Journal of Plant Growth Regulation (2023) 42:5816–5830
https://doi.org/10.1007/s00344-023-10967-4

Growth and Metabolic Response of *Glycine max* to the Plant Growth-Promoting *Enterobacter* Delta PSK and *Bradyrhizobium japonicum* Under Salinity Stress

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Received: 10 August 2022 / Accepted: 24 February 2023 / Published online: 17 March 2023
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Abstract
This study was designed to test the ability of some plant growth-promoting bacteria to adverse the effects of salinity on soybean growth. The effect of *Bacillus* MAP3 and *Enterobacter* Delta PSK, along with *Bradyrhizobium japonicum* was studied on soybean at two levels of NaCl salinization (50 and 100 mM). The physical growth parameters of bacterized soybean (21 days old), particularly plants co-inoculated with *Bradyrhizobium japonicum* + *Enterobacter* Delta PSK, were significantly enhanced compared to control plants. The shoot length, leaf area, root length, and chlorophyll a content increased by 49.58%, 78.58%, 20.19%, and 57.35%, respectively, indicating the promoting activity of this bacterial combination. After 19 days following the onset of salinity stress, the retarded growth parameters in controls improved significantly due to bacterial treatments, especially by *Bradyrhizobium japonicum* + *Enterobacter* DeltaPSK, which increased the values of all growth parameters significantly regardless of the salinity level. Additionally, electrolyte leakage, the amounts of malondialdehyde and hydrogen peroxide decreased considerably due to this combined bacterial treatment. Overall, the combination treatment of *Enterobacter* Delta PSK and the original symbiont *B. japonicum* enhanced soybean growth under salt stress, indicating the ability of *Enterobacter* Delta PSK to mitigate osmotic stress. The effect of this strain on soybean yield should be further evaluated to pave the way for its use as a biofertilizer along with *B. japonicum,* especially under salt stress.

Keywords *Bacillus* sp. · *Enterobacter* sp. · Proline · Salinity · Soybean

Introduction

Abiotic stresses, such as drought, heat, and salinity, reduce crop yield by more than 50%. Soil salinity is a worldwide problem due to global warming and climate change (Singh 2022, Wang, Han et al. 2022). Approximately 50% of the arable land is estimated to be affected by salinity by the year 2050 (Metwali et al. 2015; Shrivastava and Kumar 2015), which will likely impact the global food production.

Handling Editor: Jose M. Miguel.

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In Egypt, salinity affects 60%, 25%, and 20% of the cultivated lands in the Lower, Middle, and Upper Delta. Salinity has been reported to affect plant growth, yield, membrane integrity, chlorophylls, carotenoids and photosynthetic activity, osmotic adjustments, and water relations (Benjamin and Nielsen 2006; Akrami and Arzani 2018; Maswada et al. 2018). Saline soil drastically reduces crop yield by inhibiting seed germination, seedling growth, flowering, and fruit set (Sairam and Tyagi 2004**).** Physiological processes like respiration, photosynthesis, nitrogen fixation, and other metabolic processes are affected by salinity, resulting in stunted growth and decreased productivity (Acosta-Motos et al. 2017; Pal et al. 2021**).** Moreover, it disrupts the cellular osmotic balance and increases oxidative stress by generating reactive oxygen species (ROS), which can damage cellular components, such as proteins, lipids, and DNA, undermining vital cellular functions (Fahramand et al. 2014; El-Sheshtawy et al. 2022).

Plants have developed a range of sophisticated regulatory strategies, both enzymatic and non-enzymatic, to protect

themselves from ROS (Fang and Xiong 2015). Endophytic plant growth-promoting bacteria (PGPB) offer the potential to reduce the impact of salt stress in plants through the accumulation of osmolytes and antioxidant compounds, in addition to their direct and indirect stimulatory effect on plant growth and productivity. These compounds are associated with osmotic adjustment and stabilize cell components and work as free radical scavengers (Kumar et al. 2020). The use of PGPB aligns well with the modern demands of agricultural, economic, social, and environmental sustainability (Chaparro et al. 2012; Ha-Tran et al. 2021). Several PGPB proved the ability to alleviate the effect of salinity in cumin and wheat crops (Ramadoss et al. 2013; Moradi and Piri 2018). Other species of PGPBs can be used to control rice phytopathogens responsible for grain loss (Ngalimat et al. 2021). *Burkholderia seminalis* enhances the growth of *Arabidopsis* sp, pak choi, Chinese amaranth, lettuce, and other vegetables (Hwang et al. 2021). *Enterobacter cloacae* enhanced the growth and productivity of wheat and soybean (Ramesh et al. 2014). Both *Bacillus amyloliquefaciens* and *E. cloacae* are considered as potential PGPBs as well as bio-control agents (Chen et al. 2007a, b; Mohamed et al. 2020; Ahmed et al. 2022).

Soybean (*Glycine max* L. (Merr.) is a subtropical member of the *Leguminosae* (*Fabaceae*) family, an erect bushy annual crop with considerable morphological diversity. Soybean is a crucial strategic crop because of its variety of uses as food, feed, and raw material for industry. (Khojely et al. 2018). Besides being one of the most economically significant oilseeds and biodiesel crops today, soybean also provides a significant amount of protein and oil for both human and animal sustenance (Zhang et al. 2022). The ability of *Curtobacterium* sp. SAK1 to produce phytohormones, antioxidants, and aminocyclopropane-1-carboxylic acid deaminase alleviates the salinity stress on soybean (Khan et al. 2019). Similarly, both *Pseudomonas pseudoalcaligenes* (SRM-16) and *Bacillus subtilis* (SRM-3) could mitigate the effect of salt on hydroponically grown soybean plants (Yasmin, Naeem et al. 2020).

Considering that salinity threatens agricultural production in general and soybean in particular which is considered a glycophyte, the aim of this study was to find compatible PGPBs with the ability to support the growth of this plant under salt stress. Additionally, the study aimed to investigate the response of antioxidant systems, the accumulation of osmolytes, and membrane integrity under this condition to understand the mechanism of response to the selected bacteria.

Materials and Methods

Proline, trichloroacetic acid, and citric acid were all supplied by Sigma chemical company. Other chemicals were supplied by different local companies (mostly,Al Gomhoria company) and they all were of analytical grade.

Plant Material and Bacterial Strains

A pure and homogenous line of soybean seeds (Giza 111) was obtained from the Agriculture Research Center, Ministry of Agriculture, Egypt. Three bacterial strains were used in this experiment, two of them were previously isolated for their potential as plant growth promoters and they were molecularly identified as *Bacillus* MAP3 (accession number MG214652) and *Enterobacter* Delta PSK (accession number MT012829) (Agha, Abbas et al. 2021, M. Mowafy, S. Agha et al. 2022). *Bradyrhizobium japonicum* (accession number EMCC No.1112) was obtained from the Microbial Research Center "Cairo Mircen", Ain Shams University, Cairo, Egypt. Additionally, the Al-Aqdin inoculum (a commercial product containing *Bradyrhizobium japonicum* and other PGPB specific for soybean designed and recommended by the Agriculture Research Center, Ministry of Agriculture, Egypt) was used as a positive control in this experiment.

Experimental Design

The soybean seeds were sterilized with 0.01% $HgCl_2$ solution for 3 min, washed with sterilized distilled water, and then divided into five groups to have the following treatments: control (water treatment), *B. japonicum, B. japonicum* + *Bacillus* MAP3, *B. japonicum* + *Enterobacter* Delta PSK, and Al-Aqdin inoculum. The bacterial cultures were obtained from fresh LB (Luria–Bertani) media incubated at 28 °C with 150 rpm for 2 days and the concentration was adjusted to 10^8 CFU ml^{-1}. For mixed treatments, equal volumes were used from the cultures. The soybean seeds were pre-inoculated with the aforementioned bacterial treatment for 1 h at room temperature before sowing. The seeds soaked in sterile distilled water served as the control. Five seeds were cultivated per pot (18 cm depth and 25 cm diameter) with six Kg of soil/each. There were 15 pots for each treatment. The soil (clay: sand, 2:1 wt/wt) was mixed well before use and the clay soil was obtained from the field close to Mansoura University. The experiment was conducted in the greenhouse of the Botany Department, Faculty of Science, Mansoura University, Egypt. The temperature, relative humidity, and light intensity levels were 25–30 °C, 57–60%, and 100–200 µmol m^{-2} s^{-1}, respectively. The soil physical and chemical properties were analyzed, as shown in Table 1. After 21 days of plant growth, each of the described five treatments was subdivided into three groups to start salt treatments: control (tap water), 50 mM NaCl, and 100 mM NaCl. Plant materials were collected for analysis at two points before salinity and after it by 19 days (at 21

Table 1 Physical and Chemical properties of soil used for cultivation of Soybean in the greenhouse pot experiment

Soil properties	
Soil texture class: Clay	%
Sand	17.9
Silt	24.4
Clay	54.4
Chemical properties	
pH	8
Organic carbon %	0.83
$CaCO_3$%	1.03
Total N (mg/Kg)	48.2
Available P (mg/Kg)	6.13
Available K (mg/Kg)	202.2
Soluble Ca^{++} ($meqL^{-1}$)	3.1
Soluble Mg^{++} ($meqL^{-1}$)	3.o
Soluble Na^{+} ($meqL^{-1}$)	1.9
Soluble K^{+} ($meqL^{-1}$)	1.3
Soluble $HCO3^{-}$ ($meqL^{-1}$)	3.3
Soluble Cl^{-} ($meqL^{-1}$)	2.1
Soluble $SO4^{--}$ ($meqL^{-1}$)	3.9
Moisture content (%)	
Field capacity	40.3
Saturation percentage	80.5
Available water	20.1
Wilting point	20

and 40 days old ages). Ten plants were randomly uprooted to assess shoot and root lengths, and dry weights (g/plant) were measured after drying the fresh tissues at 70 °C for two days. The dry tissues were grounded into a fine powder using a homogenizer and stored in sealed glasses at room temperature for various analytical experiments. The water content (WC) of shoot and root was calculated from Eq. 1 (Al Hassan, Fuertes et al. 2015).

$$WC = [(FW - DW)/FW] \times 100 \quad (1)$$

where FW refers to fresh weight and DW refers to dry weight.

Germination percentage (GP) (Vibhuti et al. 2015) and Germination rate (GR) (Vashisth and Nagarajan 2010) were determined according to Eqs. 2 and 3, respectively.

$$GP = (\text{No. of germinated seeds/total cultivated seeds}) * 100 \quad (2)$$

$$GR = (a/1)+(b - a/2)+(c - b/3)+.....+(n - (n - 1)/N) \quad (3)$$

where a, b, c, …,n are numbers of germinated seeds after 1, 2, 3, …, N days from the start of imbibition.

