Research article

# Induction of tolerance to salinity in wheat genotypes by plant growth promoting endophytes: Involvement of ACC deaminase and antioxidant enzymes

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ARTICLE INFO

ABSTRACT

Plant growth-promoting endophytes (PGPEs) can colonize the internal tissues of plants and are capable of promoting plant growth. These bacteria can improve plant tolerance against various biotic and abiotic stresses via the expression of antioxidant enzymes and the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Two salt-tolerant PGPEs, *Kocuria rhizophila*: KF875448 (14ASP) and *Cronobacter sakazakii*: KM042090 (OF115), with ACC deaminase activity were investigated for their potential to ameliorate plant salinity stress. The wheat varieties Pasban 90 and Khirman were subjected to two levels of salt stress (80 and 160 mM NaCl) under greenhouse conditions by using a completely randomized design. Analyses of plant growth parameters, antioxidant enzyme activities, chlorophyll and plant mineral contents were conducted to investigate the stress tolerance induced by the PGPEs. The ACC utilization by the PGPEs directly relates to the promotion of plant growth due to the lowering of excess ethylene production under salt stress. High levels of NaCl exhibited negative effects in both varieties. However, inoculation with PGPEs increased the morphological traits and antioxidant activities of the plants while decreasing the Na$^+$ contents in all treatments compared to unin-oculated treatment. Wheat variety Pasban 90 was more tolerant than Khirman in to salt stress in all the measured morphological and biochemical parameters, while the bacterial strain OF115 performed significantly better in all morphological and biochemical parameters, such as fresh dry weight, root shoot length, proline and chlorophyll contents, compared to strain 14ASP. The K$^+$/Na$^+$ ratio in the tissues of bacterial treated plants was higher than the control, probably in order to maintain the nutrient balance. The results of our study revealed that the inoculation of plants by ACC deaminase-producing PGPEs is a potential tool for the enhancement of plant growth and stress tolerance. Moreover, endophytic bacteria allied with host plants are capable of enduring high saline conditions and can interact with plants in a very efficient way.

## 1. Introduction

Salinity stress is one of the major abiotic stresses directly responsible for stunted plant growth that eventually lead to reduced crop production. More than 6% of total land (approx. 800 million hectares) that can potentially be used for agricultural purposes is severely affected by saline conditions in arid and semi-arid regions worldwide (Sarkar et al., 2018). Elevated soil salinity not only has drastic effects on the physical and chemical properties of soil but also suppresses the growth and assortment of soil microbiota, nematodes and crop plants (Sairam et al., 2002).

Wheat (*Triticum aestivum* L.) is a major cereal crop of Pakistan, mainly cultivated in rain-fed areas (Wahid, 2006). The various growth stages of wheat are simultaneously affected by salinity and drought stresses (Zahir et al., 2009). These stresses result in stunted growth and lower biomass production due to the shifting of plant metabolism

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towards stress management and adjusting osmotic irregularities (Borrelli et al., 2018). Under high salinity, the increased sodium ($Na^+$) and chloride ($Cl^-$) concentrations lead to an ionic imbalance in plant tissues, which results in reduced nutrient uptake (Parihar et al., 2015). Furthermore, a decrease in the uptake of potassium ($K^+$) and calcium ($Ca^{++}$), the inactivation of various enzymes, the inhibition of protein synthesis, a slow rate of photosynthesis, the burning of leaves and the stem, and early leaf senescence are all noticeable symptoms during salt stress (Munns, 2002). High salinity also results in the utilization of 1-aminocyclopropane-1-carboxylic acid (ACC), an ethylene precursor, which results in elevated levels of ethylene production (S. Ali et al., 2014). At a low (10 mg/L) concentration, ethylene mediates a wide range of essential plant responses (Nadeem et al., 2010). However, elevated levels of ethylene inhibit root and shoot elongation, leaf expansion, and overall plant growth, thus promoting epinasty (Glick, 2005). Regulation of elevated production of ethylene may therefore help plants to alleviate these drastic effects and improve agricultural yield even under saline conditions (Cao et al., 2007).

Based on various agronomic and physiological markers, screening for salt tolerant/sensitive wheat lines/cultivars was executed at a seedling growth stage (Habib et al., 2016). In general, salt-tolerant wheat genotypes maintain higher $K^+/Na^+$ ratios under salinity stress compared to salt-sensitive genotypes. Furthermore, elevated levels of proline concentrations were also observed in salt-tolerant wheat genotypes, though the genotypes exhibited reduced chlorophyll contents (Hasan et al., 2015). It was noted that the acclimation treatment (2.09 dS m$^{-1}$ EC) of wheat plants at vegetative stages suppresses the inhibitory effects of high salinity stress via osmotic adjustment and stable membrane integrity, improved photosynthetic ability, and associated biomass and grain yield relative to non-acclimated plants (Maswada et al., 2018). A natural way to increase salt tolerance and yield of the crops is to use salt-tolerant microbes as a bio-augmentation procedure (Khan et al., 2016).

A group of bacteria that enhances plant growth and induces systemic resistance against both biotic and abiotic stresses is known as Plant Growth Promoting Endophytes (PGPEs) (Sofo et al., 2015). PGPEs are therefore eco-friendly attractive alternatives to chemical-based agriculture. The high concentration of stress induced due to ethylene is alleviated by ACC deaminase producing PGPEs by degrading ACC into ammonia and α-ketobutyrate. The reaction provides both nitrogen and the energy needed for the proper growth of the stressed plants (S.Z. Ali et al., 2014; Glick, 2014). Plants inoculated with ACC deaminase-producing bacteria exhibited alleviated ethylene-induced growth repression while displaying increased shoot and root lengths (Sarkar et al., 2018), possibly by positively regulating the production of proline, antioxidant enzymes, siderophores, hydrogen cyanide (HCN), and IAA and the solubilization of phosphate, all resulting in increased chlorophyll content, plant growth and subsequent crop yield (Sarkar et al., 2018).

For example, ACC deaminase-producing *Enterobacter cloacae* enhance plant growth, biomass and antioxidant enzyme activity in bio-augmented *T. aestivum* under salt and high temperature stress by efficiently inhibiting ethylene production (Cheng et al., 2007; Shaharoona et al., 2007; Ahmad et al., 2011; Barra et al., 2016). It was observed that phytohormones (auxin and abscisic acid) producing *Bacillus amyloliquefaciens* RWL-1 induced salinity stress tolerance in *Oryza sativa* and wheat (Ramadoss et al., 2013; Shahzad et al., 2013). *Zhihengliuella halotolerans*, *Staphylococcus succinus*, *Bacillus gibsonii*, *Oceanobacillus oncorhynchi*, *Halomonas* sp and *Thalassobacillus* sp. significantly promoted wheat plant growth up to 200 mM NaCl stress. Multiple studies revealed the positive impacts of IAA and ACC deaminase-producing PGPEs on reducing salt stress in wheat, rice, ryegrass, and the medicinal plant *Limonium sinense* (Bal et al., 2012).

