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Alleviation of Salt Stress in Pepper (*Capsicum annum* L.) Plants by Plant Growth-Promoting Rhizobacteria

Mi-Seon Hahm[1], Jin-Soo Son[1], Ye-Ji Hwang[1], Duk-Kee Kwon[2], and Sa-Youl Ghim[1]*

[1]*School of Life Science, Institute for Microorganisms, and BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu 41566, Republic of Korea*
[2]*Department of Biology Education, Kyungpook National University, Daegu 41566, Republic of Korea*

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**Corresponding author*
Phone: +82-53-950-5374;
Fax: +82-53-955-5522;
E-mail: ghimsa@knu.ac.kr

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In the present study, we demonstrate that the growth of salt-stressed pepper plants is improved by inoculation with plant growth-promoting rhizobacteria (PGPR). Three PGPR strains (*Microbacterium oleivorans* KNUC7074, *Brevibacterium iodinum* KNUC7183, and *Rhizobium massiliae* KNUC7586) were isolated from the rhizosphere of pepper plants growing in saline soil, and pepper plants inoculated with these PGPR strains exhibited significantly greater plant height, fresh weight, dry weight, and total chlorophyll content than non-inoculated plants. In addition, salt-stressed pepper plants that were inoculated with *B. iodinum* KNUC7183 and *R. massiliae* KNUC7586 possessed significantly different total soluble sugar and proline contents from non-inoculated controls, and the activity of several antioxidant enzymes (ascorbate peroxidase, guaiacol peroxidase, and catalase) was also elevated in PGPR-treated plants under salt stress. Overall, these results suggest that the inoculation of pepper plants with *M. oleivorans* KNUC7074, *B. iodinum* KNUC7183, and *R. massiliae* KNUC7586 can alleviate the harmful effects of salt stress on plant growth.

Keywords: Salt stress, plant growth-promoting rhizobacteria, pepper, antioxidant enzyme, proline, total soluble sugar

Introduction

Both plant growth and productivity can be negatively affected by biotic and abiotic stressors, the latter of which include high and low (*i.e.*, freezing) temperatures, drought, salinity, flooding, and heavy metal exposure. Among these stressors, salinity is one of the main factors that impede the growth, development, and yield of crop plants [1]. In particular, saline soil reduces plant productivity *via* restricted water uptake, unbalanced absorption of essential nutrients, and osmotic stress. Therefore, the development of salt-tolerant plants is a much-desired scientific goal. However, efforts have only been met with limited success, and only a few major genetic determinants of salt tolerance have been identified [2, 3].

Interestingly, as an alternative to breeding and genetic manipulation, plant salt tolerance can also be improved by the application of salt-tolerant microorganisms, and beneficial microorganisms, such as plant growth-promoting rhizobacteria (PGPR), that inhabit the rhizosphere have been investigated for their potential to alleviate salt stress. These studies have shown that the majority of PGPR, including strains of *Agrobacterium*, *Azospirillum*, *Bacillus*, *Pseudomonas*, and *Rhizobium* species, increase the salt tolerance of their host plants [4–6] and, in addition, that PGPR can stimulate plant growth through a variety of mechanisms, including the fixation of atmospheric nitrogen, solubilization of phosphate, and production of phytohormones (*e.g.*, indole-3-acetic acid (IAA), gibberellin, cytokinin, and abscisic acid), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and exopolysaccharide (EPS). For example, treatment with the ACC-producing bacterium *Pseudomonas mendocina* has been shown to enhance the uptake of essential nutrients by salt-stressed lettuce [7], and treatment with the EPS-producing bacteria *Bacillus pumilus* and *Exiguobacterium oxidotolerans* has been shown to increase the height and

bacoside A content of salt-stressed brahmi (*Bacopa monnieri*) [5]. Therefore, the application of PGPR can ameliorate salt stress in crop plants, promote plant growth [8], and control diseases [9]. Indeed, PGPR have been reported to improve the growth of a variety of crop species, including tomato [10], red pepper [11], maize [1], mung bean [4, 12], and lettuce [7], growing under saline conditions. In addition, PGPR also help plants resist salt stress by increasing the activity of both antioxidant enzymes and non-enzymatic antioxidants [13, 14].