Chlorophyll and Carotenoid Contents Measurement

The chlorophyll and carotenoid contents of the soybean leaves were determined by following the procedure described by Hiscox and Israelstam (1979). The amount of 0.1 g (FW) leaves obtained from the first leaf was extracted with 7 ml of dimethyl sulfoxide at room temperature for 24 h. The extracted solution was filtered and the absorbance was measured at 470, 644, and 662 nm by Jenway 7315 UV–VIS. Chlorophyll a and chlorophyll b amounts were calculated using the following equations (Arnon, 1949; Ibrahim et al., 2014).

$$\text{Chlorophyll a} = 12.7 \times (OD_{662}) - 2.69 \times (OD_{644}) \quad (4)$$

$$\text{Chlorophyll b} = 22.9 \times (OD_{644}) - 2.69 \times (OD_{662}) \quad (5)$$

The carotenoid content was calculated according to the following equation (Villanueva et al. 1985).

$$\text{Carotenoids} = [OD_{470} - 1.28(\text{Chl.a}) + 5.67(\text{Chl. b})] / (256 \times 0.906) \quad (6)$$

Membrane Features

Electrolyte leakage (EL) was determined in plant leaf tissue (one cm^2 piece away from the midrib from the second leaf) which was placed in test tubes containing 10 ml distilled water and the electrical conductivity (EC1) was recorded using EC meter. The tubes were placed on a shaker for 2 h and recorded the EC2. Then the tube was autoclaved at 120°C and after cooling, EC3 was recorded. Equation 7 was used to estimate electrolyte leakage on the measured values (Lutts et al. 1996).

$$\text{Electrolyte leakage (\%)} = \frac{EC2 - EC1}{EC3} x100 \quad (7)$$

Membrane Lipid peroxidation was determined by estimating malondialdehyde (MDA) formation as described by Heath and Packer (1968). One g of the fresh leaf sample (the second leaf) was macerated in 5 ml 0.1% trichloroacetic acid (TCA) and then centrifuged at 10 000 xg for 15 min. Then, 2 ml of 20% TCA and 2 ml of 0.5% thiobarbituric acid were added to 1 ml of the supernatant and the mixture was incubated at 95 °C for 30 min. The absorbance was measured spectrophotometrically at 532 and 600 nm (Jenway 7315 UV–VIS, Burlington, VT, USA). The concentration of MDA was calculated using MDA molar extinction coefficient ($\varepsilon = 155\ mM^{-1}\ cm^{-1}$).

Measurement of H_2O_2

The method of Velikova et al. (2000) was used to measure H_2O_2. First, 0.1 g of 2nd plant leaf was homogenized in 0.1% trichloroacetic acid (TCA). After centrifugation, 0.5 ml of the supernatant was mixed with 0.5 ml of 100 mM potassium phosphate buffer (pH 7.0) and 2 ml KI (1 M). The reaction was left to develop in dark for one hour, and the absorbance was measured at 390 nm (Jenway 7315 UV–VIS, Burlington, VT, USA). The standard curve was used to calculate H_2O_2 concentrations.

Assessment of Osmotic Regulators

Plant dry tissue powder was obtained by heating the fresh tissue at 70 °C for 48 h till getting constant dry weight. Osmolytes were determined in plant-water extracts prepared by incubating 0.1 g of dry tissue powder in 25 ml of distilled water at 90 °C for 60 min, followed by centrifugation. The EC of the plant-water extracts was measured to express osmotic pressure directly (Mickky et al. 2019).

In addition, citric acid was estimated as described previously (Snell and Snell 1937). To 5 ml of the extract, 15 ml of the deproteinizing solution (3 g HgCl2 and 3 g ZnSO4 in 100 ml distilled water) was added. After centrifugation, 4 ml of 10 N HCl and 1 ml of 6.2% FeCl3 were added and the absorbance was measured at 445 nm. Standard citric acid dilutions (1.0 and 100 µg) were used to construct the standard curve.

The proline content of dry leaves was estimated by the method of Bates (1973). About 0.5 g of each sample was added to sulfosalicylic acid with an equivalent amount of glacial acetic acid and ninhydrin. The samples were heated at 100 °C for 2 h. After cooling, 5 ml of toluene was added to extract the developed color. The absorbance of the toluene layer was measured at 520 nm. Standard proline dilutions were used to construct the standard curve.

Total soluble sugars (TSS) were extracted by submerging 0.2 g of the dry shoot powdered tissue overnight in 10 ml 80% ethanol at 25 °C with periodic shaking. After filtration, an aliquot of 0.1 ml was added to 3 ml of freshly prepared anthrone reagent and incubated in a boiling water bath for 10 min and the absorbance was measured at 625 nm after cooling. The amounts of TSS in plant extracts were estimated using the standard curve of glucose (Hansen and Møller 1975).

Determination of Antioxidant Capacity

The methanolic extract of the dried plant samples was prepared as described by Kosem et al. (2007). An equal volume of the freshly prepared methanolic solution of DPPH (0.1% w/v) and sample extracts were mixed and kept for 30 min at room temperature in dark. Similarly, equal proportions of methanol and the DPPH solution were mixed to take the absorbance of the control. The absorbance was measured at 517 nm by using a spectrophotometer (Jenway 7315 UV–VIS, Burlington, VT, USA). The DPPH radical scavenging activity was determined using Eq. 8 (Şengül et al. 2014).

$$\text{DPPH radical scavenging activity } \% = \left[1 - (\mathrm{A}/\mathrm{B})\right] * 100 \quad (8)$$

where A is the absorbance of the sample and DPPH mixture and B is the absorbance of the methanol and DPPH mixture.

The reducing power was determined in the methanolic extract by mixing one ml with 2.5 ml of 0.2 M potassium phosphate buffer pH 6.6 in addition to 2.5 ml potassium ferricyanide (10 g/L). The mixture was further incubated at 50 °C for 20 min. and 2.5 ml trichloroacetic acid (100 g/L) was added. After centrifugation at 3000 rpm for 10 min, 2.5 of the supernatant was mixed with 2.5 ml dist. Water and 0.5 ml $FeCl_3$ (1 g/L) then the absorbance was measured at 700 nm (Yildirim et al. 2001).

Assays of Antioxidant Enzymes

The activity of antioxidant enzymes was determined in the extracts prepared by homogenization of 2 g of liquid nitrogen frozen leaf tissue in 20 ml of 100 mM potassium phosphate buffer pH 6.8. After centrifugation, the following enzymes were assayed as described before (Oktay et al. 1995; Abd-ElGawad et al. 2020).

Catalase Activity

(CAT; EC 1.11.1.6) was assayed in 500 µl assay mixture containing 50 mM potassium phosphate buffer pH 7 and 10 mM H_2O_2. The reaction started by adding 20 µl protein extract and the absorbance was monitored for 2 min at 240 nm using the kinetic mode of Jenway 7315 UV–VIS spectrophotometer. One unit of enzyme activity is the decomposition of 1 µM H_2O_2/min/ml.

Peroxidase Activity

(POX; EC 1.11.1.7). was assayed in a 500 µl reaction mixture containing 50 mM potassium phosphate buffer pH 7, 50 mM pyrogallol, and 0.03% H_2O_2. The increase in absorbance was monitored at 420 nm after starting the reaction with 20 µl protein extract.

Polyphenol Oxidase PPO

(EC 1.14.18.1.) assay was committed in 1 ml reaction mixture containing 50 mM potassium phosphate buffer pH 7 and

50 mM pyrogallol. The measurement started by adding 50 µl protein extract and absorbance was monitored at 420 nm.

Estimation of Secondary Metabolites

The methanolic extract used for the estimation of total phenols and flavonoids was prepared by incubating 0.1 g plant dry powder in 10 ml methanol (80%) for one week at room temperature. After centrifugation, the supernatant was kept at 4 °C for analysis. Total phenolic content was estimated using Folin–Ciocalteu reagent, as described earlier (Dhungana et al. 2015; Zeitoun et al. 2017). A volume of 50 µL extract was mixed with 1000 µl 2% Na_2CO_3. After 3 min, 50 µL of 1 N Folin-Ciocalteu reagent was added and the mixture was left at room temperature for 30 min. The absorbance was measured at 750 nm. The total polyphenol content was expressed in microgram gallic acid equivalents per gram dry weight of the sample (µg GAE/g). The total flavonoid content was measured by adding 30 µl of 5% $NaNO_2$ to 300 µL of the methanolic extract then 60 µL of $AlCl_3$ (10%) was added to the mixture followed by 200 µL of 1 M NaOH and the absorbance was measured at 500 nm. The total flavonoid content was measured and expressed in microgram quercetin equivalents (QE) per gram dry weight of the sample (µg QE/g) (De Souza et al. 2018).

Statistical Analysis

Measurements were gathered from 10 samples of the biological replicates (physical growth parameters) in a completely randomized method. Three samples were analyzed for the technical replicates (pigment content, membrane features along with ROS, osmolytes, antioxidant capacity, antioxidant enzymes, phenols, and flavonoids). The statistical analysis was conducted using COSTAT. With a 95% confidence level, Fisher's One- and Two-way analyses of variance (ANOVA) were used for the measurements made on samples from the first (before salinity) and second stages (after salinity application) respectively. The Levene test (normality), a parametric distribution was used. Using COSTAT software, Fisher's test was performed with a probability level of $P < 0.05$.

Results

Bio-priming with the used PGPBs significantly affected the germination parameters of soybean. Compared with the control, the highest values of GP and GR were recorded for *B. japonicum* + Delta PSK-treated seeds. Significant differences were observed in the growth parameters of soybean in response to the used bacterial treatments after 21 days (Table 2). Shoot length increased by 6.3%, 31.13%, 49.58%, and 37.2%, while the dry weight increased by 8%, 20%, 40%, and 20% in response to *B. japonicum, B. japonicum* + MAP3, *B. japonicum* + Delta PSK, and Al-Aqdin inoculum, respectively. Leaf area, root length, and dry weight significantly increased by 78.58%, 20.19%, and 93.54% after *B. japonicum* + Delta PSK treatment, which also induced a remarkable increase in water contents of both shoot and root. Compared with the control, the highest increases in the contents of Chl. a, Chl. b, Chl. a/Chl. b, and carotenoids were obtained in response to *B. japonicum* + Delta PSK (57.35%, 46.26%, 7.33%, and 49.19%, respectively) (Table 3).

