, Quaid-i-Azam University 45320, Islamabad.

In vitro characterization of bacterial strain

Drought endurance assay: The resistance of the strain to the restricted water supply was evaluated in Trypticase Soy Broth amended with a final concentration of PEG-6000 (10, 20, 30, 40% w/v) (Marulanda *et al.,* 2009). 1% of bacterial cultures raised in trypticase soy broth were added as initial inoculums. Each treatment was replicated thrice, and absorbance of each replicate was recorded at 600 nm using a spectrophotometer (Agilent 8453 UV-visible Spectroscopy System). Evaluation of bacterial growth kinetics in drought stress was done as described by Ramadoss *et al.,* (2013).

Screening of bacterial strain for PGP activities: The production of siderophore was investigated using chrome azrolsulphate indicator dye (Amna *et al.,* 2019). Method of (Amna *et al.,* 2019) was followed to detect HCN production using nutrient agar amended with glycine (4.4 g/L). The indole acetic acid production was qualitatively observed using salkowski reagent (Bric *et al.,*1991). The potential of the strain to solubilize inorganic phosphate was tested by solubilization zone assay using modified pikovskaya's agar plates (Gupta *et al.,* 1994). The formation of the halo zone indicated the capability of strain to solubilize inorganic phosphate. To analyze ammonia production, culture was raised in peptone water and essler's reagent was added to each sample for the color indication (Cappuccino and Sherman, 2008). Catalase activity was recorded following the method of Iwase *et al*., (2013).

Profiling for intrinsic resistance to antibiotics: The strain was tested against following antibiotics of known potency by disc diffusion method (Bauer *et al.,* 1966). Tetracyclin (30µg), Clindamycin (2µg), Rifampicin (5µg), Streptomycin (10µg), Neomycin (10µg), Fosomycin (50µg), Kanamycin (30 mg), Penicillin (10 units), Lincomycin (15µg), Erythromycin (15µg), Chloramphanicol (30 µg), Spectinomycin (25µg), Gentamycin (10µg), Ampicillin (10µg), Ciprofloxacin (30µg), Cloxacillin (5µg), Nitrofurantoin (300µg), Amikacin (30µg), Colistin (10µg), Norfloxacin (10µg), Tobramycin (10µg), -Piperacillin (100µg), Carbenicillin (100µg), Ceftazidime (30µg) and Cephoxitin (30 µg).

Exopolysaccharide production: The potential for exopolysaccharide production was determined on ATCC medium no. 14 (Subair, 2015). The bacterial colonies were streaked on ATCC medium no. 14 and incubated for three days at 28ºC. Slime formation around bacterial colonies was taken as positive for EPS production.

ACC deaminase efficacy: The method of Ali *et al.,* (2014) was followed for the determination of ACC deaminase activity of bacteria. For qualitative screening, the cell pellet of culture raised in TSB was collected and washed with 0.1 M Tris-HCl twice. After washing, the pellet was re-suspended in sterile 0.1 M tris-HCl and spot inoculated on solid DF salt minimal medium containing 3mM ACC (Dworkin & Foster, 1958). DF medium with and without ammonium sulphate was kept as positive and negative controls, respectively.

For the quantitative assay, late log phase culture was used to induce ACCD activity. The pellet was washed with 0.1M Tris-HCl having pH 7.5. The cells were then supplemented with DF minimal medium containing 3mM ACC with or without amended with PEG-6000 and were incubated with shaking for 72 hours. Cell pellets were labilized in 5% toluene (v/v) supplemented with 0.3M ACC to determine the concentration of α-ketobutyrate. Negative control 50 µl from these toluinized cells without adding ACC. 0.56 N HCl (500 µl) was added in each sample by vortexing and cells were centrifuged for 5 minutes at 12000 rpm. The supernatant (500 µl) from each sample was supplemented with DNF solution and 0.56 N HCl. After given an incubation of half an hour, absorbance was recorded at 540 nm. One ml of 2N NaOH was added to

each sample at the time of absorbance. A standard curve of α-ketobutyrate was constructed by using the values of α-KB concentration versus absorbance values for each sample. Protein concentration in toluinized cells was estimated according to Bradford (1976).

Inoculation assay and pot experiment: Seeds of two maize genotypes TP 30 and TPSSWD obtained from National Agricultural Research Institute (NARC), Islamabad, were surface sterilized (Khalid *et al.,* 2004) to remove any contaminants. For seed inoculation, *E. cloacae* 2WC2 culture with a uniform density of 10^8CFU mL^{-1} was prepared, and disinfected seeds were immersed in the culture for three hours before sowing for initial seed priming. The pot experiment comprising three factors (PGPE, drought levels, and maize genotypes) was arranged in a completely randomized design (CRD) under a factorial arrangement. The experiment comprised of twelve treatments each in triplicate set. Surface sterilized seeds of both non inoculated and inoculated treatments were sown in plastic pots containing 5 kg mixture of soil, sand, and compost (1:1:1). Prior to pot experiment, a composite soil sample was collected and analyzed for various physico-chemical parameters following standard protocol as mentioned in ICARDA manual (Table 3). After germination, three plants were maintained in each respective pot. Drought conditions were maintained in the form of irrigations at 100% FC (well-watered control), 75% FC (mild stress), and 40% FC (severe stress). At the end of experiment plants were carefully harvested and analyzed for various plant growth parameters, photosynthetic pigments, and antioxidant enzyme production.

Physiological and biochemical status of plants: Immediately after harvesting, various agronomic measurements were made. Relative water content (RWC) of leaves was determined according to the method described by Ahmed *et al.,* (2016). Measurement for electrolyte leakage was made using an EC meter (Talaat *et al.,* 2015). Photosynthetic pigments from various treatments were extracted in acetone (80%). The absorbance of the extracts was recorded at wavelengths 663 nm, 645 nm, and 470 nm for Chl a, Chl b, and carotenoids, respectively (Amna *et al.,* 2019).

Antioxidant enzyme activities: SOD activity was recorded using standard methods (Beauchamp & Fridovich, 1971). The absorbance of reaction mixture made in Phosphate buffer (pH 7.8) was recorded at 560 nm. The modified method of (Reddy *et al.,* 1985) was followed to assess peroxidase activity. A modified protocol was opted to determine catalase activity (Luck, 1974). The absorbance of extracts mixed with H_2O_2 was recorded at 240 nm. The quantity of proline accumulated in inoculated and non-inoculated plants was estimated (Talaat *et al.,* 2015) using 3% sulphosalicylic acid and acidic ninhydrin.

Reisolation of the bacterial strain: After harvesting of plants, the bacterial strain was reisolated on DF minimal medium supplemented with ACC (Niu *et al.,* 2018). Colonies representing the same inoculated strain were verified by antibiotic resistance, gram staining, morphological and biochemical characteristics.

Statistical analysis

Data recorded were analyzed statistically by Fisher's analysis of variance techniques using Statistix 9.0 (Steel and Torrie, 1980). A comparison among mean values was made using LSD (Least Significant Difference) at 5% probability level. Treatments were compared using 2-way ANOVA.