Pepper (*Capsicum annum* L.; Solanaceae) is a popular and important crop in South Korea and is used in many traditional Korean foods, including kimchi (a traditional fermented vegetable) [15]. In Korea, peppers are repeatedly cultivated in greenhouses and fields for a number of years, a practice that can increase soil salinity through the accumulation of organic fertilizers and pesticides, and as a result, many agricultural lands have become highly saline, thus reducing plant yield and causing major economic losses. The application of PGPR can enhance plant growth and productivity by eliciting salt-stress tolerance in host plants [16, 17]. Although many studies have reported that the application of PGPR is an eco-friendly and sustainable agricultural strategy, only a few studies have examined the ability of PGPR to induce salt-stress tolerance in pepper [11]. Therefore, the present study was conducted to investigate the effects of three PGPR strains (*Microbacterium oleivorans* KNUC7074, *Brevibacterium iodinum* KNUC7183, and *Rhizobium massiliae* KNUC7586) on the growth of pepper plants grown under salt-stress conditions.

Materials and Methods

Plant Material and Growth Conditions

Pepper (*Capsicum annum* L.) seeds (Bugang, Dongbu Farm Hannong, Korea) were surface sterilized with 1.2% hypochlorite for 30 min, rinsed with tap water for 30 min, and dried at room temperature. The seeds were then sown in 50-hole plastic tray pots (7 cm diameter) that were filled with an autoclaved commercial soil mixture (14.5 g per pot), and the resulting pepper plants were grown in a growth room with a 12 h:12 h light:dark cycle, temperature of 25°C, and relative humidity of 50%. After 3 weeks, the early four-leaf-stage seedlings were transplanted to plastic pots (9 cm diameter; 22.5 g per pot) and then kept in the growth room.

PGPR Strains and Experimental Design

Three PGPR strains that possess plant growth-promoting attributes, like phosphate solubilization and the production of IAA, siderophore, and ACC deaminase, were previously isolated from the from the rhizospheric soil of pepper plants growing under saline conditions in Mir-Yang, Republic of Korea [18]. Cultures of the three PGPR strains were grown in 500 ml of tryptic soy broth at 30°C. Then, after 2 days, the cultures were centrifuged at 6,200 ×*g* for 10 min at 4°C, and the resulting pellets were washed twice with sterilized distilled water and resuspended in sterilized distilled water to an optical density of 0.8 at 600 nm (approximate cell density of 1×10^8 to 1×10^9 CFU/ml).

At 2 days after the seedlings had been transplanted, each plant was inoculated with either 30 ml of one of the PGPR cell suspensions or with 30 ml of sterilized distilled water (negative controls), and at 5 days after inoculation, the individual plants were exposed to salt stress through the application of 20 ml of 200 mM NaCl for 2 weeks (salt-stress treatment) or 20 ml of sterilized tap water for 2 weeks (non-stress control). The experiment was performed using a completely randomized block design, with 15 replicates per treatment, and was repeated five times.

Measurement of Plant Growth, Leaf Water Content, and Chlorophyll Content

After 2 weeks, the pepper plants were harvested. The dry weight was measured by oven-drying the seedlings to constant weight at 65°C for 5 days, and the leaf relative water content (LRWC) was measured according to González and González-Vilar [19]. In addition, the LRWC of individual leaves was calculated using the following equation: LRWC (%) = [(FW – DW)/(TW – DW)] × 100, where FW is the fresh weight, TW is the turgid fresh weight after 24 h, and DW is the dry weight after drying at 65°C for 24 h. The concentrations of chlorophyll (Chl) *a* and *b* in the pepper leaves were also determined, using spectrophotometry as described by Arnon [20]. Briefly, Chl *a* and Chl *b* were extracted from 0.1 g pepper leaves in 80% acetone at 4°C for 24 h in the dark, and then the contents of Chl *a* and Chl *b* were determined by measuring the absorbance of the extracts at 663 and 645 nm, respectively. This experiment was repeated three times, with three replications per treatment.