The lowest value of electrolyte leakage was recorded in plants treated with *B. japonicum* + Delta PSK, which also caused lipid peroxidation and malondialdehyde (MDA) content to decrease as well as hydrogen peroxide (Table 4). Levels of citric acid and proline contents were at the lowest values; however, total soluble sugars were at the highest level in response to that combined treatment. The measured osmotic pressure was at the lowest value for plants bacterized with *B. japonicum* + Delta PSK. Compared with the control, the total antioxidant content of soybean shoots increased significantly after bacterial treatment. Plants

Table 2 Effect of different bacterial treatments on germination percentage and rate, shoot and root lengths and dry weights, leaf area, and relative water contents of soybean after 21 days of growth

Treatments	Germination percentage (GP) %	Germination rate (GR)	Shoot length (cm)	Shoot dry wt. (g)	Shoot water percentage %	Leaf area	Root Length (cm)	Root Dry wt. (g)	Root water percentage (%)
Control	80 ± 1.16^{c}	7.9 ± 0.29^{b}	13.17 ± 0.17^{e}	0.25 ± 0.01^{c}	70 ± 0.62^{d}	15.27 ± 0.72^{d}	10.4 ± 0.31^{d}	0.031 ± 0.003^{d}	54 ± 0.02^{b}
B. japonicum	82 ± 1.16^{c}	8.37 ± 0.06^{b}	14 ± 0.29^{d}	0.27 ± 0.02^{c}	73 ± 0.75^{c}	20.73 ± 0.14^{c}	11.33 ± 0.44^{c}	0.035 ± 0.002^{cd}	56 ± 0.02^{b}
B. japonicum + MAP3	84 ± 2.31^{c}	8.61 ± 0.34^{b}	17.27 ± 0.15^{c}	0.3 ± 0.03^{b}	78 ± 0.91^{b}	23.4 ± 0.25^{b}	11.33 ± 0.33^{c}	0.04 ± 0.002^{c}	58 ± 0.04^{ab}
B. japonicum + Delta PSK	99.33 ± 0.67^{a}	10.41 ± 0.19^{a}	19.7 ± 0.15^{a}	0.35 ± 0.04^{a}	82 ± 0.53^{a}	27.27 ± 0.72^{a}	12.5 ± 0.29^{a}	0.06 ± 0.001^{a}	64 ± 0.01^{a}
Al-Aqdin inoculum	92.67 ± 1.77^{b}	9.7 ± 0.24^{a}	18.07 ± 0.32^{b}	0.3 ± 0.02^{b}	81 ± 0.71^{a}	26.02 ± 0.09^{a}	11.83 ± 0.44^{b}	0.05 ± 0.003^{b}	62 ± 0.01^{a}

Values listed represent the mean ± standard error (SE). Different superscript letters refer to significant differences within a column/trait

bacterized with *B. japonicum* + Delta PSK showed the lowest values for total antioxidants, reducing power, and DPPH free radical scavenging activity (Table 5). The activities of antioxidant enzymes catalase, peroxidase, and polyphenol oxidase decreased significantly in response to bacterization and *B. japonicum* + Delta PSK combined treatment led to the lowest activity of these enzymes.

The growth parameters and pigment contents, assessed after 19 days of salinity treatment decreased considerably according to the stress level. Generally, soybean growth was reduced as far as NaCl conc increased, however, bacterization particularly with *B. japonicum* + Delta PSK, considerably alleviated this deleterious impact of both salinity levels (Table 6). The contents of both chlorophylls (a and b) and

Table 3 Effect of different bacterial treatments on Chl.a, Chl.b, Chl a/Chl b, and carotenoid contents of soybean after 21 days of growth

Treatments	Chl. a (mg/g fresh wt.)	Chl. b (mg/g fresh wt.)	Chl. a/Chl. b	Carotenoids (mg/g fresh wt.)
Control	11.63 ± 1.05[de]	5.36 ± 0.55[b]	2.18 ± 0.04[b]	5.57 ± 0.47[d]
B. japonicum	12.14 ± 1.12[d]	5.42 ± 0.41[b]	2.23 ± 0.04[ab]	5.63 ± 0.55[d]
B. japonicum + MAP3	13.3 ± 2.21[c]	5.88 ± 0.92[b]	2.25 ± 0.03[ab]	6.2 ± 1.02[c]
B. japonicum + Delta PSK	18.3 ± 1.49[a]	7.84 ± 0.72[a]	2.34 ± 0.02[a]	8.31 ± 0.78[a]
Al-Aqdin inoculum	16.12 ± 1.15[b]	7.07 ± 0.44[ab]	2.28 ± 0.04[ab]	7.02 ± 0.48[b]

Values listed represent the mean ± standard error (SE). Different superscript letters refer to significant differences within a column/trait

Table 4 Effect of different bacterial treatments on electrolyte leakage, lipid peroxidation, hydrogen peroxide, osmotic pressure, citric acid, proline, and total soluble sugar contents of soybean after 21 days of growth

Treatments	Electrolyte leakage (%)	Lipid peroxidation (μ mol MDA g^{-1} F. wt)	Hydrogen peroxide (mM^{-1} cm^{-1})	Osmotic pressure (EC in mS)	Citric acid (mg g^{-1} D.wt)	Total soluble sugar (mg/g dwt.)	Proline (mg g^{-1} D. wt)
Control	51.6 ± 2.85[a]	2.37 ± 0.04[a]	1.17 ± 0.01[a]	0.43 ± 0.01[b]	11.28 ± 1.5[a]	106.58 ± 2.3[d]	3.2 ± 0.22[b]
B. japonicum	33.61 ± 2.17[b]	2.2 ± 0.12[a]	1.16 ± 0.05[a]	0.5 ± 0.02[a]	10.87 ± 1.01[ab]	149.67 ± 2.85[c]	3.75 ± 0.23[a]
B. japonicum + MAP3	26.03 ± 1.75[c]	2.09 ± 0.11[a]	0.95 ± 0.03[b]	0.44 ± 0.01[b]	9.82 ± 1.2[bc]	145.92 ± 1.86[c]	3.72 ± 0.17[a]
B. japonicum + Delta PSK	15.45 ± 0.84[d]	1.65 ± 0.15[b]	0.74 ± 0.04[c]	0.33 ± 0.01[c]	9.13 ± 0.69[c]	186.83 ± 2.95[a]	2.58 ± 0.24[c]
Al-Aqdin inoculum	25.65 ± 0.96[c]	2 ± 0.17[ab]	0.96 ± 0.06[b]	0.44 ± 0.07[b]	9.07 ± 0.78[c]	172.33 ± 1.25[b]	2.11 ± 0.2[c]

Values listed represent the mean ± standard error (SE). Different superscript letters refer to significant differences within a column/trait

Table 5 Effect of different bacterial treatments on the antioxidant capacity, antioxidant enzymes activity, and the secondary metabolites of soybean plant after 21 days of growth

Treatments	TAC	RP (Abs. at 700 nm)	DPPH (%)	TPC (mg / g dry wt.)	TFC (mg /g dry wt.)	CAT (Unit g^1 F wt.)	POX (Unit g^1 F wt.)	PPO (Unit g^1 F wt.)
Control	122.44 ± 3.88[d]	0.57 ± 0.01[b]	12.25 ± 0.07[c]	22.76 ± 1.33[d]	15.34 ± 0.71[b]	12.6 ± 1.53[a]	8.8 ± 0.25[a]	0.7 ± 0.1[a]
B. japonicum	146.79 ± 2.84[b]	0.59 ± 0.02[a]	12.99 ± 0.07[b]	27.4 ± 1.63[b]	18.71 ± 1.11[ab]	17.6 ± 1.53[a]	5.32 ± 0.07[bc]	0.48 ± 0.04[b]
B. japonicum + MAP3	144.49 ± 2.24[b]	0.59 ± 0.03[a]	12.45 ± 0.11[c]	26.98 ± 1.82[c]	16.56 ± 1.46[ab]	10.21 ± 2.08[c]	6.18 ± 0.16[b]	0.44 ± 0.07[b]
B. japonicum + Delta PSK	138.27 ± 1.54[c]	0.45 ± 0.06[c]	11.23 ± 0.15[d]	28.99 ± 3.35[a]	19.91 ± 3.35[ab]	6.46 ± 0.94[d]	2.4 ± 0.56[d]	0.3 ± 0.02[c]
Al-Aqdin inoculum	165.5 ± 2.34[a]	0.6 ± 0.02[a]	15.23 ± 0.15[a]	27.64 ± 1.47[b]	20.94 ± 1.03[a]	12.61 ± 0.85[b]	4.85 ± 0.49[c]	0.44 ± 0.09[b]

TAC Total antioxidant capacity, *RP* Reducing power, DPPH (1,1-diphenyl-2-picrylhydrazyl), *TPC* Total phenolic contents, *TFC* Total flavonoid contents, *CAT* catalase, *POX* peroxidase and *PPO* Polyphenol oxidase. Values listed represent the mean ± standard error (SE). Different superscript letters refer to significant differences within a column/trait

Table 6 Effect of different bacterial treatments in combination with three salinity levels (0, 50, and 100 mM NaCl) on shoot and root lengths and dry weights, leaf area, relative water contents, and number of nodules of soybean after 40 days of growth