Results

***In vitro* screening of endophytic *E. cloacae* 2WC2:** The strain showed prolific growth at all concentrations of PEG-6000 (Fig. 1A) indicating its osmo-adaptive ability. However, a decline in growth with a gradual increase in stress was observed. Steady growth with a long stationary phase was found at higher concentrations of PEG-6000 (20% & 30%) as compared to lower levels of stress (Fig. 1B). Bacterial strain was observed gram-positive while under water-stressed conditions clumps of bacterial cells with shrinked cell size was noticed (Fig. 2). *E. cloacae* were found resistant to 15 antibiotics out of 25 (Table 1). Moreover, bacteria produced watery colonies with no odor on ATCC medium no. 14 and found positive for exopolysaccharide production.

The capability of producing IAA, ammonia, catalase, and phosphate solubilization indicated its plant growth-promoting potential (Table 2). ACC deaminase activity was also observed via enrichment method on DF solid medium. Furthermore, the quantification assay confirmed high ACC deaminase activity in *E. Cloacae* (0.95 µM/mg protein/h) under induced drought-stress (30% PEG-6000 induced stress) (Fig. 3).

Response of drought-stressed maize genotypes towards *E. cloacae* inoculation

Agronomic responses: Differential response to water stress in both genotypes was observed in terms of growth parameters. Drought stress (40% FC) significantly ($p<0.05$) impaired maize growth in terms of all growth parameters. However, both varieties performed well at 75% FC water regime than 100% regime in terms of root length, shoot length and fresh weight (Fig. 4A-C). However, leaf area and number of leaves per plant remained unaffected at mild stress level. Inoculation with *E. cloacae* 2WC2 in well-watered plants significantly enhanced maize growth in comparison to non-inoculated well-watered controls (Fig. 4). Bacterial inoculated TP 30 genotype showed significantly increased root length (26%; T2 & 39.2%; T4) with a similar trend in shoot length. Similarly, T2 and T4 increased root length by 10.3% and 32.5% in maize genotype TPSSWD. Inoculated plants exhibited a considerable increase in shoot length up to 56.5-62.9% in T2 and 8.4-12.6% (T4) as compared to their respective stressed non-inoculated controls. Bacterial treatment imparts 18.9% and 17.5% (TP 30 genotype) and 12.4% and 47.5% (TPSSWD genotype) increment in fresh plant weight at mild and severe stress, respectively.

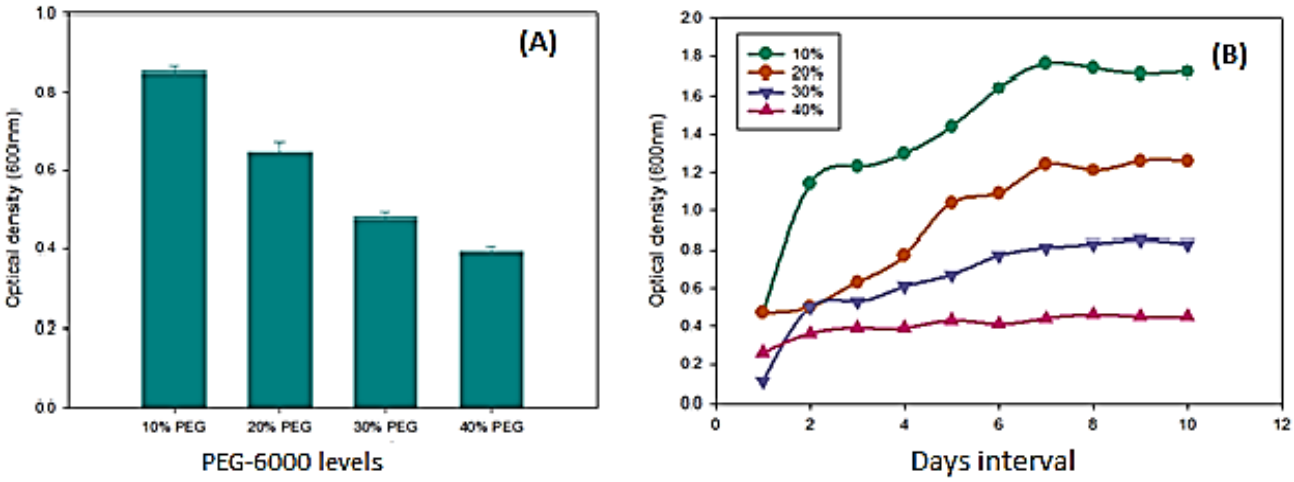

Fig. 1. Growth (A) and growth pattern (B) of *E. cloacae* 2WC2 under various PEG-6000 induced drought levels (n=3).

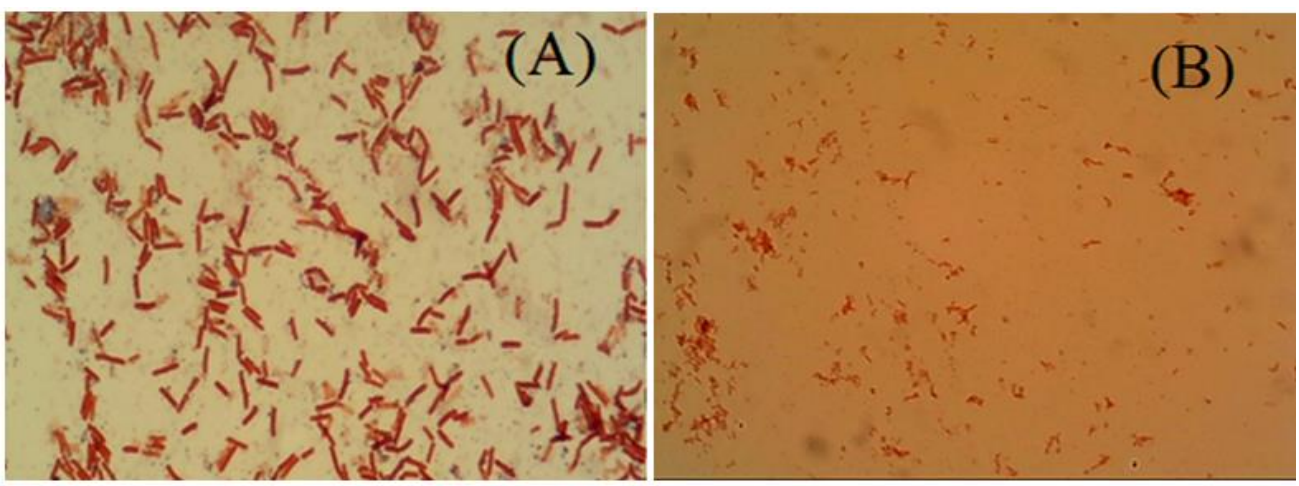

Fig. 2. Morphology of *E. cloacae* 2WC2 in A) non-stressed and B) Stressed condition.

Table 1. Antibiotic resistance pattern of *E. cloacae* 2WC2.