Antioxidant Enzyme Assays

Fresh leaves (0.5 g) were frozen in liquid nitrogen and homogenized in either 2 ml of extraction buffer (0.05 M Tris-HCl, pH 7.6, 3 mM $MgCl_2$, and 1 mM ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate) with 2 mM ascorbate, for the analysis of ascorbate peroxidase (APX) activity, or 2 ml of extraction buffer without ascorbate, for the analysis of catalase (CAT) and guaiacol peroxidase (GPX) activities. All the enzyme assays were performed on ice (4°C). The crude homogenates were centrifuged at 9,600 ×*g* for 10 min at 4°C, and the supernatants were used for the enzyme assays. The protein content was estimated using a Pierce BCA protein assay kit (Thermo Fisher Scientific, USA).

The activity of APX (E.C. 1.11.1.11) was measured using the methods described by Asada [21]. Briefly, reaction mixtures of 50 mM potassium phosphate buffer, 1 mM EDTA, 5 mM ascorbate, 1 mM H_2O_2, and individual enzyme extracts were prepared, and

the enzyme activity was measured as the H_2O_2-dependent oxidation of ascorbate, which was calculated as the decrease in absorbance (ΔE, $min^{-1} \cdot g^{-1} \cdot$FW) at 290 nm.

The activity of CAT (E.C. 1.11.1.6) was assayed using the methods described by Upadhyay *et al.* [22]. Briefly, reaction mixtures of 50 mM phosphate buffer that contained 0.1 mM EDTA, 3% H_2O_2, and individual enzyme extracts were prepared, and the enzyme activity was measured as the H_2O_2-dependent oxidation of hydrogen peroxide, which was calculated as the decrease in absorbance (ΔE, $min^{-1} \cdot g^{-1} \cdot$FW) at 240 nm.

The activity of GPX (E.C. 1.11.1.7) was estimated using the methods described by Hammerschmidt *et al.* [23]. Briefly, reactions mixtures of 50 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 50 mM H_2O_2, and individual enzyme extracts were prepared, and the enzyme activity was measured as the H_2O_2-dependent oxidation of guaiacol, which was calculated as the increase in absorbance (ΔE, $min^{-1} \cdot g^{-1} \cdot$FW) at 470 nm.

Determination of Proline and Total Soluble Sugar (TSS) Contents

The proline content was estimated using the method of Bates *et al.* [24]. Briefly, fresh leaves (0.5 g) were frozen and homogenized in 10 ml of 3% sulfosalicylic acid using a chilled mortar and pestle. The homogenates were filtered through Whatman No. 2 paper (Toyo Roshi Kaisha, Ltd., Japan), and the resulting filtrates were mixed with a 1:1:1 mixture of glacial acetic acid, acid, and ninhydrin in individual glass tubes. The mixtures were incubated at 90°C for 1 h, after which the reactions were stopped by transferring the tubes to ice. Toluene (1 ml) was then added to each sample, and the solutions were mixed vigorously for 30 sec and left at room temperature until the two phases separated. The absorbance of the extracted upper phase (toluene) was then measured at 520 nm, and the amount of proline (μmol/g FW) was determined using a standard curve [24].

To determine the TSS content, 0.1 g of fresh leaf tissue was frozen in liquid nitrogen and homogenized in a glass tube that contained 5 ml of preheated 80% (v/v) ethanol. Then, 1.5 ml aliquots of the homogenates were transferred to 2-ml centrifuge tubes and incubated for 30 min at 80°C in a heat block. Afterward, the homogenates were centrifuged at 16,200 ×*g* for 10 min at 4°C, and the supernatants were collected in 15-ml Falcon tubes for TSS analysis, which was performed using a slight modification of the anthrone-reagent method described by Irigoyen *et al.* [25]. The absorbance was measured at 620 nm, and the TSS content (μg/g FW) was calculated based on a constructed standard curve that ranged from 0 to 10 mg glucose (Sigma-Aldrich, USA).