Treatments	Salt conc. (mM)	Shoot Length (cm/plant)	Shoot Dry wt. (g/plant)	RWC (%)	Leaf area	Root Length (cm/plant)	Root Dry wt. (g/plant)	Number of nodules /plants
Control	0 mM	14.67 ± 0.33^{hi}	0.85 ± 0.12^{bcd}	67.26 ± 1.1^{a}	25.09 ± 1.83^{abc}	18.67 ± 1.67 ^{c}	0.12 ± 0.01^{bcd}	20.67 ± 1.2^{fg}
B. japonicum		16.43 ± 0.3^{fg}	0.91 ± 0.06^{bcd}	53.46 ± 1.7^{d}	27.76 ± 3.63^{ab}	19 ± 1.16 ^{c}	0.13 ± 0.02^{abc}	24.33 ± 1.2^{cd}
B. japonicum + MAP3		19.67 ± 0.33^{c}	0.97 ± 0.07^{ab}	52.87 ± 0.94^{d}	28.91 ± 3.19 ^{a}	21 ± 2.89 ^{b}	0.16 ± 0.01^{abc}	26 ± 0.58^{bcd}
B. japonicum + Delta PSK		23.33 ± 0.33^{a}	1.19 ± 0.07^{a}	41.93 ± 1.64^{g}	30.89 ± 3.92^{a}	22.67 ± 1.45 ^{a}	0.21 ± 0.04^{a}	31 ± 0.58^{a}
Al-Aqdin inoculum		20.47 ± 0.29^{c}	1 ± 0.06^{ab}	43.08 ± 1.05^{fg}	29.68 ± 0.29^{a}	22 ± 0.58 ^{a}	0.15 ± 0.01^{abc}	27.67 ± 0.88^{b}
Control	50 mM	13.33 ± 0.33^{j}	0.73 ± 0.04 cd	46.46 ± 0.45^{ef}	16.58 ± 1.48^{c}	16.67 ± 1.45 ^{e}	0.09 ± 0.01^{bcd}	18 ± 0.58^{g}
B. japonicum		15.17 ± 0.6^{h}	0.83 ± 0.02^{bcd}	63.02 ± 0.92^{c}	18.05 ± 2.28^{bc}	17.67 ± 4.1 ^{d}	0.10 ± 0.02^{bcd}	21.33 ± 0.33^{ef}
B. japonicum + MAP3		17.67 ± 0.33^{de}	0.88 ± 0.07^{bcd}	73.04 ± 0.69^{b}	21.11 ± 2.5^{abc}	20.33 ± 2.97 ^{b}	0.16 ± 0.01^{abc}	25 ± 0.58^{bcd}
B. japonicum + Delta PSK		21.50 ± 0.5^{b}	0.95 ± 0.1^{bc}	73.92 ± 1.24^{ab}	24.55 ± 1.28^{abc}	21 ± 3.06 ^{b}	0.17 ± 0.03^{ab}	26.33 ± 0.88^{bc}
Al-Aqdin inoculum		18 ± 0.29^{d}	0.84 ± 0.07^{bcd}	74.02 ± 0.43^{ab}	18.27 ± 1.96^{bc}	20.67 ± 1.2 ^{b}	0.12 ± 0.02^{bcd}	25.67 ± 1.2^{bcd}
Control	100 mM	11.17 ± 0.44^{k}	0.69 ± 0.08^{d}	37.23 ± 1.45^{h}	16.36 ± 1.99^{c}	13.67 ± 0.88 ^{e}	0.06 ± 0.02^{d}	14 ± 0.58^{h}
B. japonicum		13.93 ± 0.3^{ij}	0.70 ± 0.04^{d}	60.65 ± 0.84^{c}	18.38 ± 3.29^{bc}	16 ± 1.53 ^{e}	0.08 ± 0.02^{cd}	18 ± 0.58^{g}
B. japonicum + MAP3		15.5 ± 0.29^{gh}	0.85 ± 0.04^{bcd}	41.2 ± 1.36^{g}	18.27 ± 2.4^{bc}	18.67 ± 2.67 ^{c}	0.12 ± 0.01^{bcd}	20.67 ± 0.88^{fg}
B. japonicum + Delta PSK		17.33 ± 0.17^{def}	0.88 ± 0.12^{bcd}	56.33 ± 1.65^{d}	21.27 ± 2.35^{abc}	19.33 ± 2.41^{c}	0.11 ± 0.02^{bcd}	23.67 ± 0.88^{cde}
Al-Aqdin inoculum		16.77 ± 0.15^{ef}	0.8 ± 0.04^{bcd}	47.61 ± 1.42^{e}	18.60 ± 1.44^{bc}	17.33 ± 1.77 ^{d}	0.08 ± 0^{bcd}	23.33 ± 1.2^{def}
Salinity (S)		***	ns	***	ns	***	ns	***
Treatment (T)		*	**	**	**	*	*	*
Interaction (S × T)		***	*	***	***	*	*	**

Values listed represent the mean ± standard error (SE). Different superscript letters refer to significant differences within a column/trait. Fisher's test at $p < 0.05$ reveals significant variations in means (± standard error), which are different letters (a-k) on the same bars. *, **, and *** imply significance levels of 0.01, 0.05, and 0.001, respectively

carotenoids considerably decreased according to salinity level. This effect was retarded particularly in response to the combined bacterial treatment (*B. japonicum* + Delta PSK) as shown in Table 7. However, the ratio of Chl. a/ Chl. b did not significantly change in response to different treatments. As indicated in Tables 6 and 7, salinity affected significantly most of the measured growth parameters except dry weights, and leaf area, however, different bacterial treatments considerably affected all the growth parameters. Salinity and bacterial treatments affected pigments but Chl. a/ Chl. b was not considerably affected. The combined effect of salinity and bacterial treatments was considerable on growth parameters, chlorophylls, and carotenoids.

According to NaCl conc, electrolyte leakage, MDA, and hydrogen peroxide values increased, however, this effect was reduced considerably in response to different bacterial treatments (Table 8). The levels of citric acid, total soluble sugars, proline, and consequently osmotic pressure increased in response to salinity. Both NaCl, bacterial treatments, and their combinations considerably affected these parameters.

The antioxidant capacity, reducing power, DPPH free radical scavenging activity, and total phenolic and flavonoid contents in soybean plants increased significantly, particularly in the presence of *B. japonicum* + Delta PSK at different salinity levels (Fig. 1). Bacterial treatments considerably affected all these parameters but salinity did not show a significant effect on phenols and flavonoids. The combination between salinity and bacterial treatments developed a considerable change in these parameters. The activity of antioxidant enzymes (catalase, peroxidase, and polyphenol

Table 7 Effect of different bacterial treatments in combination with three salinity levels (0, 50, and 100 mM NaCl) on Chl. a, Chl. b, Chl. a/Chl. b, and carotenoid contents of soybean after 40 days of growth

Treatments	Salt conc. (mM)	Chl. a (mg/g F wt.)	Chl. b (mg/g F wt.)	Chl. a /Chl. b (mg/g F wt.)	Carotenoids (mg/g F wt.)
Control	0 mM	11.02 ± 1.28^{f}	6.67 ± 0.81^{d}	1.65 ± 0.24^{a}	5.87 ± 0.26^{d}
B. japonicum		12.29 ± 0.29^{e}	6.72 ± 1.68^{d}	1.83 ± 0.46^{a}	6.75 ± 0.68^{c}
B. japonicum + MAP3		17.89 ± 1.36^{b}	8.17 ± 0.25^{b}	2.19 ± 0.2^{a}	7.47 ± 0.94^{b}
B. japonicum + Delta PSK		18.42 ± 0.89^{a}	9.48 ± 0.89^{a}	1.94 ± 0.09^{a}	8.06 ± 0.59^{a}
Al-Aqdin inoculum		16.62 ± 0.71^{b}	8.26 ± 0.63^{b}	2.01 ± 0.24^{a}	7.12 ± 0.86^{b}
Control	50 mM	14.08 ± 1.95^{d}	5.39 ± 0.59^{e}	2.61 ± 0.3^{a}	4.29 ± 0.35^{e}
B. japonicum		14.19 ± 2.72^{d}	6.58 ± 0.12^{d}	2.16 ± 0.37^{a}	6.57 ± 0.68^{c}
B. japonicum + MAP3		15.66 ± 2.2^{c}	7.61 ± 0.79^{c}	2.06 ± 0.39^{a}	7.44 ± 0.16^{b}
B. japonicum + Delta PSK		15.11 ± 2.38^{c}	8.16 ± 1.11^{b}	1.85 ± 0.59^{a}	8.04 ± 1.1^{a}
Al-Aqdin inoculum		14.67 ± 0.63^{d}	6.82 ± 0.17^{d}	2.15 ± 0.06^{a}	6.77 ± 1.08^{c}
Control	100 mM	8.35 ± 0.88^{i}	4.15 ± 0.33^{f}	2.01 ± 0.07^{a}	3.96 ± 0.14^{f}
B. japonicum		9.23 ± 0.19^{h}	4.60 ± 0.09^{f}	2 ± 0.07^{a}	4.27 ± 0.2^{e}
B. japonicum + MAP3		10.38 ± 1.08^{g}	5.38 ± 0.51^{e}	1.93 ± 0.34^{a}	5.73 ± 0.44^{d}
B. japonicum + Delta PSK		14.75 ± 2.39^{d}	7.67 ± 1.23^{c}	1.92 ± 0.11^{a}	6.45 ± 0.8^{c}
Al-Aqdin inoculum		10.73 ± 1.31^{g}	5.24 ± 0.15^{e}	2.05 ± 0.23^{a}	4.94 ± 0.46^{e}
Salinity (S)		***	***	ns	***
Treatment (T)		**	**	ns	*
Interaction (S × T)		**	**	ns	*

Values listed represent the mean ± standard error (SE). Different superscript letters refer to significant differences within a column/trait. Fisher's test at $p < 0.05$ reveals significant variations in means (± standard error), which are different letters (a-k) on the same bars. *, **, and *** imply significance levels of 0.01, 0.05, and 0.001, respectively

oxidase) increased progressively, particularly in the presence of *B. japonicum* + Delta PSK (Figs. 1 and 2) along with salt treatments. Salinity, bacterial treatments, and their combinations significantly affected the activity of these enzymes.

Discussion

Beneficial plant bacteria have been shown to have a dual role in improving plant responses to abiotic and biotic stressors in addition to promoting the growth and productivity of plants (Badawy et al. 2022). Their interaction with plant metabolism and hormonal balance directs the synthesis of secondary metabolites required to alleviate stress. These effects represent PGPBs as good solutions for salinity problems (Dawood, Sofy et al. 2022) and effective alternatives for chemical fertilizers (Abu-Shahba, Mansour et al. 2022). The data represented in the current study show the impact of such beneficial organisms and their role in mitigating salinity stress on soybean.

In this study, the physical growth parameters recorded after 21 and 40 days of growth (Tables 2 and 6, respectively) showed that soybean-bacterized plants, especially those that received *B. japonicum* + *Enterobacter* Delta PSK with and without stress, surpassed control plants in their growth. It is apparent that salinity stress directs plants toward metabolism to produce the materials needed to counter these conditions. The *Enterobacter* strain used in this study was selected because of its ability to promote plant growth (Agha, Abbas et al. 2021). Additionally, several reports identified *Enterobacter* as a potential PGPB (Panigrahi et al. 2020, Sheteiwy et al. 2021, Pérez-Rodriguez, Pontin et al. 2022). *B. japonicum* is regarded as a compatible symbiont of soybean (Ramongolalaina et al. 2018). The ability of *B. japonicum* + *Enterobacter* Delta PSK to produce high amounts of indole-3-acetic acid and gibberellic acid might be responsible for stimulating growth. This ability remained even in the presence of salt (Agha, Abbas et al. 2021). The production of indole acetic acid (IAA) supports root growth and proliferation (Tables 2 and 6). *Enterobacter* has a high ability to colonize plant roots, especially the primary roots, and specifically in the elongation zone. It also could colonize and re-open stomatal cells in the host plant, indicating the ability to establish even under severe conditions (Synek et al. 2021). The ACC deaminase activity reported for *Enterobacter* (Singh, Pandey et al. 2022) might be among the mechanisms for its ability to alleviate salt stress by reducing ethylene levels.