In rice and cucumber inoculated with ACC deaminase-producing PGPR, the chlorophyll content was less compromised than in the uninoculated plants under salt stress (Bal et al., 2012). A reduced

chlorophyll content is an indicator that a plant is undergoing stress, and vice versa (Habib et al., 2016; Singh et al., 2015), and negatively impacts the photosynthetic rate. Reduced photosynthetic rates in plants under salinity stress are correlated with excessive concentrations of accumulated $Na^+$ and/or $Cl^-$ and reduced water potential in the chloroplasts and chlorophyll, which directly impact plant health (Parihar et al., 2015). Furthermore, chlorophyll content is damaged by the generation of excessive reactive oxygen species (ROS) in response to salinity stress.

To protect the plant from oxidative damage, however, plants produce antioxidant enzymes, which quench excessive ROS. These enzymes include superoxide dismutase (SOD), peroxidase (POD), polyphenyl oxidases (PPOs) and catalase (CAT) (Habib et al., 2016; Singh et al., 2015). Additionally, PGPEs can produce antioxidant enzymes such as PODs, SODs, CATs, and PPOs, which scavenge excess ROS generated during salinity stress in plants (Han and Lee, 2005). Additionally, the efficient use of PGPEs can lower the use of chemical fertilizers for the sustainable management of salt-stressed areas and can lead to an increase in crop production. This phenomenon is based on the fact that PGPEs enhance root colonization and nutrients uptake, alter root structure and size, maintain osmotic balance, limit $Na^+$ uptake, and regulate nitrogen metabolism (Compant et al., 2005; Forni et al., 2017). PGPEs, therefore, improve plant growth by evading different environmental stresses and can serve as a promising alternative to other costly techniques (Pastor-Bueis et al., 2017).

The production of antioxidant enzymes and osmo-protectants by PGPEs induce various physiological changes, such as alterations in total protein, IAA concentration, and sugar and ethylene contents, which all culminate in induced systemic tolerance (IST) in plants (Yang et al., 2009; Sarkar et al., 2018). PGPEs improve the morphological traits of germ inability, seedling vigor index, root and shoot lengths, and fresh and dry biomass (Bal et al., 2012).

Wheat is a staple food worldwide, including in Pakistan. Wheat faces various biotic and abiotic stresses, which severely compromise the wheat crop productivity. Among the abiotic stresses, high salinity stress has a strongly negative effect on crop production at different developmental stages under field conditions. The use of PGPEs for ameliorating salinity stress in plants is a more eco-friendly, highly culturally feasible, and relatively straight-forward and quick approach compared with conventional breeding, introgression, or recombinant DNA technology, for the engineering of salt-tolerant gene(s) in wheat. The PGPEs isolated from *Oxalis corniculata* have been reported as abiotic stress reducers. *K. rhizophila* was reported as a biosorbent of Cd and Cr, and this species can remove metal ions from aqueous solutions (Haq et al., 2016). Heavy metal-resistant bacteria increase plant growth and enhance the accumulation of heavy metals in plant organs with the co-application of ethylenediamine tetraacetic acid (EDTA) (Ali et al., 2018; Kotrba et al., 1999).

In the present study, both *K. rhizophila* (14ASP) and *C. sakazakii* (OF115), which also produce ACC deaminase, were evaluated against two wheat genotypes, Pasban 90 and Khirman, to ameliorate salinity stress and the associated parameters under greenhouse conditions. The *in vitro* screening of these endophytes for biochemical characterization and plant growth promoting activities was performed.

## 2. Materials and methods

Bacterial strain 14ASP was initially isolated from *Oxalis corniculata* (Mufti et al., 2015), while OF115 was isolated from *Olea ferruginea* Royle (Plant-Microbe Interaction lab, Quaid-i-Azam University, Islamabad 45320, Pakistan). Both of the strains (14ASP and OF115) were deposited to National Center for Biotechnology Information (NCBI) with accession numbers KF875448 and KM042090 respectively.

## 2.1. Salt tolerance assay

The salt tolerance of two halobiont bacterial strains, 14ASP and OF115, was estimated on the basis of the population density of these strains at different concentrations of NaCl [(ranging from 0% 5%, 10%, and 15% (w/v)] in LB medium. Sterilized flasks containing 25 mL LB medium with different NaCl concentrations were used to analyze the bacteria. The LB medium was inoculated with 10 µl of freshly prepared bacterial broth of both strains and incubated at 28 ± 2 °C and 120 rpm in a shaking incubator. Ten ml of sterilized broth with both NaCl concentrations was incubated as an uninoculated control. After 24 h of incubation, the optical density of the culture was measured at $\lambda = 600$ nm using a spectrophotometer (Agilent 8453 UV–visible Spectroscopy System) and adjusted to 0.5 to obtain a uniform population of bacteria [$10^8$–$10^9$ colony-forming units (CFU) mL$^{-1}$] for inoculation.

## 2.2. Bacterial growth conditions and seed inoculations

For seed inoculation, bacterial strains were grown in 250 mL LB broth for 48 h (Sun Gene GmbH, Innova 4430, NJ, USA). Bacterial growth was measured at 600 nm utilizing a spectrophotometer and adjusted to 1 to obtain a uniform population of bacteria [$10^8$–$10^9$ colony-forming units (CFU) mL$^{-1}$]. Seeds were surface-sterilized with 75% ethanol for 5 min, followed by HgCl$_2$ (0.1%) for 1 min and then washed 3–5 times with autoclaved distilled water (Ahmad et al., 2016). Seed inoculation was performed by soaking seeds in bacterial suspensions for 3–4 h, while seeds soaked in distilled auto-claved water were used as a control (Adesemoye et al., 2008; Chen et al., 2013).

## 2.3. In vitro screening of K. rhizophila and C. sakazakii for biochemical characterization and plant growth promoting activities

Both strains were further screened for plant growth-promoting activities.

### 2.3.1. Phosphate solubilization activity

Phosphate solubilization on Pikovskaya (PVK) agar plates (10 g/l glucose, 5 g/l calcium phosphate, 5 g/l magnesium chloride, 0.25 g/l magnesium sulphate, 0.2 g/l potassium chloride, 0.1 g/l ammonium sulphate, 15 g/l agar, pH-7) containing insoluble tri-calcium phosphate was conducted. Bacteria were spot inoculated on PVK agar plates and incubated at 28 ± 2 °C for 24-phosphate solubilization (Gupta et al., 1994).

### 2.3.2. Test for indole production

Indole-3-acetic acid production was estimated by colorimetric assay (Loper and Schroth, 1986). A nutrient broth amended with 0.1% DL-tryptophan was inoculated with 500 µl of overnight grown bacterial culture. The culture broth was incubated in the shaker at 180 rpm for 48 h in the dark at 28 ± 2 °C. The bacterial culture was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant (1 ml) was mixed with 4 mL Salkowski reagent and the appearance of a pink color indicated the production of IAA (Gordon and Weber, 1951). The absorbance of the final pink color solution was measured after 30 min at 535 nm in UV/Visible spectrophotometer.