Statistical Analysis

Statistical analysis was performed using SPSS software ver. 22.0 (SPSS, Inc., USA). Treatment means were compared using analysis of variance, followed by Duncan's multiple range test, with a 95% confidence level.

Results

Characterization of PGPR Strains

In the present study, we used three PGPR strains that were previously isolated from the rhizosphere of pepper plants grown under highly saline conditions [18]. These isolates were *M. oleivorans* KNUC7074, *B. iodinum* KNUC7183, and *R. massiliae* KNUC7586. All three strains could produce both ACC deaminase and siderophore, and both *M. oleivorans* KNUC7074 and *R. massiliae* KNUC7586 could produce IAA and solubilize phosphate, whereas *B. iodinum* KNUC7183 could not (Table 1).

Effect of Saline Conditions on Plant Growth, LRWC, and Chlorophyll Content

The present study investigated the effect of PGPR application on the growth of pepper plants grown under normal and saline conditions. The pepper plants treated with each of the PGPR strains possessed significantly greater heights, total fresh weights, and total dry weights than non-inoculated (control) plants, regardless of growing conditions (normal or saline; Table 2). More specifically, the heights of plants inoculated with *M. oleivorans* KNUC7074, *B. iodinum* KNUC7183, and *R. massiliae* KNUC7586 were 12.6%, 14.9%, and 15.8% higher, respectively, than that of the control plants, and the root fresh weights of pepper plants inoculated with *M. oleivorans* KNUC7074, *B. iodinum* KNUC7183, and *R. massiliae* KNUC7586 were 33.2%, 50.8%, and 50.4% greater, respectively, than that of the control

Table 1. Plant growth-promoting (PGP) characteristics of the three selected isolates.

Isolate	Identity (%)	PGP characteristics[a]				GenBank Accession No.
		Phosphate solubilization	Siderophore production	IAA concentration (μg/ml)	ACC deaminase activity	
KNUC7074	*M. oleivorans* (100%)	+	+	25.26	+	KU891044
KNUC7183	*B. iodinum* (99%)	–	+	3.82	+	KU891043
KNUC7586	*R. massiliae* (99%)	+	+	11.63	+	KU891045

[a]Data obtained from Hahm *et al.* [18].

IAA, indole-3-acetic acid; ACC, 1-aminocyclopropane-1-carboxylate.

Table 2. Effects of three plant growth-promoting rhizobacterial strains on the height, fresh weight, and dry weight of pepper plants grown under normal and saline conditions.

NaCl concentration	Treatment	Height (cm)			Fresh weight (g)			Dry weight (g)		
		Total	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root
0 mM	Non-inoculated	31.96 A	13.56 A	17.63 A	5.64 A	4.99 A	0.64 A	0.564 A	0.468 A	0.107 A
	KNUC7074	35.98 B	15.69 B	20.37 BC	7.20 B	6.32 B	0.85 B	0.699 B	0.532 B	0.175 B
	KNUC7183	36.73 B	16.06 B	20.88 BC	7.54 B	6.68 B	0.96 BC	0.694 B	0.551 B	0.156 B
	KNUC7586	36.73 B	15.82 B	21.12 C	7.50 B	6.63 B	0.96 BC	0.713 B	0.549 B	0.166 B
200 mM	Non-inoculated	29.31 a	9.71 a	18.35 a	3.22 a	2.69 a	0.55 a	0.443 a	0.324 a	0.084 a
	KNUC7074	32.75 ab	12.69 b	19.83 a	4.38 b	3.56 ab	0.83 b	0.528 b	0.447 b	0.113 b
	KNUC7183	34.50 b	13.27 b	19.71 a	4.77 c	3.73 b	0.94 b	0.545 b	0.470 b	0.118 b
	KNUC7586	33.42 b	13.08 ab	19.42 a	4.47 bc	3.63 b	0.81 b	0.528 b	0.465 b	0.116 b

Values are the means of five replicated experiments. Ten plants were tested per treatment.
Mean values followed by different letters in a column are significantly different, according to Duncan's multiple range test ($p < 0.05$).

plants.