Due to osmotic stress and ion toxicity, salinity dramatically reduces the ability of plant roots to absorb water (Alsaeedi et al. 2019). Furthermore, ROS are generated more rapidly (ALHaithloul, Khan et al. 2022). Under salt stress, the mechanism of photosynthesis and its efficacy are inevitably compromised and damaged (Agha, Abbas

Table 8 Effect of different bacterial treatments in combination with three salinity levels (0, 50, and 100 mM NaCl) on electrolyte leakage, lipid peroxidation, hydrogen peroxide, osmotic pressure, citric acid, proline, and total soluble sugar contents of soybean after 40 days of growth

Treatments	Salt conc. (mM)	Electrolyte leakage (%)	Lipid peroxidation (µ mol MDA g^{-1} F wt.)	Hydrogen peroxide (mM^{-1} cm^{-1})	Osmotic pressure (EC in mS)	Citric acid (mg g^{-1} D wt.)	Total soluble sugar (mg/g D wt.)	Proline (mg g^{-1} D wt.)
Control	0 mM	22.65 ± 0.73^{c}	2.88 ± 0.03^{d}	1.23 ± 0.01^{d}	0.66 ± 0.01^{g}	15.51 ± 0.2^{f}	124.54 ± 1.53^{g}	4.68 ± 0.1^{f}
B. japonicum		21.26 ± 0.21^{c}	2.83 ± 0.03^{d}	1.22 ± 0.04^{d}	0.75 ± 0.02^{f}	16.19 ± 0.44^{d}	156.5 ± 1.41^{d}	6.31 ± 0.57^{d}
B. japonicum + MAP3		14.77 ± 0.56^{d}	2.6 ± 0.01^{e}	1.01 ± 0.04^{e}	0.7 ± 0.02^{f}	16.54 ± 0.44^{d}	158.42 ± 2.1^{d}	6.96 ± 0.27^{d}
B. japonicum + Delta PSK		7.14 ± 0.73^{e}	2.02 ± 0.11^{g}	0.75 ± 0.04^{g}	0.8 ± 0.02^{gh}	17.6 ± 0.63^{c}	193.13 ± 3.34^{a}	7.04 ± 0.05^{d}
Al-Aqdin inoculum		7.09 ± 0.87^{e}	2.48 ± 0.17^{f}	1.01 ± 0.07^{e}	0.7 ± 0.02^{f}	16.61 ± 0.3^{d}	178 ± 1.58^{b}	6.98 ± 0.59^{d}
Control	50 mM	24.96 ± 0.36^{b}	3.18 ± 0.14^{c}	1.46 ± 0.09^{c}	1.54 ± 0.02^{d}	18.36 ± 1.45^{b}	121.33 ± 1.38h	5.57 ± 0.09^{e}
B. japonicum		14.77 ± 0.18^{d}	3.09 ± 0.17^{c}	1.41 ± 0.07^{c}	1.72 ± 0.01^{b}	18.85 ± 0.24^{b}	145.56 ± 1.28^{e}	7.1 ± 0.02^{d}
B. japonicum + MAP3		15.48 ± 0.52^{d}	2.67 ± 0.22^{e}	1.21 ± 0.05^{d}	1.56 ± 0.01^{d}	19.43 ± 0.32^{a}	167.25 ± 1.87^{c}	8.48 ± 0.1^{c}
B. japonicum + Delta PSK		7.39 ± 0.65^{e}	2.69 ± 0.21^{e}	1.1 ± 0.01^{f}	1.76 ± 0.01^{b}	19.92 ± 0.83^{a}	170.58 ± 0.58^{c}	9.73 ± 0.12^{b}
Al-Aqdin inoculum		25.99 ± 0.19^{ab}	2.98 ± 0.13^{d}	1.23 ± 0.08^{d}	1.58 ± 0.02^{d}	18.58 ± 0.25^{b}	149.67 ± 0.8^{e}	8.13 ± 0.04^{c}
Control	100 mM	25.58 ± 0.74^{b}	3.32 ± 0.11^{a}	1.49 ± 0.06^{a}	1.62 ± 0.01^{c}	16.89 ± 0.26^{d}	109.21 ± 1.2^{g}	7.64 ± 0.06^{cd}
B. japonicum		27.34 ± 0.53^{a}	3.12 ± 0.11^{abc}	1.44 ± 0.04^{ab}	1.67 ± 0.01^{c}	18.74 ± 0.25^{b}	130.46 ± 1.57^{f}	8.16 ± 0.12^{c}
B. japonicum + MAP3		15.85 ± 0.47^{d}	$2.75 \pm 0.09^{b-f}$	1.32 ± 0.13^{abc}	1.68 ± 0.01^{c}	18.14 ± 0.99^{b}	154.74 ± 1.2^{c}	10.38 ± 0.34^{b}
B. japonicum + Delta PSK		14.4 ± 0.49^{d}	2.6 ± 0.13^{e}	1.4 ± 0.03^{abc}	1.86 ± 0.01^{a}	19.14 ± 0.3^{a}	155.04 ± 1.41^{d}	14.92 ± 0.44^{a}
Al-Aqdin inoculum		25.2 ± 0.34^{b}	$2.9 \pm 0.12^{a-e}$	1.4 ± 0.05^{abc}	1.63 ± 0.01^{c}	18.54 ± 0.08^{b}	135.83 ± 0.84^{f}	9.91 ± 0.36^{b}
Salinity (S)		ns	***	***	***	***	***	***
Treatment (T)		***	**	*	*	*	**	**
Interaction (S × T)		***	***	*	**	*	*	*

Values listed represent the mean ± standard error (SE). Different superscript letters refer to significant differences within a column/trait. Fisher's test at $p < 0.05$ reveals significant variations in means (± standard error), which are different letters (a-k) on the same bars. *, **, and *** imply significance levels of 0.01, 0.05, and 0.001, respectively

et al. 2021). In this study, bacterization particularly with *B. japonicum* + *Enterobacter* Delta PSK increases the chlorophylls contents even under salinity stress indicating the ability of this strain to alleviate the oxidative and ionic stress of NaCl (Tables 3 and 7). The same result was observed in tomato plants (Pérez-Rodriguez, Pontin et al. 2022) in response to *Enterobacter*. Both *Bacillus subtilis* and *Pseudomonas fluorescens* have exhibited substantial potential for enhancing photosynthetic pigment biosynthesis under salt stress in pea plants (Sofy et al. 2021). In salt-stressed *Vigna radiata* plants, *Enterobacter cloaca* has been found to positively affect photosynthesis and the levels of chlorophyll, carotenoids, and all photosynthetic pigments (Bhise et al. 2017). Enhanced P, N, and K absorption might underlie PGPB's stimulation of pigment production in soybean plants (Zhao et al. 2018). The prominent increase in total soluble sugars in response to the combination treatment supports this speculation (Tables 4 and 8). Furthermore, the significant increase in carotenoids in response to bacterization supports the ability of Delta PSK to activate plant antioxidant defenses (Tables 3 and 7). The same response was recorded in *Enterobacter*-treated salinity-stressed tomato plants (Pérez-Rodriguez, Pontin et al. 2022).

Salinity decreased the number of nodules (Table 6), but combination-treated plants showed higher nodule numbers, probably due to the ability of the used *Enterobacter* to produce siderophores (M. Mowafy, S. Agha et al. 2022) that make iron available, which is essential for nodule formation.

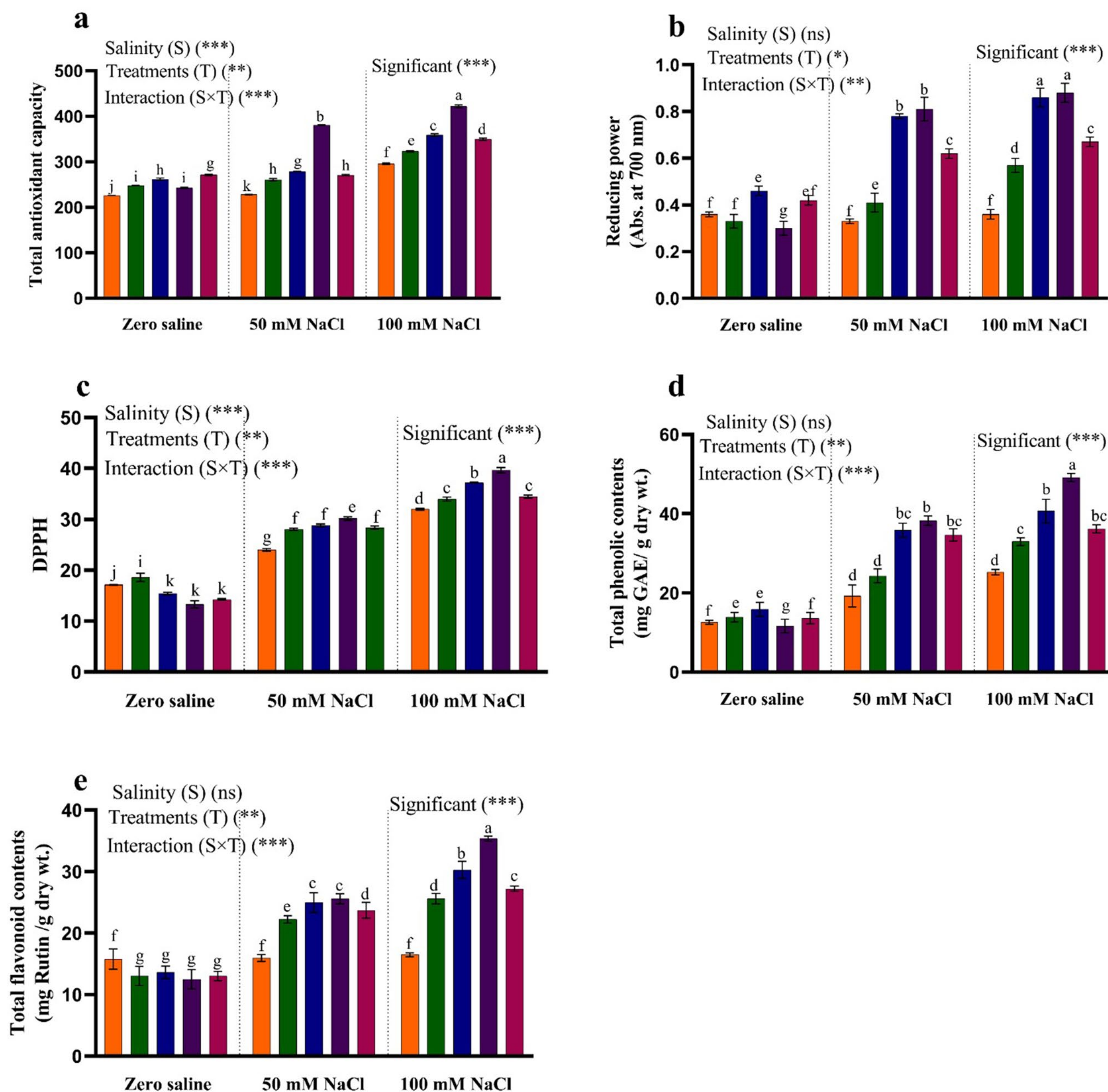

Fig. 1 Effect of different bacterial treatments in combination with three salinity levels (0, 50, and 100 mM NaCl) on **a** total antioxidant capacity, **b** reducing power, **c** DPPH (1,1-diphenyl-2-picrylhydrazyl), **d** total phenolic contents, and **e** total flavonoid contents of soybean after 40 days of growth. Fisher's test at $p < 0.05$ reveals significant variations in means (± standard error), which are different letters (a–k) on the same bars. *, **, and *** imply significance levels of 0.01, 0.05, and 0.001, respectively

The effect of PGPB on nodulation under salinity stress has been previously reported (Elsheikh and Wood 1990).

