https://doi.org/10.48048/tis.2022.6311

Evaluation of the Effect of ACC Deaminase and Exopolysaccharides Producing Bacteria in Maize (*Zea mays*) under Heat Stress

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Received: 11 October 2021, Revised: 15 January 2022, Accepted: 25 January 2022, Published: 31 October 2022

Abstract

Heat stress or global warming is a continuous temperature fluctuation that affects the environment and damage plant tissues because of the hormonal imbalances in plants. Yield losses resulting from heat stress are a major threat to global food security. Plant growth-promoting bacteria (PGPB) may be utilized to lessen this loss in yield. PGPB containing aminocyclopropane-1-carboxylic acid (ACC) deaminase activity can enhance plant growth that various abiotic stresses inhibit. This work was conducted to evaluate the effect of ACC deaminase and exopolysaccharides producing bacteria on maize plants grown under heat stress. The stressed plants were kept at 45 °C, while non-stressed plants were grown at a temperature of 28 - 35 °C. In 45 days of the growing period under heat stress, the plant growth and activities were decreased, however, in the presence of PGPB (isolated from soil and plant tissues in Muzaffagarh, Pakistan) containing ACC deaminase activity, the plant activities and biomass were increased compared to their respective control. The ACC deaminase-producing bacteria played a significant role by enhancing the physiological activities of the plants like chlorophyll a and b, carotenoid pigments, and proline content. Enzymatic activities like superoxide dismutase (81 %), peroxidase (57.8 %), and catalase (50.27 %) were increased. The relative water content of the maize plants was increased in Treatment one (T1) with 300, and 200 % for non-heat and heat, respectively, while the control was having 220, and 200 % for non-heat and heat, respectively. Soluble sugar content was improved with T1 having the highest values (4,000 and 5,700 g/mol) for heat and non-heat, respectively. The control was having 900, and 2300 g/mol for heat and non-heat, respectively. The application of ACC deaminase-producing bacteria on maize can help to overcome the adverse effects of heat stress and help the plant to survive under stress condition.

Keywords: ACC deaminase, Enzymes, Exopolysaccharides, Heat stress, Maize

Introduction

High temperature is the major risk for the agricultural zone around the globe [1]. The fluctuations in temperature cause an imbalance in the plant hormones and consequently affect their growth [1]. It was reported that PGPB containing ACC deaminase enzyme showed better performance when exposed to a daily warmness system. For example, *Bacillus globiosporous* had increased the shoot and root length of various plants [2]. Plants under heat stress show different metabolic processes and mechanisms for surviving in the environment under these conditions. Under heat stress conditions, when plants are exposed to heat stress during initial developmental stages, it decreases crop yield [3]. Heat stress shows a strong action on the physiological process of plant cells and tissues; hence, plants use several defense mechanisms for protection and adaptation [4]. When temperature fluctuation occurs in the environment, the plants may tolerate different temperatures to mimic normal processes for everyday life [5]. The intolerable processes during the response to stress, solutes compatibility, free ions, and transport scavengers are important [6]. At several different points like cellular organs, in cytoplasmic fluids

metabolic reaction, the effects of high temperature are shown [7]. The major process that manages heat stress is gene expression, and high temperature promptly changes the gene expression process [8]. An increase in temperature is the main reason for the reduction in crop yield, and this is also due to a reduction in the photosynthesis process [9]. Plant responses significantly change in different times and temperature ranges [10]. Different types of microorganisms like protozoa, algae, fungi and bacteria are present abundantly in soil. In 1 g of soil, the number of bacteria is approximately 10^8 - 10^9 [11]. Bacteria are classified into 3 classes based on their mode of action and plant response. PGPB has 2 types of mechanisms (direct and indirect mechanisms). PGPB in the direct mechanism shows a mutualistic correlation with plants. Phosphorus is more significant for plants and is present in insoluble form therefore, plants directly cannot use it. The insoluble forms Phosphomonoester, Phosphotriesters phytate and apatite are included in insoluble phosphate form [12]. Gluconic acid and citric acids are those organic acids that have lower molecular weight, and soil bacteria can produce these organic acids. The insoluble form of phosphate is converted into the soluble state after many reactions, foresters of phosphate mineralization, the soil bacteria hold special phosphatases [13]. By changing phytohormones, the plants maintain their physiology and biochemistry under stress conditions [14]. It is reported that the PGPB produced hormones including gibberellin, auxin and cytokinin. The plant benefits from phytohormones produced through PGPB (0.6 - 1 mg/mL), and its combination is also paramount for plant growth [15]. Many PGPB increases plant growth with the activity of the ACC deaminase enzyme, which is multimeric and cleaves the ACC into α-ketobutyrate and ammonia, ACC is the precursor of ethylene and the level of ethylene is increased when plants are under stress [16,17]. Ethylene is an important hormone for plants under different environmental conditions and stress signaling [18]. ACC deaminase enzyme isolates which are ethylene precursors, and subsequently low ethylene levels in different plant parts help to promote plant growth when the plants are more sensitive and unable to survive in stress conditions [19,20]. During indirect mechanism plant, antioxidant enzymes such as SOD, POX, PPO and CAT play a significant role in keeping the plant under several stress conditions. SOD help in catalyzing the unbalanced O_2 radicals into oxygen and hydrogen peroxide [21]. However, hydrogen peroxide depreciates into oxygen and water through peroxidase or catalase. When plant tissues are harmed, the polyphenol oxides and the phenolic mixes [22]. EPS plays a significant role in fighting against different antibiotics, plant tissues, antimicrobial complexes, and bacteriophages [23]. In cereal crops, maize is the third major crop in productivity [24], after wheat and rice, it is cultivated worldwide, in Pakistan Maize is grown in 2 seasons spring and autumn [24]. Maize's optimum temperature in the day is from 22 to 32 °C and its night optimum temperature is approximately from 16.7 to 24 °C. Photosynthesis becomes more rapid if ranges of temperature are in between these ranges, and consequently, respiration promotes the growth of the plants. Photosynthetic apparatus becomes inactive when temperature increases above 32 °C and decreases less than 5 °C, some negative effects on the growth of maize have been detected [25]. The highest germination of maize plants was observed at 20 - 30 °C, and its optimal growth temperature was 18 - 21 °C. If temperature increases above 40 °C it negatively affects the appearance and germination of the maize [26]. At temperature above 38 °C, thermal enzyme inactivation occur, which burden the net photosynthesis process [27]. In the heat stress period, the maize grain yield reduces 1.59 times than the normal yield production [24]. It is estimated that in 2020, the maize requirement for developing countries was expected to increase up to 504 million tons [28,29].