### 2.3.3. Ammonia production by PGPEs

The production of ammonia was analyzed by following the protocol of Ahmad et al. (2005). Bacteria were inoculated in specific media for ammonia production (Peptone water 10 g/L, NaCl 5 g/L). Tubes were placed in a shaking incubator for three days at 37 °C. After incubation, 3–4 drops of Nessler's reagent was added. The appearance of a yellow to brown color in the plates represents the ammonia production.

### 2.3.4. Catalase enzyme activity

A bacterial colony (24 h old) was mixed with a drop of 3% H$_2$O$_2$ on a glass slide. The appearance of bubbles exhibited the production of the catalase enzyme. (Joseph et al., 2012).

### 2.3.5. Production of siderophore

The screening of bacterial isolates for siderophore production was performed by using CAS (Chrome azurol S) agar media (Schwyn and Neilands, 1987). For the preparation of CAS agar, 60.5 mg of CAS was dissolved in 50 ml of distilled water. Furthermore, 10 mL of Fe$^{+3}$ solution (1 mM FeCl$_3$.6H$_2$O) and 40 mL of HDTMA (Hexadecyl-trimethylammonium bromide) (72.9 mg in 40 ml dH$_2$O) were dissolved in a previously made CAS solution. Fifteen grams of agar was added in the resultant dark blue solution and autoclaved. After the inoculation of the bacteria, plates were placed in an incubator for 7 days at 28 °C. The development of orange zones around the bacterial inoculation revealed positive results for siderophore production.

## 2.4. Screening of K. rhizophila and C. sakazakii for ACC deaminase activity

### 2.4.1. Qualitative assay

Bacterial strains were analyzed for the utilization of ACC as a nitrogen source (Glick et al., 1995). Isolates were grown in 5 mL of TSB medium at 28 °C for 24 h with continuous shaking at 120 rpm. Then, the cells were centrifuged at 3000 g for 5 min, washed twice and re-suspended in 0.1 M Tris-HCl (pH 7.5). Cultures were then spot inoculated on petri plates containing DF media (Dworkin and Foster, 1958) (supplemented with and without ACC). Plates with ammonium sulphate were used as a positive control. After three days of incubation at 28 ± 2 °C, the growth on ACC-supplemented plates was compared to positive and negative controls.

### 2.4.2. Quantification assay

After confirming the ability of the isolates to utilize ACC as a nitrogen source, a quantification assay was performed. Late log phase bacterial cultures were used in this assay. After washing in 0.1 M Tris HCl, the pellet was suspended in a DF medium enriched with 3 mM ACC with or without salt stress. Samples were then incubated for 72 h. These induced bacterial cultures were further used for the quantification of ACC by measuring the alpha ketobutyric acid produced by the cleavage of ACC by ACC deaminase (Penrose and Glick, 2003).

## 2.5. Protein estimation of bacteria

For specific enzyme activity, the concentration of protein in labialized cells was determined according to (Bradford, 1976). A standard for protein calibration curve was set up using Bovine serum albumin (Penrose and Glick, 2003).

## 2.6. Biochemical characterization

Gram staining and biochemical characteristics of *K. rhizophila* and *C. sakazakii* (24 h old culture broth) were tested by microbial identification kits QTS-24. The details of the tests and results are shown in Table 1.

## 2.7. Experimental setup

The soil for this experiment was collected from the National Agricultural Research Centre (Islamabad, Pakistan) located at 33° 43′ 11.9784″ N, 73° 5′ 45.7764″ E (altitude of 518 m above sea level). The electrical conductivity (EC) of the soil was 13.7 dS m$^{-1}$, the pH was 8.5, the soil organic matter (SOM) was 0.78%, the total N was 71.24 ppm, the available P was 1.14 ppm, and the available K was 103 ppm. The soil was clay-like in nature with 27%, 32%, and 45% of clay sand, and silt, respectively. A pot experiment was conducted under semi-control

**Table 1**
Biochemical characterization of *K. rhizophila* and *C. sakazakii*.

| Bacterial strains | ONPG | CIT | MALO | ADH | ODC | TDA | IND | VP | GEL | GLU | MALT | SUC | MANN | ARAB | RHAM | SORB | INOS | MEL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *K. rhizophila* | + | - | - | - | + | + | + | - | - | + | + | + | + | - | - | - | - | - |
| *C. sakazakii* | + | + | + | + | + | + | - | - | - | + | + | - | + | + | - | - | + | + |

ONPG,ortho-nitrophenyl-β-galactoside: CIT, Sodium citrate: MALO, Sodium malonate: ADH, Arginine dihydrolase: ODC, Ornithine decarboxylase: TDA, Tryptophane deaminase: IND, indole: VP, Acetion: GEL, Gelatin hydrolysis: GLU NO3/NO2= Acid from glucose,:MALT, Acid from maltose: SUC, Acid from sucrose: MANN, Acid from mannitol: ARAB, Acid from arabinose: RHAM, Acid from rhamnose: SORB, Acid from sorbitol: INOS, Acid from inositol: MEL, Acid from melibiose

conditions (Temp: 25 °C to 35 ± 3 °C; 10 h photoperiod with 80 μM S$^{-1}$ m$^{-2}$ light intensity; RH: 60 ± 10%). Inoculated and uninoculated wheat seeds were sown in separate pots containing sterilized soil and sand (3:1), which were further thinned to three seedlings after 10 days of germination. Three separate pots were used as replicates for each treatment. Plants were well watered with distilled water to maintain the moisture content in the pots (Khan and Bano, 2016). Salt stress was applied to the plants after 21 days of germination once a day with 30 mM NaCl increments to the plants until reaching final concentrations of 80 and 160 mM NaCl in order to avoid osmotic shock. The excess solution drained from holes in the bottom of pots was occasionally collected. The EC (electrical conductivity) of the leached water was equal to the irrigated saline water. The experiment was performed in triplicate for each treatment. Plants were harvested after 45 days of germination and used as separate replicates.

### 2.8. Measurement of growth parameters

After 45 days of experiment, plants were harvested and the roots were washed with water to remove the debris and soil. The root shoot lengths and fresh dry weight of plants grown under different treatments were measured by using a standard measuring scale.

### 2.9. Determination of relative water content

Determination of fresh biomass, dry biomass, and moisture contents were based on weights calculated before and after oven drying of leaf samples. Leaf relative water content (RWC) was estimated according to (Balestri et al., 2014), and the equation used was:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

### 2.10. Leaf chlorophyll content determination

Leaf chlorophyll content was monitored at third leaf stage before and after applying the salt stress to the plants with the help of a SPAD meter (SPAD-502, Minolta Camera Co., Ltd., Japan). A middle leaf was randomly selected and labeled, and the chlorophyll content of both control and treated leaves were determined both before and after salinity stress.

