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ORIGINAL PAPER

Inoculating maize (*Zea mays* L.) seeds with halotolerant rhizobacteria from wild halophytes improves physiological and biochemical responses of seedlings to salt stress

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Abstract
Salinity stress is a major environmental factor that poses a significant constraint to plant growth, threatening agricultural productivity and sustainability. This study investigated the potential of halotolerant bacteria, isolated from the rhizosphere of wild halotolerant plants in Turkey's Salt Lake basin, to enhance salt stress tolerance in maize (*Zea mays* L.). The rhizospheres of 17 different wild halotolerant plants were selected for bacterial isolation, resulting in the identification of 22 halotolerant bacteria using 16S rRNA sequence analysis. Among these, 19 isolates were found to possess positive activity for 1-aminocyclopropane-1-carboxylate (ACC) deaminase and nitrogen fixation. When the maize seeds inoculated with these 19 isolates were grown under normal conditions, four isolates—TG-4 (*Halomonas arcis*), TG-8 (*Marinococcus tarigensis*), TG-12 (*Halobacillus dabanensis*), and TG-20 (*Halomonas eurihalina*)–significantly stimulated seedling growth and development. To evaluate the effect of these four isolates on salt tolerance, inoculated seeds were grown under various salt conditions (0.0, 75, 150, and 250 mM NaCl). The responses of plants to salt stress were analyzed by evaluating growth parameters, membrane damage, photosynthetic pigment and proline content, reactive oxygen species and lipid peroxidation levels, and antioxidant enzyme activities. According to the parameters, the results indicated that TG-4, TG-8, and TG-12, in particular, have the potential to function as plant growth-promoting rhizobacteria and effectively enhance salt stress tolerance in the maize seedlings. Overall, this research highlights the potential of halotolerant bacteria to improve salt stress tolerance in maize plants through multifaceted mechanisms, offering valuable insights for sustainable agriculture in saline environments.

Keywords Maize · Rhizosphere · Antioxidant enzyme · Halotolerant bacteria · Halophyte · Salt stress

Introduction

Agricultural production is increasingly failing to meet the nutritional needs of the growing global population, and this gap is expected to widen in the future (Shabaan et al. 2022). Soil salinity, a significant abiotic stress factor limiting crop growth and productivity, results from factors such as rising temperatures, decreasing rainfall, high evaporation rates, deforestation, improper irrigation, and certain agricultural practices (Jha et al. 2019; Corwin 2021; Kaleh et al. 2022). Approximately 833 million hectares (Mha) of soil worldwide are affected by salinity, representing about 8.7% of the Earth's surface. Additionally, 20–50% of irrigated lands globally are salt-affected, significantly impacting agricultural productivity (Ullah et al. 2021). The continuous increase in salt accumulation expands the extent of salt-affected soils, negatively impacting plant growth, yield, and the agricultural economy (Mukhopadhyay et al. 2020; Hernández 2019). High salt levels reduce plant turgor, causing osmotic stress and increased production of reactive oxygen species (ROS). This leads to DNA mutations, protein degradation, membrane damage, and cell death (El Ghazali 2020). Key ROS types include hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide anion (O_2^-), and hydroxyl radical ($OH^·$) (Nadarajah 2020). Plants possess both non-enzymatic (glutathione, ascorbate, α-tocopherol,

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carotenoids) and enzymatic (peroxidase, catalase, superoxide dismutase, glutathione reductase) antioxidants to scavenge ROS under stress (Gill and Tuteja 2010; Nadarajah 2020).

Organisms living in symbiosis with plants, especially certain bacteria, play a crucial role in the enhancement of plant tolerance to biotic and abiotic stresses. Plant growth-promoting rhizobacteria (PGPR) reside around plant roots and support plant growth either directly or indirectly by releasing various chemicals (Belbahri et al. 2017; Aslam and Ali 2018; Kaleh et al. 2022). PGPR enhance tolerance to abiotic stress by increasing phosphorus solubility, synthesizing plant hormones (abscisic acid and indole acetic acid), fixing nitrogen, and inducing the secretion of siderophores (Slama et al. 2019; Kerbab et al. 2021). Halotolerant bacteria, which thrive in high-salt environments, are especially effective in mitigating this stress (Castiglione et al. 2021). For instance, studies have shown that PGPR, such as *Enterobacter asburiae*, *Bacillus aquimaris*, *Bacillus thuringiensis*, *Pseudomonas stutzeri*, *Enterobacter cloacae*, and *Ochrobactrum anthropi*, significantly improve plant yield under saline conditions (Raheem and Ali 2015; Bhise et al. 2017; Li et al. 2019). Additionally, many PGPRs possess nitrogen-fixing capabilities and produce the enzyme 1 aminocyclopropane-1-carboxylate (ACC) deaminase, which suppresses ethylene hormone synthesis by degrading ACC, the precursor of ethylene (Nadeem et al. 2007; Zahir et al. 2009; Shahzad et al. 2013; Zhou et al. 2017). PGPR from the rhizospheric soil of halophytes have been shown to enhance growth parameters of maize under salinity conditions, as well as improve photosynthetic parameters, stress-responsive gene expression, and antioxidant defense systems (Ullah and Bano 2015; El-Esawi et al. 2018).

Plant groups particularly susceptible to salinity stress include glycophytes, such as most cereal crops, legumes, and vegetables, while halophytes, adapted to saline environments, exhibit higher tolerance. Maize (*Zea mays* L.) is the third most widely cultivated cereal crop in the world, following wheat and rice (Ullah and Bano 2015; Li et al. 2019; Riffat and Ahmed 2020). However, maize is highly sensitive to stress factors such as salinity, drought, and high temperatures (Crafts-Brandner and Salvucci 2002; Riffat and Ahmed 2020). Therefore, it is important to develop biotechnologies that increase crop yields and improve salinity problems in saline areas in agriculture (Etesami and Maheshwari 2018). This study proposed that halotolerant rhizobacteria isolated from wild halophytic plants in the Salt Lake basin (Turkey), could enhance salt tolerance in commonly salt-sensitive cultivated plants. For this purpose, halophilic bacteria were isolated from the rhizosphere of some halophytic plants in Salt Lake, and the isolated bacteria were identified through 16S rRNA sequence analysis. Subsequently, these bacteria were applied as maize seed inoculants and their effects on salt stress tolerance of the maize seedlings were evaluated by assessing growth, physiological, and biochemical parameters.

Materials and methods

Collection of halophyte plants

In the Salt Lake basin (38° 32′ 45.2760″ and 44° 11′ 8.1348″), 17 halotolerant plant specimens were collected at 6 different stations during the vegetation period. Root samples (3 replicates, each approximately 5 cm in length) containing rhizosphere soil from each specimen were transferred in sterile tubes (15 mL) containing 10 mL of sterile physiological water (0.9% NaCl) (Tindall 1991). The tubes were transported to the laboratory under refrigerated conditions at + 4 °C within 24 h. After the herbarium of the collected plant specimens, they were transported to the laboratory and their species identification was conducted by a plant systematics expert.

Isolation and purification from root rhizosphere

The plant root samples were gently shaken for 8 h at 4 °C and 90 rpm to facilitate the transition of rhizospheric bacteria into the physiological solution. A 2 mL aliquot of the solution was inoculated onto sterile solid halophilic agar plates using the spread plate method in Petri dishes. They were then incubated at 25 °C for 7 days. The halophilic agar (1 L, pH 7.2) contained 200 g NaCl, 2 g KCl, 1.5 g $CaCl_2$, 20 g $MgSO_4$-$7H_2O$, 3 g tri-sodium citrate, 10 g yeast extract, 7.5 g casein hydrolysate, 36 mg $FeCl_2$-$4H_2O$, 0.36 mg $MnCl_2$-$4H_2O$, and 20 g nutrient agar (Tindall 1991). The resulting bacterial cultures were purified and stored as pure isolates in horizontal agar tubes containing halophilic medium at 4 °C.

Identification of bacteria

We isolated 22 halotolerant bacteria from 17 different plants, identifying them through 16S rRNA gene sequencing. Genomic DNA extraction was carried out using a bacterial genomic DNA isolation kit. For PCR, 150 ng of bacterial genomic DNA was used in a 50 µL reaction containing 1X Taq buffer, 0.2 mM dNTPs, 16S universal primers (27F and 1492R), 1.5 mM $MgCl_2$, and 0.2 U ThermoHot Start Taq polymerase. PCR conditions included an initial denaturation at 95 °C for 5 min, followed by 30 cycles of (94 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s), with a final extension step at 72 °C for 5 min. DNA sequencing utilized the BigDye Cycle Sequencing Kit v3.1 and ABI 3100 XL Genetic Analyzer

(Siddikee et al. 2010). Phylogenetic analysis was performed using Mega software.

Characteristics of isolated bacteria

We tested the bacteria using the Gram reaction test according to Halebian et al. (1981). The presence of ACC deaminase in a specific bacterial strain was determined by culturing it on Dworkin and Foster salt medium (DFSM). To assess the nitrogen fixation abilities of the bacterial isolates, a nitrogen-free medium was used. Any isolates that grew on this medium at the end of the incubation period were considered positive for nitrogen fixation abilities ((Li et al. 2000; Shahzad et al. 2013).

Isolate selection

To determine the most suitable bacterial isolate among the 22 strains for promoting the growth and development of maize plants, we conducted preliminary experiments during the germination stage. The isolates were cultured in Tryptic Soy Broth (TSB) medium to create bacterial inoculum suspensions, which were then applied to maize seeds at a specific concentration. Based on root-shoot length and dry weight tests during germination, four isolates (*Halomonas arcis*, TG4; *Marinococcus tarijensis*, TG8; *Halobacillus dabanensis*, TG12; *Halomonas eurihalina*, TG20) showed a positive impact on maize seedling growth. These selected isolates were then used in pot experiments.

Selected bacterium inoculation and seedling growth

Sterilized maize seeds were immersed in suspensions of four bacterial isolates (*H. arcis* TG4, *M. tarijensis* TG8, *H. dabanensis* TG12, and *H. eurihalina* TG20) for 8 h at room temperature (approximately 25 °C). Sterile distilled water was used as a control. Both inoculated and non-inoculated seeds were sown, ten seeds per pot, with five replicates for each of the four salt media (0.0, 75, 150, and 250 mM NaCl). The experiments were conducted in sterile plastic pots containing a mixture of sterile sand and perlite (3:1). Seed germination and seedling development were carried out under $15{,}000 \pm 100$ lx fluorescent light with an 8/16 h night/day cycle at 25 ± 2 °C for 15 days. The pots were irrigated daily with distilled water until the 9th day, and with 50% Hoagland solution starting on the 10th day. Plant organs (roots and shoots) were harvested on the 15th day for analysis.

