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MITIGATION OF DROUGHT STRESS IN MAIZE THROUGH INOCULATION WITH DROUGHT TOLERANT ACC DEAMINASE CONTAINING PGPR UNDER AXENIC CONDITIONS

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Abstract

Drought is one of abiotic factors that hampers the growth and yield of crops via an elevated level of ethylene too, inspite of limited nutrients supply. The ACC deaminase containing PGPR can mitigate drought stress in crops by decreasing the synthesis and accumulation of ethylene. As maize is widely cultivated cereal and fodder crop, a glass jar study was conducted for screening of drought-tolerant ACC deaminase containing PGPR under axenic condition. Under various levels (i.e., 0, 10 and 20%) of polyethylene glycol (PEG) induced drought stress, some of the ACC deaminase containing PGPR significantly enhanced shoot and root length, shoot fresh and dry weight and root fresh and dry weight in maize seedlings. Further, a significant improvement in photosynthetic pigments formation and nutrients concentrations i.e., NPK in maize shoot validated the efficacious functioning of PGPR strains, *Pseudomonas aeruginosa*, *Enterobacter cloacae, Achromobacter xylosoxidans and Leclercia adecarboxylata* regarding reduction in ethylene accumulation in maize seedlings under drought. *Pseudomonas aeruginosa*, *Enterobacter cloacae* and *Achromobacter xylosoxidans* are previously documented but *Leclercia adecarboxylata* is a new drought tolerant ACC deaminase containing PGPR that might have the potential to alleviate drought stress by improving root elongation, NPK uptake and possibility decreasing ethylene in plants.

Key words: Chlorophyll pigments, Stress, Crops, Morphological attributes, Rhizobacteria.

Introduction

Limited availability of water is a major agricultural problem for the cultivation of field crops (Aslam *et al*., 2015). Drought is considered one of the most critical environmental abiotic stresses that decrease the production of crops (Lambers *et al*., 2008; Danish *et al*., 2019). It is predicted through climatic models that the ongoing changes in climate are going to increase the frequency and severity of drought in near future (IPCC, 2007; Farooq *et al*., 2009). The higher rate of evapotranspiration and low precipitation lead towards the development of drought condition (Mishra & Cherkauer, 2010). It is expected that 10% demand for water will be increased in 2050 for the cultivation of crops (Wada *et al*., 2013).

Under drought condition, most plants are unable to uptake ample water which is required for normal growth (Manivannan *et al*., 2008). Less uptake of water results in loss of turgor, decrease in leaf water potential, enzymes impairment, reduction in cell division and elongation (Kiani *et al*., 2007; Farooq *et al*., 2009; Hussain *et al*., 2009; Taiz & Zeiger, 2010). Stress degenerated by drought also decreases the duration of growing cycle in crops by disturbing their phenology (Desclaux & Roumet, 1996).

Reduction in the concentration of nitrogen, phosphorus and potassium in shoot and root is a general phenomenon in crops under drought stress (Subramanian *et al*., 2006; Danish & Zafar-ul-Hye, 2019; Zafar-ul-Hye *et al.,* 2019). Due to changes in the physiological and biochemical processes under drought stress, the productivity of crops may be reduced up to 50% (Hoekstra *et al*., 2001; Anjum *et al*., 2011; Zafar-ul-Hye *et al*., 2014). Plants which were grown under the drought stress usually have a low leaf area which decreases the intake of CO_2. This reduction in CO_2, impair the ATP and carboxylation enzymes resulting in the destruction of photosynthesis mechanism (Yamane *et al*., 2003).

Higher biosynthesis and accumulation of ethylene under drought stress is an established fact, that has been reported by many scientists (Mayak *et al*., 2004; Zahir *et al*., 2008; Zafar-ul-Hye *et al*., 2014). Severe drought stimulates the production of 1-aminocyclopropane-1-carboxylic acid (ACC) that increases ethylene (Wang *et al*., 2003). Due to the accumulation of more ethylene root becomes thick and shortened (Knight & Crocker, 1913). Less supply of energy and limited water availability at imbibition phase significantly decreased the germination of seeds due to poor development of the root (Taiz & Zeiger, 2010; Ricardo, 2012). Stomatal closure, high transpiration rate, less biological nitrogen fixation, inhibition of abscisic acid activity and evoking of physiological responses are some of the major drawbacks of higher ethylene accumulation in the plants beside poor root growth (Tamimi & Timko, 2003; Wang *et al*., 2003; Tanaka *et al*., 2005).

Most of the plant growth promoting rhizobacteria (PGPR) not only enhance the productivity of crops but also protect them from abiotic stresses (Saleem *et al*., 2007; Saraf *et al*., 2010; Ngumbi & Kloepper, 2016; Vurukonda *et al*., 2016; Zafar-ul-Hye *et al*., 2018). However, there are some PGPR that can mitigate abiotic stresses via the activity of ACC deaminase (Shahzad *et al*., 2013). The polymeric ACC deaminase enzyme is dependent on pyridoxal 5-phosphate (PLP) (Honma & Shimomura, 1978) that is efficacious to mitigate drought stress by decreasing the ethylene level in plants (Mayak *et al*., 2004; Zahir *et al*., 2008; Zafar-ul-Hye *et al*., 2014). This enzyme hydrolyzes ethylene into α-ketobutyrate and ammonia (Glick *et al*., 1997) thus, improve the stomatal conductance and photosynthesis too (Jiang *et al*., 2012).

Maize in Pakistan, is 3rd leading cereal crop after wheat and rice. It has highest amount of energy i.e., ME 3350 Kcal/kg among all cereals. Maize is highly polymorphic and holds maximum amount of genetic variability (Carpici *et al.*, 2010). Drought stress may decrease 17% of maize yield (loss of 24 million tons yr^{-1}) as compared to well-watered production (Edmeades *et al.*, 1993). Keeping in mind the loss of maize growth under drought, a glass jar experiment was conducted under the axenic condition to isolate the ACC deaminase producing PGPR to alleviate the drought stress in maize. The aim of the study was to find some new drought tolerant ACC deaminase producing PGPR for mitigation of drought stress.

Materials and Methods

Collection of rhizosphere: The maize rhizospheric soil of different sites of Multan, Pakistan was collected. Sterilized bags were used to bring the rhizospheric soil in Department of Soil Science, Bahauddin Zakariya University, Multan. The adhered soil was removed using a sterilized spatula. Homogenized soil was used for the isolation of PGPR.

Isolation, incubation and purification of PGPR isolates: For the isolation of PGPR, 1.0 g homogenized rhizospheric soil was taken and serial dilutions (10^{-1} to 10^{-7}) were made. Using ACC (nitrogen source), DF minimal salt medium was prepared for the isolation of ACC deaminase producing PGPR (Dworkin & Foster, 1958). The petri dishes containing the isolates were kept at 25°C for 48 hours. For purification, 55 isolates were picked and streaked again and again on DF media to get pure strains.

Selection of drought-tolerant isolates: For the selection of drought-tolerant ACC deaminase producing PGPR, Polyethylene Glycol 6000 (PEG) was added at the rate of 20% in the DF media. There were 37 isolates which successfully grew on 20% PEG containing DF media. These isolates were considered as drought tolerant PGPR.

Statistical design and site of experiment: A hydroponic glass jar (3-inch diameter, 6-inch length) experiment was started at 15th of December, 2017 under axenic condition on maize for the screening of most effective drought-tolerant ACC deaminase producing PGPR for maize. There were 38 treatments applied at 3 levels of PEG (0, 10 and 20%) with 3 replications following CRD design.

Seeds sterilization and inoculation: The maize seeds of Kanzo 123-Hybrid were screened out manually to get healthy seeds. For the seed surface sterilization, 0.1% $HgCl_2$ was used by dipping the seeds in it for 5 min. To remove the residues of 0.1% $HgCl_2$ the seeds were washed three times using sterilized (autoclaved at 120°C for 20 min) deionized water as described by Sadiq & Ali (2013). The three sterilized seeds were placed on each autoclaved filter paper (Whatman's No. 40) and inoculum of the respective PGPR was poured. Finally, the seeds were sandwiched with another filter papers. Filter papers were rolled and placed in a sterilized glass jar. In each glass jar, 50 ml sterilized water was added and maintained throughout the experiment.

Nutrients supply: Hoagland solution was used to provide the plants with all the macro and micronutrients (Hoagland & Arnon, 1950).

Artificial drought stress: The polyethylene glycol (PEG) was used at three different rates (control = 0% (-0.08 MPa), 10% (-0.27 MPa) and 20% (-0.85 MPa) PEG) to induce artificial drought stress as described by Piwowarczyk *et al.*, (2014).

Harvesting and morphological attributes: After 21 days of sowing the seedlings were harvested by removing filter papers. The morphological growth attributes (shoot and root fresh weight, shoot and root dry weight, shoot and root length) were noted immediately. The dry weights were noted by drying the samples at 70°C for 48h on analytical grade weight balance.

Chemical analyses: The shoot samples were digested by using a di-acid mixture (HNO_3-$HClO_4$) for the analysis of phosphorus and potassium concentration in maize shoot (Chapman & Pratt, 1961). For the determination of phosphorus in the digested samples of maize shoot yellow color method was used. The absorbance on spectrophotometer (HITACHI U-2000) was taken at 420nm wavelength as described by Jones *et al.*, (1991). The potassium was determined on flame photometer (PFP-7, Jenway) as described by Nadeem *et al.*, (2013). However, nitrogen concentration was assessed in the shoot samples through H_2SO_4 digestion following Jones *et al.*, (1991). The samples were distilled according to Van Schouwenberg & Walinge, (1973) using Kjeldahl's distillation apparatus.