### 2.11. Analysis of antioxidant activity in wheat leaves

The determination of antioxidant enzymes catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in fresh leaves of wheat was done (Khan et al., 2016).

### 2.12. Analysis of proline accumulation in wheat leaves

Proline accumulation was also determined by following the method of Khan and Bano (2016).

### 2.13. Analysis of minerals in wheat shoots

For the determination of minerals (Na, Ca, Mg and K), the method described by (Khan et al., 2016) was followed. A digestion mixture of HNO3:HClO4 (3:1 v/v) was prepared. The dried ground plant shoots (1 g) was digested with 8 mL of digestion mixture and kept overnight. Then, the digestion flasks containing plants shoots were placed on a hot plate and heated until the brown fumes turned to white. Upon cooling, the digested mixtures were diluted with 40 mL of distilled water and filtered through Whatman No.42 filter paper. The collected filtrates were used for mineral analysis with the aid of an atomic absorption spectrophotometer (AAS) (Spectra AA240 FS,Varian, New Jersey, USA).

### 2.14. Recovery of inoculated bacteria

Inoculated plants were harvested after 45 days, sterilized and cut into small sections. Samples were surface sterilized and homogenized in autoclaved distilled water. The homogenized mixture was plated in nutrient agar plates. Emerging colonies were identified by their morphological characteristics; gram staining and antibiotic resistance of both bacteria were detected by the disc diffusion method. A 0.1 mL bacterial culture [10$^8$ colony forming units (CFU) mL$^{-1}$] was spread on LB agar plates. Meanwhile, antibiotic discs were positioned on the surface of the media and plates were incubated for 24 h at 27 °C. The following antibiotics were used: Erythromycin (15 μg), Rifampicin (5 μg), Tetracycline (30 μg), Cephalothin (30 μg), Clindamycin (2 μg), Lincomycin (15 μg), Streptomycin (10 μg), Kanamycin (30 μg), Ampicillin (10 μg), Spectinomycin (25 μg), Neomycin (10 μg), Penicillin (1 μg), Chloramphenicol (30 μg), Fosfomycin (50 μg) and Gentamicin (10 μg). Plates with 3 discs each were used for each antibiotic combination. The diameter of the inhibition zone was measured after 24 h, and the bacterial strains were classified as resistant (R) (< 10 mm), intermediate (I) (10–15 mm) and susceptible (S) (> 15 mm) (Qin et al., 2014; Ma et al., 2015).

### 2.15. Statistical analysis

The current experiment was arranged in a completely randomized design (CRD) in a factorial scheme (2 × 9). Two cultivars (Khirman and Pasban 90) and nine treatments (control, Seed + 80 mM NaCl, Seed + 160 mM NaCl, Seed + *K. rhizophilla*, Seed + *K. rhizophilla* + 80 mM NaCl, Seed + *K. rhizophilla* +160 mM NaCl, Seed + *C. sakazakii*, Seed + *C. sakazakii* +80 mM NaCl and Seed + *C. sakazakii* +160 mM NaCl) were analyzed. Microsoft Excel software was used to compile all results and form a database. All analysis was run in triplicates, and proper observations were made. The data were subjected to ANOVA by the F test (p ≤ 0.05), using R software (R Core Team). After evaluating the significance of the variables by F test, the means were compared by the Scott-Knott test at 5% probability.

## 3. Results

### 3.1. Biochemical characterization, screening of enzyme assays and PGP traits

*K. rhizophila* and *C. sakazakii*, when analyzed by the microbial identification kits QTS-24, showed positive results for the different tests (Table 1) and were characterized as plant growth promoting bacteria on

the basis of phosphate solubilization (zones up to16 mm and 17 mm), IAA production (0.36 mg/L and 36 mg/L) and ammonia production. The bacteria showed significant catalase activity but did not show any response relative to amylase, HCN, and protease production. *C. sakazakii,* however, displayed positive results for protease and catalase activities. Both strains appeared positive for siderophore production by the presence of orange zones around the colonies. Both bacteria show tolerance against different levels of salinity stress from 5% to 15% NaCl. Isolates were screened for their ability to use ACC as a sole nitrogen source. Both of these species grew well on DF plates with or without a nitrogen source, showing ACC deaminase positive activity. However, the growth on the DF medium without a nitrogen source was lower compared to the positive control and the plates with ACC. *K. rhizophila* and *C. sakazakii* were further assayed for quantitative ACC deaminase activity. A comparison was made both in stressed (15% NaCl) and non-stressed conditions. Both the isolates were able to utilize ACC as nitrogen source with the help of the ACC deaminase enzyme. Higher levels of ACC deaminase activity was recorded in non-stressed conditions compared to salt stressed conditions in *K. rhizophila.* However, in the case of *C. sakazakii*, more enzyme activity was observed in stressed conditions compared to the control (Fig. 1).

### 3.2. Plant growth responses

Growth parameters such as plant biomass and root and shoot lengths were significantly influenced by the inoculation of *K. rhizophila* (T1) and *C. sakazakii* (T2). Decreased growth was observed under saline conditions (i.e., 80 mM and 160 mM NaCl) compared to the respective controls in the reference soil for both the wheat cultivars (Pasban 90 and Khirman) (Table 2). Under both normal and saline conditions, the effects of both endophytic *K. Rhizophila* and *C. sakazakii* on morphological attributes were more pronounced than their respective uninoculated control soils. Inoculation of *K. rhizophila* and *C. sakazakii* enhanced the fresh and dry biomass of Pasban 90 from 25% to 57%, respectively. Similarly, both bacteria increased the fresh and dry biomass of Khirman (50%–80%) as compared to their respective control. Additionally, increase in root and shoot lengths of Pasban 90 and Khirman with the *K. rhizophila* and *C. sakazakii* treatments were observed to be in a range of 10%–27% higher than their respective controls. Remarkable plant growth differences were observed between *K. rhizophila* and *C. sakazakii* inoculated wheat cultivars. Interestingly, Pasban 90 inoculated with *C. sakazakii* showed better results than Khirman inoculated with *K. rhizophila.*
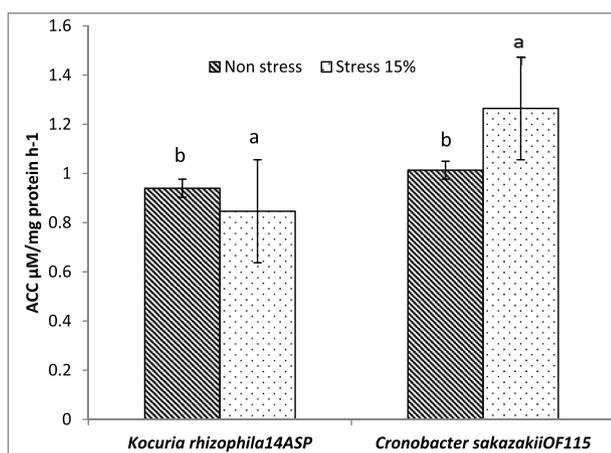


**Fig. 1.** ACC deaminase activity of *K. rhizophila* 14ASP and *C. sakazakii* OF115 under the non-stress (Control conditions and no NaCl) and salinity stress (Nacl 15%) using DF medium. Different lowercase letters above columns indicate a significant difference at P < 0.05 between isolates. Bars represent the error of the mean (n = 3).