Antibiotics	Concentration	Zone diameter (cm)	Zone diameter interpretation
Tetracycline	30 µg	16	Susceptible
Clindamycin	2 µg	0	Resistant
Rifampicin	5 µg	9	Resistant
Streptomycin	10 µg	8	Resistant
Neomycin	10 µg	0	Resistant
Fosomycin	50 µg	20	Susceptible
Kanamycin	30 mcg	18	Susceptible
Penicillin	10 µg	0	Resistant
Lincomycin	15 µg	0	Resistant
Erythromycin	15 µg	0	Resistant
Chloramphanicol	30 µg	21	Susceptible
Spectinomycin	25 µg	8	Resistant
Gentamycin	10 µg	9	Resistant
Ampicillin	10 µg	0	Resistant
Ciprofloxacin	30 µg	21	Susceptible
Cloxacillin	5 µg	8	Resistant
Nitrofurantoin	300 µg	17	Susceptible
Amikacin	30 µg	14	Intermediate
Colistin	10 µg	0	Resistant
Norfloxacin	10 µg	17	Susceptible
Tobramycin	10 µg	8	Resistant
Piperacillin	100 µg	29	Susceptible
Carbenicillin	100 µg	17	Susceptible
Ceftazidime	30 µg	0	Resistant
Cephoxitin	30 µg	0	Resistant

Resistant, <10 mm; Intermediate, 10-15 mm; Susceptible >15 mm

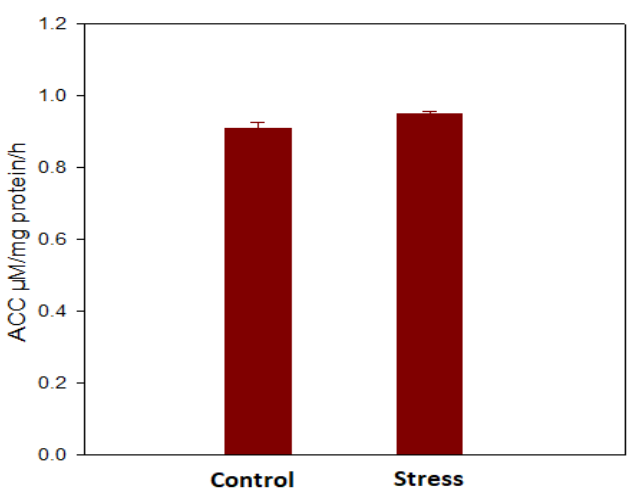

Fig. 3. ACC deaminase activity in *E. cloacae* 2WC.

Relative water content (RWC): Inoculated plants showed 41.07% (TP 30) and 29.53% (TPSSWD) increase in RWC at mild stress. Similarly, increment in RWC by 36.6% and 15.7% was also observed in inoculated stressed (40% FC) plants. Likewise, inoculated well-irrigated plants exhibited a significant increase of 6.9% (TP 30) and 27.92% (TPSSWD) (Table 4).

Electrolyte leakage: Drought stress significantly induce electrolyte leakage in both genotypes ($p<0.05$) which was counteracted with bacterial inoculation. Endophytic drought-tolerant *E. cloacae* reduced adversity of water stress in terms of electrolyte leakage by 18.9% and10.6% at mild stress and 40.8% and 9.1% at severe stress in TP 30 and TPSSWD, respectively (Table 4).

Chlorophyll contents: Chl a content remained statistically unaffected at both stress levels in TP 30, however, water deficit significantly reduced the amount of Chl a in genotype TPSSWD by 33.97% at 75% FC (mild stress) and 53.25 % in 40% FC (severe stress). A significant increase in Chl a content was observed in drought-stressed inoculated plants in both genotypes (Table 4). Chl b content in both genotypes decreased with the severity of drought stress significantly, however, non-significant differences were noticed among varieties in terms of treatments at $p < 0.05$. Furthermore, inoculation with PGPE significantly enhanced Chl b content at both stress levels than non-inoculated stressed plants. The improvement recorded was 32.6% & 28% at 75% FC and 20.5% & 29.2% at 40% FC in TP 30 and TPSSWD, respectively. Both varieties showed a differential response in carotenoid content under stress conditions. Drought stress at 40 % FC reduced carotenoids up to 20.8%. In contrast, more drastic effects were observed in TPSSWD at both stress levels i.e., 34.69%, and 44.89% reduction in carotenoids contents were observed at mild stress and severe stress, respectively. Bacterial inoculation increased carotenoid contents in TP 30 at severe stress by 28%. However, the increment was more obvious in genotype TPSSWD being 32.63% at 75% FC and34. 14% at 40% FC level (Table 4).

Differential response towards antioxidant enzymes production: Under drought stress, both genotypes showed a significant increase in SOD activity than their respective controls (Fig. 5). Bacterial inoculation significantly enhanced SOD activity in control non-stressed maize genotypes (Fig. 5A). Inoculated TPSSWD (T2) couldn't improve POD activity in stressed plants at 75% FC level, but with increased stress level, bacteria triggered POD activity by 29%. The pronounced effect of PGPB was also observed in TP 30 at both stress levels with a 16%-24% increase in POD activity (Fig. 5B). At 40% FC, a significant reduction was observed in the catalase activity of both genotypes. However, bacteria enhance catalase activity in all treatments. PGPE improved catalase activity at 75% FC level by 26.2% and 43.8%, while at 40% FC, 37.7% and 54.7% increased levels of catalase enzyme were noticed in TP 30 and TPSSWD genotypes respectively (Fig. 5C).

Proline accumulation: Both maize genotypes couldn't produce much proline in severe stress as compared to mild stress (75% FC). However, significantly increased proline activity was observed in all inoculated plants whether stressed or not. At 75% FC level *E. cloacae* show elevated proline contents up to 44.8% and 68.9% while at 40% FC it was increased by 90% and 75.6% in TP 30 and TPSSWD genotypes, respectively (Fig. 5D).

Table 2. Morphological and biochemical characteristics of bacterial strain.

Characteristics studied	Results
Colony morphology	Circular, raised, off-white, smooth colonies on LB agar at 30°C after 24 hours of incubation
Bacterial cell features	Gram negative, Microscopic view showed scattered arrangements of cells, long rods
Biochemical properties	Positive for phosphate solubilization, Indole acetic acid, catalase, ammonia, Exopolysaccharide production, ACC-deaminase activity negative for HCN and siderophore production

Table 3. Physico-chemical properties of soil used in pot experiment.

Sand	Silt	Clay	OM†	SP‡	N	P	K	EC	pH
%						mg kg^{-1} soil		dS m^{-1}	
49.3	23.7	27	0.66	29	0.05	5.98	138	2.39	7.9

†Organic matter; ‡Saturation percentage

Table 4. Photosynthetic pigments, RWC and Electrolyte leakage influenced by different treatments.