After 21 days of growth, salinity stress was not applied, indicating that the observed responses, including the profiles of citric acid, proline, total soluble sugar contents, total antioxidant, reducing power, DPPH free radical scavenging activity, the activities of CAT, POX, and PPO, total phenolic contents, and total flavonoid contents, were due to bacterization (Tables 4 and 5). The induction of phenols and proline in bacterized plants indicates the activation of the

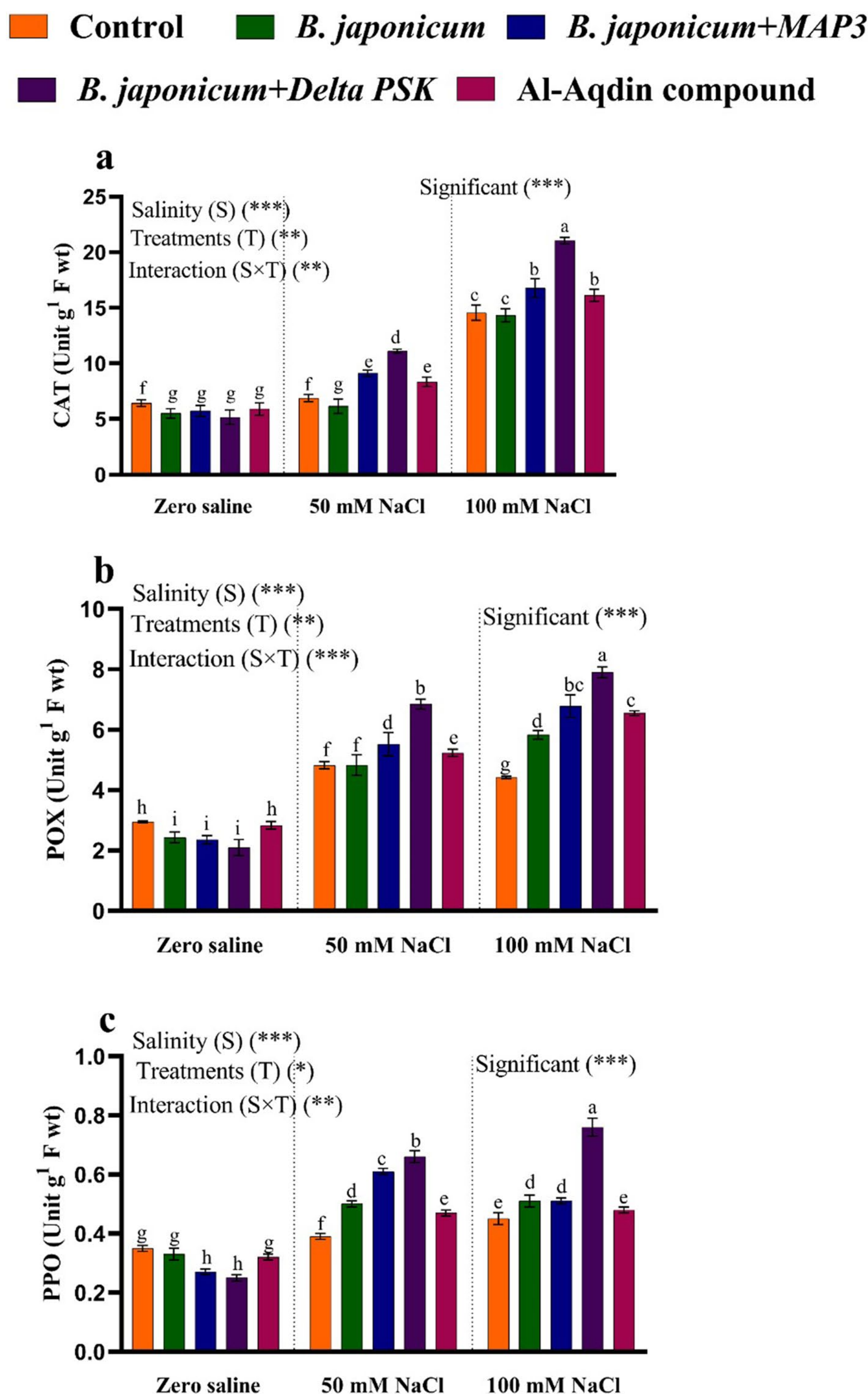

Fig. 2 Effect of different bacterial treatments in combination with three salinity levels (0, 50, and 100 mM NaCl) on the activity of **a** catalase (CAT), **b** peroxidase (POX), and **c** polyphenol oxidase (PPO) of soybean after 40 days of growth. Fisher's test at $p < 0.05$ reveals significant variations in means (± standard error), which are different letters (a–k) on the same bars. *, **, and *** imply significance levels of 0.01, 0.05, and 0.001, respectively

defense response. These results complement those recorded in grapevine plantlets in response to *Burkholderia phytofirmans* (Ait Barka et al. 2006). The detectable increase in flavins (Table 4) has also been reported in another study in *Astragalus sinicus* inoculated with *Burkholderia cepacian* under iron deficiency conditions (Zhou et al. 2018).

Due to NaCl stress, soybean produced higher levels of MDA and hydrogen peroxide and showed greater EL, which caused membrane damage and lipid peroxidation (Table 8). Membrane integrity was maintained in bacterized (*B. japonicum* + *Enterobacter* Delta PSK) plants and the detected amounts of MDA and EL were lower too. Damodaran et al. (2014) reported that membrane damage during NaCl stress

altered membrane function in mustard cultivars. The same effect was reported in *Astragalus sinicus* plants inoculated with *B. cepacian* (Zhou et al. 2018).

Although NaCl stimulates antioxidant enzymes, inoculating the plant with *B. japonicum* + Delta PSK further boosts the antioxidant process and removes toxic ROS. The same effect was recorded, although in another experimental purpose, in which *Enterobacter cloacae* antagonized the effect of *Ralstonia solanacearum* on potato plant (Mohamed et al. 2020). In the Haber–Weiss reaction, CAT is a vital antioxidant enzyme that scavenges superoxide radicals, hydroxyl radicals, and hydrogen peroxide (Sofy, Mancy et al. 2022). Therefore, CAT, POX, and PPO are upregulated in soybean plants to protect membranes from free radical damage caused by NaCl in this study. The increased POX activity in the combination-treated plants might be attributed to improved lignin biosynthesis and other antioxidant compounds that reduce oxidative stress as reported previously in *Vigna unguiculata* under salt stress Chen et al. (2007a, b).

Salinity stress for 19 days significantly affected plant growth and metabolism across the different treatment groups. The physical growth parameters were reduced with salt concentration but bacterization improved them compared with the control. Salinity induces a remarkable reduction in plant growth, pigmentation, and metabolism (Parida et al. 2004; Shabala and Cuin 2008). Various strains of PGPB alleviate this devastating effect under salinity stress (Hamdia et al. 2004; Rojas-Tapias et al. 2012), as corroborated by our results. As shown previously, the increase in the number of root nodules in bacterized plants under salinity stress supports the role of PGBP in enhancing growth and nodulation (M. Mowafy, S. Agha et al. 2022). Compared with the control, the increase in chlorophyll content in Delta PSK-treated plants is the real reason for better growth, indicating the compatibility of the *Enterobacter* strain used in this study with soybean plants and its ability to relieve salinity stress.

The ability to produce indole-3-acetic acid, siderophores, and solubilized phosphate may have conferred upon Delta PSK the ability to support soybean growth under salinity stress (Agha, Abbas et al. 2021), resulting in antioxidant responses via regulation of osmolyte accumulation and the development of free radical scavenging activities. The same conclusion was drawn in several studies (Mishra, Mishra et al. 2021; Fouda and Sofy 2022). Simultaneously, the amounts of citric acid, total soluble sugars, and proline increased leading to an elevated internal osmotic pressure in the combination-treated plants compared with the control. This was accompanied by lower lipid peroxidation and EL, indicating the integrity of the cellular membrane under such stress. Similar results have been reported in other studies (Prittesh et al. 2020, Pérez-Rodriguez, Pontin et al. 2022). Furthermore, the increase in TA capacity (Fig. 1) and antioxidant enzyme activity (Fig. 2) reflects the stimulatory effect of *B. japonicum* + Delta PSK on systematic resistance, which eventually decreases the levels of ethylene, a metabolite that accumulates due to salinity stress (Fan et al. 2020). Further, plants that received Delta PSK showed higher levels of total phenolic compounds (Fig. 1), a result reported previously for *Enterobacter*-treated tomato and maize plants (Ali et al. 2022, Pérez-Rodriguez, Pontin et al. 2022).

Conclusion

The mutualistic interactions with proper PGPB can increase plant ability to withstand abiotic stress for their numerous direct and indirect mechanisms including phytohormones production, ACC deaminase activity, and nutrient sequestration which are beneficial to plant growth and productivity. This study demonstrated the ability of *Enterobacter* Delta PSK to serve as a soybean growth promoter under salinity stress leading to improved physiological and growth parameters. The impact of these isolates on soybean yield is currently being reported. An open-field experiment is ongoing to ensure the effectiveness of *Enterobacter* Delta PSK along with *B. japonicum* on soybean under salinity stress in the local environment.

Author contribution Mona S. Agha is the PhD student who did this the experiments collected and organized the data and participate in writing. Samia A. Haroun is the main PhD supervisor in the thesis. She put the design of the experiment, followed the experiments and participated in writing. Mohamed A. Abbas is the supervisor of the thesis and he participated in writing. Mahmoud R. Sofy participated in data analysis and representation as well as writing. Amr M. Mowafy was the day by day supervisor during this study. He put the idea and plan of work and he participated in writing and data representaion.