Plant growth parameters and element analysis

The root and shoot lengths of the seedlings were measured, and their fresh weights were recorded. The same samples were then dried at 70 °C for 48 h, and their dry weights were determined. To analyze the Na^+ and K^+ content in the seedlings, the method described by Kaçar and İnal (2008) was followed. Electrolyte leakage (EL) was assessed using fresh leaf discs from randomly selected plants by measuring electrolytic conductivity. These discs were washed with sterile pure water to remove any surface electrolytes and placed in tubes containing sterile distilled water. After incubating for 12 h at room temperature, EL (µS/cm) was measured using an EC meter. The same tubes were then autoclaved, and once cooled, EL was measured again. The values were converted to EL% (Bajji et al. 2002).

Determination of proline content

For proline content, 0.5 g of leaf sample was ground in liquid nitrogen and homogenized with 7.5 mL of 3% sulphosalicylic acid. The homogenate was then centrifuged at 6,000xg for 10 min. A 1 mL aliquot of the supernatant was mixed with 1 mL of glacial acetic acid and 1 mL of acid ninhydrin, and the mixture was incubated at 100 °C for 1 h. The reaction was stopped by placing the tubes in an ice bath, followed by the addition of 4 mL of toluene. The mixture was thoroughly vortexed, and after 15 min, the absorbance of the pink upper phase was measured at 520 nm. The proline concentration was quantified using a standard calibration curve (Bates et al. 1973).

Determination of lipid peroxidation level

Lipid peroxidation (LPO) level was assessed by measuring malondialdehyde (MDA) content. For this, maize leaves (0.5 g) were first ground with liquid nitrogen and then mixed with 5 mL of 0.1% TCA (Trichloroacetic acid) solution in a mortar until fully homogenized. The homogenates were centrifuged at 13,000 rpm for 15 min. From the supernatant, 4 mL was taken, and 1 mL of 0.5% TBA (thiobarbituric acid) solution was added and mixed. The mixture was incubated in boiling water for 30 min, then cooled quickly in an ice bath. The solution was centrifuged again at 12,000 rpm for 5 min, and the absorbance of the supernatant was measured at 532 nm and 600 nm (Demiral and Türkan 2005). The MDA content was calculated in nmol/g tissue using the formula: MDA (nmol/g tissue) = (A532–A600) × 6.45 (Hodges et al. 1999).

Measurement of ROS content

The H_2O_2 content was determined by homogenizing plant material (0.5 g) in 10 mL of chilled acetone and centrifuging at 12,000 × g for 15 min at 4 °C. Subsequently, 1.5 mL of the supernatant was combined with 0.15 mL of 5% Ti(SO4)2 and 0.3 mL of 19% NH_4OH. The mixture was then subjected

to a second centrifugation under identical conditions. The resulting pellet was dissolved in 3 mL of 2 M H_2SO_4, and absorbance was measured at 415 nm. The absorbance values were converted to µg of H_2O_2 using a standard curve prepared in accordance with the methodology outlined by Gezgincioglu and Atici (2023). For O_2^- anion content, plant samples (0.5 g) were homogenized in liquid nitrogen and mixed with 2 mL of 65 mM phosphate buffer (pH 7.8). The homogenate was centrifuged at 12,000xg for 10 min at 4 °C. Then, 0.1 mL of 10 mM hydroxylamine hydrochloride and 0.9 mL of phosphate buffer were added to 1 mL of the supernatant. Next, 1 mL of 17 mM aminobenzene sulfonic acid and 1 mL of 17 mM 1-naphthylamine were added to the mixture. The absorbance of the final solution was measured at 530 nm, and the O_2^- content was quantified using a standard curve of HNO_2 concentrations (Gezgincioglu and Atici 2023).

Determination of antioxidant enzyme activities

For the enzyme extraction, fresh tissue sample (0.5 g) was homogenized using liquid nitrogen and then 5 mL of homogenate buffer (0.15 M KH_2PO_4, pH 7.0) containing 1% PVP (polyvinylpyrrolidone) and 1 mM EDTA (ethylenediaminetetraacetic acid) was introduced to the homogenate. The resultant extract was centrifuged at 12,000 rpm (+4 °C) for 15 min. The resulting supernatant was utilized as a substrate resource for evaluating all the enzyme activities (Esim et al. 2013). Catalase (CAT; EC.1.11.1.6) activity was assayed according to Kar and Mishra (1976). For this, 1.450 mL of 103 mM KH_2PO_4 buffer (pH 7) and 1.5 mL of 40 mM H_2O_2 solution were added to a spectrophotometer cuvette, followed by the addition of 50 µL of enzyme extract. The absorbance was read at 0.5-min intervals for 3 min at 240 nm. To calculate activity, average absorbance values were converted to µmol of H_2O_2 per minute using a standard curve prepared with pure H_2O_2. The amount of enzyme that reduces 1 µmol of substrate per minute at 25 °C is defined as 1 enzyme unit. Ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined based on the decrease in absorbance at 290 nm (Nakano and Asada, 1987). The enzyme activity is measured by using a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 250 µM ascorbic acid (ASA), 5 mM H_2O_2, and 100 µL of enzyme extract. APX activity is calculated using an epsilon coefficient of 2.8 mM^{-1} cm^{-1} for ASA at 290 nm. For guaiacol peroxidase (GPX; EC 1.11.1.7), the reaction mixture (3 mL) contained 100 mL of 0.1 M KH_2PO_4 (pH 5.5), 5 mM guaiacol, and 10 µL of enzyme extract. The absorbance increase was recorded at 470 nm for 5 min with 1-min intervals. One enzyme unit was defined as the amount of enzyme that increased the absorbance by 0.01 per minute at 25 °C (Esim et al. 2013). For superoxide dismutase (SOD; EC 1.15.1.1), the reaction mixture (3 mL) contained 50 mM KH_2PO_4 (pH 7.8), 13 mM methionine, 75 µM NBT (nitroblue tetrazolium), 2 µM riboflavin, 0.1 mM EDTA, and 100 µL enzyme extract. The reaction was initiated by adding riboflavin into the tube and the tube was placed in front of a white light source for 15 min, and then the reaction was stopped by turning off the light source. The intensity of NBT reduction was measured against a blank at 560 nm. One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of NBT reduction observed at 560 nm (Esim et al. 2013). The enzyme activity results were reported as enzyme units per mg of protein.

Determination of non-enzymatic antioxidants

Glutathione (GSH) content were determined enzymatically by using the method of Griffith (1980). Fresh tissue (0.2 g) was homogenized in 2 mL of 5% meta-phosphoric acid and centrifuged at 12,000 × g for 20 min. The reaction mixture consisted of 150 µL of the supernatant and 1850 µL of KH_2PO_4 (50 mM, pH 7.5) including 2.5 mM EDTA, 1 mM DTNB (Ellman's Reagent; 5,5-dithio-bis-(2-nitrobenzoic acid)), 0.3 U glutathione reductase, and 1 mM NADPH. The increase in absorbance at 412 nm was monitored for 3 min at 25 °C. Ascorbic acid (AsA) content were determined as described by Gezgincioglu and Atici (2023). Briefly, a 0.2 g of powdered sample was extracted in 2 mL of 5% TCA. The homogenate was centrifuged at 12,000 × g for 20 min at 4 °C. The reaction mixture consisted of 1 mL of supernatant and 1.5 mL of KH_2PO_4 (pH 7.4) including 10 mM dithiothreitol (DTT), 0.014% N-ethylmaleimide, 2.6% TCA, 11.7% H_3PO_4, 1% 2,2'-dipyridyl, and 0.3% $FeCl_3$.

Determination of total chlorophyll content

The levels of chlorophyll (Chl) in the leaves were determined using the method from Lichtenthaler and Buschmann (2001). For this, 0.5 g of fresh aerial tissue was extracted with 10 mL of 80% cold acetone, centrifuged at 12,000 rpm for 10 min, and then measured for optical density (OD) of supernatant at 645 and 663 nm using a UV–visible spectrophotometer. Total chlorophyll (mg/g) tissue = [(20.2*(OD645) + 8.02(OD663)]* (V/1000*W). In the equations: OD is the absorbance, V is the final volume, W is the fresh weight (g) of the extracted tissue.

Statistical analysis

Two-way ANOVA was performed using SPSS 18.0 to determine the main and interaction effects of bacterial treatments and salt concentrations on seedling parameters. Post-hoc tests (Duncan's) identified group differences at $p < 0.05$.

ANOVA F(d1, d2) values for each effect (main and interaction) are presented in the tables in the results section.

Results

Bacteria and properties

Table S1 provides information on 17 wild halophytic plants, including 5 endemic species, and the halotolerant bacteria isolated from these plants; Figure S1, in turn, illustrates the kinship relationships among these bacteria. According to the 16S rRNA sequence analysis, *Halobacillus dabanensis* was the most common bacterial species with 8 isolates (Table S1 and Figure S1). Additionally, all the bacterial isolates except for *H. arcis* (TG-4), *H. elongate* (TG-9) and *H. caseinilytica* (TG-19) were positive for ACC deaminase activity (Table S2). When evaluating the bacteria in terms of nitrogen fixation ability, all the isolates were positive except for *M. tarijensis* (TG-8), *H. caseinilytica* (TG-11), and *H. caseinilytica* (TG-19). In our preliminary studies, it was determined that 4 bacterial isolates (*Halomonas arcis*, TG-4; *Marinococcus tarijensis*, TG-8; *Halobacillus dabanensis*, TG-12; and *Halomonas eurihalina*, TG-20) stimulated growth parameters in maize seeds germinated under control conditions (0.0 M NaCl). Therefore, these 4 isolates were used in studies planned to increase salt stress tolerance of maize plants. From these 4 isolates, *H. arcis (TG-4)* were isolated from *S. europaea* (Amaranthaceae), *M. tarijensis* (TG-8) from *F. falcarioides* (Apiaceae), *H. dabanensis* (TG-12) from *T. compressum* (Santalaceae), and *H. eurihalina* (TG-20) from *P. convolute* (Poaceae).

Effect of bacteria application on seedling growth

The TG-4, TG-8, TG-12, and TG-20 bacterial isolates were evaluated for their ability to mitigate the effects of salt stress on the maize seedlings. Seeds were inoculated with these isolates and grown in media containing 0, 75, 150, and 250 mM NaCl. Salt treatments (S) alone significantly ($p < 0.05$) reduced seedling growth (16.4%, 22% and 39% in roots, and 6%, 17% and 30% in shoots, respectively) in a dose-dependent manner (Table 1). However, bacterial applications (B) improved seedling growth under salt stress. Specifically, all the isolates increased shoot length compared to salt treatment alone, while TG-4, TG-8, and TG-12 were particularly effective in enhancing root length (Table 1). The salt stress alone caused a decrease in the dry weight of roots and shoots in parallel with the increase in dose compared to the control; this decrease was 31% and 30% at 250 mM salt in roots and shoots, respectively (Table 2). Nonetheless, all the bacterial isolates improved shoot dry weights under salt stress, particularly at 150 mM and 250 mM NaCl. Among the isolates, TG-4 and TG-8 uniquely enhanced root dry weight compared to salt-only treatments (Table 2). These findings suggest that TG isolates, particularly TG-4 and TG-8, offer potential for mitigating the adverse effects of salinity on maize growth.