Maize varieties	Treatments	Chlorophyll a	Chlorophyll b	Carotenoids	Electrolyte leakage %	RWC*
TP-30	T0	0.39 ± 0.006^{de}	0.38 ± 0.01^{c}	1.68 ± 0.03^{c}	74.58 ± 9.6^{bc}	42.8 ± 0.99^{c}
	T1	0.42 ± 0.008^{cd}	0.33 ± 0.02^{d}	1.96 ± 0.02^{ab}	75.08 ± 11.8^{bc}	29.49 ± 0.40^{fg}
	T2	0.46 ± 0.01^{a}	0.52 ± 0.01^{a}	2.01 ± 0.05^{a}	60.88 ± 13.45^{cde}	50.03 ± 1.28^{a}
	T3	0.38 ± 0.009^{e}	0.33 ± 0.004^{d}	1.33 ± 0.03^{d}	82.53 ± 4.9^{abc}	28.61 ± 0.31^{g}
	T4	0.46 ± 0.01^{ab}	0.39 ± 0.02^{c}	1.85 ± 0.07^{b}	48.89 ± 1.3^{de}	45.14 ± 3.04^{bc}
	T5	0.46 ± 0.01^{a}	0.42 ± 0.01^{c}	2.01 ± 0.02^{a}	42.02 ± 0.42^{e}	45.97 ± 0.81^{bc}
TPSSWD	T0	0.41 ± 0.01^{cd}	0.41 ± 0.003^{c}	1.96 ± 0.04^{ab}	65.49 ± 2.7^{cde}	36.04 ± 1.15^{de}
	T1	0.27 ± 0.008^{g}	0.33 ± 0.008^{d}	1.28 ± 0.06^{d}	79.28 ± 1.64^{abc}	33.97 ± 1.14^{e}
	T2	0.39 ± 0.009^{de}	0.47 ± 0.01^{b}	1.91 ± 0.03^{ab}	70.89 ± 7.54^{bcd}	48.21 ± 0.94^{ab}
	T3	0.19 ± 0.007^{h}	0.3 ± 0.01^{d}	1.08 ± 0.06^{e}	82.11 ± 3.61^{a}	32.88 ± 0.67^{ef}
	T4	0.34 ± 0.01^{f}	0.41 ± 0.01^{c}	1.64 ± 0.04^{c}	91.26 ± 5.65^{ab}	39.03 ± 0.67^{d}
	T5	0.43 ± 0.008^{bc}	0.47 ± 0.01^{b}	2.04 ± 0.04^{a}	61.78 ± 7.97^{cde}	50.00 ± 0.55^{a}

*Relative water content. Means with ± SE sharing a common letter don't differ significantly from each other ($p<0.05$). T0, 100% FC; T1, 75% FC; T2, 75% FC+ PGPE; T3, 40% FC; T4, 40% FC+ PGPE; T5, PGPE

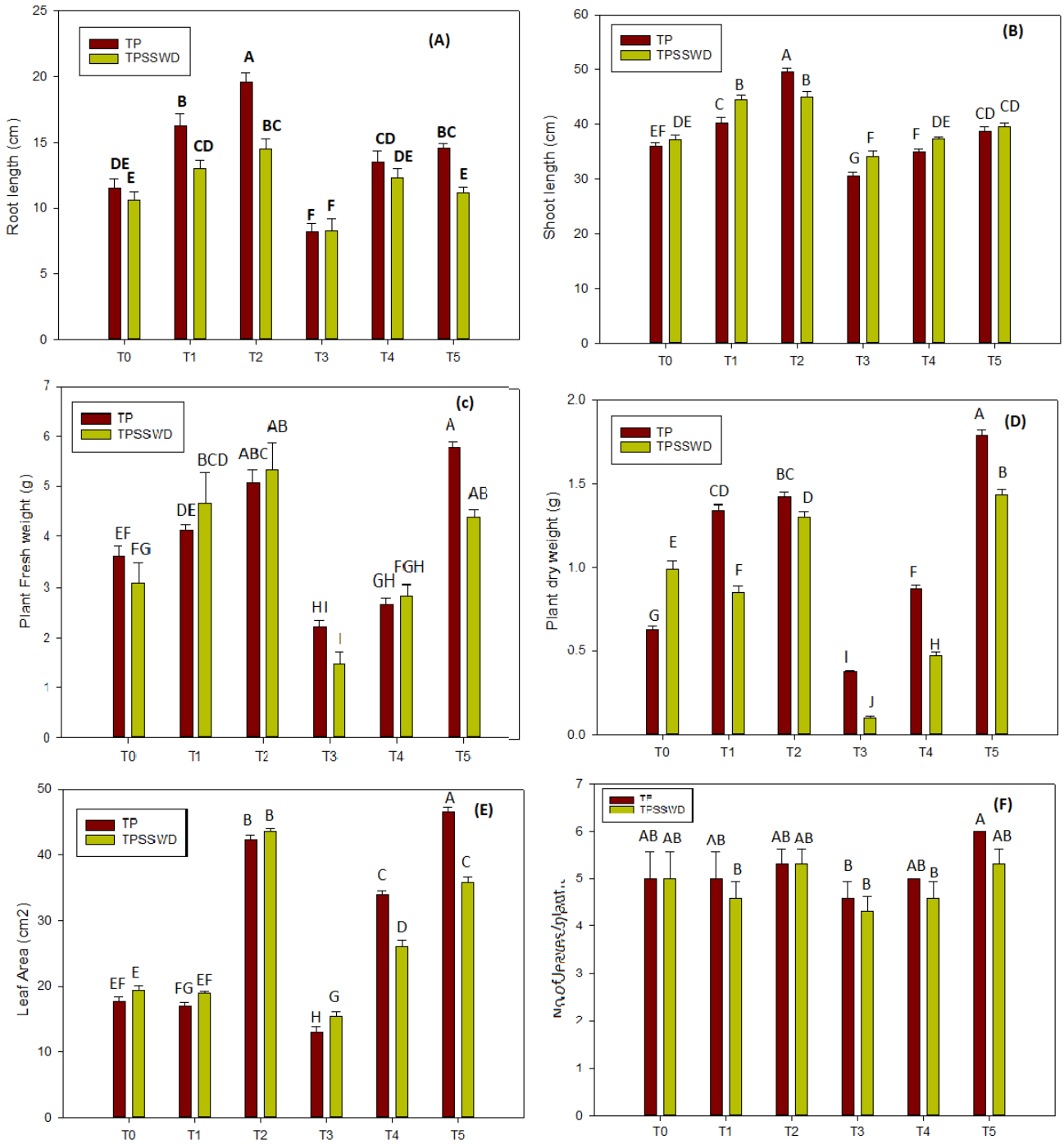

Fig. 4. Effect of *E. cloacae* 2WC2 on growth parameters of water stressed *Zea mays* L. A) Root length (cm) B) Shoot length (cm) C) Fresh weight (g) D) Dry weight (g) E) Leaf area (cm^2) F) Number of leaves/ plant. Each treatment shows mean of three replicates. Means with different letters indicate significant differences among treatments at 5% probability level (T0, Control; T1, 75%FC; T2. 75%FC+ PGPE; T3, 40% FC; T4, 40% FC+PGPE; T5, PGPE).