Funding Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

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References

Abd-ElGawad AM, Rashad YM, Abdel-Azeem AM, Al-Barati SA, Assaeed AM, Mowafy AM (2020) *Calligonum polygonoides* L. shrubs provide species-specific facilitation for the understory plants in coastal ecosystem. Biology 9(8):232. https://doi.org/10.3390/biology9080232

Abu-Shahba MS, Mansour MM, Mohamed HI, Sofy MR (2022) Effect of biosorptive removal of cadmium ions from hydroponic solution containing indigenous garlic peel and mercerized garlic peel on lettuce productivity. Sci Horticul 293:110727. https://doi.org/10.1016/j.scienta.2021.110727

Acosta-Motos JR, Ortuño MF, Bernal-Vicente A, Diaz-Vivancos P, Sanchez-Blanco MJ, Hernandez JA (2017) Plant responses to salt stress: adaptive mechanisms. Agronomy 7(1):18. https://doi.org/10.3390/agronomy7010018

Agha MS, Abbas MA, Sofy MR, Haroun SA, Mowafy AM (2021) Dual inoculation of *Bradyrhizobium* and *Enterobacter* alleviates the adverse effect of salinity on *Glycine max* seedling. Notul Bota Horti Agro Cluj-Nap 49(3):12461. https://doi.org/10.15835/nbha49312461

Ahmed W, Zhou G, Yang J, Munir S, Ahmed A, Liu Q, Zhao Z, Ji G (2022) *Bacillus amyloliquefaciens* WS-10 as a potential plant growth-promoter and biocontrol agent for bacterial wilt disease of flue-cured tobacco. Egy J Biol Pest Con 32(1):1–14. https://doi.org/10.1186/s41938-022-00527-5

Ait Barka E, Nowak J, Clément C (2006) Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia phytofirmans* strain PsJN. Appl Environm Microbiol 72(11):7246–7252. https://doi.org/10.1128/AEM.01047-06

Akrami M, Arzani A (2018) Physiological alterations due to field salinity stress in melon (*Cucumis melo* L.). Acta Physiol Plantarum 40(5):1–14. https://doi.org/10.1007/s11738-018-2657-0

Al Hassan M, Fuertes MM, SÁNCHEZ FJR, Vicente O, Boscaiu M (2015) Effects of salt and water stress on plant growth and on accumulation of osmolytes and antioxidant compounds in cherry tomato. NotuL Bota Horti Agro Cluj-Nap 43(1):1–11. https://doi.org/10.15835/nbha4319793

ALHaithloul HAS, Khan MI, Musa A, Ghoneim MM, ALrashidi AA, Khan I, Azab E, Gobouri AA, Sofy MR, El-Sherbiny M (2022) Phytotoxic effects of *Acacia saligna* dry leachates on germination, seedling growth, photosynthetic performance, and gene expression of economically important crops. PeerJ 10:e13623. https://doi.org/10.7717/peerj.13623

Ali B, Wang X, Saleem MH, Hafeez A, Afridi MS, Khan S, Ullah I, Amaral Júnior ATd, Alatawi A, Ali S (2022) PGPR-mediated salt tolerance in maize by modulating plant physiology, antioxidant defense, compatible solutes accumulation and bio-surfactant producing genes. Plants 11(3):345. https://doi.org/10.3390/plants11030345

Alsaeedi A, El-Ramady H, Alshaal T, El-Garawany M, Elhawat N, Al-Otaibi A (2019) Silica nanoparticles boost growth and productivity of cucumber under water deficit and salinity stresses by balancing nutrients uptake. Plant Physiol Bioch 139:1–10. https://doi.org/10.1016/j.plaphy.2019.03.008

Badawy IH, Hmed AA, Sofy MR, Al-Mokadem AZ (2022) Alleviation of Cadmium and Nickel Toxicity and Phyto-Stimulation of Tomato Plant L. by Endophytic *Micrococcus luteus* and *Enterobacter cloacae*. Plants 11(15):2018. https://doi.org/10.3390/plants11152018

Bates L (1973) Waldren RP andTeare ID 1973 rapid determination of free proline for water stress studies. Plant Soil 39:205–208. https://doi.org/10.1007/BF00018060

Benjamin J, Nielsen D (2006) Water deficit effects on root distribution of soybean, field pea and chickpea. Field Crops Res 97(2–3):248–253. https://doi.org/10.1016/j.fcr.2005.10.005

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. Jof Plant Growth Regul 36(1):215–226. https://doi.org/10.1007/s00344-016-9631-0

Chaparro JM, Sheflin AM, Manter DK, Vivanco JM (2012) Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils 48(5):489–499. https://doi.org/10.1007/s00374-012-0691-4

Chen C, Tao C, Peng H, Ding Y (2007a) Genetic analysis of salt stress responses in asparagus bean (*Vigna unguiculata* (L.) ssp. sesquipedalis Verdc.). J Heredity 98(7):655–665. https://doi.org/10.1093/jhered/esm084

Chen XH, Koumoutsi A, Scholz R, Eisenreich A, Schneider K, Heinemeyer I, Morgenstern B, Voss B, Hess WR, Reva O (2007b) Comparative analysis of the complete genome sequence of the plant growth–promoting bacterium *Bacillus amyloliquefaciens* FZB42. Nature Biotechnol 25(9):1007–1014. https://doi.org/10.1038/nbt1325

Damodaran T, Rai R, Jha S, Kannan R, Pandey B, Sah V, Mishra V, Sharma D (2014) Rhizosphere and endophytic bacteria for induction of salt tolerance in gladiolus grown in sodic soils. J Plant Inter 9(1):577–584. https://doi.org/10.1080/17429145.2013.873958

Dawood MF, Sofy MR, Mohamed HI, Sofy AR, Abdel-kader HA (2022) Hydrogen Sulfide Modulates Salinity Stress in Common Bean Plants by Maintaining Osmolytes and Regulating Nitric Oxide Levels and Antioxidant Enzyme Expression. J Soil Sci Plant Nut. https://doi.org/10.1007/s42729-022-00921-w

De Souza MM, Mendes CR, Doncato KB, Badiale-Furlong E, Costa CS (2018) Growth, phenolics, photosynthetic pigments, and antioxidant response of two new genotypes of sea asparagus (*Salicornia neei* Lag.) to salinity under greenhouse and field conditions. Agriculture 8(7):115. https://doi.org/10.3390/agriculture8070115

Dhungana S, Kim BR, Son JH, Kim HR, Shin DH (2015) Comparative study of CaMsrB2 gene containing drought-tolerant transgenic rice (*Oryza sativa* L.) and non-transgenic counterpart. J Agr Crop Sci 201(1):10–16. https://doi.org/10.1111/jac.12100

Elsheikh EAE, Wood M (1990) Effect of salinity on growth, nodulation and nitrogen yield of chickpea (*Cicer arietinum* L.). J Exper Bot 41(10):1263–1269. https://doi.org/10.1093/jxb/41.10.1263

El-Sheshtawy HS, Mahdy HM, Sofy AR, Sofy MR (2022) Production of biosurfactant by *Bacillus megaterium* and its correlation with lipid peroxidation of *Lactuca sativa*. Egy J Petr 31(2):1–6. https://doi.org/10.1016/j.ejpe.2022.03.001

Fahramand M, Mahmoody M, Keykha A, Noori M, Rigi K (2014) Influence of abiotic stress on proline, photosynthetic enzymes and growth. Int Res J Appl Basic Sci 8(3):257–265

Fan D, Subramanian S, Smith DL (2020) Plant endophytes promote growth and alleviate salt stress in *Arabidopsis thaliana*. Sci Rep 10(1):1–18. https://doi.org/10.1038/s41598-020-69713-5

Fang Y, Xiong L (2015) General mechanisms of drought response and their application in drought resistance improvement in plants. Cellular Molecul Life Sci 72(4):673–689. https://doi.org/10.1007/s00018-014-1767-0

Fouda HM, Sofy MR (2022) Effect of biological synthesis of nanoparticles from *Penicillium chrysogenum* as well as traditional salt and chemical nanoparticles of zinc on canola plant oil productivity and metabolic activity. Egy J Chem 65(3):1–2. https://doi.org/10.21608/ejchem.2021.95120.4469

Hamdia M, Shaddad M, Doaa MM (2004) Mechanisms of salt tolerance and interactive effects of *Azospirillum brasilense* inoculation on maize cultivars grown under salt stress conditions. Plant

Growth Regul 44(2):165–174. https://doi.org/10.1023/B:GROW.0000049414.03099.9b

Hansen J, Møller I (1975) Percolation of starch and soluble carbohydrates from plant tissue forquantitative determination with anthrone. Analy Bioch 68(1):87–94. https://doi.org/10.1016/0003-2697(75)90682-X

Ha-Tran DM, Nguyen TTM, Hung S-H, Huang E, Huang C-C (2021) Roles of plant growth-promoting rhizobacteria (PGPR) in stimulating salinity stress defense in plants: a review. I International J Molecul Sci 22(6):3154. https://doi.org/10.3390/ijms22063154

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Bioch Biophy 125(1):189–198. https://doi.org/10.1016/0003-9861(68)90654-1

Hiscox J, Israelstam G (1979) Different methods of chlorophyll extraction. Can J Bot 57:1332–1332. https://doi.org/10.1139/b79-163

Hwang H-H, Chien P-R, Huang F-C, Hung S-H, Kuo C-H, Deng W-L, Chiang E-PI, Huang C-C (2021) A plant endophytic bacterium, *Burkholderia seminalis* strain 869T2, promotes plant growth in *Arabidopsis*, Pak Choi, Chinese Amaranth, Lettuces, and other vegetables. Microorganisms 9(8):1703. https://doi.org/10.3390/microorganisms9081703

Khan MA, Asaf S, Khan AL, Ullah I, Ali S, Kang S-M, Lee I-J (2019) Alleviation of salt stress response in soybean plants with the endophytic bacterial isolate *Curtobacterium* sp. SAK1. Ann Microbiol 69(8):797–808. https://doi.org/10.1007/s13213-019-01470-x

Khojely DM, Ibrahim SE, Sapey E, Han T (2018) History, current status, and prospects of soybean production and research in sub-Saharan Africa. The Crop J 6(3):226–235. https://doi.org/10.1016/j.cj.2018.03.006

Kosem N, Han Y-H, Moongkarndi P (2007) Antioxidant and cytoprotective activities of methanolic extract from *Garcinia mangostana* hulls. Sci Asia 33(1):283–292. https://doi.org/10.2306/scienceasia1513-1874.2007.33.283

Kumar A, Singh S, Gaurav AK, Srivastava S, Verma JP (2020) Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants. Front Microbiol 11:1216. https://doi.org/10.3389/fmicb.2020.01216