Electrolyte leakage in leaves

The results of the two-way ANOVA for EL% content are summarized in Table S3. Significant main effects were observed for NaCl level ($F(4, 60) = 259.268$, $p < 0.05$) and group ($F(4, 60) = 110.580$, $p = 0.05$). The interaction

Table 1 Effects of bacterial treatments on the seedling root and shoot lengths under different salt concentrations

Groups	0.0 mM NaCl	75 mM NaCl	150 mM NaCl	250 mM NaCl
Root length (cm.seedling^{-1})				
Control	$17.73 \pm 0.37^{a,AB}$	$14.83 \pm 0.54^{b,B}$	$13.82 \pm 0.10^{b,B}$	$10.81 \pm 0.60^{c,B}$
TG-4	$16.83 \pm 0.54^{a,A}$	$15.23 \pm 0.15^{b,A}$	$15.02 \pm 0.56^{b,A}$	$11.58 \pm 0.72^{c,A}$
TG-8	$16.93 \pm 0.20^{a,AB}$	$15.86 \pm 0.38^{ab,A}$	$14.40 \pm 0.72^{b,AB}$	$11.53 \pm 0.64^{c,A}$
TG-12	$18.06 \pm 0.26^{a,A}$	$15.60 \pm 0.81^{b,A}$	$13.26 \pm 0.13^{c,B}$	$11.43 \pm 0.44^{d,A}$
TG-20	$17.36 \pm 0.63^{a,B}$	$14.10 \pm 0.67^{b,B}$	$11.66 \pm 0.38^{c,C}$	$8.83 \pm 0.38^{d,C}$
F & p values	$F(4, 60) = 91.83$ $p < 0.05$	$F(4, 60) = 518.5p < 0.05$	$F(4, 60) = 33.07$ $p < 0.05$	$F(4, 60) = 4.9$ $p < 0.05$
Shoot length (cm.seedling^{-1})				
Control	$26.74 \pm 0.79^{a,B}$	$25.16 \pm 0.24^{a,BC}$	$22.15 \pm 0.80^{b,A}$	$18.72 \pm 0.64^{c,C}$
TG-4	$30.24 \pm 0.56^{a,A}$	$28.30 \pm 0.36^{b,AB}$	$24.77 \pm 0.57^{c,A}$	$20.37 \pm 0.55^{d,A}$
TG-8	$29.23 \pm 0.28^{a,AB}$	$29.13 \pm 0.09^{a,A}$	$23.76 \pm 1.01^{b,A}$	$19.73 \pm 2.36^{b,B}$
TG-12	$28.00 \pm 0.50^{a,AB}$	$27.37 \pm 0.48^{a,BC}$	$24.70 \pm 0.90^{a,A}$	$20.66 \pm 1.77^{b,A}$
TG-20	$27.93 \pm 1.26^{a,AB}$	$26.26 \pm 0.65^{a,C}$	$23.70 \pm 0.56^{a,A}$	$19.30 \pm 0.97^{b,B}$
F & p values	$F(4, 60) = 31.26$ $p < 0.05$	$F(4, 60) = 175.3$ $p < 0.05$	$F(4, 60) = 3.57$ $p < 0.05$	$F(4, 60) = 1.2$ $p = 0.332$

Lowercase and uppercase letters indicate significant differences for rows and columns, respectively. F values indicate the ratio of between-group variance to within-group variance and p values assess the statistical significance ($p < 0.05$) of these differences. ± represents standard error

Table 2 Effects of bacterial treatments on the dry weight of seedlings under different salt conditions

Groups	0.0 mM NaCl	75 mM NaCl	150 mM NaCl	250 mM NaCl
Root dry weight (mg.seedling^{-1})				
Control	$0.052 \pm 0.010^{a,B}$	$0.048 \pm 0.000^{a,C}$	$0.042 \pm 0.004^{b,A}$	$0.036 \pm 0.011^{b,B}$
TG-4	$0.058 \pm 0.003^{a,A}$	$0.057 \pm 0.003^{a,B}$	$0.054 \pm 0.006^{a,A}$	$0.046 \pm 0.004^{b,A}$
TG-8	$0.063 \pm 0.005^{a;A}$	$0.057 \pm 0.004^{a,B}$	$0.052 \pm 0.002^{b,A}$	$0.035 \pm 0.001^{c,B}$
TG-12	$0.056 \pm 0.007^{a,A}$	$0.044 \pm 0.002^{b,C}$	$0.039 \pm 0.005^{b,B}$	$0.032 \pm 0.000^{c,B}$
TG-20	$0.063 \pm 0.006^{a,A}$	$0.064 \pm 0.012^{a,A}$	$0.040 \pm 0.004^{b,A}$	$0.041 \pm 0.012^{b,A}$
F & p values	$F(4, 60) = 251.6\ p < 0.05$	$F(4, 60) = 175.3\ p < 0.05$	$F(4, 60) = 13.6\ p < 0.05$	$F(4, 60) = 1.18$ p = 0.332
Shoot dry weight (mg.seedling^{-1})				
Control	$0.056 \pm 0.000^{a,A}$	$0.055 \pm 0.005^{a,A}$	$0.041 \pm 0.010^{b,A}$	$0.039 \pm 0.008^{c,A}$
TG-4	$0.065 \pm 0.003^{a,A}$	$0.054 \pm 0.004^{b,A}$	$0.054 \pm 0.005^{b,A}$	$0.040 \pm 0.002^{c,A}$
TG-8	$0.067 \pm 0.008^{a,A}$	$0.063 \pm 0.001^{a,A}$	$0.055 \pm 0.002^{b,A}$	$0.046 \pm 0.008^{a,A}$
TG-12	$0.063 \pm 0.002^{a,A}$	$0.054 \pm 0.001^{b,A}$	$0.051 \pm 0.005^{b,A}$	$0.043 \pm 0.005^{c,A}$
TG-20	$0.059 \pm 0.004^{a,A}$	$0.052 \pm 0.003^{a,A}$	$0.046 \pm 0.001^{b,A}$	$0.042 \pm 0.004^{b,A}$
F & p values	$F(4, 60) = 308.6\ p < 0.05$	$F(4, 60) = 294.8\ p < 0.05$	$F(4, 60) = 31.32\ p < 0.05$	$F(4, 60) = 9.03\ p < 0.05$

Lowercase and uppercase letters indicate significant differences for rows and columns, respectively. F values indicate the ratio of between-group variance to within-group variance and p values assess the statistical significance ($p < 0.05$) of these differences. ± represents standard error

effect (NaCl Level × Group) was marginally significant ($F(4, 60) = 0.094$, $p < 0.05$), suggesting that the effect of NaCl concentration on electrolyte leakage varied depending on the group applied. For instance, the salt treatments alone caused an increase in EL% in seedling leaves, and this increase was proportional to the dose of S applied (Table 3). The EL% specifically rose by 12%, 27%, and 28% in the 75, 150, and 250 mM S treatments, respectively, compared to the treatments with salt alone. However, the application of the TG-8 and TG-12 isolates significantly ($p < 0.05$) decreased the EL% value in seedlings under salt conditions, while TG-4 and TG-20 were not successful enough in lowering EL% compared to the S treatments alone. For example, TG-8 and TG-12 isolates decreased EL% value by 12% and 8%, respectively, compared to the S treatments alone (Table 3). These results indicate that both NaCl concentration and bacterial groups significantly influence electrolyte leakage, with group-specific differences under varying NaCl levels.

Table 3 Effects of bacterial treatments on electrolyte leakage (EL) and proline content in seedlings under different salt conditions

Groups	0.0 mM NaCl	75 mM NaCl	150 mM NaCl	250 mM NaCl
EL% (µS.cm^{-1})				
Control	$11.97 \pm 1.37^{c,A}$	$13.36 \pm 0.63^{b,A}$	$15.15 \pm 0.44^{a,A}$	$15.31 \pm 1.72^{a,A}$
TG-4	$09.18 \pm 0.53^{b,C}$	$13.19 \pm 0.46^{c,A}$	$14.71 \pm 0.59^{b,AB}$	$15.31 \pm 1.67^{a,A}$
TG-8	$10.76 \pm 0.83^{c,BA}$	$12.02 \pm 1.38^{b,B}$	$13.31 \pm 0.35^{a,C}$	$13.49 \pm 0.28^{a,C}$
TG-12	$10.28 \pm 1.37^{a,B}$	$12.25 \pm 0.69^{b,B}$	$14.00 \pm 1.50^{a,B}$	$14.15 \pm .68^{a,BC}$
TG-20	$09.12 \pm 1.51^{c,C}$	$13.21 \pm 0.38^{b,A}$	$13.90 \pm 0.46^{b,CB}$	$15.33 \pm 0.37^{a,A}$
F & p values	$F(4, 60) = 276.7\ p < 0.05$	$F(4, 60) = 259.3p < 0.05$	$F(4, 60) = 110.6\ p < 0.05$	$F(4, 60) = 0.094$ p = 0.05
Proline content (µmol.g^{-1} FW)				
Control	$20.44 \pm 2.31^{c,B}$	$27.14 \pm 0.31^{b,A}$	$29.49 \pm 2.53^{a,C}$	$30.58 \pm 1.61^{a,B}$
TG-4	$19.72 \pm 2.09^{c,C}$	$25.51 \pm 1.88^{b,B}$	$33.29 \pm 1.75^{a,A}$	$32.93 \pm 3.63^{a,A}$
TG-8	$24.06 \pm 1.73^{c,A}$	$28.59 \pm 2.22^{b,A}$	$28.04 \pm 3.63^{b,DC}$	$33.11 \pm 2.35^{a,A}$
TG-12	$20.08 \pm 0.63^{c,CB}$	$20.99 \pm 1.81^{bc,C}$	$21.17 \pm 0.00^{b,E}$	$27.68 \pm 0.83^{a,C}$
TG-20	$21.71 \pm 2.82^{c,B}$	$27.86 \pm 4.86^{b,A}$	$31.48 \pm 2.37^{a,B}$	$32.57 \pm 0.83^{a,A}$
F & p values	$F(4, 100) = 295.7\ p < 0.05$	$F(4, 100) = 331.13\ p < 0.05$	$F(4, 100) = 368.05\ p < 0.05$	$F(4, 60) = 0.042$ p = 0.05

Lowercase and uppercase letters indicate significant differences for rows and columns, respectively. F values indicate the ratio of between-group variance to within-group variance and p values assess the statistical significance ($p < 0.05$) of these differences. ± represents standard error

Na^+ and K^+ contents

Applying S alone led to an increase in Na^+ content in both the roots and shoots of seedlings, proportionate to the salt dose applied, compared to the control (Table 4). At a 250 mM S treatment, for instance, Na^+ content in both the roots and shoots of seedlings increased by 47% and 21%, respectively. However, the B isolates generally had the effect of reducing Na^+ content in the seedlings, with TG-4, TG-8, and TG-12 isolates, showing the most significant reduction in Na^+ content in both root and shoot organs exposed to the salt doses. Specifically, TG-8 was most effective in reducing Na^+ content in the roots, while TG-20 showed the highest effectiveness in reducing Na^+ content in the shoots. Additionally, compared to the control, the B applications alone, as well as the S applications alone except at 75 mM, increased K^+ content in the roots. However, all the S applications reduced K^+ content in the shoots, with the extent of reduction varying based on the amount of S applied. The B isolates studied counteracted these effects of S applications on K^+ content in both roots and shoots, except in roots treated with 75 mM NaCl.