Materials and methods

Acquisition of Bacterial isolates for ACC deaminase activity and exopolysaccharides production

Forty previously isolated bacterial strains from soil and plant tissues (S-1, S-3, S-7, S-8, S-16, S-17, S-20, S-25, S-27, S-41, S-41, S-44, S-47, S-49, S-58, S-59, S-60, S-75, S-77, S-94, S-96, S-100, S-101, S-108, S-118, Pb3a-1, Jp1a-2, Dp74, Dp4c, New, New*, New-2, New-3, Dp1a, Pb4a, Dp7c-2, Rp2a, Rp3b, Rp4b and Rp3c) were obtained from the laboratory of Plant-microbe interaction, Quaid e Azam University Islamabad, Pakistan. The isolates were identified using microbiological and biochemical methods. These isolates were identified as *Azospirillum brasilense*, *Pseudomonas putida*, *P. fluorescens*, *Paenibacillus polymyxa* and *Serratia marcescens.* All the 40 strains were screened for ACC deaminase activity using qualitative and quantitative methods.

Qualitative ACC deaminase activity

All the bacterial strains were screened for ACC deaminase activity qualitative methods. For checking this activity, tryptic soy broth containing the concentration of Trypton 17 g, Peptone 3 g, NaCl 5

g, Dipotassium hydrogen phosphate 2.5 g, and Glucose 2.5 g was prepared and mixed in 1,000 mL distilled water. Each bacterial strain was inoculated in 5 mL of tryptic soy broth in a shaking incubator at 120 rpm and temperature of 28 °C. After 24 h, the cells were centrifuged for 5 min at 3,000 rpm, the supernatant was discarded, and the pellet was washed 2 times with 0.1 M Tris HCl autoclaved (pH 7.5). One mL of 0.1 M Tris HCl (pH 7.5) was added and mixed well using a micropipette on Petri plates. The inoculation was done using DF minimal salt medium [30] containing Glucose of 2.0 g, Gluconic acid of 2.0 g, Citric acid of 2.0 g, KH_2PO_4 of 4.0 g, Na_2HPO_4 of 6.0 g, $MgSO_4.7H_2O$ of 0.2 g, chemicals and its concentration for micronutrients solution. $CaCl_2$ of 0.200 g, $FeSO_4.7H_2O$ of 0.200 g, H_3BO_3 of 0.015 g, $ZnSO_4.7H_2O$ of 0.020 g, Na_2MoO_4 of 0.010 g, KI of 0.010 g, NaBr of 0.010 g, $MnCl_2$ of 0.010 g, $CoCl_2$ of 0.005 g, $CuCl_2$ of 0.005 g, $AlCl_3$ of 0.002 g, and $NiSO_4$ of 0.002 g were added in 1,000 mL distilled water. Ten mL of micronutrient solution was added in 990 mL of distilled water and together with 3 mM ACC as the main nitrogen source.

Quantification of ACC deaminase activity

For measuring ACC deaminase activity, the isolated bacterial strains were grown in tryptic soy broth with 5 mL concentration at a temperature of 28 °C for 2 to 3 days. The cells were harvested through centrifugation for 5 min at 3,000 rpm, the supernatant was discarded and the pellet was washed 2 times with 0.1 M TrisHCl (pH 7.5), this was followed by the addition of modified DF minimal salts medium with 3 mM ACC final concentration at a temperature of 28 °C, incubation was done in a shaker for 2 to 3 days.

The flow-through centrifugation cells were harvested for 5 min at 3,000 rpm, and the harvested cells were washed 2 times, the 1st wash was using 0.1 M Tris HCl to maintain a pH of 7.5, and the 2nd one was using 0.1 M Tris HCl having pH 8.5 and re-suspended in 200 µL of 0.1 M Tris HCl having pH 8.5 Then the cells were labialized through 5 % toluene (v/v) and vortexed for 20 to 30 s. Finally, 50 µL of this suspension was incubated with 5 µL of 0.3 M ACC in Eppendorf at a temperature of 28 °C for 30 min.

For comparison (negative control), 50 µL of the labilized cells without ACC were used. The samples were mixed with 500 µL of 0.56 N HCl through vortexing for 30 s, and the debris of cells was removed through centrifugation for 5 min at 12,000 rpm, 500 µL of the supernatant was transferred into an autoclaved test tubes and mixed well with 400 µL of 0.56 N HCl, and 150 µL DNF solution (0.1 g of 2,4 dinitrophenyl hydrazine in 100 mL of 2 N). NaOH was added to the samples before measuring the absorbance at 540 nm.