Photosynthetic pigments determination: The concentrations of chlorophyll a and b were examined following the methodology given by Arnon, (1949). The shoot samples were ground in the mortar by taking 80% acetone for the extraction of pigments from leaves. Spectrophotometer (HITACHI U-2000) was used to record the absorbance at 663 and 645nm wavelength. The final calculations were made using the equations:

$$\text{Chlorophyll a (mg g}^{-1}\text{ leaf fresh weight)} = \frac{12.7\ (\text{OD } 663) - 2.69\ (\text{OD } 645)\ \text{V}}{1000\ (\text{W})}$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ leaf fresh weight)} = \frac{22.9\ (\text{OD } 645) - 4.68\ (\text{OD } 663)\ \text{V}}{1000\ (\text{W})}$$

$$\text{Total chlorophyll (mg g}^{-1}\text{ leaf fresh weight)} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

where,
V = final volume made
W = grams of fresh leaf sample

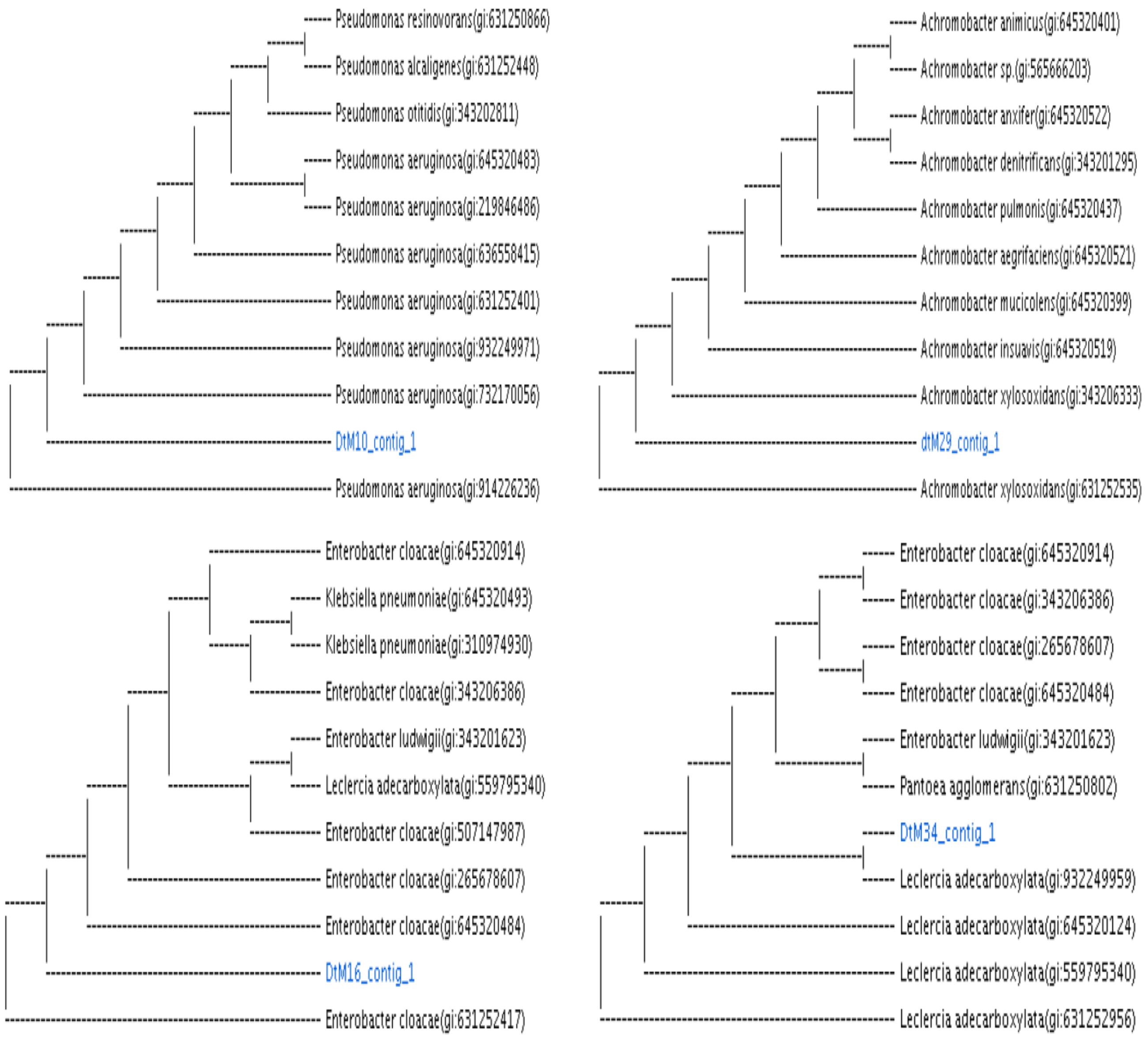

Fig. 1. Phylogenetic tree obtained from 16S rDNA sequence alignment for most effective drought tolerant ACC deaminase producing isolates collected from maize rhizosphere.

Molecular identification of effective drought tolerant PGPR: The 16S rRNA genes sequence was done for the molecular identification of most effective drought tolerant ACC deaminase containing PGPR. The PCR primers 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3' and 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3' were used. However, the gene sequencing primers 907R 5' (CCG TCA ATT CMT TTR AGT TT) 3' and 785F 5' (GGA TTA GAT ACC CTG GTA) 3' were used. The BLAST analysis (at NCBI) was done to align and deduce the affiliations of 16S rRNA gene sequences (Siddikee *et al.*, 2010). The most effective drought tolerant ACC deaminase containing PGPR were identified as *Pseudomonas aeruginosa* (DtM_{10}), *Enterobacter cloacae* (DtM_{16}), *Achromobacter xylosoxidans* (DtM_{29}) and *Leclercia adecarboxylata* (DtM_{34}) (Fig. 1).

Biochemical characterization of most efficient PGPR: For the determination of indole acetic acid (IAA) production with and without L-tryptophan (L-TRP; Sigma) the protocol by Sarwar *et al.*, (1992) and Glickmann and Dessaux (1995) were followed. Pikovskaya's medium was used to assess the phosphorus solubilizing activity of PGPR according to Vazquez *et al.*, (2000). The protocol stated by Setiawati and Mutmainnah (2016) was followed to assess the potassium solubilizing ability in the PGPR. For determination of ACC deaminase activity methodology of El-Tarabily, (2008) and Honma and Shimomura, (1978) was used. The characteristics of efficient drought tolerant ACC deaminase producing PGPR is provided in Table 5.

Statistical analysis

Statistical analysis of maize morphological attributes, chlorophyll and nutrients in the shoot was carried out using statistical software SPSS version 18.0 (Steel *et al.*, 1997). The treatments were compared using 2-factorial ANOVA followed by Tukey's test at $p \leq 0.05$.

Results

Main and interactive effects of PGPR and various levels of drought (D) remained significantly different for shoot and root length of maize seedlings. The strains, DtM2, DtM3, DtM10, DtM14, DtM16, DtM25, DtM26, DtM29, DtM32, DtM33, DtM34 and DtM35 proved significantly better from control at 0% PEG for shoot length. Inoculation with DtM29 proved significantly better than other strains at 10% PEG for shoot length. At 20% PEG level, the isolates DtM10, DtM16, DtM27, DtM28, DtM29, DtM32 and DtM34 performed significantly better for shoot length (Table 1). Maximum increase, 0.84, 1.21 and 3.22-fold in shoot length was noted over control at 0, 10 and 20% PEG respectively where DtM29 was used. In case of root length, DtM9, DtM10, DtM16, DtM18, DtM26, DtM28, DtM29 and DtM33 differed significantly from control at 0% PEG for root length. The DtM16 and DtM29 remained statistically alike with each other but found to be significantly better at 10% PEG for root length. At 20% PEG, the DtM16 remained significantly better as compared to control for root length (Table 1). Maximum increase of 0.94-fold in root length was recorded over control (No PGPR) at 0% PEG where DtM29 was applied. However, at 10 and 20%, PEG maximum increase of 1.10 and 1.35-fold in root length was noted over control, where DtM16 was applied.

Main effects of PGPR and various levels of D were significantly different but interaction remained statistically similar for shoot fresh weight. For shoot dry weight both main and interactive effects of PGPR and various levels of D differed significantly. The strains, DtM2, DtM4, DtM6, DtM9, DtM10, DtM14, DtM16, DtM27, DtM29, DtM33 and DtM34 differed significantly from control for shoot fresh weight. At 0% PEG, the shoot fresh weight was significantly higher as compared to 10% and 20% PEG (Table 2). Maximum increase of 1.33-fold in shoot fresh weight was noted over control where DtM16 was used. For shoot dry weight, the strain DtM29 performed significantly better at 0, 10 and 20% PEG over control (Table 2). Maximum increase of 0.80, 0.93 and 1.25-fold in shoot dry weight was noted at 0, 10 and 20% PEG induced drought respectively from control where DtM29 was applied.