Lipid peroxidation level (as MDA)

Significant main effects were observed for salt concentration ($F(4, 100) = 508.468$, $p < 0.05$) and group ($F(4, 100) = 93.444$, $p < 0.05$). Additionally, the interaction between salt concentration and group was significant ($F(4, 100) = 3.444$, $p = 0.05$), indicating that the effect of salt concentration on MDA content varied depending on the group applied. For example, the salt stress alone increased MDA

Table 4 Effects of bacterial treatments on Na^+ and K^+ levels in root and shoot of seedlings under different salt conditions

	Groups	0.0 mM NaCl	75 mM NaCl	150 mM NaCl	250 mM NaCl
Root (μmol.g^{-1} DW)					
Na^+	Control	$72.50 \pm 0.05^{d,B}$	$85.90 \pm 0.05^{c,B}$	$98.40 \pm 0.05^{b,A}$	$106.30 \pm 0.05^{a,A}$
	TG-4	$70.50 \pm 0.01^{d,B}$	$74.60 \pm 0.05^{c,E}$	$91.20 \pm 0.01^{a,C}$	$93.10 \pm 0.01^{b,C}$
	TG-8	$71.90 \pm 0.01^{d,B}$	$78.80 \pm 0.01^{c,C}$	$80.90 \pm 0.01^{a,D}$	$76.20 \pm 0.01^{b,D}$
	TG-12	$78.60 \pm 0.01^{c,A}$	$100.90 \pm 0.01^{a,A}$	$93.90 \pm 0.01^{b,B}$	$101.60 \pm 0.00^{d,B}$
	TG-20	$69.50 \pm 0.01^{d,C}$	$77.20 \pm 0.01^{c,D}$	$98.40 \pm 0.01^{b,A}$	$106.40 \pm 0.01^{a,A}$
F & p values	$F(4, 60) = 11{,}500.4$ $p < 0.05$	$F(4, 60) = 5280.7$ $p < 0.05$	$F(4, 60) = 1176.9$ $p < 0.05$	$F(4, 60) = 0.00$ $p = 0.05$	
	Control	$18.60 \pm 0.05^{c,D}$	$13.70 \pm 0.05^{d,D}$	$31.30 \pm 0.05^{a,A}$	$28.70 \pm 0.05^{b,A}$
	TG-4	$25.50 \pm 0.05^{a,A}$	$24.70 \pm 0.01^{b,A}$	$9.30 \pm 0.01^{d,D}$	$12.90 \pm 0.01^{c,C}$
K^+	TG-8	$23.30 \pm 0.01^{a,C}$	$13.40 \pm 0.01^{b,D}$	$13.10 \pm 0.01^{c,C}$	$9.90 \pm 0.01^{d,E}$
	TG-12	$22.90 \pm 0.01^{a,C}$	$19.00 \pm 0.01^{b,B}$	$19.10 \pm 0.01^{b,B}$	$13.60 \pm 0.01^{c,B}$
	TG-20	$24.30 \pm 0.01^{a,B}$	$17.70 \pm 0.01^{c,C}$	$21.20 \pm 0.01^{b,B}$	$11.70 \pm 0.01^{d,D}$
F & p values	$F(4, 60) = 7593.5$ $p < 0.05$	$F(4, 60) = 8227.6$ $p < 0.05$	$F(4, 60) = 5731.6$, $p = p < 0.05$	$F(4, 60) = 0.00$ $p = 0.05$	
Shoot (μmol.g^{-1} DW)					
Na^+	Control	$79.90 \pm 0.05^{d,A}$	$82.20 \pm 0.11^{c,A}$	$94.00 \pm 0.05^{b,C}$	$96.40 \pm 0.05^{a,C}$
	TG-4	$79.03 \pm 0.05^{c,B}$	$81.05 \pm 0.05^{a,B}$	$70.50 \pm 0.01^{d,D}$	$79.20 \pm 0.01^{b,E}$
	TG-8	$39.60 \pm 0.01^{d,E}$	$66.50 \pm 0.01^{c,C}$	$97.10 \pm 0.01^{b,B}$	$108.80 \pm 0.01^{a,A}$
	TG-12	$53.50 \pm 0.01^{d,D}$	$53.70 \pm 0.01^{c,D}$	$56.60 \pm 0.01^{b,E}$	$79.90 \pm 0.01^{a,D}$
	TG-20	$53.40 \pm 0.01^{c,C}$	$50.40 \pm 0.01^{d,D}$	$99.20 \pm 0.01^{a,A}$	$98.40 \pm 0.01^{b,B}$
F & p values	$F(4, 60) = 14{,}891.8$ $p < 0.05$	$F(4, 60) = 15{,}470.8$ $p < 0.05$	$F(4, 60) = 5573.5$ $p < 0.05$	$F(4, 60) = 0.00$ $p = 0.05$	
	Control	$142.00 \pm 0.05^{a,D}$	$136.10 \pm 0.05^{b,C}$	$127.60 \pm 0.05^{d,C}$	$132.50 \pm 0.05^{c,C}$
	TG-4	$149.80 \pm 0.06^{d,C}$	$157.40 \pm 0.06^{c,B}$	$175.20 \pm 0.01^{a,D}$	$162.60 \pm 0.01^{b,D}$
K^+	TG-8	$190.80 \pm 0.01^{a,A}$	$170.70 \pm 0.01^{b,A}$	$145.00 \pm 0.58^{d,B}$	$159.60 \pm 0.0^{c,B}$
	TG-12	$177.37 \pm 0.03^{a,B}$	$137.10 \pm 0.01^{c,C}$	$152.10 \pm 0.01^{b,E}$	$137.20 \pm 0.01^{c,E}$
	TG-20	$179.90 \pm 0.01^{b,B}$	$166.80 \pm 0.01^{c,A}$	$156.80 \pm 0.01^{d,A}$	$192.60 \pm 0.01^{a,A}$
F & p values	$F(4, 60) = 15{,}431.7$ $p < 0.05$	$F(4, 60) = 16{,}122.7$ $p < 0.05$	$F(4, 60) = 1517.6$ $p < 0.05$	$F(4, 60) = 0.00$ $p = 0.05$	

Lowercase and uppercase letters indicate significant differences for rows and columns, respectively. F values indicate the ratio of between-group variance to within-group variance and p values assess the statistical significance ($p < 0.05$) of these differences. ± represents standard error. DW: Dry weight

content by 9% and 20.6% and 26.8% at 75, 150 and 250 mM salt, respectively, compared with the control (Fig. 1). In contrast, bacterial applications generally reduced MDA levels, demonstrating their potential to alleviate oxidative damage induced by salt stress. Furthermore, bacteria + the salt (BS) applications (75, 150, and 250 mM) significantly decreased MDA levels compared to S application alone. Under high salt conditions (250 mM), TG-4, TG-8, TG-12, and TG-20 isolates reduced MDA content by 31%, 15%, 19%, and 37%, respectively, compared to S application alone.

ROS content

The level of H_2O_2 in the salt-stressed plants was increased by 30% and 37% at 150 and 250 mM salt, respectively (Fig. 1). In contrast, in the BS treatments, all the isolates showed a significant ($p < 0.05$) reduction in H_2O_2 levels compared to those observed at 150 and 250 mM salt concentrations. Notably, TG-4 exhibited a 30% reduction at 250 mM, while TG-12 demonstrated a 27% decrease at 150 mM salt. The increase in O_2^- levels in salt-treated plants was similar to the increase in H_2O_2 levels (Fig. 1). Significant main effects were observed for salt concentration ($F(4, 100) = 727.387$, $p < 0.05$) and group ($F(4,100) = 1160.145$, $p < 0.05$). Additionally, the interaction between salt concentration and group was significant ($F(4, 100) = 28.287$, $p < 0.05$), indicating that the effect of salt concentration on cellular H_2O_2 levels varied depending on the group applied (Table S3). Moreover, in BS treatments, TG-4, TG-8, and TG-20 isolates notably suppressed O_2^- content compared to controls across all the S concentrations applied. For instance, shoot O_2^- content was 4.69 $ng.g^{-1}$ at 250 mM S alone, but decreased by 39% to 3.06 $ng.g^{-1}$ with the TG-20 isolate treatment. Significant main effects were observed for salt concentration ($F(4, 100) = 781.341$, $p < 0.05$) and group ($F(4, 100) = 55.264$, $p < 0.05$). Additionally, the interaction between salt concentration and group was significant (F(4,