### 3.3. Relative water content (RWC)

Uninoculated plants had significantly lower leaf water potential than inoculated plants. For example, there was a 10–15% increase in the water potential of Pasban 90 when inoculated with *K. rhizophila* and *C. sakazakii* as compared to the control. For Khirman, *K. rhizophila* and *C. sakazakii* significantly increased the water potential of the leaves in the studied soil samples by 11%–12% compared with its respective control (Table 3).

### 3.4. Chlorophyll contents

Leaf chlorophyll content significantly decreased with the application of salt stress. However, the application of *K. rhizophila* and *C. sakazakii* enhanced the leaf chlorophyll content of Pasban 90 by 10–17% and that of Khirman by 11%, compared to control (Table 3). Higher chlorophyll content (44.65 SPAD) was noted in Pasban 90 than Khirman when inoculated with *C. sakazakii.*

### 3.5. Antioxidants in fresh leaves

The activities of antioxidants (POD, SOD, and CAT) were evaluated in uninoculated and inoculated plants with different salinity treatments (Table 4). Lower antioxidant activity was observed in uninoculated plant tissues than the inoculated ones, when salinity stress was applied. The activity of POD was observed to be higher in inoculated plants than in uninoculated plants in both wheat varieties. The SOD and CAT activities were higher in both varieties under salt stress when inoculated with *K. rhizophila* and *C. sakazakii* as compared to the uninoculated plants, although the activity of SOD was higher in Pasban 90 than Khirman at 160 mM NaCl. Furthermore, the activities of these antioxidants were higher in Pasban 90 than in Khirman. The overall ratio of antioxidant activities was higher in Pasban 90 than in Khirman. In general, the order of antioxidant activities of the enzymes relative to PGPEs was observed as *C. sakazakii* > *K. rhizophila* > Control.

### 3.6. Proline contents

The proline accumulation in leaves of both wheat varieties was significantly increased in both the inoculated and uninoculated plants under salt stress conditions. The accumulation of proline, however, was regulated more by *C. sakazakii* than *K. rhizophila* in both Khirman and Pasban 90 at 80 and 160 mM NaCl. The increase in proline contents was upto 72% and 83% in Khirman inoculated with *K. rhizophila* and *C. sakazakii,* respectively (Table 4).

Decreased proline contents were observed in the reference soil compared to the wheat plants grown in soils with 80 and 160 mM NaCl.

### 3.7. Analysis of ionic elements

The effects of bacterial inoculation on $Na^+$ and $K^+$ concentration and the $K^+/Na^+$ ratio under various salinity levels was measured (Table 5). Salinity stress significantly decreased the $K^+$, $Mg^{++}$ and $Ca^{++}$ contents and increased the $Na^+$ concentration in uninoculated plant shoots. However, the *K. rhizophila* and *C. sakazakii* inoculation decreased the $Na^+$ concentration and increased the $K^+$, $Mg^{2+}$ and $Ca^{2+}$ contents in the shoots of both Pasban 90 and Khirman under salinity stress. The $K^+/Na^+$ ratio, however, was higher in Pasban 90 than in Khirman at 80 and 160 mM NaCl relative to the reference soil.

Taken together, the F test for the interaction between the factors (species and treatments) did not show any significant difference for chlorophyll before stress, proline, SOD, $Na^+$, $K^+$, $Ca^{++}$ and $Mg^{++}$ (Table 4). Chlorophyll content (without stress), proline, SOD and $Mg^{++}$ exhibited the highest value/best results in cultivar Pasban 90 as opposed to Khirman, while $Na^+$, $K^+$ and $Ca^{++}$ showed the best results in cultivar Khirman rather than Pasban 90.

**Table 2**

Effect of bacterial isolates *K. rhizophilla* and *C. sakazakii* on growth parameter of two wheat varieties (Pasban 90 and Khirman) under various salt and bacterial inoculated treatment.

| Treatments | SL (cm) | | RL (cm) | | FW (g) | | DW(g) | |
|---|---|---|---|---|---|---|---|---|
| | Pasban90 | Khirman | Pasban90 | Khirman | Pasban90 | Khirman | Pasban90 | Khirman |
| Seed (Control) | 25.66 ± 0.57Bcd | 28.66 ± 0.23Ad | 27.13 ± 0.57Ac | 18.66 ± 0.66Bd | 6.04 ± 0.02Ad | 3.71 ± 0.32Be | 0.22 ± 0.00Afg | 0.04 ± 0.06Bbc |
| Seed + 80 mMNaCl | 21.33 ± 0.51Bf | 25.33 ± 0.66Ae | 22.35 ± 0.15Ae | 16.66 ± 0.57Bf | 4.01 ± 0.52Aef | 2.67 ± 0.33Bf | 0.21 ± 0.12Af | 0.03 ± 0.04Bcd |
| Seed + 160 mM NaCl | 18.14 ± 0.28Bg | 23.11 ± 0.57Af | 20.72 ± 0.17Af | 15.90 ± 0.21Bg | 3.15 ± 0.09Af | 2.20 ± 0.06Bf | 0.13 ± 0.01Ah | 0.03 ± 0.33Bcd |
| Seed + K. rhizophilla | 26.10 ± 0.19Bbc | 30.29 ± 0.27Ac | 31.44 ± 0.42Ab | 22.88 ± 0.47Bb | 6.93 ± 0.19Ac4 | .83 ± 0.42Bcd | 0.36 ± 0.41Ab | 0.05 ± 0.15Bb |
| Seed + K. rhizophilla + 80 mMNaCl | 24.33 ± 0.57Bde | 28.70 ± 0.34Acd | 24.88 ± 0.22Ad | 22.10 ± 0.88Bb | 5.67 ± 0.67Ad | 4.14 ± 0.06Bde | 0.25 ± 0.30Ae | 0.04 ± 0.09Bbc |
| Seed + K. rhizophilla +160 mMNaCl | 22.66 ± 0.22Aef | 23.29 ± 0.51Af | 21.33 ± 0.88Aef | 17.69 ± 0.47Be | 4.63 ± 0.22Ae | 3.67 ± 0.33Be | 0.20 ± 0.13Ag | 0.03 ± 0.08Bcd |
| Seed + C. sakazakii | 29.20 ± 0.88Ba | 34.11 ± 0.39Aa | 34.11 ± 0.16Aa | 24.66 ± 0.03Ba | 9.38 ± 0.13Aa | 7.99 ± 0.44Ba | 0.44 ± 0.39Aa | 0.06 ± 0.21Ba |
| Seed + C. sakazakii +80 mMNaCl | 27.44 ± 0.33Bb | 29.60 ± 0.67Acd | 25.29 ± 0.08Ad | 22.28 ± 0.13Bb | 8.37 ± 0.12Ab | 5.87 ± 0.37Bb | 0.32 ± 0.49Ac | 0.04 ± 0.23Bbc |
| Seed + C. sakazakii +160 mMNaCl | 26.66 ± 0.44Bbc | 32.17 ± 0.77Ab | 27.66 ± 0.33Ac | 20.56 ± 0.93Bc | 7.21 ± 0.17Ac | 5.67 ± 0.67Bbc | 0.28 ± 0.02Ad | 0.03 ± 0.11Bcd |