Lutts S, Kinet J, Bouharmont J (1996) Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Plant Growth Regul 19(3):207–218

Maswada HF, Djanaguiraman M, Prasad P (2018) Response of photosynthetic performance, water relations and osmotic adjustment to salinity acclimation in two wheat cultivars. Acta Physiol Plantarum 40(6):1–15. https://doi.org/10.1007/s11738-018-2684-x

Metwali EM, Abdelmoneim TS, Bakheit MA, Kadasa NM (2015) Alleviation of salinity stress in faba bean ('*Vicia faba*'L.) plants by inoculation with plant growth promoting rhizobacteria (PGPR). Plant Omics 8(5):449–460

Mickky BM, Abbas MA, Sameh NM (2019) Morpho-physiological status of fenugreek seedlings under NaCl stress. J King Saud Uni-Sci 31(4):1276–1282. https://doi.org/10.1016/j.jksus.2019.02.005

Mishra P, Mishra J, Arora NK (2021) Plant growth promoting bacteria for combating salinity stress in plants–recent developments and prospects: a review. Microbiol Res 252:126861. https://doi.org/10.1016/j.micres.2021.126861

Mohamed BF, Sallam N, Alamri SA, Abo-Elyousr KA, Mostafa YS, Hashem M (2020) Approving the biocontrol method of potato wilt caused by *Ralstonia solanacearum* (Smith) using Enterobacter cloacae PS14 and Trichoderma asperellum T34. Egy J Biol Pest Con 30(1):1–13. https://doi.org/10.1186/s41938-020-00262-9

Moradi A, Piri R (2018) Plant growth promoting rhizobactria enhance salinity stress tolerance in Cumin (*Cuminum cyminum* L.) during germination stage. Jof Plant Proc and Funct 6(22):47–54

Mowafy M, A, M S. Agha, S A. Haroun, M A. Abbas and M Elbalkini, (2022) Insights in nodule-inhabiting plant growth promoting bacteria and their ability to stimulate Vicia faba growth. E Egy J Basic Appl Sci 9(1):51–64. https://doi.org/10.1080/2314808X.2021.2019418

Ngalimat MS, Mohd Hata E, Zulperi D, Ismail SI, Ismail MR, Mohd Zainudin NAI, Saidi NB, Yusof MT (2021) Plant growth-promoting bacteria as an emerging tool to manage bacterial rice pathogens. Microorganisms 9(4):682. https://doi.org/10.3390/microorganisms9040682

Oktay M, Küfreviolu I, Kocaçalişkan I, Şaklrolu H (1995) Polyphenoloxidase from Amasya apple. J of Food Sci 60(3):494–496. https://doi.org/10.1111/j.1365-2621.1995.tb09810.x

Pal KK, Dey R, Sherathia DN, Mangalassery S, Kumar A, Rupapara RB, Mandaliya M, Rawal P, Bhadania RA, Thomas M (2021) Alleviation of Salinity Stress in Peanut by Application of Endophytic Bacteria. Front Microbiol 12:791. https://doi.org/10.3389/fmicb.2021.650771

Panigrahi S, Mohanty S, Rath C (2020) Characterization of endophytic bacteria *Enterobacter cloacae* MG00145 isolated from *Ocimum sanctum* with Indole Acetic Acid (IAA) production and plant growth promoting capabilities against selected crops. South Afr J of Bot 134:17–26. https://doi.org/10.1016/j.sajb.2019.09.017

Parida AK, Das A, Mittra B (2004) Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*. Trees 18(2):167–174. https://doi.org/10.1007/s00468-003-0293-8

Pérez-Rodriguez MM, Pontin M, Piccoli P, Lobato Ureche MA, Gordillo MG, Funes-Pinter I, Cohen AC (2022) Halotolerant native bacteria *Enterobacter* 64S1 and *Pseudomonas* 42P4 alleviate saline stress in tomato plants. Physiol Plant 174(4):e13742. https://doi.org/10.1111/ppl.13742

Prittesh P, Avnika P, Kinjal P, Jinal HN, Sakthivel K, Amaresan N (2020) Amelioration effect of salt-tolerant plant growth-promoting bacteria on growth and physiological properties of rice (*Oryza sativa*) under salt-stressed conditions. Arch Microbiol 202(9):2419–2428. https://doi.org/10.1007/s00203-020-01962-4

Ramadoss D, Lakkineni VK, Bose P, Ali S, Annapurna K (2013) Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. Springerplus 2(1):1–7. https://doi.org/10.1186/2193-1801-2-6

Ramesh A, Sharma SK, Sharma MP, Yadav N, Joshi OP (2014) Plant growth-promoting traits in *Enterobacter cloacae* subsp. dissolvens MDSR9 isolated from soybean rhizosphere and its impact on growth and nutrition of soybean and wheat upon inoculation. Agr Res 3(1):53–66. https://doi.org/10.1007/s40003-014-0100-3

Ramongolalaina C, Teraishi M, Okumoto Y (2018) QTLs underlying the genetic interrelationship between efficient compatibility of *Bradyrhizobium* strains with soybean and genistein secretion by soybean roots. PLoS ONE 13(4):e0194671

Rojas-Tapias D, Moreno-Galván A, Pardo-Díaz S, Obando M, Rivera D, Bonilla R (2012) Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (Zea mays). Applied Soil Ecol 61:264–272. https://doi.org/10.1016/j.apsoil.2012.01.006

Sairam R, Tyagi A (2004) Physiology and molecular biology of salinity stress tolerance in plants. Curr sci 86(3):407–421

Şengül M, Yildiz H, Kavaz A (2014) The effect of cooking on total polyphenolic content and antioxidant activity of selected vegetables. Int J Food Prop 17(3):481–490. https://doi.org/10.1080/10942912.2011.619292

Shabala S, Cuin TA (2008) Potassium transport and plant salt tolerance. Physiol Plant 133(4):651–669. https://doi.org/10.1111/j.1399-3054.2007.01008.x

Sheteiwy MS, Abd Elgawad H, Xiong YC, Macovei A, Brestic M, Skalicky M, Shaghaleh H, Alhaj Hamoud Y, El-Sawah AM (2021)

Inoculation with *Bacillus amyloliquefaciens* and mycorrhiza confers tolerance to drought stress and improve seed yield and quality of soybean plant. Physiol Plant 172(4):2153–2169. https://doi.org/10.1111/ppl.13454
Shrivastava P, Kumar R (2015) Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi Biol Sci 22(2):123–131. https://doi.org/10.1016/j.sjbs.2014.12.001
Singh A (2022) Soil salinity: a global threat to sustainable development. Soil Use Manag 38(1):39–67. https://doi.org/10.1111/sum.12772
Singh RP, Pandey DM, Jha PN, Ma Y (2022) ACC deaminase producing rhizobacterium Enterobacter cloacae ZNP-4 enhance abiotic stress tolerance in wheat plant. PloS one 17(5):e0267127. https://doi.org/10.1371/journal.pone.0267127
Snell CT, Snell CT (1937) Colorimetric methods of analysis. Soil Sci. https://doi.org/10.1097/00010694-195907000-00018
Sofy MR, Aboseidah AA, Heneidak SA, Ahmed HR (2021) ACC deaminase containing endophytic bacteria ameliorate salt stress in *Pisum sativum* through reduced oxidative damage and induction of antioxidative defense systems. Environ Sci Poll Res 28(30):40971–40991. https://doi.org/10.1007/s11356-021-13585-3
Sofy MR, Mancy AG, Alnaggar AEAM, Refaey EE, Mohamed HI, Elnosary ME, Sofy AR (2022) A polishing the harmful effects of Broad Bean Mottle Virus infecting broad bean plants by enhancing the immunity using different potassium concentrations. Not Bot Horti Agro Cluj-Nap 50(1):12654. https://doi.org/10.15835/nbha50112654
Synek L, Rawat A, L'Haridon F, Weisskopf L, Saad MM, Hirt H (2021) Multiple strategies of plant colonization by beneficial endophytic *Enterobacter* sp. SA187. Environ Microbiol 23(10):6223–6240. https://doi.org/10.1111/1462-2920.15747
Vashisth A, Nagarajan S (2010) Effect on germination and early growth characteristics in sunflower (*Helianthus annuus*) seeds exposed to static magnetic field. J Plant Physiol 167(2):149–156. https://doi.org/10.1016/j.jplph.2009.08.011
Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci 151(1):59–66. https://doi.org/10.1016/S0168-9452(99)00197-1
Vibhuti CS, Bargali K, Bargali S (2015) Seed germination and seedling growth parameters of rice (*Oryza sativa* L.) varieties as affected by salt and water stress. Ind J Agric Sci 85(1):102–108
Villanueva MC, Muniz BF, Tames RS (1985) Effects of glyphosate on growth and the chlorophyll and carotenoid levels of yellow nutsedge (*Cyperus esculentus*). Weed Sci 33(6):751–754. https://doi.org/10.1017/S0043174500083284
Wang C-F, Han G-L, Yang Z-R, Li Y-X, Wang B-S (2022) Plant salinity sensors: current understanding and future directions. Front in plant sci. https://doi.org/10.3389/fpls.2022.859224
Yasmin H, Naeem S, Bakhtawar M, Jabeen Z, Nosheen A, Naz R, Keyani R, Mumtaz S, Hassan MN (2020) Halotolerant rhizobacteria *Pseudomonas pseudoalcaligenes* and *Bacillus subtilis* mediate systemic tolerance in hydroponically grown soybean (*Glycine max* L.) against salinity stress. PLoS One 15(4):e0231348. https://doi.org/10.1371/journal.pone.0231348
Yildirim A, Mavi A, Kara A (2001) Determination of antioxidant and antimicrobial activities of Rumer crispus L extracts. J of Agri and food chem 49(8):4083–4089. https://doi.org/10.1021/jf0103572
Zeitoun M, Mansour HM, Ezzat S, El Sohaimy S (2017) Effect of pretreatment of olive leaves on phenolic content and antioxidant activity. Am J Food Technol 12(2):132–139. https://doi.org/10.3923/ajft.2017.132.139
Zhang M, Liu S, Wang Z, Yuan Y, Zhang Z, Liang Q, Yang X, Duan Z, Liu Y, Kong F (2022) Progress in soybean functional genomics over the past decade. Plant Biotechnol J20(2):256. https://doi.org/10.1111/pbi.13682
Zhou C, Zhu L, Ma Z, Wang J (2018) Improved iron acquisition of *Astragalus sinicus* under low iron-availability conditions by soil-borne bacteria *Burkholderia cepacia*. J Plant Int 13(1):9–20. https://doi.org/10.1080/17429145.2017.1407000

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