Main effects of PGPR and various level of D were significantly different but the interaction remained similar for root fresh weight. For root dry weight, both main and interactive effects of PGPR and D remained significantly different. The strains, DtM7, DtM10, DtM16, DtM18, DtM26, DtM27, DtM29 and DtM34 differed significantly as compared to rest of the strains for root fresh weight (Table 3). Maximum increase of 3.31-fold in the root fresh weight was noted over control where DtM29 was applied. Maximum and significant increase i.e., 0.82, 0.83 and 1.56-fold in the root dry weight was noted over control at 0, 10 and 20% PEG respectively through the DtM29.

Both main and interactive effects of PGPR and D were significantly different for the chlorophyll a and total chlorophyll content in maize seedlings. For chlorophyll b, the main effect of PGPR and D remained significantly different but the interaction was non-significant. The strain DtM16 remained significantly better over control at 0% PEG for chlorophyll a content. Maximum increase i.e., 1.27-fold in the chlorophyll a was noted over control at 0% PEG where DtM16 was used. In the case of chlorophyll b content, DtM10, DtM14, DtM16, DtM19, DtM25, DtM26, DtM27, DtM29, DtM30, DtM31 and DtM34 performed significantly better as compared to rest of the strains (Table 4). Maximum increase of 2.39-fold in the chlorophyll b was noted from control in DtM29. For total chlorophyll content, DtM16 and DtM29 differed significantly at 0% PEG. At 10% PEG-induced drought, DtM29 performed significantly better for total chlorophyll content. All the treatments remained statistically similar to each other at 20% PEG for total chlorophyll.

Both main and interactive effects of PGPR and D were significantly different for shoot nitrogen, phosphorus and potassium concentration. For nitrogen (Fig. 2) and phosphorus concentration (Fig. 3), the strains DtM29 and DtM34 remained significantly better over control at 0% and 10% PEG. Maximum increase of 2.56 and 2.33-fold in the shoot nitrogen and phosphorus concentrations were noted at 0% PEG respectively over control where DtM29 was used. For shoot potassium concentration (Fig. 4), the strain DtM10 and DtM34 differed significantly at 0 % PEG over control. At 10% PEG, the DtM16 was significantly better as compared to control for shoot potassium. Maximum increase of 0.38-fold in the maize shoot potassium concentration was noted at 0% PEG as compared to control where DtM10 was used.

Discussion

The experiment was conducted to examine the effect of drought-tolerant ACC deaminase PGPR on growth attributes, pigments synthesis and nutrients concentration in maize seedlings under drought stress. The data revealed that the growth, pigments synthesis and nutrient concentration in maize seedlings were significantly different without ACC deaminase containing PGPR under drought stress. Higher biosynthesis and accumulation of ethylene ultimately induced negative changes in the development phases and decreased plant growth (Arshad *et al.*, 2008). In the current experiment, there were four ACC deaminase containing PGPR (DtM_{10}, DtM_{16}, DtM_{29} and DtM_{34}) that significantly enhanced shoot and root length of maize seedlings. The improvement in shoot and root length might be due to reduction in ethylene biosynthesis by the activity of ACC deaminase produced by *Pseudomonas aeruginosa* (DtM_{10}), *Enterobacter cloacae* (DtM_{16}), *Achromobacter xylosoxidans* (DtM_{29}) and *Leclercia adecarboxylata* (DtM_{34}). The findings of Zafar-ul-Hye *et al.*, (2014) supported our argument regarding improvement in the growth of crops by inoculation with ACC deaminase containing PGPR. Zahir *et al.*, (2009) also suggested the inhibition of ethylene accumulation by ACC deaminase PGPR as one of the factors that promote plant growth. According to Glick *et al.*, (1999), the ACC deaminase enzyme breaks the ethylene into NH_3 and α-ketobutyrate.

Table 1. Effect of ACC deaminase containing PGPR on shoot length (cm) and root length (cm) of maize seedlings under various levels of PEG induced drought.