Standard curve for α-ketobutyrate

For each of the prepared bacterial samples, the α-ketobutyrate concentration was determined. This process was done through a standard curve. The sample was compared with different concentrations (0.01, 0.05, 0.1, 0.2, 0.5, 0.75, 1.0 and 1.5 mM), the addition of aliquot followed this in each sample, 500 µL concentration of 0.4 mL of N HCl and DNF solution was added in each of the samples. Two N NaOH in 1 mL concentration was poured. The optical value was measured at the wavelength of 540 nm. The absorbance value in mM concentration of alpha-ketobutyrate was determined [30].

Estimation of protein concentration

For determination of protein concentration, [31] method was used, for ACC deaminase assay determination, the bacterial cells were labialized with toluene (26.5 µL) and diluted with 173.5 µL of 0.1 M Tris-HCl with pH of 8.0. Then the solution was heated at boiling point after the addition of 200 µL of 0.1 N NaOH for 10 min and the bacterial cell samples were cooled at room temperature, the addition of 200 µL of Bradford's reagent solution was followed and mixed well for estimation of protein concentration, the absorbance was measured at the wavelength of 595 nm.

Standard curve

Bovine serum albumin (BSA) was used for the standard curve.

Production of exopolysaccharides

Qualitative

To determine the production of the exopolysaccharides, the bacterial streaking was carried out in Petri plates containing (4) ATCC no. 14 having Sucrose 20 g, Yeast extract 0.5 g, $FeCl_3$ 0.002 g, $CaSO_4.2H_2O$ 0.1 g, K_2HPO_4 0.8 g, KH_2PO_4 0.2 g, $MgSO_4.7H_2O$ 0.1 g, and $Na_2MoO_4.2H_2O$ in trace amount, and maintain at 7.2 pH at 28 °C, the round colonies of the bacteria with slimy layer showed the formation of exopolysaccharide production [32].

Quantification

For checking the production of exopolysaccharides quantitatively, the strains were grown in ATCC no. 14 50 mL liquid medium. The bacterial strains were incubated at 28 °C for 72 h in a shaking incubator at 2,000 rpm. The cells were harvested through centrifugation at 10,000 rpm for 20 min. Then 2 volumes of cold acetone were added and kept overnight at 40 °C temps [13] after that, the centrifugation for 20 min at 10,000 rpm followed. The presence of pellet indicated the production of the exopolysaccharide [32].

Experimental design and statistical analysis

To study the effect of ACC deaminase and exopolysaccharide producing bacteria against heat stress on maize plants, a pot experiment was conducted in the growth chamber for 30 days. A total of 32 pots were used for the selected Maize plant; each pot contains 385.27 g of sieved sterilized soil, and 4 surface-sterilized seeds in each of the 15 cm long pots. After that, the untreated soil was considered as a control. Untreated pots were grown at optimum temperature, and the heat-stressed pots containing maize seeds were grown at 45 °C. After 15 days of germination, the temperature increases from 35 to 45 °C with 3 h time interval, and symptoms appear on the maize plants at 45 °C. After 30 days, the plants were harvested and used for further analysis. A total of 4 treatments (T1, T2, T3 and control) in comparison were structured in the completely randomized design (CRD) with 3 replications for each treatment. Statistical analysis was conducted using analysis of variance (2-way ANOVA). Duncan's multiple range test (DMRT) at $P \leq 0.05$ was performed to check the significant difference among the treatments.

Table 1 Treatments used for the experiments.

Treatments	Heat stress	Non Heat stress
C	Control	Control
T1	B1	B1
T2	B2	B2
T3	B1 + B2	B1 + B2

Photosynthetic pigments estimation

For chlorophyll a, chlorophyll b, and carotenoids estimation, 1 g leaf sample from the control and each treated plant was used. These samples were surface sterilized and ground with the help of pestle and mortar in 10 mL of 80 % acetone. The estimation was done using the method of [33]. The equation below was used as described by [33].

Chlorophyll a = 1.07 (OD 663 nm) – 0.09 (OD 645 nm) (1)
Chlorophyll b = 1.77 (OD 645 nm) – 0.28 OD 663 nm)
Carotenoids = (OD 470 nm)×4

Electrolyte leakage

For electrolyte leakage (EL), the determination was done as described by [34].

Malondialdehyde (MDA) or Lipid peroxidation activity

The Malondialdehyde activity was determined using the method of [35]. The equivalents of Malondialdehyde was calculated by using the following formula [36]:

MDA = 6.45 (A532 - A600) – 0.56 A440. (2)

Relative water content

For measuring the relative water content (RWC), approximately 20 leaves from each sample of the treated plants were used, after harvesting the plants, they were kept in double-distilled water for 4 h and turgid weight (TW) was calculated. The leaves were dried in an oven (80 °C) for 1 day, and the dry

weight (DW) values were obtained. RWC was measured using the method of [37]. The following formula was used to calculate the relative water content;

Relative water content (RWC) = (FW) – (DW)/(TW) – (DW)×100 (3)

Antioxidant activities

The SOD activity was determined by following the method of [38]. There following formula was used for the calculation of SOD activity:

R1 = O.D of reference (4)
R2 = O.D of blank
R3 = O.D of sample
R4 = R3 – R2
Final = R4/A

The method of [39] was used to determine peroxidase activity. The catalase activity was determined by using the method of [40] with some modifications.

Plant proline content

For the determination of maize proline content, the methods of [41], and [42] were used. From the standard curve, the proline concentration was determined based on the fresh weight and calculated using the formula below:

Proline µg/g = k value×dilution factor×absorbance/sample weight (5)
Values

K= 17.52
Dilution factor = 2
Sample weight = 0.1 g

Results and discussion

Results

Effects of ACC deaminase, Exopolysaccharides (EPS) producer bacterial strains and its consortium on maize plant under heat stress

After characterization, 19 out of the 40 microbial strains show positive results against ACC Deaminase activity under normal conditions but the seven strains range from 1.08 - 1.15 µM/mg protein/h under heat stress. One strain (New*) showed the highest with 1.6 µM/mg protein/h (**Figure 1**).