Growth was measured at 45 days after seed germination under different salt concentration regimes. SL–shoot length, RL–root length, FW–fresh weight, DW–dry weight. Averages with matching lower case letters with in the same column in upper case letter with in the same row are not significantly different according to the LSD grouping test ($p \leq 0.05$).

**Table 3**

Influences of *K. rhizophilla* and *C. sakazakii* on chlorophyll contents in leaves under salinity stress.

| Treatments | Chlorophyll before stress | | Chlorophyll after stress | | Relative Water Content | |
|---|---|---|---|---|---|---|
| | Pasban90 | Khirman | Pasban90 | Khirman | Pasban90 | Khirman |
| Seed (Control) | 40.74 ± 0.37Ab | 39.08 ± 0.32Abc | 38.80 ± 0.34Ab | 33.54 ± 0.27Bc | 49.48 ± 0.17Adc | 47.61 ± 0.21Bb |
| Seed + 80 mM NaCl | 37.72 ± 0.32Ac | 35.82 ± 0.70Be | 34.04 ± 0.54Ac | 31.13 ± 0.56Bd | 47.12 ± 0.71Ade | 45.55 ± 0.77Bbc |
| Seed + 160 mM NaCl | 34.87 ± 0.34Ad | 32.77 ± 0.32Bf | 32.37 ± 0.63Ad | 28.44 ± 0.33Be | 46.80 ± 0.20Ade | 40.51 ± 0.37Bd |
| Seed + K. rhizophilla | 41.55 ± 0.68Ab | 39.71 ± 0.59Bb | 38.49 ± 0.49Ab | 36.32 ± 0.21Bb | 54.87 ± 0.29Ab | 53.01 ± 0.57Aa |
| Seed + K. rhizophilla + 80 mMNaCl | 40.37 ± 0.52Ab | 37.89 ± 0.36Bde | 35.14 ± 0.57Ac | 34.14 ± 0.39Ac | 47.95 ± 0.55Ad | 45.71 ± 0.67Bbc |
| Seed + K. rhizophilla +160 mMNaCl | 38.28 ± 0.66Ac | 36.78 ± 0.19Bde | 33.82 ± 0.60Ac | 33.47 ± 0.74Ac | 41.07 ± 0.36Af | 42.85 ± 0.87Ac |
| Seed + C. sakazakii | 44.65 ± 0.68Aa | 44.31 ± 0.65Aa | 41.79 ± 0.47Aa | 38.58 ± 0.30Ba | 58.33 ± 0.68Aa | 54.32 ± 0.17Ba |
| Seed + C. sakazakii +80 mMNaCl | 40.19 ± 0.57Ab | 38.26 ± 0.65Bbcd | 39.56 ± 0.29Ab | 36.29 ± 0.43Bb | 52.27 ± 0.45Abc | 47.95 ± 0.07Bb |
| Seed + C. sakazakii +160 mMNaCl | 41.34 ± 0.67Ab | 36.78 ± 0.23Bde | 39.37 ± 0.25Ab | 34.22 ± 0.41Bc | 51.11 ± 0.57Abc | 44.33 ± 0.44Bbc |

Averages with matching lower case letters with in the same column in upper case letter with in the same row are not significantly different according to the LSD grouping test ($p \leq 0.05$).

## 4. Discussion

The current study describes the efficacy of salt-tolerant PGPEs, the production of ACC deaminase, osmolytes and antioxidant enzyme activity on the growth of wheat plants under various salinity regimes. *In vitro* and *in vivo* screening revealed that wheat plants inoculated with both PGPEs, *K. rhizophila* and *C. sakazakii,* had higher morphological variables (fresh and dry root masses, shoot length, number of leaves), relative water contents, $K^+/Na^+$ ratios, chlorophyll contents and antioxidant activities, inoculation with both PGPEs reduced the drastic effects of salinity stress (Tables 2–5). Enhanced nutrient uptake, plant growth and development by halotolerant bacteria have also been previously reported in wheat, rice, meadows, cucumber, tomato and maize plants (Bal et al., 2012; Nadeem et al., 2014; Akram et al., 2016). These findings suggest that the screening of PGPEs for salt stress tolerance and growth promotion is an effective measure for higher growth and the yield of wheat.

The ethylene produced during salinity stress lowers the seed germination and root development, which hinders plant growth. In the present study, both *K. rhizophila* and *C. sakazakii* displayed the production of ACC deaminase, which cleaved ACC to α-ketobutyrate and ammonia and lowered the drastic effects of ethylene on plant health under salinity stress (Table 2).

Furthermore, these microbes induced salt tolerance and promoted growth in both the Pasban 90 and Khirman wheat varieties. The inoculation of ACC deaminase-producing bacteria *Bacillus cereus* strain Y5, *Bacillus* sp. Y14 and *Bacillus subtilis* strain Y16 improved wheat growth in saline soil (Khan et al., 2017). *Klebsiella* sp. SBP-8 had a significant impact on plant growth under salinity and temperature stresses. The findings in the present study showed a significant increase in the salt-treated plants as a result of PGPEs (*K. rhizophila* and *C. sakazaki*), with an inoculation of wheat similar to previously reported results (Barra et al., 2016). Taken together, these studies indicate the positive effect of PGPEs on plants growth and development under salinity stress.