PGPR	Shoot length (cm)				Root length (cm)			
	Various levels of PEG induced drought							
	IE (PGPR × D)			+ ME	IE (PGPR × D)			+ ME
	0%	10%	20%		0%	10%	20%	
Control	24.6 $^{\text{i-v}}$	17.5 $^{\text{r-w}}$	7.70 $^{\text{w}}$	16.6 $^{\text{M}}$	15.5 $^{\text{u-O}}$	13.2 $^{\text{E-U}}$	9.20 $^{\text{Q-V}}$	12.6 $^{\text{MN}}$
DtM1	29.1 $^{\text{b-p}}$	26.4 $^{\text{f-t}}$	25.5 $^{\text{f-u}}$	27.0 $^{\text{E-K}}$	14.0 $^{\text{B-S}}$	12.1 $^{\text{J-V}}$	9.10 $^{\text{R-V}}$	11.7 $^{\text{N}}$
DtM2	35.4 $^{\text{a-h}}$	33.5 $^{\text{b-l}}$	24.5 $^{\text{i-v}}$	31.1 $^{\text{B-H}}$	22.5 $^{\text{d-l}}$	15.3 $^{\text{u-P}}$	14.2 $^{\text{A-R}}$	17.3 $^{\text{F-K}}$
DtM3	35.9 $^{\text{a-f}}$	25.2 $^{\text{g-u}}$	22.5 $^{\text{m-v}}$	27.9 $^{\text{D-K}}$	20.1 $^{\text{g-v}}$	13.1 $^{\text{F-V}}$	9.10 $^{\text{S-V}}$	14.1 $^{\text{L-N}}$
DtM4	35.0 $^{\text{a-j}}$	33.2 $^{\text{b-l}}$	27.0 $^{\text{f-s}}$	31.7 $^{\text{B-G}}$	18.2 $^{\text{k-E}}$	15.7 $^{\text{u-O}}$	15.5 $^{\text{u-O}}$	16.5 $^{\text{H-L}}$
DtM5	29.1 $^{\text{b-p}}$	21.2 $^{\text{o-v}}$	7.50 $^{\text{w}}$	19.3 $^{\text{LM}}$	23.9 $^{\text{c-h}}$	14.5 $^{\text{y-P}}$	8.20 $^{\text{UV}}$	15.5 $^{\text{KL}}$
DtM6	34.6 $^{\text{a-k}}$	30.0 $^{\text{b-p}}$	26.2 $^{\text{f-t}}$	30.3 $^{\text{B-I}}$	17.5 $^{\text{l-I}}$	16.1 $^{\text{t-M}}$	12.1 $^{\text{J-V}}$	15.2 $^{\text{K-M}}$
DtM7	32.2 $^{\text{b-n}}$	30.4 $^{\text{b-o}}$	27.3 $^{\text{f-s}}$	30.0 $^{\text{B-I}}$	21.0 $^{\text{f-t}}$	20.0 $^{\text{g-w}}$	16.1 $^{\text{t-M}}$	19.0 $^{\text{E-H}}$
DtM8	26.9 $^{\text{f-s}}$	25.3 $^{\text{f-u}}$	21.1 $^{\text{o-v}}$	24.4 $^{\text{J-L}}$	15.0 $^{\text{w-P}}$	14.6 $^{\text{y-P}}$	12.9 $^{\text{G-V}}$	14.2 $^{\text{L-N}}$
DtM9	32.2 $^{\text{b-n}}$	29.8 $^{\text{b-p}}$	17.4 $^{\text{r-w}}$	26.5 $^{\text{F-K}}$	26.1 $^{\text{a-f}}$	18.1 $^{\text{k-F}}$	13.2 $^{\text{E-U}}$	19.1 $^{\text{E-G}}$
DtM10	39.1 $^{\text{ab}}$	30.5 $^{\text{b-o}}$	30.0 $^{\text{b-p}}$	33.2 $^{\text{B-D}}$	25.9 $^{\text{a-f}}$	21.6 $^{\text{f-q}}$	20.3 $^{\text{g-u}}$	22.6 $^{\text{BC}}$
DtM11	30.0 $^{\text{b-p}}$	28.2 $^{\text{c-q}}$	25.0 $^{\text{g-u}}$	27.7 $^{\text{D-K}}$	21.4 $^{\text{f-s}}$	20.1 $^{\text{g-v}}$	19.4 $^{\text{h-y}}$	20.3 $^{\text{C-E}}$
DtM12	27.9 $^{\text{d-r}}$	27.1 $^{\text{f-s}}$	24.6 $^{\text{i-v}}$	26.5 $^{\text{F-K}}$	17.5 $^{\text{l-I}}$	13.6 $^{\text{B-T}}$	13.4 $^{\text{C-T}}$	14.8 $^{\text{K-M}}$
DtM13	33.3 $^{\text{b-l}}$	27.6 $^{\text{e-s}}$	27.0 $^{\text{f-s}}$	29.3 $^{\text{B-J}}$	17.2 $^{\text{n-I}}$	16.0 $^{\text{t-M}}$	12.7 $^{\text{H-V}}$	15.3 $^{\text{KL}}$
DtM14	39.1 $^{\text{ab}}$	31.3 $^{\text{b-o}}$	22.1 $^{\text{n-v}}$	30.8 $^{\text{B-H}}$	17.7 $^{\text{k-H}}$	15.8 $^{\text{u-N}}$	14.1 $^{\text{A-S}}$	15.9 $^{\text{KL}}$
DtM15	27.1 $^{\text{f-s}}$	24.2 $^{\text{k-v}}$	23.6 $^{\text{l-v}}$	25.0 $^{\text{I-K}}$	16.6 $^{\text{q-K}}$	14.3 $^{\text{z-Q}}$	13.4 $^{\text{C-T}}$	14.8 $^{\text{K-M}}$
DtM16	38.6 $^{\text{a-c}}$	33.8 $^{\text{b-l}}$	29.1 $^{\text{b-p}}$	33.8 $^{\text{AB}}$	29.3 $^{\text{ab}}$	27.7 $^{\text{ab}}$	21.6 $^{\text{f-q}}$	26.2 $^{\text{A}}$
DtM17	17.2 $^{\text{s-w}}$	17.1 $^{\text{s-w}}$	15.9 $^{\text{t-w}}$	16.7 $^{\text{M}}$	13.7 $^{\text{B-S}}$	11.7 $^{\text{K-V}}$	10.6 $^{\text{O-V}}$	12.0 $^{\text{N}}$
DtM18	27.6 $^{\text{e-s}}$	25.1 $^{\text{g-u}}$	22.3 $^{\text{n-v}}$	25.0 $^{\text{I-K}}$	26.7 $^{\text{a-e}}$	21.6 $^{\text{f-r}}$	10.9 $^{\text{N-V}}$	19.7 $^{\text{D-G}}$
DtM19	32.0 $^{\text{b-n}}$	29.9 $^{\text{b-p}}$	19.5 $^{\text{p-v}}$	27.1 $^{\text{E-K}}$	22.1 $^{\text{e-p}}$	19.3 $^{\text{h-z}}$	10.4 $^{\text{P-V}}$	17.3 $^{\text{G-K}}$
DtM20	34.1 $^{\text{b-l}}$	25.8 $^{\text{f-t}}$	24.4 $^{\text{j-v}}$	28.1 $^{\text{D-K}}$	17.9 $^{\text{k-G}}$	16.5 $^{\text{r-L}}$	14.4 $^{\text{y-P}}$	16.3 $^{\text{I-L}}$
DtM21	26.6 $^{\text{f-s}}$	26.6 $^{\text{h-v}}$	24.8 $^{\text{h-v}}$	26.0 $^{\text{H-K}}$	18.4 $^{\text{j-C}}$	16.0 $^{\text{t-M}}$	11.4 $^{\text{L-V}}$	15.3 $^{\text{KL}}$
DtM22	29.7 $^{\text{b-p}}$	22.1 $^{\text{n-v}}$	17.3 $^{\text{r-w}}$	23.0 $^{\text{KL}}$	24.6 $^{\text{b-g}}$	19.4 $^{\text{h-y}}$	12.5 $^{\text{I-V}}$	18.8 $^{\text{E-I}}$
DtM23	29.7 $^{\text{b-p}}$	27.2 $^{\text{f-s}}$	22.1 $^{\text{n-v}}$	26.3 $^{\text{G-K}}$	22.2 $^{\text{e-n}}$	19.1 $^{\text{h-A}}$	14.8 $^{\text{y-P}}$	18.7 $^{\text{E-J}}$
DtM24	27.2 $^{\text{f-s}}$	26.7 $^{\text{f-s}}$	24.1 $^{\text{k-v}}$	26.0 $^{\text{H-K}}$	21.4 $^{\text{f-s}}$	18.3 $^{\text{k-D}}$	8.60 $^{\text{T-V}}$	16.1 $^{\text{J-L}}$
DtM25	35.1 $^{\text{a-i}}$	31.2 $^{\text{b-o}}$	15.0 $^{\text{u-w}}$	27.1 $^{\text{E-K}}$	22.3 $^{\text{e-m}}$	17.0 $^{\text{p-J}}$	8.10 $^{\text{V}}$	15.8 $^{\text{KL}}$
DtM26	38.9 $^{\text{ab}}$	32.6 $^{\text{b-n}}$	14.2 $^{\text{v-w}}$	28.6 $^{\text{B-J}}$	27.4 $^{\text{a-d}}$	22.3 $^{\text{e-m}}$	16.4 $^{\text{s-M}}$	22.0 $^{\text{B-D}}$
DtM27	35.1 $^{\text{a-i}}$	33.0 $^{\text{b-m}}$	29.2 $^{\text{b-p}}$	32.4 $^{\text{B-E}}$	17.1 $^{\text{o-J}}$	15.7 $^{\text{u-O}}$	13.2 $^{\text{D-U}}$	15.3 $^{\text{KL}}$
DtM28	33.1 $^{\text{b-m}}$	31.5 $^{\text{b-o}}$	31.4 $^{\text{b-o}}$	32.0 $^{\text{B-E}}$	27.1 $^{\text{a-e}}$	24.1 $^{\text{c-h}}$	19.9 $^{\text{g-x}}$	23.7 $^{\text{AB}}$
DtM29	45.2 $^{\text{a}}$	38.7 $^{\text{a-c}}$	32.5 $^{\text{b-n}}$	38.8 $^{\text{A}}$	30.0 $^{\text{a}}$	27.1 $^{\text{a-e}}$	10.6 $^{\text{O-V}}$	22.6 $^{\text{BC}}$
DtM30	27.2 $^{\text{f-s}}$	24.6 $^{\text{i-v}}$	23.5 $^{\text{l-v}}$	25.1 $^{\text{I-K}}$	17.3 $^{\text{m-I}}$	15.7 $^{\text{u-O}}$	15.1 $^{\text{v-P}}$	16.0 $^{\text{KL}}$
DtM31	30.5 $^{\text{b-o}}$	29.2 $^{\text{b-p}}$	25.3 $^{\text{f-u}}$	28.3 $^{\text{C-K}}$	15.8 $^{\text{u-M}}$	14.9 $^{\text{x-P}}$	14.1 $^{\text{A-S}}$	14.9 $^{\text{K-M}}$
DtM32	39.1 $^{\text{ab}}$	30.5 $^{\text{b-o}}$	30.0 $^{\text{b-p}}$	33.2 $^{\text{B-D}}$	24.1 $^{\text{c-h}}$	17.7 $^{\text{k-H}}$	14.5 $^{\text{y-P}}$	18.8 $^{\text{E-I}}$
DtM33	38.0 $^{\text{a-e}}$	32.1 $^{\text{b-n}}$	25.9 $^{\text{f-t}}$	32.0 $^{\text{B-E}}$	25.0 $^{\text{a-g}}$	22.6 $^{\text{d-k}}$	14.9 $^{\text{x-P}}$	20.8 $^{\text{C-E}}$
DtM34	38.3 $^{\text{a-d}}$	33.1 $^{\text{b-m}}$	29.7 $^{\text{b-p}}$	33.7 $^{\text{A-C}}$	23.6 $^{\text{c-i}}$	23.4 $^{\text{c-j}}$	19.9 $^{\text{g-x}}$	22.3 $^{\text{B-D}}$
DtM35	35.6 $^{\text{a-g}}$	33.2 $^{\text{b-l}}$	26.9 $^{\text{f-s}}$	31.9 $^{\text{B-F}}$	21.4 $^{\text{f-s}}$	18.6 $^{\text{i-B}}$	17.8 $^{\text{k-G}}$	19.3 $^{\text{E-G}}$
DtM36	32.4 $^{\text{b-n}}$	31.7 $^{\text{b-o}}$	30.7 $^{\text{b-o}}$	31.6 $^{\text{B-G}}$	21.0 $^{\text{f-t}}$	12.7 $^{\text{H-V}}$	11.4 $^{\text{M-V}}$	15.0 $^{\text{K-M}}$
DtM37	31.2 $^{\text{b-o}}$	28.8 $^{\text{b-p}}$	18.0 $^{\text{q-w}}$	26.0 $^{\text{H-K}}$	23.4 $^{\text{c-j}}$	22.1 $^{\text{e-o}}$	14.2 $^{\text{A-R}}$	19.9 $^{\text{D-F}}$
*ME	32.5 $^{\text{A}}$	28.6 $^{\text{B}}$	23.4 $^{\text{C}}$		21.1 $^{\text{A}}$	17.8 $^{\text{B}}$	13.6 $^{\text{C}}$	

*ME = Main effect of drought; +ME = Main effect of PGPR; IE = Interactive effect

Table 2. Effect of ACC deaminase containing PGPR on shoot fresh weight (g) and shoot dry weight (g) of maize seedlings under various levels of PEG induced drought.