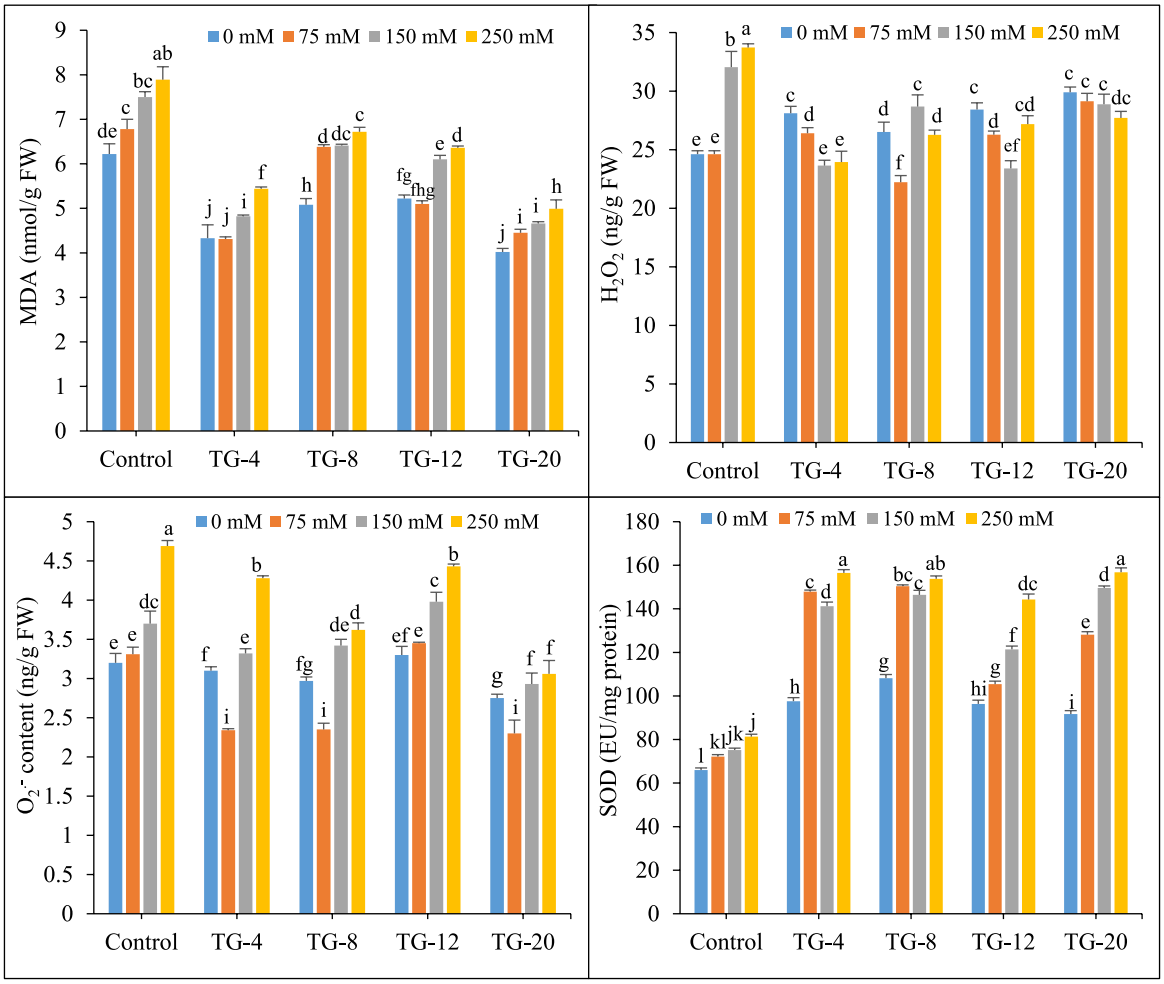

Fig. 1 Effects of salt and bacteria + salt application on ROS content and SOD activity. Differences between groups with the same letters are insignificant according to Duncan's Multiple Comparison test ($p < 0.05$)

100) = 2.732, p = 0.05), indicating that the effect of salt concentration on O_2^- levels varied depending on the group applied (Table S3).

Antioxidant enzyme activities

Significant main effects were observed for salt concentration and group for all enzymatic activities ($p < 0.05$), suggesting that both factors influenced the activity levels (Table S3). Additionally, the interaction effects (NaCl Level × Group) were significant ($p < 0.05$) for all enzymes, indicating that the influence of salt concentration on enzymatic activity levels varied depending on the treatment group. These findings highlight the complex interplay between salinity and treatment on the antioxidant defense mechanisms in plants. For instance, SOD activity was higher ($p < 0.05$) in all S applications compared to the control group. TG-4 and TG-8 isolates exhibited the highest activity rates at 75 and 250 mM S concentrations, respectively (Fig. 1). At 250 mM salt, TG-12 isolate showed an activity rate of 144.3 EU.mg^{-1}, which was 77% higher than the control, while TG-20 isolate had an activity rate of 149.6 EU.mg^{-1}, 99% higher than the control at 150 mM. These findings indicate that SOD activity varies with different isolates and salt concentrations, with TG-4, TG-8, TG-12, and TG-20 isolates significantly enhancing SOD activity under specific conditions.

CAT activity significantly ($p < 0.05$) decreased in S applications alone compared to the control group. For instance, CAT activity in the control was 1.55 EU.mg^{-1}, which dropped by 35% to 1.04 EU.mg^{-1} at 250 mM S concentration. B applications generally reduced CAT activity at 75 and 150 mM salt concentrations but increased it at 250 mM S application. Under 250 mM salt, TG-4, TG-8, and TG-12 isolates increased CAT activity by 29%, 20%, and 15%, respectively, while TG-20 showed a 14% decrease (Fig. 2). These findings suggest that S concentration significantly impacts CAT activity, with higher S levels tending to reduce it. Furthermore, the effect of B applications on CAT activity varies depending on the B isolate and S concentration."

While POX activity was 357.25 EU.mg^{-1} in the control, it decreased by 10% to 20% with increasing salt concentrations. BS applications generally reduced POX activity, with the highest decrease (19%) observed in the 250 mM + TG-20 isolate (Fig. 2). At 75 mM NaCl, a slight

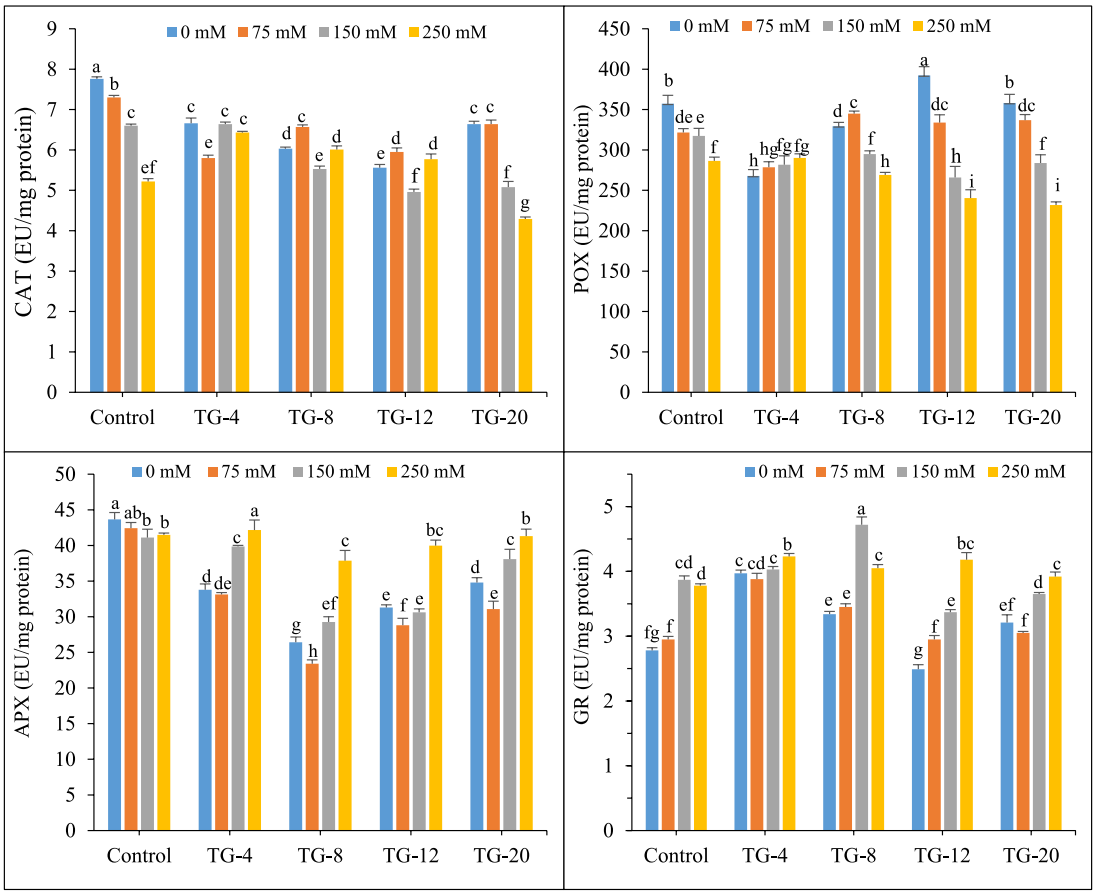

Fig. 2 Effects of salt and bacteria + salt application on CAT, POX, APX, and GR activities. Differences between groups with the same letters are insignificant according to Duncan's Multiple Comparison test ($p < 0.05$)

increase in POX activity was observed in TG-8, TG-12, and TG-20 isolates. However, it was observed that this increase was statistically significant ($p < 0.05$) only in TG-8, whereas the increases in TG-12 and TG-20 did not reach statistical significance. These findings suggest that higher salt concentrations negatively impact POX activity. However, at 75 mM salt, B isolates TG-8, TG-12, and TG-20 showed minor increases in POX activity, though not statistically significant. Overall, the addition of B in the presence of S generally led to reduced POX activity, with TG-20 being the most affected at 250 mM salt concentration.

BS applications generally reduced APX activity. For example, TG-4, TG-8, TG-12, and TG-20 exhibited reductions of 21%, 44%, 31%, and 26%, respectively, at 75 mM S compared to controls. At 150 mM S, TG-8 isolate decreased APX activity by 29%, while TG-12 isolate showed a 25% reduction (Fig. 2). These findings indicate that different isolates respond variably to treatments, resulting in differing extents of APX activity reduction at various S concentrations.

While B and S applications alone generally increased GR activity compared to the control, BS applications exhibited varied effects across different groups. Notably, TG-4 and TG-8 isolates increased GR activity at all S concentrations. TG-4 showed the highest GR activity, with a 29% increase at 75 mM S compared to the control group. Conversely, the lowest GR activity was observed in TG-12 isolate at 150 mM S, with a 16% decrease compared to the control group (Fig. 2).

Non-enzymatic antioxidant levels

Significant main effects were observed for salt concentration and group for all four parameters ($p < 0.05$), indicating that both factors influenced their levels (Table S3). Additionally, the interaction effects (NaCl Level × Group) were significant for all parameters ($p < 0.05$), highlighting that the influence of salt concentration on AsA, DHA, GSH, and GSSG levels was group-dependent. The salt and the bacteria applications alone increased GSH content compared to the control (Fig. 3). The GSH content in the control group was 275.02 ng.g^{-1}, which increased to 285.25 ng.g^{-1} at 75 mM, 410.61 ng.g^{-1} at 150 mM, and 465.61 ng.g^{-1} at 250 mM S. In the BS groups, all isolates increased GSH content at 75 mM S, but no consistent increase was observed at other concentrations. For example, while TG-8 showed increases at all concentrations, TG-4 led to a decrease at 150 mM and TG-12 at 250 mM S compared to the control group. S applications alone significantly increased AsA content compared to the control, especially at 150 and 250 mM NaCl. The AsA content in the control group was 746.01 g.g^{-1}, which increased by 10% to 820.96 ng.g^{-1} at 250 mM NaCl. While B applications alone did not significantly increase AsA levels, BS applications generally caused a notable increase at all concentrations compared to S alone. The most significant