Discussion

Among abiotic stresses, drought is a major constraint to plant growth and development causing nutrient deficiencies and imbalance in phytohormones resulting in less vigor and low yield of plants (Asghar *et al.,* 2015). Therefore, the isolation, selection and screening of drought-tolerant bacterial strains and their application to agriculture could significantly enhance crop productivity as well as food security. Under current circumstances, we have studied the potential of an endophytic *Enterobacter cloacae* to induce drought resilience and growth promotion in two maize genotypes under stressed conditions. Previously, two endophytic bacterial strains namely *Enterobacter* sp. FD17 and *Burkholderia phytofirmans* PsJN has been reported to induce drought tolerance to maize cultivars under hydric stress (Naveed *et al.,* 2014). Some bacterial strains show PEG-6000 induced drought stress tolerance up to 40% and 25%, respectively (Asghar *et al.,* 2015; Hussain *et*

al., 2014). Similarly, a drought-tolerant *Pseudomonas sp.* could tolerate PEG concentration of up to 40.5% (Kumar *et al.,* 2014). The ability to withstand water deficit conditions may be attributed to the production of osmoprotectants like QACs (Quaternary ammonium compounds) to abide by the adverse effects of drought as well as by EPS production that leads to biofilm formation (Vanderlinde *et al.,* 2010). EPS mediated protection of *A. brasilense* Sp245 in water deprivation (Konnova *et al.,* 2001) and a correlation between the amount of exopolysaccharide produced and desiccation tolerance of *Bradyrhizobium* strains was well reported (Hartel & Alexander, 1986). EPS acts as a microenvironment that has water retention capability thus protecting bacterial cells from exposure to water stress (Naseem *et al.,* 2018). Our bacterial strain showed resistance to fifteen antibiotics tested which could be attributed to competing benefits among other bacteria in the nutrient challenging rhizospheric environment. A correlation between antibiotic resistance and abiotic stress has been previously reported (Wani *et al.,* 2009). Gene mutation and transfer of genes responsible for antibiotic resistance between neighboring cells are the main reasons for developing antibiotic resistance (Wani & Khan, 2014). Bacterial strains possessing more than one PGP traits are capable to enhance plant growth both under normal and stressed conditions (Yang *et al.,* 2010). PGP substances have capability to maintain nutritional status under water deprivation. IAA is responsible for stress tolerance along with plant growth promotion (Marulanda *et al.,* 2009). Our strain being positive for phosphate solubilization indicated the presence of phosphatase and organic acids. Some bacteria capable of phosphorous solubilization aids in yield improvement under stressed environments (Banerjee *et al.,* 2010). Catalase enzyme protects effected organism from toxic free radicals under stressed conditions. Application of microbes possessing ACC deaminase activity can induce drought stress tolerance to plants by keeping the ethylene levels to an optimal extent. ACC deaminase containing bacteria act via binding to the seed coats and play a key role in the deamination of ACC via activity of ACC deaminase enzyme in drought stressed surroundings (Amna *et al.,* 2019). ACC deaminase production under drought stress was first reported in *Klebsiella oxytoca*, *Klebsiella variicola* (Zheng *et al.,* 2014) however, various other genera can also potential production of ACCD under stressed conditions (Mayak *et al.,* 2004; Toklikishvili *et al.,* 2010).

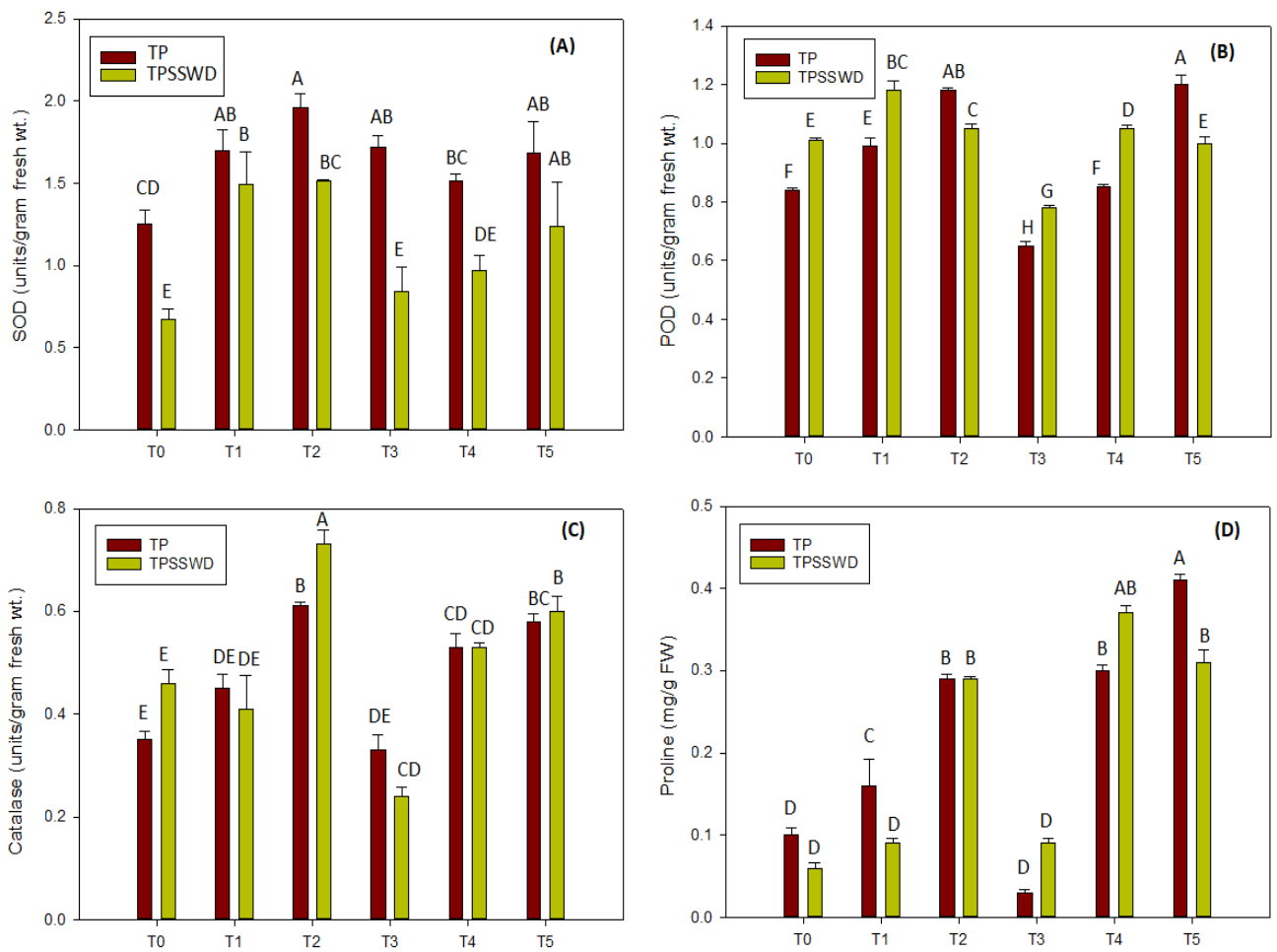

Fig. 5. Effect of drought tolerant *E. cloacae* 2WC2 on antioxidants and proline content of maize genotypes A) Superoxide dismutase B) Peroxidase C) Catalase D) Proline. Each treatment shows mean of three replicates (T0, Control; T1, 75%FC; T2. 755%FC+ PGPE; T3, 40% FC; T4, 40% FC+PGPE; T5, PGPE).