PGPR	Shoot fresh weight (g)				Shoot dry weight (g)			
	Various levels of PEG induced drought							
	IE (PGPR × D)			+ME	IE (PGPR × D)			+ ME
	0%	10%	20%		0%	10%	20%	
Control	0.41	0.26	0.15	0.27 ^{G}	0.050 $^{c-e}$	0.040 $^{e-g}$	0.020 $^{h-j}$	0.037 $^{E-H}$
DtM1	0.54	0.46	0.31	0.44 $^{A-G}$	0.050 $^{c-e}$	0.040 $^{e-g}$	0.010 ^{j}	0.033 $^{F-I}$
DtM2	0.61	0.57	0.36	0.51 $^{A-F}$	0.040 $^{e-g}$	0.040 $^{e-g}$	0.020 $^{h-j}$	0.033 $^{F-I}$
DtM3	0.59	0.41	0.32	0.44 $^{A-G}$	0.050 $^{c-e}$	0.030 $^{f-i}$	0.010 ^{j}	0.030 $^{G-J}$
DtM4	0.58	0.53	0.52	0.54 $^{A-E}$	0.050 $^{c-e}$	0.030 $^{f-i}$	0.030 $^{f-i}$	0.037 $^{E-H}$
DtM5	0.58	0.50	0.26	0.45 $^{A-G}$	0.040 $^{e-g}$	0.020 $^{h-j}$	0.010 ^{j}	0.023 ^{IJ}
DtM6	0.63	0.53	0.52	0.56 $^{A-D}$	0.060 ^{bc}	0.040 $^{e-g}$	0.030 $^{f-i}$	0.044 $^{B-E}$
DtM7	0.53	0.49	0.40	0.47 $^{A-G}$	0.050 $^{c-e}$	0.040 $^{e-g}$	0.030 $^{f-i}$	0.040 $^{C-G}$
DtM8	0.37	0.34	0.33	0.35 $^{E-G}$	0.030 $^{f-i}$	0.020 $^{h-j}$	0.015 ^{ij}	0.021 ^{J}
DtM9	0.72	0.55	0.49	0.59 $^{A-C}$	0.070 ^{b}	0.050 $^{c-e}$	0.030 $^{f-i}$	0.051 ^{B}
DtM10	0.72	0.55	0.49	0.59 $^{A-C}$	0.060 $^{b-d}$	0.050 $^{c-e}$	0.040 $^{e-g}$	0.050 ^{BC}
DtM11	0.60	0.32	0.25	0.39 $^{C-G}$	0.040 $^{e-g}$	0.030 $^{f-i}$	0.010 ^{j}	0.027 $^{H-J}$
DtM12	0.49	0.50	0.36	0.45 $^{A-G}$	0.030 $^{f-i}$	0.030 $^{f-i}$	0.020 $^{h-j}$	0.027 $^{H-J}$
DtM13	0.52	0.52	0.38	0.47 $^{A-G}$	0.050 $^{c-e}$	0.020 $^{h-j}$	0.010 ^{j}	0.027 $^{H-J}$
DtM14	0.61	0.50	0.40	0.50 $^{A-F}$	0.030 $^{f-i}$	0.030 $^{f-i}$	0.010 ^{j}	0.023 ^{IJ}
DtM15	0.42	0.26	0.17	0.28 ^{G}	0.050 $^{c-e}$	0.040 $^{e-g}$	0.010 ^{j}	0.033 $^{F-I}$
DtM16	0.70	0.62	0.56	0.63 ^{A}	0.070 ^{b}	0.050 $^{c-e}$	0.030 $^{f-i}$	0.051 ^{B}
DtM17	0.34	0.31	0.20	0.28 ^{G}	0.050 $^{c-e}$	0.040 $^{e-g}$	0.010 ^{j}	0.033 $^{F-I}$
DtM18	0.35	0.30	0.29	0.31 ^{FG}	0.070 ^{b}	0.040 $^{e-g}$	0.020 $^{h-j}$	0.043 $^{B-F}$
DtM19	0.54	0.52	0.13	0.40 $^{C-G}$	0.050 $^{c-e}$	0.050 $^{c-e}$	0.030 $^{f-i}$	0.043 $^{B-F}$
DtM20	0.64	0.38	0.30	0.44 $^{A-G}$	0.060 ^{bc}	0.040 $^{e-g}$	0.030 $^{f-i}$	0.044 $^{B-E}$
DtM21	0.42	0.40	0.29	0.37 $^{D-G}$	0.040 $^{e-g}$	0.040 $^{e-g}$	0.020 $^{h-j}$	0.033 $^{F-I}$
DtM22	0.48	0.34	0.21	0.34 $^{E-G}$	0.050 $^{c-e}$	0.030 $^{f-i}$	0.020 $^{h-j}$	0.033 $^{F-I}$
DtM23	0.38	0.32	0.31	0.34 $^{E-G}$	0.030 $^{f-i}$	0.030 $^{f-i}$	0.010 ^{j}	0.023 ^{IJ}
DtM24	0.52	0.50	0.23	0.42 $^{A-G}$	0.050 $^{c-e}$	0.030 $^{f-i}$	0.010 ^{j}	0.030 $^{G-J}$
DtM25	0.68	0.56	0.17	0.47 $^{A-G}$	0.050 $^{c-e}$	0.040 $^{e-g}$	0.020 $^{h-j}$	0.038 $^{D-G}$
DtM26	0.58	0.57	0.22	0.46 $^{A-G}$	0.050 $^{c-e}$	0.030 $^{f-i}$	0.020 $^{h-j}$	0.033 $^{F-I}$
DtM27	0.69	0.45	0.43	0.52 $^{A-F}$	0.060 $^{b-d}$	0.030 $^{f-i}$	0.030 $^{f-i}$	0.040 $^{C-G}$
DtM28	0.55	0.47	0.31	0.44 $^{A-G}$	0.030 $^{f-i}$	0.020 $^{h-j}$	0.010 ^{j}	0.020 ^{J}
DtM29	0.84	0.59	0.41	0.61 ^{AB}	0.090 ^{a}	0.077 ^{ab}	0.045 $^{c-f}$	0.071 ^{A}
DtM30	0.54	0.52	0.31	0.46 $^{A-G}$	0.040 $^{e-g}$	0.030 $^{f-i}$	0.030 $^{f-i}$	0.033 $^{F-I}$
DtM31	0.49	0.47	0.22	0.39 $^{C-G}$	0.040 $^{e-g}$	0.040 $^{e-g}$	0.020 $^{h-j}$	0.033 $^{F-I}$
DtM32	0.49	0.39	0.34	0.41 $^{B-G}$	0.060 ^{bc}	0.050 $^{c-e}$	0.030 $^{f-i}$	0.048 $^{B-D}$
DtM33	0.74	0.44	0.40	0.53 $^{A-E}$	0.070 ^{b}	0.040 $^{e-g}$	0.030 $^{f-i}$	0.048 $^{B-D}$
DtM34	0.76	0.55	0.53	0.61 ^{AB}	0.070 ^{b}	0.043 $^{d-f}$	0.030 $^{f-i}$	0.050 $^{B-D}$
DtM35	0.73	0.53	0.36	0.54 $^{A-E}$	0.040 $^{e-g}$	0.040 $^{e-g}$	0.010 ^{j}	0.030 $^{G-J}$
DtM36	0.55	0.45	0.27	0.42 $^{A-G}$	0.060 $^{b-d}$	0.030 $^{f-i}$	0.030 $^{f-i}$	0.040 $^{C-G}$
DtM37	0.46	0.45	0.21	0.37 $^{D-G}$	0.050 $^{c-e}$	0.040 $^{e-g}$	0.020 $^{h-j}$	0.037 $^{E-H}$
*ME	0.56 ^{A}	0.46 ^{B}	0.33 ^{C}		0.051 ^{A}	0.037 ^{B}	0.022 ^{C}	

*ME = Main effect of drought; +ME = Main effect of PGPR; IE = Interactive effect

Table 3. Effect of ACC deaminase containing PGPR on root fresh weight (g) and root dry weight (g) of maize seedlings under various levels of PEG induced drought.