The PGPEs also have the potential to increase water uptake in saline conditions, thereby alleviating the suppression of photosynthesis under salinity stress (Hashem et al., 2016). Moreover, the chlorophyll contents of wheat were elevated by PGPEs inoculation when treated with 80 and 160 mM NaCl, respectively (Khan et al., 2016). These results are in accordance to the findings of Nadeem et al. (2014) at a low salt concentration (1.46 dS m$^{-1}$). The chlorophyll contents were enhanced at 5 dS m$^{-1}$ by *Pseudomonas putida* (W2) strain, while strain *Pseudomonas fluorescens* (W17) performs efficiently at 10 and 15 dS m$^{-1}$ salt levels. In the present experiment, the relative water content in the salt-treated plants significantly increased in inoculated plants compared to uninoculated plants. The application of ACC deaminase-producing bacteria in plants is associated with abiotic stress tolerance (Chen et al., 2017). The possible underlying mechanisms controlled by these bacteria involve reducing the endogenous ethylene, thus facilitating the uptake of maximum nutrients by enhancing root growth (Shahzad et al., 2013). Furthermore, a combination of PGPEs increased the dry biomass compared to single bacterial inoculation. Thus, applied either alone or in combination, PGPEs positively influence plants for nutrient

**Table 4**
Influence of *K. rhizophila* and *C. sakazakii* on antioxidants (POD, SOD, CAT) production and proline accumulation in the leaves of wheat varieties Pasban 90 and Khirman under salinity stress.

| Treatments | POD ($\mu$molg-1 FW min$^{-1}$) | | SOD ($\mu$molg-1 FW min$^{-1}$) | | CAT ($\mu$molg-1 FW min$^{-1}$) | | Proline ($\mu$molg-1 FW min$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | Pasban90 | Khirman | Pasban90 | Khirman | Pasban90 | Khirman | Pasban90 | Khirman |
| Seed (Control) | 0.40 ± 0.05Ad | 0.40 ± 0.05Ad | 0.32 ± 0.03Ad | 0.29 ± 0.00Ac | 0.62 ± 0.00Ag | 0.62 ± 0.00Ag | 16.11 ± 1.07Ae | 9.81 ± 1.07Be |
| Seed + 80 mM NaCl | 0.78 ± 0.45Acd | 0.58 ± 0.01Aabcd | 1.20 ± 0.26Abcc | 0.39 ± 0.34Aab | 0.73 ± 0.00Ae | 0.68 ± 0.00Be | 18.39 ± 0.61Ad | 16.1 ± 1.07Bd |
| Seed + 160 mM NaCl | 1.00 ± 0.20Abc | 0.80 ± 0.01Aabcd | 1.48 ± 0.25Abc | 0.82 ± 0.33Babc | 0.82 ± 0.00Ac | 0.77 ± 0.00Bc | 31.53 ± 1.21Ad | 24.2 ± 0.61Bb |
| Seed + K. rhizophila | 1.18 ± 0.03Aabc | 0.50 ± 0.00Bbcd | 0.88 ± 0.06Acd | 0.59 ± 0.27Babc | 0.66 ± 0.02Af | 0.63 ± 0.00Bfg | 26.16 ± 0.84Ad | 17.0 ± 0.84Bd |
| Seed + K. rhizophilla + 80 mMNaCl | 1.42 ± 0.24Aa | 0.87 ± 0.00Babc | 1.95 ± 0.54Aab | 0.73 ± 0.36Babc | 0.77 ± 0.00Ad | 0.71 ± 0.00Bd | 37.14 ± 0.81Ac | 23.5 ± 0.84Bbc |
| Seed + K. rhizophilla +160 mMNaCl | 1.44 ± 0.03Aa | 0.95 ± 0.01Ba | 2.41 ± 0.30Aa | 1.09 ± 0.08Bab | 0.87 ± 0.00Ab | 0.81 ± 0.00Bb | 37.84 ± 5.62Ac | 33.6 ± 1.61Ba |
| Seed + C. sakazakii | 1.13 ± 0.17Aabc | 0.47 ± 0.00Bcd | 0.81 ± 0.03Acd | 0.35 ± 0.00Bbc | 0.67 ± 0.00Af | 0.65 ± 0.00Bf | 29.06 ± 0.42Ad | 18.0 ± 0.22Bcd |
| Seed + C. sakazakii +80 mMNaCl | 1.26 ± 0.10Aab | 0.81 ± 0.00Babc | 1.47 ± 0.13Abc | 0.63 ± 0.01Aabc | 0.82 ± 0.00Ac | 0.73 ± 0.00Bd | 45.78 ± 1.01Ab | 36.6 ± 0.29Ba |
| Seed + C. sakazakii +160 mMNaCl | 1.34 ± 0.03Aab | 0.89 ± 0.00Bab | 2.28 ± 0.27Aa | 1.15 ± 0.39Ba | 0.96 ± 0.01Aa | 0.87 ± 0.00Ba | 52.56 ± 0.81Aa | 36.6 ± 0.29Ba |

The effect of NaCl treatments under different salt concentration regimesPOD– peroxidase, SOD– superoxide dismutase, CAT– catalase and Proline–dry weight. Averages with matching lower case letters with in the same column in upper case letter with in the same row are not significantly different according to the LSD grouping test ($p \leq 0.05$).

uptake and subsequent growth.

Enhancing the uptake of essential elements is important for plant growth. The increased $K^+$ uptake helps to mitigate the oxidative stress imposed by higher salinity (Etesami and Beattie, 2018). In the current study, a higher $K^+/Na^+$ ratio in PGPE-inoculated wheat was observed compared to the uninoculated control under salinity stress in both cultivars. The highest $K^+/Na^+$ ratio modulated by *C. sakazakii* was detected at 0 mM NaCl, followed by the ratio at 60 mM NaCl. Wheat yield was inversely proportional to high $Na^+$ concentrations, and it was directly proportional to the high $K^+$ concentrations (Nio et al., 2018). In addition, the inoculation also increased $Ca^{++}$ and $Mg^{++}$concentrations, which exerted a positive impact on wheat growth. These results suggest that PGPEs are important for optimally maintaining the balance of essential ions needed for wheat growth.

Antioxidants are one of the indicators of a plant's tolerance to stresses. Catalase, SOD and POD enzymes are low molecular weight antioxidants produced by plants to confer salt stress tolerance (Xu et al., 2015). A majority plant species, however, do not produce an adequate amount of antioxidants to cope with the drastic effects of salinity stress. The increased enzyme activities of POD, CAT, SOD and proline indicates the mitigation of the oxidative stress caused by high NaCl in maize plants (Akram et al., 2016) Gladiolus plants can tolerate salt stress by increased production of proline, POX and other defense enzymes (Panuccio et al., 2014; Khan et al., 2016). The important role of high concentrations of SOD (1.72), induced by *K. rhizophila* and *C. sakazakii*, in conferring salt tolerance (160 mM NaCl) to both wheat varieties was confirmed in the current study.

Furthermore, proline accumulation was 41.23 when the seeds of Pasban 90 were inoculated with *K. rhizophila* and tested at 80 mM NaCl, and proline accumulation was 44.62 when the Pasban 90 seeds were inoculated with *C. sakazakii* and treated at 160 mM NaCl. The increased amount of proline in Pasban 90 compared to Khirman might be the reason why Pasban 90 is more salt tolerant than Khirman (Table 4), suggesting a role for proline as a marker for salt tolerance (Younesi and Moradi, 2014; Khan et al., 2016). Endophytes can significantly colonize the internal tissues of higher plants without any negative effects, and can also be isolated from the plant tissues by surface-sterilized methods. Various plant growth-promoting endophytic Actinobacteria have been reported from wheat, tomatoes, rice, potatoes and carrots, as well as from some medicinal plants (Coombs and Franco, 2003; Strobel, 2003; Tian et al., 2007; Qin et al., 2009, 2014; Trujillo et al., 2010; Zhao et al., 2011; Li et al., 2012; Rungin et al., 2012; Khan et al., 2016). Thus, PGPEs prime salt tolerance in wheat via regulating cellular antioxidants.