For the screening of microbial isolates against exopolysaccharides production, the D4c strain showed positive results under heat stress. All the bacterial strains have shown colony colour of white turbid. Based on the bacterial strains' dry weight, the isolate (Dp4c) was found to be the highest with 3.12 mg/mL, while the least strain was found to be S-101 with 2.42 mg/mL as presented in **Table 1**.

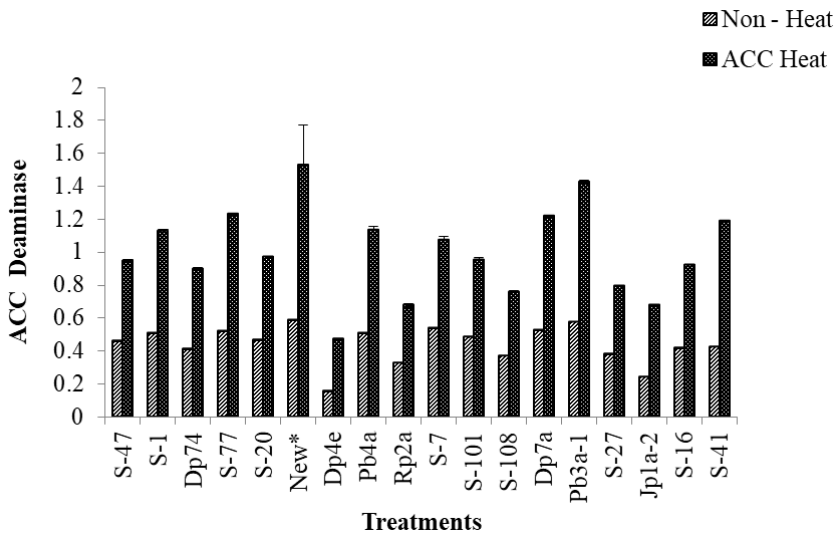


Figure 1 Quantification of ACC deaminase (µM/mg) producing rhizobacteria under normal and heat stress conditions.

Table 2 Production of exopolysaccharides (EPS) under heat stress condition.

Sr. No.	Bacterial strains	Colour of colony	Dry material weight (mg/mL)
1	Dp4c	White Turbid	3.1191
2	Pb3a-1	White Turbid	2.8801
3	S-77	White Turbid	3.051
4	S-101	White Turbid	2.418

Physiological parameters

For physiological parameters, the photosynthetic pigments chlorophyll a (Chl a) and its consortium improved the content. For Chl a, the highest values were observed in T1 with 0.59, and 0.78 µg/mL for heat and non-heat, respectively. The lowest values (control) were 0.21, and 0.42 µg/mL for heat and non-heat, respectively. For Chl b, the highest values were observed in T1 with 0.57, and 0.67 µg/mL for heat and non-heat, respectively. The lowest values (control) were 0.25, and 1.36 µg/mL for heat and non-heat, respectively. Based on the Chl b, the isolate New* was found to have a more positive effect. Carotenoid was also found to have more positive effects with the highest values of 2.53, and 4.14 µg/mL for heat and non-heat, respectively for T1 (**Figures 2**(**A**) - **2**(**C**)). The control was having the lowest values of 1.28, and 2.34 µg/mL for heat and non-heat, respectively.

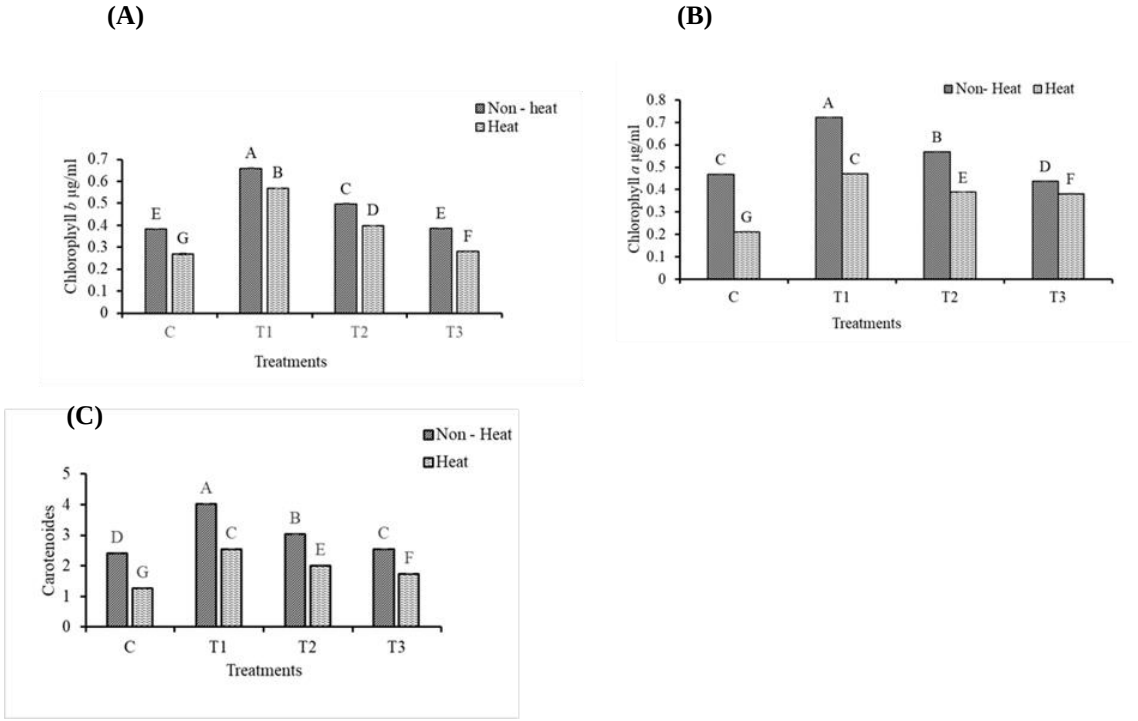


Figure 2 Photosynthetic pigments under normal and heat stress: (A) Chlorophyll b, (B) Chlorophyll a and (C) Carotenoids by using 2-way ANOVA.