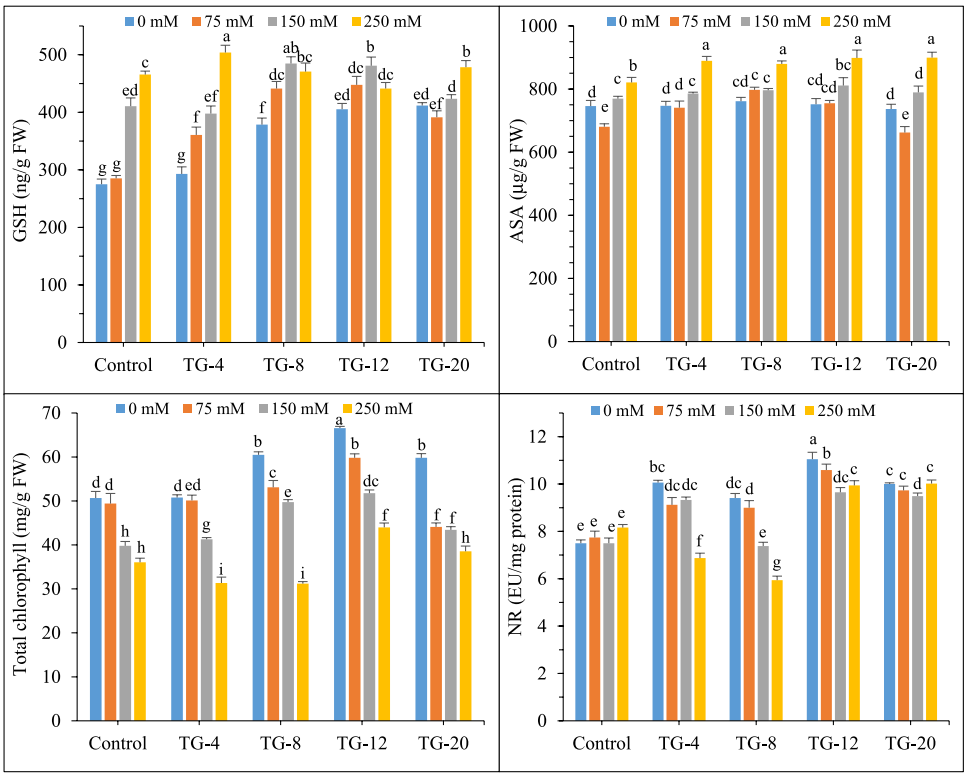

Fig. 3 Effects of salt and bacteria + salt application on GSH, ASA, total chlorophyll, and NR activity. Differences between groups with the same letters are insignificant according to Duncan's Multiple Comparison test ($p < 0.05$)

increase was observed in TG-20 isolate at 250 mM NaCl (Fig. 3). The highest increase in AsA content was at 250 mM S, with increases of 8.4%, 7%, 8%, and 9.6% in TG-4, TG-8, TG-12, and TG-20 isolates, respectively. These findings underscore the complex regulatory mechanisms plants employ to manage oxidative stress under saline conditions, with variations in antioxidant capacity observed across different treatments and salt levels.

Nitrate reductase activity

Significant main effects were observed for salt concentration ($F(4, 100) = 520.954$, $p < 0.05$) and group ($F(4, 100) = 97.186$, $p < 0.05$). Additionally, the interaction effect (NaCl Level × Group) was significant ($F(4, 100) = 20.658$, $p < 0.05$), indicating that the effect of salt concentration on nitrate reductase (NR) activity varied depending on the group applied (Table S3). NR activity in the leaves of the control plant was 7.50 EU, which increased by 9% to 8.16 EU at 250 mM S concentration (Fig. 3). In the roots, NR activity was 8.86 EU in the control plant and increased by 6% to 9.53 EU.g^{-1} with 250 mM S application. BS applications generally increased NR activity in the leaves but caused insignificant ($p < 0.05$) decreases in the roots. For instance, under 150 mM S conditions, TG-4, TG-12, and TG-20 isolates increased NR activity by 24%, 29%, and 27%, respectively, compared to the control plant, while TG-8 isolate showed a 2% decrease. Under 250 mM S conditions, TG-12 and TG-20 isolates increased NR activity, whereas TG-4 and TG-8 isolates decreased it. These findings suggest that both salt concentration and bacterial treatments influence NR activity, with distinct variations between treatment groups under saline conditions.

Total chlorophyll content

Significant main effects were observed for salt concentration ($F(4, 100) = 518.672$, $p < 0.05$) and group ($F = 36.159$, $p < 0.05$). Additionally, the interaction effect (NaCl Level × Group) was significant ($F(4, 100) = 4.323$, $p < 0.05$), indicating that the effect of salt concentration on total chlorophyll content varied depending on the group applied (Table S3). For instance, salt applications alone resulted in decreased total chlorophyll levels compared to the control group (Fig. 3). However, B applications alone increased total chlorophyll levels. In addition, BS applications also generally increased total chlorophyll levels compared to the control group. Specifically, TG-12 isolate increased total chlorophyll by 21%, 30%, and 22% with 75, 150, and 250 mM S applications, respectively. These findings demonstrate that both salt stress and bacterial treatments significantly influence chlorophyll levels, highlighting group-specific differences under varying salt concentrations.

Discussion

Twenty-two halotolerant bacterial strains were identified from the rhizosphere of 17 halotolerant wild plants in the Salt Lake basin (Turkey) (Table S1 and Figure S1). The most commonly found species was *Halobacillus dabanensis*, with 8 isolates. When evaluated for ACC deaminase activity and nitrogen fixation capacity, the majority of the isolates were positive for both of these properties. However, there were also some isolates that were negative for ACC deaminase (*H. arcis, H. elongate,* and *H. caseinilytica*) and nitrogen fixation ability (*M. tarijensis, H. caseinilytica, H. caseinilytica*) (Table S2). These properties have been suggested to enhance the ability of these bacteria to alleviate the symptoms of salt stress in crop plants (Nadeem et al. 2007; Siddikee et al. 2010). For example, the inoculation of bacterial isolates with ACC deaminase activity to maize (Nadeem et al. 2006 and 2007) and wheat (Zahir et al. 2009) resulted in increased growth compared to uninoculated ones. However, it is worth mentioning that there were also some isolates that were negative for ACC deaminase and nitrogen fixation ability indicating that not all the isolates found in this study may have the same level of PGPR potential. This suggests that different bacterial isolates employ distinct mechanisms to confer salt tolerance, and the absence of these traits does not preclude their efficacy as PGPR. For example, some isolates may enhance stress tolerance through the production of osmoprotectants, phytohormones, or other metabolites that were not evaluated in this study. El-Esawi et al. (2018) and Li et al. (2020) have shown that maize seeds inoculated with halotolerant strains of *Serratia liquefaciens* and *Kocuria rhizophila*, respectively, resulted in increased seed germination rate and growth performance as well as biomass production in developing seedlings. In these studies, the researchers isolated halotolerant bacteria from agricultural soils where maize was cultivated. However, the bacterial isolates examined in this study were isolated from the rhizosphere of different wild halophyte plants belonging to various plant families (Table S2). This suggests that halotolerant bacteria isolated from different wild halophyte plant roots may be useful in ameliorating seedling growth in cultivated plants under saline conditions.

The four bacterial isolates (*Halomonas arcis* TG-4, *Marinococcus tarigensis* TG-8, *Halobacillus dabanensis* TG-12, and *Halomonas eurihalina* TG-20) were selected among all the bacteria studied for their ameliorative effect on germination and seedling growth in maize (*Zea mays* L., cv. Hido) under non-saline conditions. These four isolates were then used to increase the salt stress tolerance of maize seedlings. Of these, TG-12 and TG-20 were positive for both ACC deaminase activity and nitrogen fixation

ability, while TG-4 and TG-8 were positive for at least one of these properties (Table S2). This property may allow these isolates to partially offset the nutritional deficiencies that occur under salt stress. This suggests that PGPR bacteria may use different mechanisms and pathways to tolerate salt stress, and that ACC deaminase activity or nitrogen fixation ability may not always be necessary for salt tolerance (Zhou et al. 2017). The effects of seed inoculation with these four isolates were evaluated on growth and development, cell membrane damage (as electrolyte leakage), and Na^+ and K^+ content in maize seedlings grown for 15 days under 0.0, 75, 150, and 250 mM NaCl salt conditions. These salt doses caused approximately 25%, 50%, and 75% inhibition of germination and seedling growth, respectively. Similar to the findings, Menezes-Benavente et al. (2004) determined that a salt level higher than 250 mM NaCl severely damaged seedling growth in maize plants. The salt applications alone significantly decreased seedling growth and plant biomass compared to the control, and these decreases were proportional to the increase in salt doses (Tables 1 and 2). Certain studies report that salt stress reduces growth and development in crop plants (Menezes-Benavente et al. 2004; Hassen et al. 2014). When the effects of the four bacterial isolates were studied on the same parameters, the bacterial inoculation alone did not generally affect the length and biomass yield of the roots while increasing those of the shoots, compared to the control. Under salt conditions, however, all the isolates except for TG-20 stimulated seedling growth in almost all the S treatments compared to the S application alone (Table 3). The improved growth observed in inoculated plants could be attributed to the reduction of stress-induced ethylene via ACC deaminase activity, which may have allowed better allocation of energy to growth processes. All the isolates also increased the shoot dry weight of seedlings, especially under 150- and 250 mM salt conditions compared to the S applications alone (Table 4). These findings indicate that the seed inoculations by TG-4, TG-8, and TG-12 isolates can improve the seedling growth in maize exposed to salt conditions. Certain studies have shown that maize plants can benefit from inoculation with plant growth-promoting rhizobacteria (PGPR) (Nadeem et al. 2006 and 2007; Shahzad et al. 2013). The growth increase observed in the maize seedlings treated with these isolates may be a result of ACC deaminase activity and nitrogen fixation. Similarly, PGPR obtained from wheat rhizosphere were found to be more effective on shoot growth than root growth in wheat seedlings (Majeed et al. 2015). These studies have isolated halotolerant bacteria from agricultural soils where maize is cultivated. However, the bacterial isolates examined in this study were isolated from the rhizosphere of different wild halophyte plants belonging to various plant families (Table S2). This suggests that halotolerant bacteria isolated from different wild halophyte plant roots may be useful in ameliorating seedling growth in cultivated plants under saline conditions.

The EL in maize seedlings under salt stress conditions was measured as an indicator of cell membrane damage. Salt stress increased the EL in seedlings compared to the control, and these increases were proportional to the salt levels applied. The bacterial inoculations reduced EL under all salt treatments compared to the S application alone (Table 3). Among the four isolates, TG-12 and TG-8 were the most effective in reducing EL under 150 and 250 mM NaCl conditions, while TG-4 and TG-20 showed relatively lower effects. The decrease in EL by bacterial inoculations indicates that bacterial isolates may help maintain cell membrane integrity in maize seedlings under salt conditions. The reduction in membrane damage may be linked to bacterial-mediated synthesis of antioxidants or compatible solutes that protect against oxidative stress induced by high salt concentrations. This is consistent with previous studies that have shown the beneficial effects of plant growth-promoting bacteria on plants grown in saline conditions. Researches indicate that exposure to salt stress can lead to a significant increase in the EL in maize seedlings, with an approximate 100% increase compared to control (Kaya et al. 2010 and 2013). However, Hasanuzzaman et al. (2022) showed that the application of *Bacillus subtilis* decreased EL and increased the activity of antioxidant enzymes in soybean seedlings grown in saline soil.