PGPR	Root fresh weight (g)				Root dry weight (g)			
	Various levels of PEG induced drought							
	IE (PGPR × D)			+ ME	IE (PGPR × D)			+ ME
	0%	10%	20%		0%	10%	20%	
Control	0.17	0.14	0.09	0.13^{G}	0.022^{cd}	0.018^{de}	0.009^{fg}	0.016^{E-G}
DtM1	0.44	0.23	0.19	0.29^{B-G}	0.022^{cd}	0.018^{de}	0.013^{ef}	0.018^{C-E}
DtM2	0.31	0.30	0.19	0.27^{B-G}	0.018^{de}	0.018^{de}	0.009^{fg}	0.015^{E-G}
DtM3	0.36	0.13	0.10	0.20^{D-G}	0.022^{cd}	0.013^{ef}	0.004^{g}	0.013^{F-H}
DtM4	0.30	0.29	0.29	0.29^{B-G}	0.022^{cd}	0.013^{ef}	0.013^{ef}	0.016^{E-G}
DtM5	0.35	0.33	0.10	0.26^{B-G}	0.018^{de}	0.009^{fg}	0.004^{g}	0.010^{HI}
DtM6	0.38	0.24	0.24	0.29^{B-G}	0.026^{bc}	0.018^{de}	0.013^{ef}	0.019^{B-D}
DtM7	0.48	0.40	0.27	0.38^{A-E}	0.022^{cd}	0.018^{de}	0.013^{ef}	0.018^{C-E}
DtM8	0.29	0.25	0.21	0.25^{B-G}	0.013^{ef}	0.009^{fg}	0.004^{g}	0.009^{I}
DtM9	0.46	0.27	0.13	0.29^{B-G}	0.022^{cd}	0.018^{de}	0.004^{g}	0.015^{E-G}
DtM10	0.56	0.48	0.22	0.42^{A-C}	0.031^{b}	0.022^{cd}	0.013^{ef}	0.022^{B}
DtM11	0.31	0.24	0.21	0.25^{B-G}	0.018^{de}	0.013^{ef}	0.004^{g}	0.012^{G-I}
DtM12	0.25	0.17	0.16	0.19^{E-G}	0.013^{ef}	0.013^{ef}	0.009^{fg}	0.012^{G-I}
DtM13	0.33	0.26	0.20	0.26^{B-G}	0.022^{cd}	0.009^{fg}	0.004^{g}	0.012^{G-I}
DtM14	0.32	0.23	0.18	0.24^{B-G}	0.013^{ef}	0.013^{ef}	0.004^{g}	0.010^{HI}
DtM15	0.22	0.22	0.16	0.20^{D-G}	0.022^{cd}	0.018^{de}	0.004^{g}	0.015^{E-G}
DtM16	0.52	0.44	0.33	0.43^{AB}	0.026^{bc}	0.022^{cd}	0.018^{de}	0.022^{B}
DtM17	0.35	0.15	0.09	0.20^{D-G}	0.022^{cd}	0.018^{de}	0.004^{g}	0.015^{E-G}
DtM18	0.55	0.31	0.21	0.36^{A-F}	0.031^{b}	0.018^{de}	0.009^{fg}	0.019^{B-D}
DtM19	0.35	0.30	0.05	0.23^{B-G}	0.022^{cd}	0.022^{cd}	0.013^{ef}	0.019^{B-D}
DtM20	0.33	0.24	0.21	0.26^{B-G}	0.026^{bc}	0.018^{de}	0.013^{ef}	0.019^{B-D}
DtM21	0.28	0.14	0.11	0.18^{E-G}	0.018^{de}	0.018^{de}	0.009^{fg}	0.015^{E-G}
DtM22	0.25	0.18	0.06	0.16^{FG}	0.022^{cd}	0.013^{ef}	0.009^{fg}	0.015^{E-G}
DtM23	0.22	0.18	0.10	0.17^{E-G}	0.013^{ef}	0.013^{ef}	0.004^{g}	0.010^{HI}
DtM24	0.38	0.24	0.18	0.27^{B-G}	0.022^{cd}	0.013^{ef}	0.004^{g}	0.013^{F-H}
DtM25	0.42	0.26	0.06	0.25^{B-G}	0.022^{cd}	0.018^{de}	0.009^{fg}	0.016^{E-G}
DtM26	0.52	0.46	0.33	0.44^{AB}	0.031^{b}	0.013^{ef}	0.013^{ef}	0.019^{B-D}
DtM27	0.48	0.40	0.36	0.41^{A-D}	0.026^{bc}	0.022^{cd}	0.013^{ef}	0.021^{BC}
DtM28	0.34	0.25	0.16	0.25^{B-G}	0.022^{cd}	0.013^{ef}	0.009^{fg}	0.015^{E-G}
DtM29	0.78	0.56	0.34	0.56^{A}	0.040^{a}	0.033^{ab}	0.023^{cd}	0.032^{A}
DtM30	0.39	0.33	0.32	0.35^{A-G}	0.018^{de}	0.013^{ef}	0.013^{ef}	0.015^{E-G}
DtM31	0.39	0.31	0.22	0.31^{B-G}	0.018^{de}	0.018^{de}	0.009^{fg}	0.015^{E-G}
DtM32	0.39	0.31	0.22	0.31^{B-G}	0.026^{bc}	0.022^{cd}	0.013^{ef}	0.021^{BC}
DtM33	0.25	0.20	0.17	0.21^{C-G}	0.018^{de}	0.018^{de}	0.004^{g}	0.013^{F-H}
DtM34	0.56	0.40	0.32	0.43^{AB}	0.031^{b}	0.018^{de}	0.013^{ef}	0.021^{BC}
DtM35	0.33	0.31	0.22	0.29^{B-G}	0.013^{ef}	0.009^{fg}	0.004^{g}	0.009^{I}
DtM36	0.53	0.15	0.15	0.28^{B-G}	0.026^{bc}	0.013^{ef}	0.013^{ef}	0.018^{C-E}
DtM37	0.25	0.20	0.11	0.19^{E-G}	0.022^{cd}	0.018^{de}	0.009^{fg}	0.016^{BC}
*ME	0.38^{A}	0.28^{B}	0.19^{C}		0.022^{A}	0.016^{B}	0.009^{C}	

*ME = Main effect of drought; +ME = Main effect of PGPR; IE = Interactive effect

Table 4. Effect of ACC deaminase containing PGPR on chlorophyll a (mg/g), chlorophyll b (mg/g) and total chlorophyll (mg/g) synthesis in maize seedlings under various levels of PEG induced drought.

PGPR	Chlorophyll a (mg/g)				Chlorophyll b (mg/g)				Total chlorophyll (mg/g)			
	Various levels of PEG induced drought											
	IE (PGPR × D)			+ME	IE (PGPR × D)			+ME	IE (PGPR × D)			+ME
	0%	10%	20%		0%	10%	20%		0%	10%	20%	
Control	1.13^{b-i}	0.67^{d-i}	0.49^{g-i}	0.77^{DE}	0.92	0.47	0.32	0.57^{G-J}	2.05^{c-l}	1.15^{h-l}	0.82^{kl}	1.34^{IJ}
DtM1	1.31^{a-i}	1.02^{b-i}	0.75^{d-i}	1.03^{B-E}	1.16	0.78	0.64	0.86^{D-J}	2.47^{c-l}	1.80^{e-l}	1.39^{f-l}	1.89^{E-J}
DtM2	1.42^{a-i}	0.98^{b-i}	0.67^{d-i}	1.02^{B-E}	0.98	0.73	0.60	0.77^{E-J}	2.40^{c-l}	1.71^{f-l}	1.27^{h-l}	1.79^{E-J}
DtM3	1.39^{a-i}	0.95^{b-i}	0.75^{d-i}	1.03^{B-E}	0.56	0.38	0.30	0.41^{IJ}	1.95^{d-l}	1.34^{f-l}	1.04^{j-l}	1.44^{IJ}
DtM4	1.37^{a-i}	1.23^{a-i}	1.22^{a-i}	1.27^{A-E}	0.55	0.50	0.41	0.49^{H-J}	1.92^{d-l}	1.73^{f-l}	1.63^{f-l}	1.76^{E-J}
DtM5	1.37^{a-i}	1.18^{a-i}	0.61^{f-i}	1.05^{B-E}	0.55	0.48	0.25	0.43^{IJ}	1.92^{d-l}	1.66^{f-l}	0.86^{kl}	1.48^{G-J}
DtM6	1.72^{a-i}	1.25^{a-i}	0.85^{d-i}	1.27^{A-E}	0.70	0.51	0.35	0.52^{H-J}	2.42^{c-l}	1.76^{f-l}	1.20^{h-l}	1.79^{E-J}
DtM7	1.32^{a-i}	1.23^{a-i}	1.16^{a-i}	1.23^{A-E}	0.53	0.50	0.46	0.50^{H-J}	1.85^{d-l}	1.73^{f-l}	1.62^{f-l}	1.73^{E-J}
DtM8	0.87^{c-i}	0.80^{d-i}	0.78^{d-i}	0.82^{C-E}	0.35	0.32	0.31	0.33^{J}	1.22^{h-l}	1.12^{h-l}	1.09^{i-l}	1.15^{J}
DtM9	1.37^{a-i}	1.34^{a-i}	0.52^{g-i}	1.08^{B-E}	0.55	0.55	0.21	0.44^{IJ}	1.92^{d-l}	1.89^{d-l}	0.73^{l}	1.51^{G-J}
DtM10	2.05^{a-e}	1.85^{a-g}	1.20^{a-i}	1.70^{AB}	2.14	1.58	1.25	1.66^{A-C}	4.19^{a-d}	3.43^{a-i}	2.45^{c-l}	3.36^{AB}
DtM11	1.41^{a-i}	1.23^{a-i}	0.75^{d-i}	1.13^{B-E}	0.59	0.35	0.31	0.41^{IJ}	2.00^{c-l}	1.57^{f-l}	1.06^{j-l}	1.54^{G-J}
DtM12	1.65^{a-i}	1.46^{a-i}	1.32^{a-i}	1.48^{A-D}	0.69	0.59	0.53	0.60^{G-J}	2.33^{c-l}	2.05^{c-l}	1.85^{d-l}	2.08^{D-J}
DtM13	1.27^{a-i}	1.09^{b-i}	0.89^{c-i}	1.08^{B-E}	1.13	0.90	0.77	0.93^{B-J}	2.40^{c-l}	1.99^{c-l}	1.66^{f-l}	2.01^{D-J}
DtM14	1.33^{a-i}	1.22^{a-i}	0.98^{b-i}	1.18^{A-E}	1.55	1.20	0.73	1.16^{A-I}	2.89^{a-l}	2.42^{c-l}	1.71^{f-l}	2.34^{A-J}
DtM15	2.28^{a-c}	1.73^{a-i}	1.17^{a-i}	1.73^{AB}	1.86	1.42	1.01	1.43^{IJ}	4.14^{a-e}	3.15^{a-k}	2.17^{c-l}	3.15^{A-D}
DtM16	2.57^{a}	1.66^{a-i}	1.36^{a-i}	1.86^{A}	2.52	1.00	0.89	1.47^{A-F}	5.09^{a}	2.66^{b-l}	2.25^{c-l}	3.33^{A-C}
DtM17	1.75^{a-i}	1.21^{a-i}	0.52^{g-i}	1.16^{A-E}	0.93	0.83	1.17	0.97^{B-J}	2.69^{b-l}	2.03^{c-l}	1.68^{f-l}	2.13^{C-J}
DtM18	1.82^{a-h}	1.31^{a-i}	0.75^{d-i}	1.29^{A-E}	0.82	0.91	1.13	0.95^{B-J}	2.63^{b-l}	2.23^{c-l}	1.88^{d-l}	2.25^{B-J}
DtM19	1.97^{a-f}	0.58^{f-i}	1.22^{a-i}	1.26^{A-E}	2.11	0.95	0.71	1.26^{A-H}	4.08^{a-f}	1.53^{f-l}	1.94^{d-l}	2.52^{A-I}
DtM20	1.83^{a-g}	1.32^{a-i}	1.48^{a-i}	1.54^{A-C}	1.13	1.05	0.60	0.93^{B-J}	2.96^{a-l}	2.37^{c-l}	2.08^{c-l}	2.47^{A-I}
DtM21	1.20^{a-i}	1.28^{a-i}	0.75^{d-i}	1.08^{B-E}	1.01	0.78	0.50	0.76^{E-J}	2.21^{c-l}	2.06^{c-l}	1.25^{h-l}	1.84^{E-J}
DtM22	1.46^{a-i}	1.15^{b-i}	0.51^{g-i}	1.04^{B-E}	0.49	0.87	1.07	0.81^{D-J}	1.94^{d-l}	2.01^{c-l}	1.58^{f-l}	1.85^{E-J}
DtM23	0.99^{b-i}	1.44^{a-i}	0.66^{e-i}	1.03^{B-E}	1.10	0.78	0.77	0.88^{C-J}	2.09^{c-l}	2.21^{c-l}	1.43^{f-l}	1.91^{E-J}
DtM24	0.95^{b-i}	0.77^{d-i}	1.42^{a-i}	1.05^{B-E}	0.98	0.74	1.14	0.95^{B-J}	1.93^{d-l}	1.50^{f-l}	2.57^{b-l}	2.00^{D-J}
DtM25	1.53^{a-i}	1.16^{a-i}	1.21^{a-i}	1.30^{A-E}	1.93	1.55	0.90	1.46^{A-F}	3.47^{a-h}	2.71^{b-l}	2.11^{c-l}	2.76^{A-F}
DtM26	1.39^{a-i}	1.14^{b-i}	0.96^{b-i}	1.16^{A-E}	1.74	1.48	1.30	1.51^{A-E}	3.13^{a-k}	2.62^{b-l}	2.26^{c-l}	2.67^{A-H}
DtM27	1.79^{a-i}	1.55^{a-i}	0.73^{d-i}	1.36^{A-E}	1.00	1.71	1.27	1.32^{A-G}	2.79^{a-l}	3.26^{a-k}	1.99^{c-l}	2.68^{A-G}
DtM28	1.05^{b-i}	1.53^{a-i}	1.83^{a-g}	1.47^{A-D}	1.41	0.63	0.70	0.92^{B-J}	2.46^{c-l}	2.16^{c-l}	2.53^{b-l}	2.38^{A-I}
DtM29	2.34^{ab}	1.58^{a-i}	0.79^{d-i}	1.57^{AB}	2.50	2.06	1.22	1.93^{A}	4.84^{ab}	3.64^{a-g}	2.01^{c-l}	3.49^{A}
DtM30	1.15^{a-i}	0.95^{b-i}	1.28^{a-i}	1.13^{B-E}	1.87	1.95	1.22	1.68^{AB}	3.02^{a-l}	2.90^{a-l}	2.51^{b-l}	2.81^{A-E}
DtM31	0.87^{c-i}	1.24^{a-i}	1.38^{a-i}	1.16^{A-E}	1.30	1.13	1.36	1.26^{A-H}	2.17^{c-l}	2.36^{c-l}	2.74^{a-l}	2.42^{A-I}
DtM32	0.77^{d-i}	0.58^{f-i}	0.81^{d-i}	0.72^{E}	1.71	0.56	0.83	1.03^{B-J}	2.48^{c-l}	1.14^{h-l}	1.64^{f-l}	1.75^{E-J}
DtM33	1.26^{a-i}	1.13^{b-i}	0.97^{b-i}	1.12^{B-E}	1.03	0.87	0.69	0.86^{D-J}	2.29^{c-l}	2.00^{c-l}	1.66^{f-l}	1.98^{D-J}
DtM34	2.08^{a-d}	1.34^{a-i}	1.19^{a-i}	1.54^{A-C}	2.23	1.60	0.96	1.60^{A-D}	4.32^{a-c}	2.93^{a-l}	2.16^{c-l}	3.14^{A-D}
DtM35	1.26^{a-i}	0.99^{b-i}	0.92^{b-i}	1.06^{B-E}	0.96	0.77	0.94	0.89^{C-J}	2.23^{c-l}	1.76^{f-l}	1.86^{d-l}	1.95^{D-J}
DtM36	1.28^{a-i}	0.40^{hi}	0.38^{i}	0.69^{E}	1.45	0.61	0.55	0.87^{C-J}	2.73^{b-l}	1.01^{j-l}	0.93^{j-l}	1.56^{F-J}
DtM37	1.11^{b-i}	0.77^{d-i}	0.47^{g-i}	0.78^{DE}	0.82	0.69	0.53	0.68^{F-J}	1.93^{d-l}	1.46^{f-l}	1.00^{j-l}	1.46^{H-J}
*ME	1.47^{A}	1.17^{B}	0.94^{C}		1.21^{A}	0.91^{B}	0.76^{C}		2.67^{A}	2.08^{B}	1.70^{C}	