It was also clear that the 200 mM NaCl treatment induced salt stress in the treated seedlings and that the three PGPR strains helped the pepper plants tolerate salinity stress, even though the negative effects of salt stress on plant growth and development was not eliminated completely. More specifically, the heights of the salt-stressed pepper plants were 11.7%, 17.7%, and 14.0% higher when inoculated with *M. oleivorans* KNUC7074, *B. iodinum* KNUC7183, and *R. massiliae* KNUC7586, respectively, than when treated with water alone (Table 2). Meanwhile, the root growth of the salt-stressed plants was 50.5%, 72.6%, and 47.2% greater when inoculated with *M. oleivorans* KNUC7074, *B. iodinum* KNUC7183, and *R. massiliae* KNUC7586, respectively, than when treated with water alone (Table 2). Among the three PGPR, the growth-promoting effect of *B. iodinum* KNUC7183 was greater than that of the other two strains (Table 2).

In addition, the LRWC was also considerably reduced by the salt-stress treatment. For example, under normal conditions, the LRWC of the non-inoculated control plants was 6.2%, 7.1%, and 7.1% lower than that of the plants inoculated with *M. oleivorans* KNUC7074, *B. iodinum* KNUC7183, and *R. massiliae* KNUC7586, respectively. However, under saline conditions, the LRWC of the non-inoculated control plants was 18.0%, 20.3%, and 21.7% lower than that of the plants inoculated with *M. oleivorans* KNUC7074, *B. iodinum* KNUC7183, and *R. massiliae* KNUC7586, respectively.

The Chl content of leaves was determined for pepper plants grown under both normal and saline conditions. Inoculation with all three PGPR strains resulted in Chl contents that were greater than that of the non-inoculated plants, regardless of growing conditions (Table 3). Indeed, under normal conditions, inoculation with *M. oleivorans*

Table 3. Effects of three plant growth-promoting rhizobacterial strains on the chlorophyll (Chl) contents and leaf relative water content (LRWC) of leaf tissue from pepper plants grown under normal and saline conditions.

NaCl concentration	Treatment	Chlorophyll contents (mg/g leaf tissue)		LRWC (%)
		Chl *a*	Chl *b*	
0 mM	Non-inoculated	1.213 ± 0.090 A	0.448 ± 0.036 A	87.57 ± 0.36 A
	KNUC7074	1.441 ± 0.132 AB	0.544 ± 0.053 AB	93.02 ± 0.54 AB
	KNUC7183	1.555 ± 0.069 B	0.581 ± 0.035 B	93.78 ± 0.16 B
	KNUC7586	1.514 ± 0.079 B	0.568 ± 0.032 B	93.79 ± 0.43 B
200 mM	Non-inoculated	0.590 ± 0.025 a	0.197 ± 0.016 a	53.07 ± 0.12 a
	KNUC7074	0.686 ± 0.055 a	0.240 ± 0.021 ab	62.64 ± 0.58 a
	KNUC7183	0.968 ± 0.053 b	0.379 ± 0.036 c	63.82 ± 0.62 a
	KNUC7586	0.851 ± 0.040 b	0.297 ± 0.005 b	64.59 ± 0.64 a

Values are means of five replicated experiments. Five plants were tested per treatment.
Mean values followed by different letters in a column are significantly different, according to Duncan's multiple range test ($p < 0.05$).

KNUC7074, *B. iodinum* KNUC7183, and *R. massiliae* KNUC7586 increased the total chlorophyll content by 19.5%, 28.7%, and 25.4%, respectively, and under saline conditions, inoculation increased the total chlorophyll content by 17.5%, 71.1%, and 45.4%, respectively. Among the three PGPR, the effect of *B. iodinum* KNUC7183 on total chlorophyll content was greater than that of the other two strains, regardless of growth conditions.