Reduction in maize growth in water deficit conditions has been previously documented (Anjum *et al.,* 2017). Both maize genotypes exhibited efficient growth parameters at 75% FC than 100% FC level which could be an optimized level of field capacity for these genotypes. Inoculation of drought-tolerant ACC deaminase containing bacterial strain to maize genotypes significantly reduced the adverse effects of drought stress. Increased root length in PGPR inoculated plants led to efficient nutrients and water uptake from soil (Mohamed *et al.,* 2019). Similarly, maize plants inoculated with *K. oxytoca* 10MKR7, *E. sakazakii* 8MR5, and *Pseudomonas* sp. 4MKS8 exhibited improvement in various agronomic variables including root elongation (Bhattacharyya & Jha, 2012). Reduction in relative water content of drought-stressed plants may be linked to plant vigor (Valentovic *et al.,* 2011). Many investigations have shown a significant decline in relative water content and water potential in water deficit conditions (Nayyar & Gupta, 2006). Inoculation of *E. cloacae* 2WC2 helped drought-stressed plants to resume their water content that could be attributed due to improvement in the root system of inoculated plants (Casanovas *et al.,* 2002). Drought stress-induced electrolyte leakage have severe negative effects in genotype TP 30 that could be linked with elevated POD and catalase activity indicating that membrane damage might be caused by oxidative stress. Bacterial inoculation, in this regard, reduced the membrane damage, most probably, due to the augmentation of antioxidant enzymes by bacterial colonization, to ameliorate oxidative damage. *Bacillus* sp., impart membrane stability by reducing the electrolyte leakage in maize seedlings under drought stress (Vardharajula *et al.,* 2011). Production and scavenging of reactive oxygen species are balanced by antioxidant enzymes (Miller *et al.,* 2010). The antioxidant defense mechanism was upregulated in drought-stressed plants of both genotypes. In our study, inoculated plants significantly enhanced activities of ROS scavenging enzymes under drought stress. The findings are in line with the previous study, where elevation in enzymatic activities was observed in Okra plants (Habib *et al.,* 2016) and potato (Gururani *et al.,* 2013) under stress condition, when inoculated with ACC deaminase producing PGPR.

Increased levels of proline in stress via the upregulation of the proline biosynthesis pathway keep plants safe from stress by membrane protection and maintaining cell water content (Sandhya *et al.,* 2010). In agreement with previous findings (Ansary *et al.,* 2012; Armada *et al.,* 2014), bacterial inoculation considerably enhanced proline contents in water-stressed plants. Chlorophyll content is an indicator of stability under stress. The decline in chlorophyll content (a, b) in water stress has already been evaluated (Efeoğlu *et al.,* 2009). Reduction in chlorophyll content is an indication of photo-oxidation (Rahdari *et al.,* 2012). Bacterial inoculation considerably improved chlorophyll content in all stressed and non-stressed plants. Greater chlorophyll content in inoculated plants was previously reported (Kang *et al.,* 2010). Carotenoid contents were noticeably increased in both inoculated and non-inoculated plants of genotype TP 30 (Sohrabi *et al.,* 2012) attributed high carotenoid content to genotype tolerance since they are responsible for the breakdown of singlet oxygen. Since carotenoids are found in association with reaction centers (Efeoğlu *et al.,* 2009) and loss of photosynthetic reaction center was observed in acute water deficiency, the decline in carotenoid content was expected. Thus, our results indicate that endophytic stress-tolerant bacterial strains having promising PGP attributes both under stressed and non-stressed condition can be used to develop bio-fertilizers to increase soil fertility and plant growth promotion under drought conditions.

Conclusion

It is inferred that rhizobacterial endophytes could be bio-prospective for drought stress tolerance and plant growth promotion and may be applied as biofertilizer to increase the fitness of crop plants under stressful environments. In the current study, both maize genotypes showed a positive response towards the inoculation of *E. cloacae* that enhanced agronomic traits, antioxidant activities, and chlorophyll content leading to drought tolerance. The tolerance mechanism of the strain involved ACC deaminase activity to optimize the ethylene levels, various inherent PGP compounds, and exopolysaccharide production in axenic conditions. Microbial assisted maize plants optimized water status by increasing the relative water content of leaves, thus ameliorate oxidative stress under drought conditions. Conclusively, ACC deaminase possessing endophytes could be utilized to crop plants as a sustainable agro-technology to combat drought stress.

References

Ahmed, N., M.A. Chowdhry, I. Khaliq and M. Maekawa. 2016. The inheritance of yield and yield components of five wheat hybrid populations under drought conditions. *Indones. J. Agric. Sci.,* 8: 53-59.

Ali, S.Z., V. Sandhya and L.V. Rao. 2014. Isolation and characterization of drought-tolerant ACC deaminase and exopolysaccharide-producing fluorescent Pseudomonas sp. *Ann. Microbiol.,* 64: 493-502.

Amna, S., B. Sarfraz, Y. Din, M.A. Xia, M.T. Kamran, T. Javed Sultan and H.J. Chaudhary. 2019. Mechanistic elucidation of germination potential and growth of wheat inoculated with exopolysaccharide and ACC-deaminase producing bacillus strains under induced salinity stress. *Eco. Toxicol. Environ. Saf*., 183: 109466.

Anjum, S.A., U. Ashraf, M. Tanveer, I. Khan, S. Hussain, B. Shahzad, A. Zohaib, F. Abbas, M.F. Saleem and I. Ali. 2017. Drought induced changes in growth, osmolyte accumulation and antioxidant metabolism of three maize hybrids. *Front. Plant. Sci.,* 8.

Ansary M.H., H.A. Rahmani, M.R. Ardakani, F. Paknejad, D. Habibi and S. Mafakheri. 2012. Effect of Pseudomonas fluorescent on proline and phytohormonal status of maize (*Zea mays* L.) under water deficit stress. *Ann. Biol. Res.,* 3: 1054-1062.

Armada, E., G. Portela, A. Roldán and R. Azcón. 2014. Combined use of beneficial soil microorganism and agrowaste residue to cope with plant water limitation under semiarid conditions. *Geoderma.,* 232: 640-648.

Asghar, H.N., Z.A. Zahir, M.A. Akram, H.T. Ahmad and M.B. Hussain. 2015. Isolation and screening of beneficial

bacteria to ameliorate drought stress in wheat. *Soil. Environ.*, 34: 100-110.

Banerjee, G., J.S. Scott-Craig and J.D. Walton. 2010. Improving enzymes for biomass conversion: a basic research perspective. *Bioenergy. Res.*, 3: 82-92.

Bashan, Y. and G. Holguin. 1998.Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. *Soil. Biol. Biochem.*, 30: 1225-1228.

Bauer, A., W. Kirby, J.C. Sherris and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Amer. J. Clin. Pathol*., 45(4): 493

Beauchamp, C. and I. Fridovich. 1971.Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.*, 44: 276-287.

Bhattacharyya, P. and D. Jha. 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World. J. Microbiol. Biotechnol.*, 28: 1327-1350.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248-254.

Bric, J.M., R.M. Bostock and S.E. Silverstone.1991. Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane.*Appl. Environ. Microbiol.*, 57: 535-538.

Bryan, E., C. Ringler, B. Okoba, C. Roncoli, S. Silvestri and M. Herrero. 2013.Adapting agriculture to climate change in Kenya: Household strategies and determinants. *J. Environ. Manag.*, 114: 26-35.

Cairns, J.E., J. Crossa, P. Zaidi, P. Grudloyma, C. Sanchez, J.L. Araus and A. Menkir. 2013. Identification of drought, heat, and combined drought and heat tolerant donors in maize. *Crop. Sci.*, 53: 1335-1346.