*ME = Main effect of drought; +ME = Main effect of PGPR; IE = Interactive effect

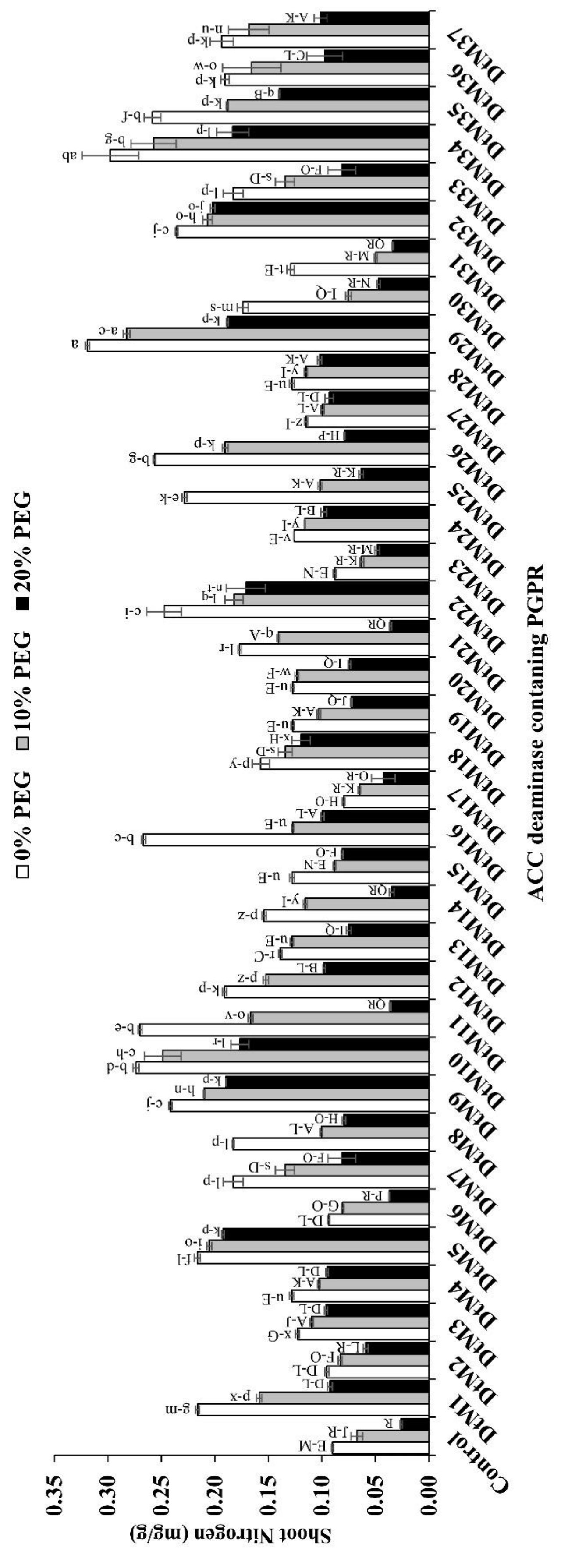

Fig. 2. Effect of ACC deaminase containing PGPR on nitrogen concentration in shoot of maize seedlings under various levels of PEG induced drought. Different letters on bars showed significant difference at $p \leq 0.05$ compared by Tukey's test.