Effect of Saline Conditions on Antioxidant Enzyme Activity

In the present study, we assessed the activity of three antioxidant enzymes (APX, CAT, and GPX) in the extracts of leaves from both PGPR-inoculated and non-inoculated pepper plants grown under normal and saline conditions. Under saline conditions, inoculation with all three PGPR strains increased the APX, CAT, and GPX activities (Fig. 1). More specifically, the APX activities of pepper plants inoculated with *M. oleivorans* KNUC7074, *B. iodinum* KNUC7183, and *R. massiliae* KNUC7586 were 83.0%, 85.6%, and 105.0% higher, respectively, than that of non-inoculated control plants, whereas under normal conditions, the antioxidant enzyme activities were only slightly affected. Similarly, the CAT activities of plants inoculated with *M. oleivorans* KNUC7074, *B. iodinum* KNUC7183, and *R. massiliae* KNUC7586 increased under saline conditions and were 36.0%, 109.3%, and 49.2% greater, respectively, than that of the control plants, and a similar trend was observed for the plants grown under normal conditions (Fig. 1). The GPX activity also increased in the PGPR-inoculated plants, regardless of growth conditions. However, the activity increased only slightly, when compared with the changes observed for the other two antioxidant enzymes. Taken together, these results suggest that the enhanced antioxidant enzyme activity of PGPR-inoculated plants could contribute to the plants' improved tolerance of salt stress.

Effect of Saline Conditions on the Accumulation of Proline and TSS

Proline and TSS are common antioxidants in higher plants and accumulate in response to stress. In the present study, we measured the proline content of leaves from PGPR-inoculated and non-inoculated pepper plants growing under normal and saline conditions. Both the proline and TSS contents were significantly higher in the PGPR-inoculated plants than in the untreated control plants (Table 4), and the inoculated plants accumulated greater levels of proline and TSS under saline conditions than under normal conditions. The TSS contents of salt-stressed pepper plants inoculated with *M. oleivorans* KNUC7074 and *B. iodinum* KNUC7183 were similar to that of the non-inoculated control plants, whereas the TSS content of salt-stressed plants treated with *R. massiliae* KNUC7586 was significantly lower (Table 4). In addition, the plants inoculated with *B. iodinum* KNUC7183 possessed the highest contents of both proline and TSS when grown under saline conditions (Table 4).