Cappuccino, J.G. and N. Sherman. 2008. Microbiology: a laboratory manual, Vol 9 (Pearson/Benjamin Cummings).

Casanovas, E.M., C.A. Barassi and R.J. Sueldo. 2002. Azospirillum inoculation mitigates water stress effects in maize seedlings. *Cereal. Res. Commun.*, 30: 343-350.

Schittenhelm, S. 2010. Effect of drought stress on yield and quality of maize/sunflower and maize/sorghum intercrops for biogas production. *J. Agron Crop Sci.*, 196: 253-261.

Compant, S., C. Clément and A. Sessitsch. 2010. Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil. Biol. Biochem.*, 42: 669-678.

Dworkin, M. and J. Foster. 1958. Experiments with some microorganisms which utilize ethane and hydrogen. *J. Bacteriol*., 75(5): 592.

East, R. 2013. Microbiome: soil science comes to life. *Nature*, 501: 18-19.

Edmeades, G.O. 2008. Drought Tolerance in Maize: an Emerging Reality. *Isaaa,* doi: 978-1-892456-44-3

Efeoğlu, B., Y. Ekmekci and N. Cicek. 2009. Physiological responses of three maize cultivars to drought stress and recovery. *S. Afr. J. Bot.*, 75: 34-42.

Figueiredo, C.A., J.G. Barroso, L.G. Pedro and J.J.C. Scheffer. 2008. Factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavour. Fragr. J.*, 23: 213-26.

Foley, J.A., N. Ramankutty, K.A. Brauman, E.S. Cassidy, J.S. Gerber, M. Johnston, N.D. Mueller, C. O'Connell, D.K. Ray and P.C. West. 2011.Solutions for a cultivated planet. *Nature,* 478: 337-342.

Gururani, M.A., C.P. Upadhyaya, V. Baskar, J.Venkatesh, A. Nookaraju and S.W. Park. 2013. Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. *J. Plant. Growth. Regul.*, 32: 245-58.

Glick, B.R. 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiol. Res.*, 169: 30-39.

Gupta, R., R. Singal, A. Shankar, R.C. Kuhad and R.K. Saxena. 1994. A modified plate assay for screening phosphate solubilizing microorganisms. *J. Gen. Appl. Microbiol.*, 40: 255-260.

Habib, S.H., H. Kausar and H.M. Saud. 2016.Plant growth-promoting rhizobacteria enhance salinity stress tolerance in okra through ROS-scavenging enzymes. *Biomed. Res. Int.*, 2016.

Hartel, P.G. and M. Alexander. 1986. Role of extracellular polysaccharide production and clays in the desiccation tolerance of cowpea Bradyrhizobia. *Soil. Sci. Soc. Amer. J.*, 50: 1193-1198.

Honma, M. and T. Shimomura. 1978. Metabolism of 1-aminocyclopropane-1-carboxylic acid. *Agric. Biol. Chem.*, 42: 1825-1831.

Hussain, M.B., Z.A. Zahir, H.N. Asghar and S. Mahmood. 2014. Scrutinizing rhizobia to rescue maize growth under reduced water conditions. *Soil. Sci. Soc. Amer. J.*, 78: 538-545.

Iwase, T., A. Tajima, S. Sugimoto, K.I. Okuda, I. Hironaka, Y. Kamata and Y. Mizunoe. 2013. A simple assay for measuring catalase activity: a visual approach. *Scientific reports*, 31-4.

Jaleel, C.A., P. Manivannan, A. Wahid, M. Farooq, H.J. Al-Juburi, R. Somasundaram and R. Panneerselvam. 2009. Drought stress in plants: a review on morphological characteristics and pigments composition. *Int. J. Agric. Biol.*, 11: 100-105.

Kang, B.G., W.T. Kim, H.S. Yun and S.C. Chang. 2010. Use of plant growth-promoting rhizobacteria to control stress responses of plant roots. *Plant. Biotechnol. Rep.*, 4: 179-183.

Kasim, W.A., M.E. Osman, M.N. Omar, I.A.A. El-Daim, S. Bejai and J. Meijer. 2013.Control of drought stress in wheat using plant-growth-promoting bacteria. *J. Plant.Growth. Reg.*, 32: 122-130.

Kavamura, V.N., S.N. Santos, J.L. Silva, M.M. da Parma, L.A. Ávila, A. Visconti, T.D. Zucchi, R.G. Taketani, F.D. Andreote and I.S. Melo. 2013. Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. *Microbiol. Res.*, 168: 183-191.

Khalid, A., M. Arshad and Z. Zahir. 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *J. App. Microbiol*., 96(3): 473-480.

Khan, A.L., B.A. Halo, A. Elyassi, S. Ali, K. Al-Hosni, J. Hussain, A. Al-Harrasi and J.J. Lee. 2016. Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum*. *Electron. J. Biotechnol.*, 21: 58-64.

Kim, Y.C., B.R. Glick, Y. Bashan and C.M. Ryu. 2012. Enhancement of plant drought tolerance by microbes. In "Plant responses to drought stress", pp. 383-413. Springer.

Koh, J., G. Chen, M.J. Yoo, N. Zhu, D. Dufresne, J.E. Erickson, H. Shao and S. Chen. 2015. Comparative proteomic analysis of Brassica napus in response to drought stress. *J. Proteom. Res.*, 14: 3068-3081.

Kohler, J., J.A. Hernández, F. Caravaca and A. Roldán. 2008. Plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungi modify alleviation biochemical mechanisms in water-stressed plants. *Funct. Plant Biol.*, 35(2): 141-151.

Konnova, S., O. Brykova, O. Sachkova, I. Egorenkova and V. Ignatov. 2001. Protective role of the polysaccharide-containing capsular components of Azospirillum brasilense. *Microbiology.*, 70: 436-440.

Kour, D., K.L. Rana, I. Sheikh, V. Kumar, A.N. Yadav, H.S. Dhaliwal and A.K. Saxena. 2019. Alleviation of drought stress and plant growth promotion by *Pseudomonas libanensis* EU-LWNA-33, a drought-adaptive phosphorus-solubilizing bacterium. Proceedings of the National Academy of Sciences, India Section B: *Biological Sciences*, 1-11.

Kumar, P.G., M.H.S.K. Ahmed, S. Desai, L.D.E. Amalraj and A. Rasul. 2014. *In vitro* screening for abiotic stress tolerance in potent biocontrol and plant growth promoting strains of *Pseudomonas* and *Bacillus* spp. *Int. J. Bacteriol.*,doi:10.1155/2014/195946

Lobell, D.B., M.J. Roberts, W. Schlenker, N. Braun, B.B. Little, R.M. Rejesus and G.L. Hammer. 2014.Greater sensitivity to drought accompanies maize yield increase in the US Midwest. *Science*, 344: 516-519.

Luck, H. 1974. Estimation of catalase activity. Methods of enzymology. Academic Press, New York, 885.

Marulanda, A., J.M. Barea and R. Azcón. 2009.Stimulation of plant growth and drought tolerance by native microorganisms (AM fungi and bacteria) from dry environments: mechanisms related to bacterial effectiveness. *J. Plant. Growth. Reg.*, 28: 115-124.