EL plays a vital role in reducing MSI, with T3 having the highest values of 80.73 and 20.57 % for heat and non-heat, respectively. The control had 82.15, and 38.75 % for heat and non-heat, respectively (**Figure 3**(**A**)). For MDA, the isolate New* showed an increase under heat stress with T1 having the highest values of 2.29, and 1.68 $nmolg^{-1}FW$ for heat and non-heat, respectively (**Figure 3**(**B**)). The control had 0.68 and 0.17 $nmolg^{-1}FW$ for heat and non-heat, respectively.

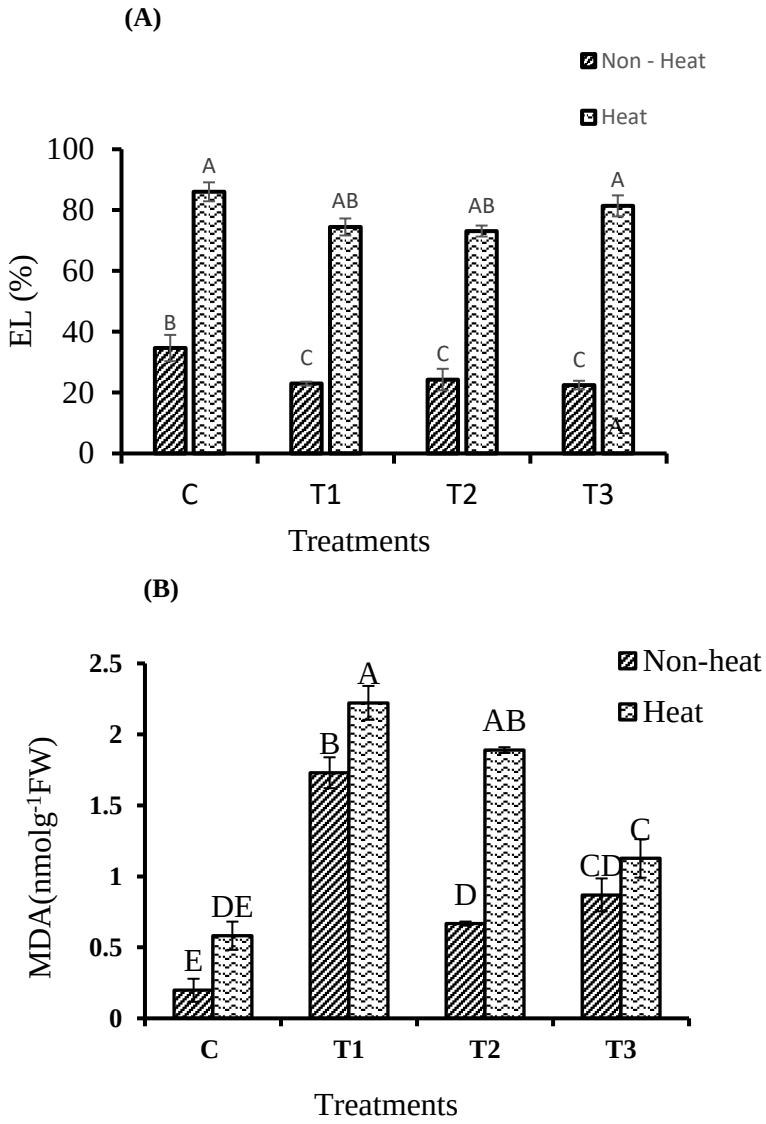


Figure 3 (A) EL of maize seedlings under normal and heat stress and (B) Malondialdehyde activity of maize seedlings under normal and heat stress by using 2-way ANOVA. The bars for the figures represent the standard errors.

In heat stress, the membrane stability index (MSI) was found to be decreased with T3 having the values of 81.90, and 23.5 % for heat and non-heat, respectively (**Figure 4**). The control had 82.46 and 37.74 % for heat and non-heat, respectivly.

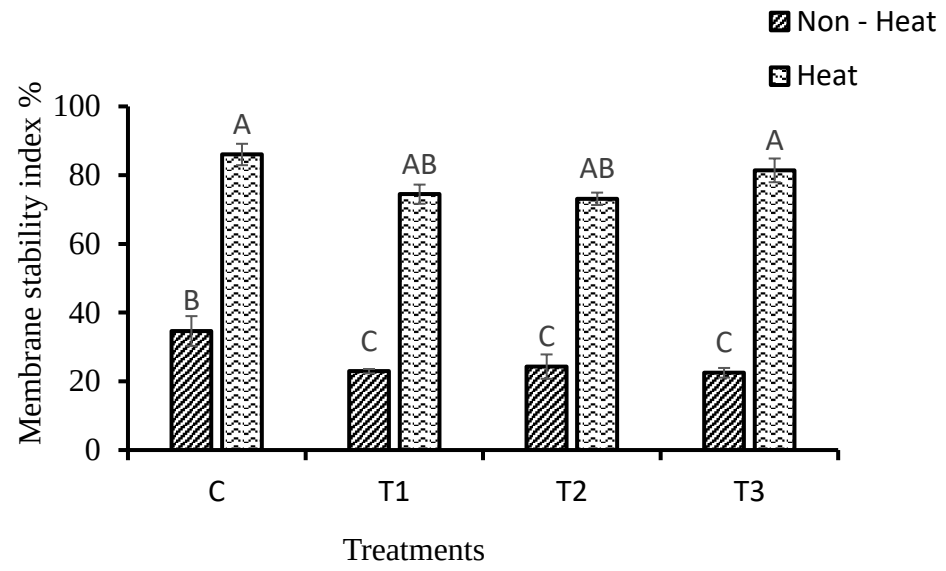


Figure 4 Membrane stability index of maize seedlings under normal and heat stress by using 2-way ANOVA. The bars for the figures represent the standard errors.