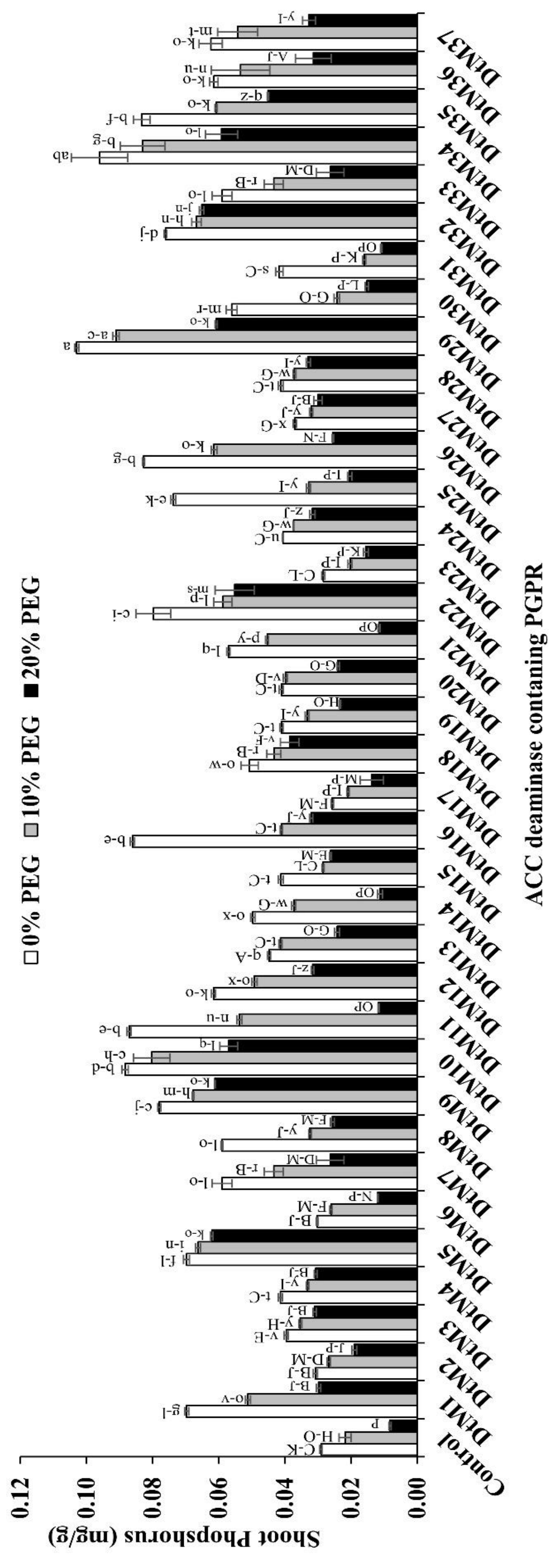

Fig. 3. Effect of ACC deaminase containing PGPR on phosphorus concentration in shoot of maize seedlings under various levels of PEG induced drought. Different letters on bars showed significant difference at $p \leq 0.05$ compared by Tukey's test.

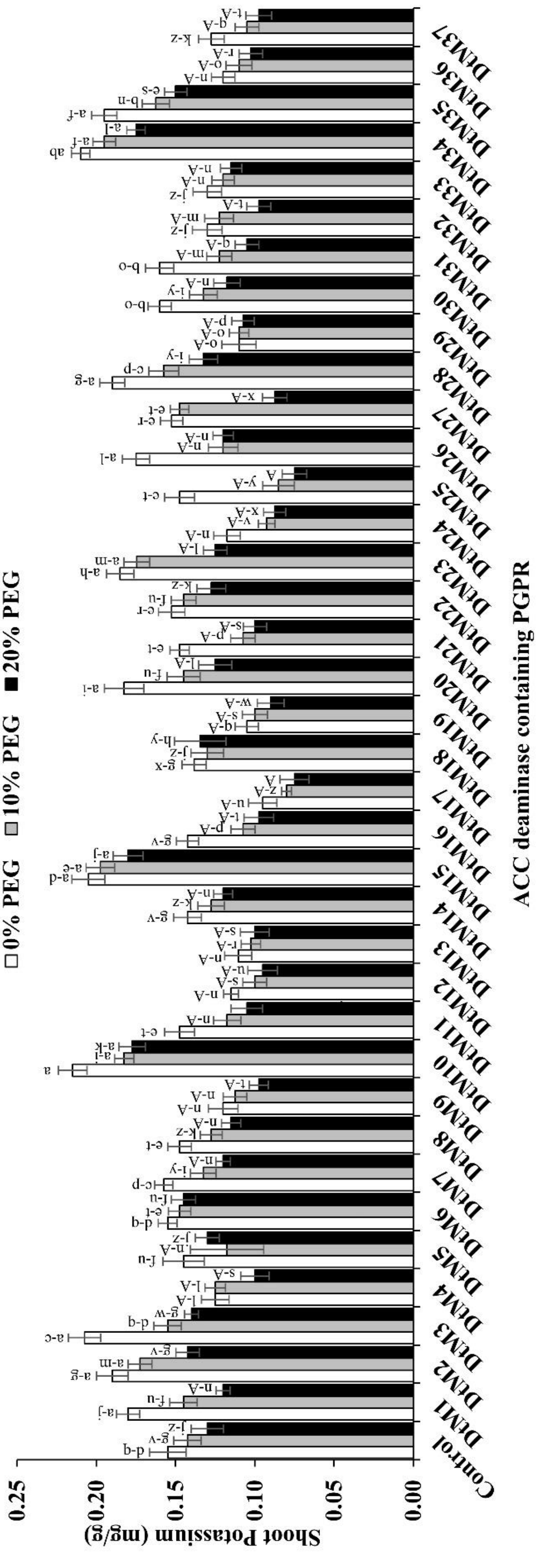

Fig. 4. Effect of ACC deaminase containing PGPR on potassium concentration in shoot of maize seedlings under various levels of PEG induced drought. Different letters on bars showed significant difference at $p \leq 0.05$ compared by Tukey's test.

Diffusion of root ethylene in rhizosphere along concentration gradient significantly decreased the accumulation of ethylene in plants (Glick, 2004; Siddikee *et al*., 2011). Xie *et al.*, (1996) stated that the root elongation might also be due to IAA production by the PGPR. Danish *et al.*, (2019) also noted similar kind of improvements by sole inoculation and co-application of PGPR and biochar. They argued that ACC deaminase activity, synthesis of IAA and better NPK uptake due to root elongation by inoculation of drought tolerant ACC deaminase producing PGPR are primarily linked with better growth of wheat under osmotic stress. The improvement in adventitious and lateral root was also noted by Gamalero & Glick (2011) and Mohite (2013) due to IAA secretion by the PGPR. The most effective drought tolerant ACC deaminase containing PGPR strains, in the current experiment were also found to be capable to secret IAA with and without L-tryptophan that might be an allied factor for improvement in maize growth under drought (Table 5). In the current study a significant improvement in shoot N, P and K, was noted which might be due to better root elongation. According to Reid & Renquist (1997), the better elongation of roots helps the plants to uptake relatively more water that improves water use efficiency under drought (Zahir *et al*., 2008). Safronova *et al*., (2006) also reported the better nutrients uptake in pea plants when seeds were inoculated with ACC deaminase containing PGPR *P. brassicacearum* and *P. marginalis*. However, chlorophyll a and b were significantly decreased where no PGPR was used at 10 and 20% PEG. This reduction in synthesis of chlorophyll content, might be due to limited availability of NPK under PEG induced osmotic stress. Matile *et al*., (1997) also reported a similar reduction in the synthesis of chlorophyll in plants as a result of higher biosynthesis of ethylene under stress. They stated that the outburst of ethylene under stress condition degraded the lipid which had resulted in the loss of chloroplast cell membrane integrity. In chloroplast, the chlorophyllase (chlase) gene gets stimulated by higher ethylene accumulation which starts degradation when comes in contact with chlorophyll (Matile *et al*., 1997). A significant improvement in the synthesis of chlorophyll a, chlorophyll b and total chlorophyll due to reduction in ethylene level might have developed resistance in maize plants against drought. In addition to the above argument, Stefan *et al*., (2013) also suggested the activity of IAA as an allied factor which might have improved the synthesis of chlorophyll. The findings of Danish & Zafar-ul-Hye (2019) also supported the results of improvement in the synthesis of chlorophyll a, chlorophyll b and total chlorophyll via inoculation of PGPR.

Conclusion

The ACC deaminase producing PGPR, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Achromobacter xylosoxidans* and *Leclercia adecarboxylata* were found to be drought tolerant and possessed capability of providing resistance to plants against drought stress. *Leclercia adecarboxylata* is a new drought tolerant ACC deaminase producing PGPR. Further investigation is suggested in this regard to declare *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Achromobacter xylosoxidans* and *Leclercia adecarboxylata* as drought tolerant ACC deaminase producing PGPR for better growth of maize under drought stress.

Table 5. Characterization of ACC deaminase containing PGPR.

Source	**Maize isolated rhizobacteria**			
PGPR experiment code	DtM_{10}	DtM_{16}	DtM_{29}	DtM_{34}
No. of nucleotide	6317050	1480	6813182	1527
Closet type strain and its accession number	CP012001.1 *Pseudomonas aeruginosa*	CP001918.1 *Enterobacter cloacae*	LN831029.1 *Achromobacter xylosoxidans*	NR_104933.1 *Leclercia adecarboxylata*
P-Solubilization (µg/ml)	29.1 ± 1.19	66.3 ± 0.38	77.4 ± 0.98	20.1 ± 1.29
K-Solubilization (mg/ml)	12.6 ± 0.92	19.1 ± 0.82	24.5 ± 0.42	16.4 ± 1.40
IAA (Tryptophan) (µg/ml)	21.3 ± 0.37	78.8 ± 0.35	61.2 ± 0.14	61.6 ± 0.20^{a}
IAA (No Tryptophan) (µg/ml)	2.94 ± 0.49	3.39 ± 0.41	5.52 ± 0.79	2.11 ± 0.17^{a}
ACCD activity (µmol α-ketobutyrate g $^{-1}$protein h^{-1})	115.2 ± 16.1	402.1 ± 27.3	381.17 ± 11.7	296.1 ± 21.7

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