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.

Meena, K.K., A.M. Sorty, U.M. Bitla, K. Choudhary, P. Gupta, A. Pareek, D.P. Singh, R. Prabha, P.K. Sahu and V.K. Gupta. 2017. Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. *Front.Plant. Sci.*, 8.

Miller, G., N. Suzuki, S. Ciftciyilmaz and R. Mittler. 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant. Cell. Environ.*, 33: 453-467.

Mohamed, I., K.E. Eid, M.H. Abbas, A.A. Salem, N. Ahmed, M. Ali and C. Fang. 2019. Use of plant growth promoting Rhizobacteria (PGPR) and mycorrhizae to improve the growth and nutrient utilization of common bean in a soil infected with white rot fungi. *Ecotoxicol. Environm. Saf.*, 171: 539-548.

Nadeem, S.M., M. Ahmad, Z.A. Zahir, A. Javaid and M. Ashraf. 2014.The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnol. Adv.*, 32: 429-448.

Naseem, H., M. Ahsan, M.A. Shahid and N. Khan. 2018. Exopolysaccharides producing rhizobacteria and their role in plant growth and drought tolerance. *J. Basic Microbiol.*, 58: 1009-1022.

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* sp. FD17. *Environ. Exp. Bot.*, 97: 30-39.

Nayyar, H. and D. Gupta. 2006. Differential sensitivity of C 3 and C 4 plants to water deficit stress: association with oxidative stress and antioxidants. *Environ. Exp. Bot.*, 58: 106-113.

Niu, X., L.Song, Y. Xiao and W. Ge. 2018. Drought-tolerant plant growth-promoting rhizobacteria associated with foxtail millet in a semi-arid agroecosystem and their potential in alleviating drought stress. *Front. Microbiol.*, 8: 2580.

Rahdari, P., S. Tavakoli and S.M. Hosseini. 2012. Studying of salinity stress effect on germination, proline, sugar, protein, lipid and chlorophyll content in purslane (*Portulaca oleracea* L.) leaves. *J. Stress. Physiol. Biochem.*, 8: 182-193.

Ramadoss, D., V.K. Lakkineni, P. Bose, S. Ali and K. Annapurna. 2013. Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. *Springerplus.*,2, 6.doi: 10.1186/2193-1801-2-6

Rashid, S., T.C. Charles and B.R. Glick. 2012. Isolation and characterization of new plant growth-promoting bacterial endophytes. *Appl. Soil. Ecol.*, 61: 217-224.

Reddy, K., S. Subhani, P. Khan and K. Kumar. 1985. Effect of light and benzyladenine on dark-treated growing rice (*Oryza sativa*) leaves II. Changes in peroxidase activity. *Plant.Cell. Physiol.*, 26: 987-994.

Saikia, J., R.K. Sarma, R. Dhandia, A.Yadav, R. Bharali, V.K. Gupta and R. Saikia. 2018. Alleviation of drought stress in pulse crops with ACC deaminase producing rhizobacteria isolated from acidic soil of Northeast India. *Sci. Rep.*, 8(1): 1-16.

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.

Santoyo, G., G. Moreno-Hagelsieb, M. del Carmen Orozco-Mosqueda and B.R. Glick. 2016. Plant growth-promoting bacterial endophytes. *Microbiol. Res.*, 183: 92-99.

Sarma, R.K. and R. Saikia. 2014. Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* GGRJ21. *Plant Soil*, 377(1-2): 111-126.

Singh, R. 2014. Microorganism as a tool of bioremediation technology for cleaning environment: a review. *Proceedings of the International Academy of Ecology and Environmental Sciences*, 4, 1.

Sohrabi, Y., G. Heidari, W. Weisany, K.G. Golezani and K. Mohammadi. 2012. Changes of antioxidative enzymes, lipid peroxidation and chlorophyll content in chickpea types colonized by different Glomus species under drought stress. *Symbiosis*, 56: 5-18.

Sorensen, J. and A. Sessitsch. 2015. Plant-associated bacteria lifestyle and molecular interactions (Eds.): J.D. van Elsas *et al*., Modern Soil Microbiology (2nd edn.), CRC Press, pp. 211-236.

Steel, R.G. and J.H. Torrie. 1980. Principle and procedures of statistic: A biometrical approach. New York: McGraw-Hill.

Subair, H. 2015. Isolation and Screening Bacterial Exopolysaccharide (EPS) from Potato Rhizosphere in Highland and the Potential as a Producer Indole Acetic Acid (IAA). *Procedia Food Sci.*,3: 74-81.

Talaat, N.B., B.T. Shawky and A.S. Ibrahim. 2015. Alleviation of drought-induced oxidative stress in maize (*Zea mays* L.) plants by dual application of 24-epibrassinolide and spermine. *Environ. Exp. Bot.*, 113: 47-58.

Timmusk, S., I.A.A. El-Daim, L. Copolovici, T. Tanilas, A. Kännaste, L. Behers, E. Nevo, G. Seisenbaeva, E. Stenström 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.

Toklikishvili, N., N. Dandurishvili, A. Vainstein, M. Tediashvili, N. Giorgobiani, S. Lurie, E. Szegedi, B. Glick and L. Chernin. 2010. Inhibitory effect of ACC deaminase producing bacteria on crown gall formation in tomato plants infected by *Agrobacterium tumefaciens* or *A. vitis*. *Plant. Pathol.*, 59: 1023-1030.

Valentovic, M., J.G. Ball and J.M. Brown. 2011. Cisplatin mediated alterations in oxidative stress enzymes are modulated by resveratrol.*FASEB J.*,25: 1087-14.

Vanderlinde, E.M., J.J. Harrison, A. Muszyński, R.W. Carlson, R.J. Turner and C.K. Yost. 2010. Identification of a novel ABC transporter required for desiccation tolerance, and biofilm formation in Rhizobium leguminosarum bv. viciae 3841. *FEMS Microbiol. Ecol.*,71: 327-340.

Vardharajula, S., Z.S. Ali, 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. Int.*, 6: 1-14.

Vinocur, B. and A. Altman. 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Curr. Opin. Biotechnol.*, 16: 123-132.

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.

Wang, W., B. Vinocur and A. Altman. 2003. Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. *Planta.*, 218: 1-14.

Wani, P.A. and M.S. Khan. 2014. Screening of multiple metal and antibiotic resistant isolates and their plant growth promoting activity. *Pak. J. Biol. Sci.*, 17: 206-212.

Wani, P.A., A. Zaidi and M.S. Khan. 2009. Chromium reducing and plant growth promoting potential of *Mesorhizobium* species under chromium stress. *Bioremediat. J.*,13: 121-129.

Yang, S., B. Vanderbeld, J. Wan and Y. Huang. 2010. Narrowing down the targets: towards successful genetic engineering of drought-tolerant crops. *Mol. Plant.*, 3: 469-490.

Zheng, P.E.N.G., L. Zhang, L. Tian, L. Zhang, F.U.C.A.I. Chen, B.Z. Li and Z. Cui. 2014. Isolation and characterization of novel bacteria containing ACC Deaminase from the rhizosphere resource on Dry-Farming Lands. *Pak. J. Bot.*, 46: 1905-1910.

(Received for publication 2 May 2019)