This study opens future directions for researchers to investigate the genetic mechanisms involved in the induction of salt tolerance by PGPEs in wheat and other crop plants. A study on expression profiling of stress-responsive genes in wheat in response to PGPE inoculation might be helpful for understanding the molecular cross-talk between plants and bacterial strains. Treatment with PGPEs stimulates different molecular mechanisms in plants to protect them from soil-borne diseases, to increase salt stress tolerance and plant growth and development. Such mechanisms include the increased production of phytohormones and antifungal metabolites, increased availability of plant nutrients, decreased production of stress-induced ethylene and the induction of systemic resistance (Khan and Bano, 2016). According to previous studies, the regulation of mineral uptake and the increase in the antioxidant enzyme activities may be the two key mechanisms involved in the alleviation of salt stress.

To date, the knowledge regarding the screening and exploration of potential ACC deaminase-producing plant growth-promoting endophytes is scarce (Glick, 2014; Kruasuwan and Thamchaipenet, 2018). Our results demonstrate that endophytic strains have promising PGP attributes and can be used as biofertilizers to promote soil fertility and plant growth, even under saline conditions.

**Table 5**
Influence of *K. rhizophilla* and *C. sakazakii* on Na$^+$, K$^+$,Ca$^{++}$ and Mg$^{++}$ ion concentrations in shoot tissues under salinity stress.

| Treatments | Na$^+$ (mg g$^1$Dw) | | K$^+$ (mg g$^1$Dw) | | Ca$^{++}$ (mg g$^1$ Dw) | | Mg$^{++}$ (mg g$^1$Dw) | |
|---|---|---|---|---|---|---|---|---|
| | Pasban90 | Khirman | Pasban90 | Khirman | Pasban90 | Khirman | Pasban90 | Khirman |
| Seed (Control) | 3.23 ± 0.14Ade | 3.20 ± 0.05Ad | 3.21 ± 0.06Aabc | 3.19 ± 0.04Aab | 3.12 ± 0.12Aa | 3.08 ± 0.01Ba | 2.99 ± 0.06Aab | 2.93 ± 0.10Aab |
| Seed + 80 mMNaCl | 3.71 ± 0.08Aab | 3.68 ± 0.05Aabc | 3.11 ± 0.06Abc | 3.08 ± 0.06Aab | 3.11 ± 0.00Aa | 3.05 ± 0.02Ba | 2.87 ± 0.09Abc | 2.73 ± 0.03Abc |
| Seed + 160 mM NaCl | 3.86 ± 0.21Aa | 3.87 ± 0.03Aa | 3.05 ± 0.04Ac | 3.05 ± 0.06Ab | 3.07 ± 0.05Aa | 3.05 ± 0.06Ba | 2.72 ± 0.05Ac | 2.66 ± 0.03Ac |
| Seed + *K. rhizophila* | 3.19 ± 0.06Ae | 3.18 ± 0.10Ad | 3.28 ± 0.08Aab | 3.23 ± 0.06Ab | 3.17 ± 0.02Aa | 3.14 ± 0.01Ba | 3.09 ± 0.00Aab | 2.94 ± 0.07Aab |
| Seed + *K. rhizophilla* + 80 mMNaCl | 3.50 ± 0.03Abc | 3.56 ± 0.02Abc | 3.18 ± 0.05Aabc | 3.15 ± 0.08Aab | 3.10 ± 0.10Aa | 3.09 ± 0.04Ba | 3.02 ± 0.08Aab | 2.89 ± 0.07Aabc |
| Seed + *K. rhizophilla* +160 mMNaCl | 3.72 ± 0.02Aab | 3.75 ± 0.03Aab | 3.16 ± 0.08Aabc | 3.12 ± 0.06Aab | 3.09 ± 0.04Aa | 3.07 ± 0.03Ba | 2.97 ± 0.03Aab | 2.81 ± 0.10Aabc |
| Seed + *C. sakazakii* | 3.15 ± 0.02Ae | 3.15 ± 0.12Ad | 3.31 ± 0.06Aa | 3.26 ± 0.07Aa | 3.17 ± 0.02Aa | 3.18 ± 0.02Ba | 3.12 ± 0.10Aa | 2.97 ± 0.10Aa |
| Seed + *C. sakazakii* + 80 mMNaCl | 3.44 ± 0.05Acd | 3.47 ± 0.03Ac | 3.20 ± 0.01Aabc | 3.16 ± 0.07Aab | 3.15 ± 0.08Aa | 3.15 ± 0.14Ba | 3.09 ± 0.09Aab | 2.93 ± 0.08Aab |
| Seed + *C. sakazakii* +160 mMNaCl | 3.61 ± 0.06Abc | 3.63 ± 0.03Aabc | 3.18 ± 0.04Aabc | 3.11 ± 0.05Aab | 3.15 ± 0.08Aa | 3.12 ± 0.03Ba | 3.01 ± 0.09Aab | 2.88 ± 0.11Aabc |

Averages with matching lower case letters with in the same column in upper case letter with in the same row are not significantly different according to the LSD grouping test (p ≤ 0.05).

## 5. Conclusion

Under salinity stress, the inoculation of the bacterial endophytes OF115 and 14ASP had a conclusive effect on the augmentation of toxic ions, increased resistance and positive biochemical responses in both wheat genotypes. These findings open the possibility for further exploration of plant-bacteria interactions under salt conditions. However, in both wheat varieties, the best response was observed by the inoculation of both ACC deaminase-producing endophytes. This result might be due to the higher colonizing competence of the bacteria, the reduced production of ethylene and the inhibited influx of toxic ions. However, the wheat variety Pasban 90 inoculated with OF115 exhibited a higher salt-tolerance and more plant growth promotion compared with the variety of Khirman inoculated with 14ASP. Thus, the strain *C. sakazakii*OF115 played a better role in maintaining the K$^+$/Na$^+$ ratio. The SOD, POD, and CAT activities were higher in OF115-inoculated Pasban 90 plants compared to14ASP-inoculated Khirman. Our findings suggest that, for ameliorating salinity stress (80 and 160 mM NaCl) and increasing wheat productivity in saline soils, the application of both *K. rhizophila* and *C. sakazakii* could be a sustainable solution.

### 5.1. Future prospects

Evaluation from the current study suggests that *K. rhizophila* and *C. sakazakii* can potentially be utilized as a promising alternative and environmentally friendly approach to facilitate the growth and salt tolerance of wheat. Moreover, the positive role of these species as bio-inoculants still needs to be investigated under field conditions.

## Contribution

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.03.041.

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