The accumulation of Na^+ and Cl^- in different plant tissues elevates linearly with salinity levels, resulting in ion imbalances in plant cells. Plant cells are generally more sensitive to Na^+ accumulation than that Cl^- accumulation (Farooq et al. 2015). The results of this study indicate that the application of S alone led to an increase in the Na^+ content in both the roots and shoots of seedlings in proportion to the salt dose applied, as previously reported in literature (Farooq et al. 2015; Hu et al. 2022). For example, at a S treatment of 250 mM, the Na^+ content in the roots and shoots of the seedlings increased by 47% and 21%, respectively (Table 4). However, the four bacterial isolates studied generally had the effect of reducing the Na^+ content in the seedlings, with the TG-4, TG-8, and TG-12 isolates showing the greatest success in reducing Na^+ content in both the root and shoot organs exposed to the salt doses. Specifically, the isolate TG-8 was found to be most effective in reducing Na^+ content in the roots, while TG-20 was most effective in reducing Na^+ content in the shoots. This is in line with previous researches (Nadeem et al. 2006; Chahal et al. 2022) which have shown that certain bacterial isolates can play a role in reducing the negative effects of salt stress on the plants including maize. Additionally, the results showed that the application of salt alone, with the exception of 75 mM NaCl, led to an increase

in K^+ content in the roots compared to the control. In contrast, all of the S applications resulted in a reduction in K^+ content in the shoots, with the extent of reduction varying depending on the amount of S applied. This is consistent with previous findings (El-Esawi et al. 2018) that salt stress can lead to changes in the uptake and distribution of mineral nutrients in plants. For example, 2 tolerant hybrids among 10 hybrids of maize under high salt levels exhibited high K^+/Na^+, maintaining high K^+ content in roots (Akram et al. 2010). The bacterial isolates studied were found to reverse these effects of S applications on K^+ content in both the roots and shoots, with the exception of roots at a salt dose of 75 mM NaCl. Based on the results, it can be suggested that the inoculation of bacterial isolates to maize seeds increases salt tolerance by improving the K^+/Na^+ ratio. As a result, it was evaluated that the isolates studied here contributed significantly to the improvement in root and shoot length and biomass of seedlings under salt stress by regulating the K^+/Na^+ balance in the seedlings. This suggests that these bacterial isolates may have the potential to improve the salt tolerance of plants by regulating the uptake and distribution of mineral nutrients.

The remarked effects of bacterial isolates on corn plants under salt stress were not limited to physical growth parameters. Additionally, the study also investigated the effect on the levels of ROS and antioxidant enzymes in plants exposed to salt stress. LPO, as MDA level, is an indicator used to determine the tolerance of plants to stress. ROS can cause lipid peroxidation in cells, leading to cellular damage. Lipid peroxidation negatively affects cell metabolism by disrupting cellular membranes and dysfunction of biological membranes (Khan et al. 2019). The present study recorded an increase in MDA content at three different salt concentrations, but bacterial isolates decreased MDA levels in BS applications (Fig. 1). Similar results have been reported in wheat plants treated with PGPR under salt stress and in plants inoculated and grown under saline conditions. A significant decrease in MDA content was noted in wheat plants inoculated with PGPR (BR5, OR15, and RB13) under unstressed and salt-stressed conditions when comparing inoculated control groups (Kerbab et al. 2021). An increase in ROS levels, which causes harmful effects on plant cells due to salt stress, was detected, similar to the findings of previous research (Aslam and Ali 2018; Nozari et al. 2022). In addition, the treated bacterial isolates reduced this harmful effect, causing a significant decrease in O_2^- and H_2O_2 levels compared to controls at high salt concentrations. These results show that the applied bacterial isolates affect the reduction of oxidative stress in plants under salt stress.

Antioxidant enzyme activities in treated corn plants show different reactions. CAT, POX, and APX activities exhibited a consistent decrease with increasing salt concentrations. In contrast to these enzymes, SOD activity was generally higher in the presence of salt; this suggests a potential adaptive response to salt-induced stress. Some bacterial isolates caused a decrease in CAT, POX, and APX activities under sure salt concentrations. Remarkable increases in SOD activity were observed. Besides these, GR activity showed an interesting dilemma. While B and S treatments alone generally increased GR activity, the combined effect varied among isolates. Isolates TG-4 and TG-8 consistently increased GR activity at salt concentrations. Against this, TG-12 exhibited a concentration-dependent decrease in GR activity. These observations highlight the complex interplay between salt stress and microbial interactions in determining the oxidative stress response in plants. Moreover, it shows that S application alone increases GSH concentration in a dose-dependent manner. However, the effect of adding bacteria on GSH content varied depending on the isolate and salt concentration. According to the findings, it appears to have a positive result on ASA content, especially at higher concentrations such as 150 mM and 250 mM. Although B treatments alone did not have a notable result on ASA content, when combined with salt, they generally increased ASA content compared to the control group. TG-20 was determined to have the highest result on increasing ASA levels at a salt concentration of 250 mM. These findings suggest that the combination of S and B could potentially increase ASA production in the study. Previous studies have found that the induction of CAT, POX, and APX enzyme activities under salt stress increases with increasing salt concentration (Upadhyay et al. 2012; Siddiqui et al. 2017; Nozari et al. 2021). Activation of antioxidant enzymes by rhizobacteria has frequently been reported in plants exposed to abiotic stress, including elevated salt content. This is indicated as one of the mechanisms responsible for salinity tolerance mediated by microorganisms (Gong et al. 2020; Ali et al. 2022). Our findings were consistent with studies showing antioxidant enzymatic activities after inoculation with PGPR against salt stress in plants such as corn and wheat (Kerbab et al. 2021; Nozari et al. 2022). Increases and decreases in antioxidant activities indicate that each PGPR isolate has a specific enzymatic potential under stressful or non-stressful environmental conditions. In addition, the interactions of plant-associated microbial communities are complex and can respond differently (Etesami and Maheshwari 2018).

Our findings shed light on the complex interplay between S, B, and chlorophyll dynamics in maize plants. S applications alone caused a significant decrease in total chlorophyll levels. It is consistent with the findings of previous studies highlighting the denial effects on chlorophyll content in various plants exposed to salt stress (Rabhi et al. 2018). In contrast to the negative result of salt alone, B treatments increased chlorophyll levels compared to the control group, indicating a potential protective role against salinity-induced chlorophyll loss. Our findings coincide with previous studies

designate the positive effect of PGPRs on chlorophyll content under abiotic stress conditions (Kang et al. 2014; Kerbab et al. 2021). The most absorbing aspect of our finding lies in the synergistic effects of bacterial isolates and salt on chlorophyll content. In the BS-treated groups, an overall increase in chlorophyll was observed at all salt concentrations compared to S alone. Isolate TG-12 especially stood out, causing a significant increase in total chlorophyll levels at various salt concentrations. Similar to our results, Kumar et al. (2017) observed high chlorophyll levels in inoculated plants under salt stress and noted the role of PGPB in minimizing the effect of salinity on chlorophyll content. Moreover, our study reflects similar results to the findings of studies reporting that an increase in chlorophyll content leads to increased photosynthetic rates, thereby facilitating plant growth under salinity (Kaushal and Wani 2016; Kumar et al. 2017).

Conclusions for future biology

This study demonstrates the potential of halotolerant PGPR isolated from the rhizospheres of certain wild halophytes in the Salt Lake region to alleviate salt stress in the maize seedlings. Notably, certain isolates including TG-4 (*Halomonas arcis*), TG-8 (*Marinococcus tarigensis*), TG-12 (*Halobacillus dabanensis*), and TG-20 (*Halomonas eurihalina*) exhibited significant efficacy in enhancing maize tolerance to salinity. These isolates achieved this by optimizing physiological processes, reducing oxidative damage, and maintaining ionic balance. As such, they represent promising tools for improving crop performance in saline environments. To fully realize the potential of these findings, long-term field studies are essential to assess the performance of these bacterial isolates across different soil types and environmental conditions. Further research should also explore their effects on other crops under both controlled and field conditions, testing the isolates individually and in combination. Additionally, it is crucial to investigate the underlying molecular mechanisms through which these bacteria confer salt tolerance, including their influence on stress-responsive gene expression and signaling pathways. Scaling these results to field trials across diverse agro-climatic regions is vital for validating the applicability of these PGPR-based solutions in agricultural settings. This approach contributes to a broader understanding of microbial adaptations in extreme environments and their role in promoting sustainable agriculture. Extending the application of such microbial solutions to other economically significant crops could further address challenges associated with climate change and soil degradation, offering a sustainable path toward resilient agricultural systems.

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Declarations

Conflict of interest The authors declare no competing interests.

References

Akram M, Ashraf MY, Ahmad R (2010) Screening for salt tolerance in maize (*Zea Mays* L.) hybrids at an early seedling stage. Pak J Bot 42:141–154

Ali B, Hafeez A, Ahmad S, Javed MA, Sumaira AMS, Dawoud TM, Almaary KS, Muresan CC, Marc RA, Alkhalifah DHM, Selim S (2022) *Bacillus thuringiensis* PM25 ameliorates oxidative damage of salinity stress in maize via regulating growth, leaf pigments, antioxidant defense system, and stress responsive gene expression. Front Plant Sci 13:921668

Aslam F, Ali B (2018) Halotolerant bacterial diversity associated with *Suaeda fruticosa* (L.) Forssk. improved growth of maize under salinity stress. Agronomy 8:131

Bajji M, Kinet LM, Lutts S (2002) The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. Plant Growth Regul 36:61–70

Bates L, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Belbahri L, Chenari BA, Rekik I, Alenezi FN, Vallat A, Luptakova L, Petrovova E, Oszako T, Cherrad S, Vacher S, Rateb ME (2017) Comparative genomics of *Bacillus amyloliquefaciens* strains reveals a core genome with traits for habitat adaptation and a secondary metabolites rich accessory genome. Front Microbiol 8:1438

Bhise KK, Bhagwat PK, Dandge PB (2017) Plant growth-promoting characteristics of salt tolerant Enterobacter cloacae strain KBPD and its efficacy in amelioration of salt stress in *Vigna radiata* L. J Plant Growth Regul 36:215–226

Castiglione S, Oliva G, Vigliotta G, Novello G, Gamalero E, Lingua G et al (2021) Effects of compost amendment on glycophyte and halophyte crops grown on saline soils: isolation and characterization of rhizobacteria with plant growth promoting features and high salt resistance. Appl Sci 11:2125

Chahal GK, Kaur A, Ghai N (2022) Mitigation of salt stress with Azospirillium and Azotobacter inoculation in maize (*Zea mays* L.). Cereal Res Commun. 1:13

Corwin DL (2021) Climate change impacts on soil salinity in agricultural areas. Eur J Soil Sci 72:842–862

Crafts-Brandner SJ, Salvucci ME (2002) Sensitivity of photosynthesis in a C4 plant, maize, to heat stress. Plant Physiol 129(4):1773–1780