Soluble sugar content has been improved in both treatments with T1 having the highest value of 4,000 g/mol for heat stress and 5,700 g/mL for non-heat stress (**Figure 5**). The control had 900 and 2,300 g/mol for heat and non-heat, respectively.

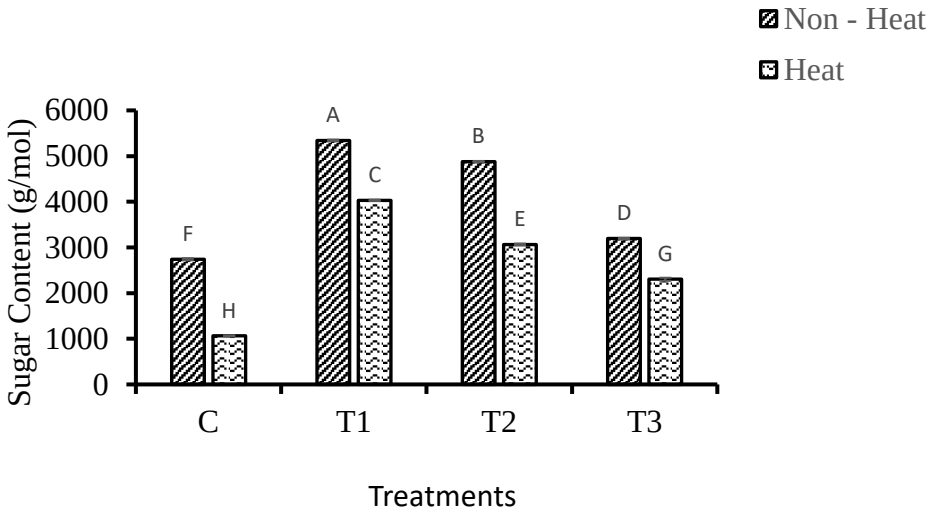


Figure 5 The membrane stability index of maize seedlings under normal and heat stress by using 2-way ANOVA. The bars for the figures represent the standard errors.

Relative water content under heat was enhanced by ACC deaminase bacterial strains. Exopolysaccharides producing strains improve the plant RWC than the consortium. The relative water content of the maize plant was increased in T1 by 300 and 200 % for non-heat and heat, respectively, while the control had 220 and 200 % for non-heat and heat, respectively (**Figure 6**).

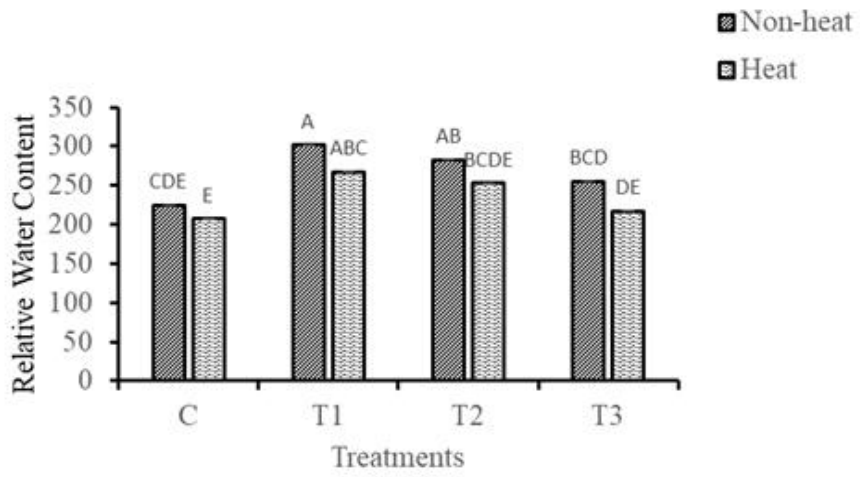
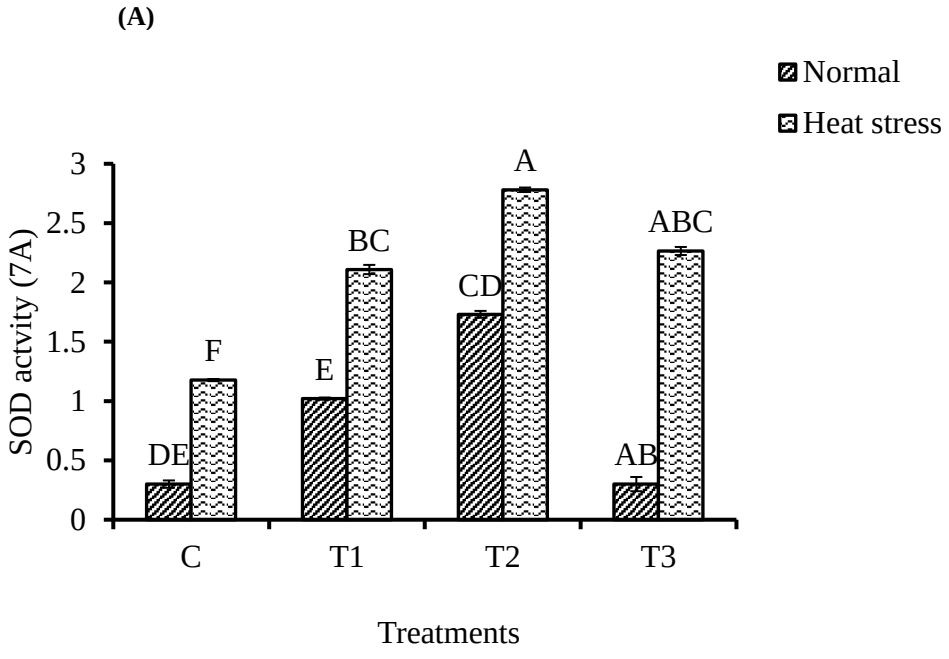


Figure 6 Relative water content of maize seedlings under normal and heat stress conditions by using 2-way ANOVA. The bars for the figures represent the standard errors.

Antioxidant activities

Antioxidant actives like superoxide dismutase (SOD) and peroxidase (POD) were determined in both heat and non-heat maize seedlings. T2 had the highest values of SOD with 1.77, and 2.93 for non-heat and heat, respectively. The control had 0.20 and 1.10 for non-heat and heat, respectively (**Figure 7**(**A**)). T1 had the highest POD values with 3.61, and 5.97 for non-heat and heat, respectively. The control had 1.21 and 2.10 for non-heat and heat, respectively (**Figure 7**(**B**)).



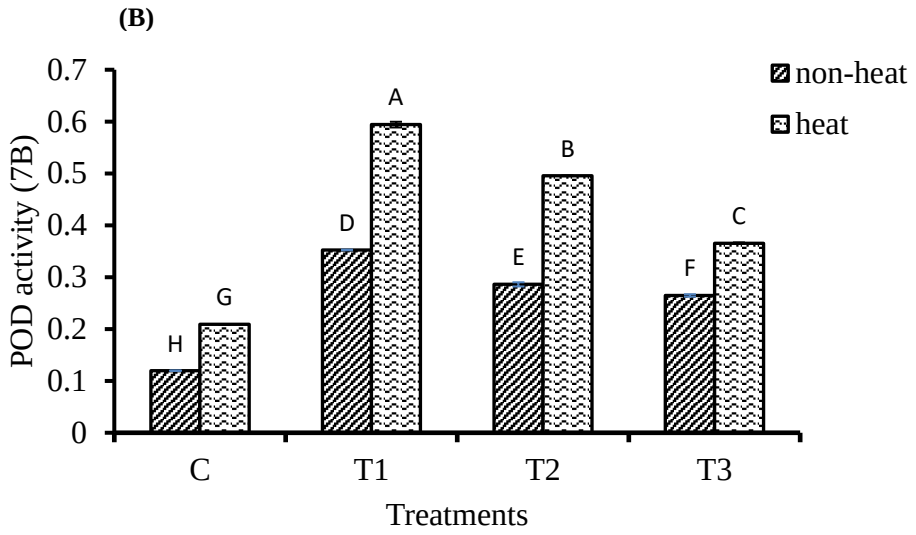


Figure 7 (A) Superoxide dismutase (SOD) activity for maize seedlings under normal and heat stress and (B) Peroxidase (POD) activity for maize seedlings under normal and heat stress by using 2-way ANOVA. The bars for the figures represent the standard errors.

In proline content, there was an increased by having the highest values of 58.98, and 72.30 µg/g for non-heat and heat, respectively. The control was having 17.51, and 36.00 µg/g for non-heat and heat, respectively (**Figure 8**). T1 was having the highest values of catalase with 2.50, and 1.78 for heat and non-heat, respectively. The control was having 1.23, and 0.16 for heat and non-heat, respectively (**Figure 9**).

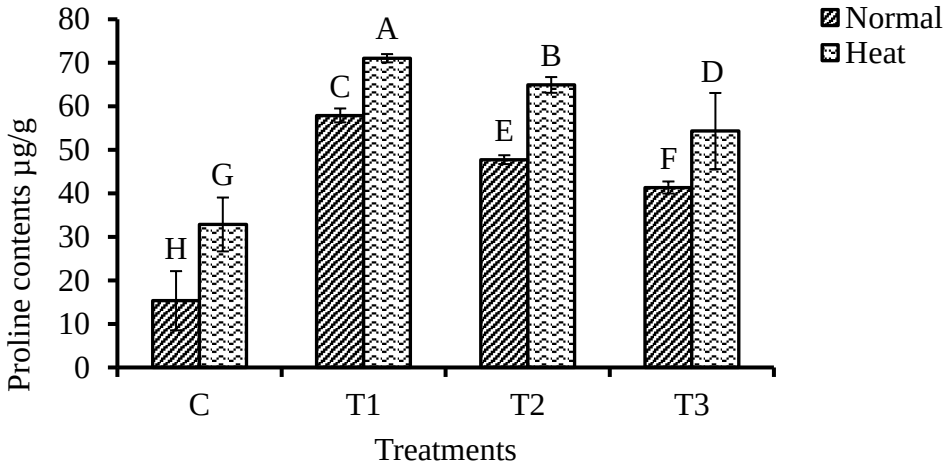


Figure 8 Proline contents for maize seedlings under normal and heat stress by using 2-way ANOVA. The bars for the figures represent the standard errors.