Demidchik V, Straltsova D, Medvedev SS (2014) Stress-induced electrolyte leakage: the role of K^+-permeable channels and

involvement in programmed cell death and metabolic adjustment. J Exp Bot 65:1259–1270
Demiral T, Türkan İ (2005) Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. EEB 53:247–257
El Ghazali GE (2020) *Suaeda vermiculata* Forssk. ex JF Gmel.: structural characteristics and adaptations to salinity and drought: a review. Int J Sci 9:28–33
El-Esawi MA, Alaraidh IA, Alsahli AA, Alzahrani SM, Ali HM, Alayafi AA, Ahmad M (2018) *Serratia liquefaciens* KM4 improves salt stress tolerance in maize by regulating redox potential, ion homeostasis, leaf gas exchange and stress-related gene expression. Int J Mol Sci 19(11):3310
Esim N, Atici O (2013) Nitric oxide alleviates boron toxicity by reducing oxidative damage and growth inhibition in maize seedlings (*Zea mays* L.). Aust J Crop Sci 7(8):1085–1092
Etesami H, Maheshwari DK (2018) Use of plant growth promoting rhizobacteria (PGPR) with multiple plant growth promoting traits in stress agriculture: action mechanisms and future prospects. Ecotoxicol Environ Saf 156:225–246
Farooq M, Hussain M, Wakeel A (2015) Salt stress in maize: effects, resistance mechanisms, and management. Rev Agron Sustain Dev 35:461–481
Gezgincioglu E, Atici Ö (2023) Chicken feather protein hydrolysate improves cold resistance by upregulating physiologic and biochemical responses of wheat (*Triticum aestivum* L.). Environ Sci Pollut Res 30(2):3593–3605
Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48(12):909–930
Gong Y, Chen LJ, Pan SY, Li XW, Xu MJ, Zhang CM, Qin S (2020) Antifungal potential evaluation and alleviation of salt stress in tomato seedlings by a halotolerant plant growth-promoting actinomycete Streptomyces sp. KLBMP5084. Rhizosphere 16:100262
Griffith OW (1980) Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem 106:207–212
Halebian S, Harris B, Finegold SM, Rolfe RD (1981) Rapid method that aids in distinguishing Gram-positive from Gram-negative anaerobic bacteria. J Clin Microbiol 13(3):444–448
Hasanuzzaman M, Raihan MRH, Nowroz F (2022) Insight into the mechanism of salt-induced oxidative stress tolerance in soybean by the application of *Bacillus subtilis*: coordinated actions of osmoregulation, ion homeostasis, antioxidant defense, and methylglyoxal detoxification. Antioxidants 11:1856
Hassen A, Maher S, Cherif H (2014) Effect of salt stress (NaCl) on germination and early seedling parameters of three pepper cultivars (*Capsicum annuum* L.). J Stress Physiol Biochem 10:14–25
Hernández JA (2019) Salinity tolerance in plants: trends and perspectives. IJMS 20:2408
Hodges DM, DeLong JM, Forney CF, Prange RK (1999) Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207:604–611
Hu D, Li R, Dong S (2022) Maize (*Zea mays* L.) responses to salt stress in terms of root anatomy, respiration and antioxidative enzyme activity. BMC Plant Biol 22:1–17
Jha UC, Bohra A, Jha R, Parida SK (2019) Salinity stress response and 'omics' approaches for improving salinity stress tolerance in major grain legumes. Plant Cell Rep 38:255–277
Kaçar B, İnal A (2008) Plant analysis. Nobel Publication, Ankara-Turkey
Kaleh AM, Singh P, Mazumdar P, Chua KO, Harikrishna JA (2022) Halotolerant rhizobacteria isolated from a mangrove forest alleviate salinestress in *Musa acuminata* cv. Berangan Microbiol Res 265:127176
Kang SM, Khan A, Waqas M, You YH, Kim JH, Kim JG, Hamayun M, Lee LJ (2014) Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. J Plant Interact 9:673–682
Kar M, Mishra D (1976) Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiol 57(2):315–319
Kaushal M, Wani P (2016) Rhizobacterial-plant interactions: strategies ensuring plant growth promotion under drought and salinity stress. Agric Ecosyst Environ 231:68–78
Kaya C, Tuna AL, Okant AM (2010) Effect of foliar applied kinetin and indole acetic acid on maize plants grown under saline conditions. Turk J Agric for 34:529–538
Kaya C, Aydemir S, Sönmez O (2013) Regulation of growth and some key physiological processes in salt-stressed maize (*Zea mays* L.) plants by exogenous application of asparagine and glycerol. Acta Bot Croat 72:157–168
Kerbab S, Silini A, Chenari Bouket A, Cherif-Silini H, Eshelli M, El Houda RN, Belbahri L (2021) Mitigation of NaCl stress in wheat by rhizosphere engineering using salt habitat adapted PGPR halotolerant bacteria. Appl Sci 11:1034
Khan MA, Asaf S, Khan AL, Ullah I, Ali S, Kang SM, Lee IJ (2019) Alleviation of salt stress response in soybean plants with the endophytic bacterial isolate *Curtobacterium* sp. SAK1. Ann Microbiol 69:797–808
Kumar K, Amaresan N, Madhuri K (2017) Alleviation of the adverse effect of salinity stress by inoculation of plant growth promoting rhizobacteria isolated from hot humid tropical climate. Ecol Eng 102:361–366
Li J, Ovakim DH, Charles TC (2000) An ACC deaminase minus mutant of Enterobacter cloacae UW4 no longer promotes root elongation. Curr Microbiol 41:101–105
Li H, Zhao Y, Jiang X (2019) Seed soaking with Bacillus sp. strain HX-2 alleviates negative effects of drought stress on maize seedlings. Chil J Agric Res 79(3):396–404
Li X, Sun P, Zhang Y (2020) A novel PGPR strain *Kocuria rhizophila* Y1 enhances salt stress tolerance in maize by regulating phytohormone levels, nutrient acquisition, redox potential, ion homeostasis, photosynthetic capacity and stress-responsive genes expression. Environ Exp Bot 174:04023
Lichtenthaler HK, Buschmann C (2001) Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Curr Protoc Food Anal Chem 1:F4–F3
Majeed A, Abbasi MK, Hameed S (2015) Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Front Microbiol 6:1–11
Menezes-Benavente L, Kernodle SP, Margis-Pinheiro M (2004) Salt-induced antioxidant metabolism defenses in maize (*Zea mays* L.) seedlings. Redox Rep 9:29–36
Mukhopadhyay R, Sarkar B, Jat HS, Sharma PC, Bolan NS (2020) Soil salinity under climate change: challenges for sustainable agriculture and food security. JEVMAW 280:111736
Nadarajah KK (2020) ROS homeostasis in abiotic stress tolerance in plants. Int J Mol Sci 21(15):5208
Nadeem SM, Zahir Z, Naveed M (2006) Variation in growth and ion uptake of maize due to inoculation with plant growth promoting rhizobacteria under salt stress. Soil Environ 25:78–84
Nadeem SM, Zahir Z, Naveed M (2007) Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. Can J Microbiol 53:1141–1149
Nakano Y, Asada K (1987) Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. Plant Cell Physiol 28(1):131–140

Nozari RM, Ortolan F, Astarita LV, Santar´em ER, (2021) *Streptomyces* spp. enhance vegetative growth of maize plants under saline stress. Braz J Microbiol 52:1371–1383

Nozari RM, Ramos LM, da Luz LA, Almeida RN, Lucas AM, Cassel E, Santarém ER (2022) Halotolerant Streptomyces spp. induce salt tolerance in maize through systemic induction of the antioxidant system and accumulation of proline. Rhizosphere 24:100623

Rabhi NEH, Silini A, Cherif-Silini H, Yahiaoui B, Lekired A, Robineau M, Esmaeel Q, Jacquard C, Vaillant-Gaveau N, Clément C et al (2018) *Pseudomonas knackmussii* MLR6, arhizospheric strain isolated from halophyte, enhances salt tolerance in *Arabidopsis thaliana*. J Appl Microbiol 125:1836–1851

Raheem A, Ali B (2015) Halotolerant rhizobacteria: Beneficial plant metabolites and growth enhancement of *Triticum aestivum* L. in salt amended soils. Arch Agron Soil Sci 61:1691–1705

Riffat A, Sajid M, Ahmad A (2020) Alleviation of adverse effects of salt stress on growth of maize (*Zea mays*) by sulfur supplementation. Pak J Bot 52(3):763–773

Shabaan M, Asghar HN, Zahir ZA, Sardar MF, ParveenAli RFQ (2022) Halotolerant rhizobacterial consortium confers salt tolerance to maize under naturally salt-affected soil. Soil Sci Soc Am J 86:1264–1279

Shahzad SM, Arif MS, Riaz M (2013) PGPR with varied ACC-deaminase activity induced different growth and yield response in maize (*Zea mays* L.) under fertilized conditions. Eur J Soil Biol 57:27–34

Siddikee MA, Chauhan PS, Anandham R (2010) Isolation, characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil. J Microbiol Biotechnol 20:1577–1584

Siddiqui MN, Mostofa MG, Akter MM, Srivastava AK, Sayed MA, Hasan MS, Tran LSP (2017) Impact of salt-induced toxicity on growth and yield-potential of local wheat cultivars: oxidative stress and ion toxicity are among the major determinants of salt-tolerant capacity. Chemosphere 187:385–394

Slama HB, Cherif-Silini H, Chenari Bouke A, Qader M, Silini A, Yahiaoui B, Alenezi FN, Luptakova L, Triki MA, Vallat A et al (2019) Screening for *Fusarium* antagonistic bacteria from contrasting niches designated the endophyte *Bacillus halotolerants* as plant warden against *Fusarium*. Front Microbiol 9:3236

Tindall BJ (1991) Cultivation and preservation of members of the family Halobacteriaceae. World J Microbiol Biotechnol 7:95–98

Ullah S, Bano A (2015) Isolation of plant-growth-promoting rhizobacteria from rhizospheric soil of halophytes and their impact on maize (*Zea mays* L.) under induced salinity. Can J Microbiol 61:307–313

Ullah A, Bano A, Khan N (2021) Climate change and salinity effects on crops and chemical communication between plants and plant growth-promoting microorganisms under stress. Front Sustain Food Syst 5:618092

Upadhyay SK, Singh JS, Saxena AK, Singh DP (2012) Impact of PGPR inoculation on growth and antioxidants status of wheat under saline conditions. Plant Biol 4:605–611

Zahir ZA, Ghani U, Naveed M (2009) Comparative effectiveness of *Pseudomonas* and *Serratia* sp. containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.) under salt-stressed conditions. Arch Microbiol 191:415–424

Zhou N, Zhao S, Tian CY (2017) Effect of halotolerant rhizobacteria isolated from halophytes on the growth of sugar beet (*Beta vulgaris* L.) under salt stress. FEMS Microbiol Lett 15(11):364