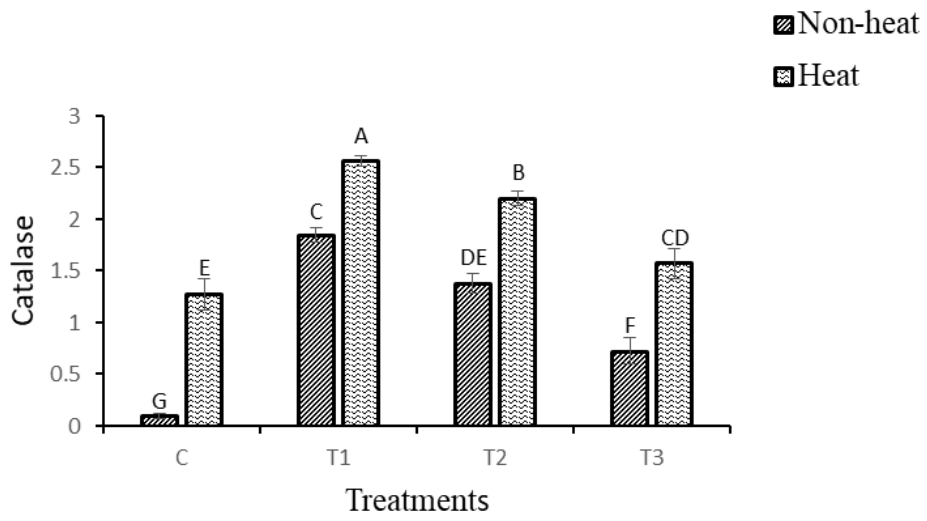


Figure 9 Catalase contents for maize seedlings under normal and heat stress by using 2-way ANOVA. The bars for the figures represent the standard errors.

Discussion

Based on the findings of this study, the results showed that the maize treated with plant growth-promoting bacteria has ACC deaminase activity and exopolysaccharides, which differs from the results of the uninoculated plants (control) under heat stress. Their growth and O.D values showed that these strains were heat tolerant as reported by [43].

Ethylene and indole acetic acid are known to control different processes involving differentiation, cell division, control different stages of growth like seed germination, formation of vascular bundles, and ACC deaminase production through root colonizers which improve the growth of the plants [44].

The current study showed that ACC deaminase-producing bacterial application on the seeds improved the germination of the seeds as compared to the un-inoculated control, a similar plant growth improvement has been reported by [44]. Furthermore, in sunflowers, the exopolysaccharides producing rhizobacteria improved the plant biomass, dry and fresh weight, root and shoot length as compared to the stressed control (un-inoculated), it also improved water uptake and the nitrogen content of the plants [45].

The exopolysaccharides producing rhizobacteria improved the soil aggregation in the root surface of wheat and ameliorated plants' growth under the stress condition [46]. In this study, exopolysaccharides producing rhizobacteria improved plant growth under stress conditions as compared to uninoculated stressed control. Upadhyay *et al*. [47] reported that the exopolysaccharides producing rhizobacteria help promote plant growth under stress conditions by reducing the level of Na^+ present for plants uptake. Kaushal and Wani [48] reported that the EPS-producing plant growth-promoting rhizobacteria have significantly improved the plant growth under drought stress and salinity stress. Dodd *et al*. [49] stated that the EPS producing plant growth-promoting rhizobacteria have hydrophilic biofilms which colonize the plants' roots and protect the plant against water deficiency.

The plant growth-promoting rhizospheric bacteria have several influences on the plant hormones, by improving hormones level in the shoot, enhancing the growth of the plant and its physiological processes under stress conditions [50].

The degradation of chlorophyll content occurred due to oxidative stress as ROS directly affects the membrane stability, resulting in a decline in the rate of photosynthesis [51]. T1 (New* bacterial) strain with ACC deaminase activity overcomes the deficiency of the chlorophyll contents under heat stress. Exopolysaccharides producing bacteria had also lessened the chlorophyll deficiency in the plant. Therefore, chlorophyll content recovery is due to the presence of ACC deaminase activity in the New* bacterial strains, which overcome the fatal effects of heat stress through low ethylene production, the same explanation was given by [52], who compared the chlorophyll content under drought stress by bacterial inoculation and without bacterial inoculation.

It was reported that the ACC deaminase producing PGPR increases the level of antioxidants in plants [53]. In the current study under heat stress at 45 °C in maize plants, the enzymatic activity like

catalase, superoxide dismutase, and peroxidase increased significantly, the same results were reported by [54], who explained that under abiotic stresses, the enzymatic activity was increased. Maize plants inoculated with NEW* bacterial strains showed a significant increase in the enzymatic activities and enhanced plant growth. They increased peroxidase activity (64.8 %), the Dp4c strains increased (57.8 %), and their consortium increase was (42.9 %) significantly T1 > T2 > T3. Similarly, the SOD activity was increased (85.7 %) by the implementation of New* strain, (84 %) by Dp4c and consortium showed (81 %) increase, T1 > T2 > T3. The catalase activity was also increased under heat stress as compared to the un-inoculated control (50.27 %) by New*, (42.10 %) by Dp4c and New*+Dp4c (consortium) showed an increase of 30.97 %. The inoculated bacterial efficacy of enzymatic activities in controlling possible oxidative damage is similar to the findings of [55,56]. Rhizospheric microorganisms like *Azotobacter, Pseudomonas, Klebsiella,* etc. enhance plant growth under abiotic and biotic stresses [56-59].

Conclusions

The PGPR can produce ACC deaminase enzyme that plays a significant role in protecting maize plants from various environmental stresses. In this study, ACC deaminase showed a significant effect (30-40 %) under heat stress on maize plants, the exopolysaccharides producing rhizobacteria also showed a positive result as compared to the control. The reason behind heat stress tolerance is that the PGPR can lower the ethylene level under stress conditions. This study showed that the application of ACC deaminase-producing bacteria helped to protect the plants from several abiotic stresses. In the future, the PGPB application would help immensely by increasing food sustainability.

Acknowledgments

The authors are grateful to the supporting and technical staff of the Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan for providing the necessary support during this research work.

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