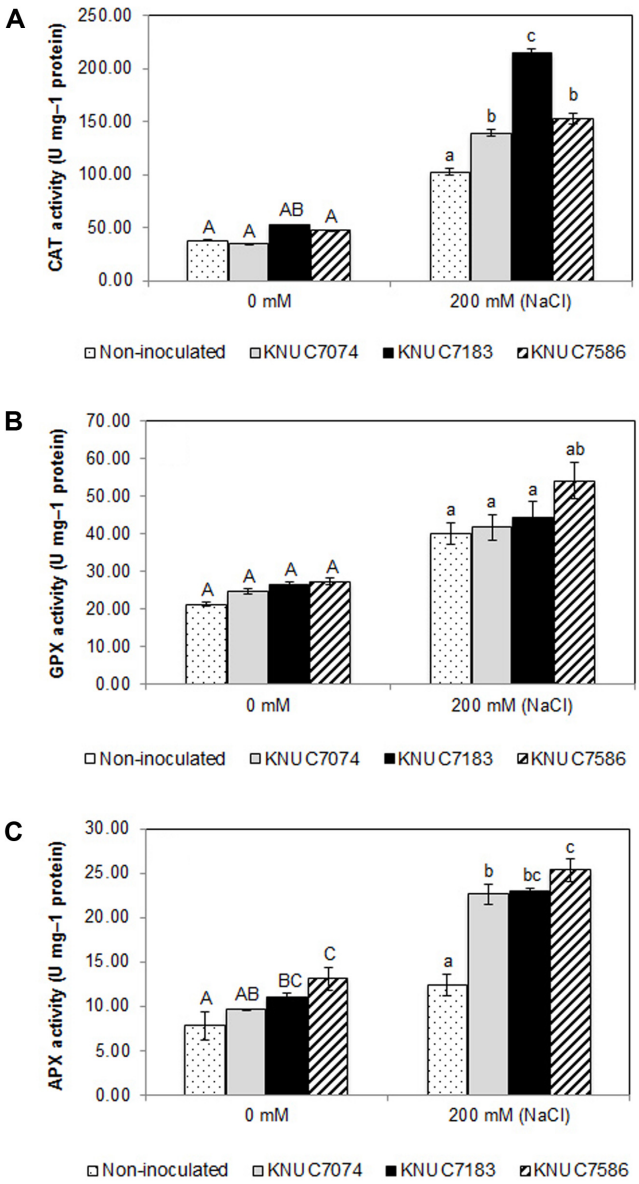

Fig. 1. Effect of saline conditions on the antioxidant enzyme activity of leaf extracts from pepper plants inoculated with plant growth-promoting rhizobacteria.
(**A**) Catalase (CAT) activity, (**B**) guaiacol peroxidase (GPX) activity, and (**C**) ascorbate peroxidase (APX) activity. The values given for each treatment are the means ± SE (n = 5).

Table 4. Proline and total soluble sugar contents of leaf tissue from pepper plants grown under normal and saline conditions.

NaCl concentration	Treatment	Proline (μmol/g fresh weight)	Total soluble sugar (μg/g fresh weight)
0 mM	Non-inoculated	0.29 ± 0.01 A	0.29 ± 0.01 A
	KNUC7074	0.47 ± 0.05 B	0.35 ± 0.05 AB
	KNUC7183	0.48 ± 0.04 B	0.39 ± 0.03 B
	KNUC7586	0.78 ± 0.05 C	0.42 ± 0.07 B
200 mM	Non-inoculated	1.79 ± 0.11a	6.59 ± 0.29 b
	KNUC7074	1.96 ± 0.09 ab	6.67 ± 0.57 b
	KNUC7183	4.09 ± 0.22 c	8.24 ± 0.06 c
	KNUC7586	3.86 ± 0.18 b	5.92 ± 0.36 a

Values are the means of five replicated experiments. Five plants were tested per treatment.
Mean values followed by different letters in a column are significantly different, according to Duncan's multiple range test ($p < 0.05$).

Discussion

The present study investigated the effect PGPR inoculation on the growth, LRWC, chlorophyll content, antioxidant enzyme activity, and proline and TSS contents of pepper plants grown under normal and saline conditions. Under stressful conditions, both plant growth and nutrient uptake have been reported to decrease [22, 26]. However, the present study demonstrates that PGPR application can ameliorate the negative effects of saline soils on plant growth. Indeed, under saline conditions, both the fresh and dry weights of pepper plants inoculated with PGPR (*M. oleivorans* KNUC7074, *B. iodinum* KNUC7183, and *R. massiliae* KNUC7586) were greater than those of non-inoculated control plants. Previous research has also shown that PGPR strains can alleviate the deleterious effects of saline soil on the growth of lettuce, maize, pepper, tomato, and wheat [22, 27–29].

Furthermore, it has been suggested that phosphate nutrition is a limiting factor for the growth of salt-stressed plants [30]. In the present study, two of the PGPR strains (*M. oleivorans* KNUC7074 and *R. massiliae* KNUC7586) were able to solubilize phosphate (Table 1) and, thus, to improved phosphate nutrition, which may have partially accounted for the ability of the PGPR-treated plants to overcome salinity stress. Indeed, previous studies have reported that phosphate-solubilizing organisms are associated with increased plant phosphate content [31, 32]. Moreover, both *M. oleivorans* KNUC7074 and *R. massiliae* KNUC7586 are able to produce IAA (Table 1). It has been suggested that the depressive effect of salinity on plant growth is related to reductions in endogenous levels of hormones [12, 32]. Therefore, the application of additional natural phytohormones, such as bacterial auxins, could positively affect plant development under high-salinity conditions [33, 34].

Previous studies have also shown that PGPR release metal-chelating substances, such as iron-chelating siderophores, into the rhizosphere and have suggested that siderophore-producing bacteria influence the plant uptake of various metals, including Fe, Zn, and Cu [35–37]. Interestingly, the PGPR strains used in the present study (*M. oleivorans* KNUC7074, *B. iodinum* KNUC7183, and *R. massiliae* KNUC7586) can produce siderophore, which suggests that PGPR and other microorganisms can also affect plant stress tolerance by influencing the bioavailability of metal ions required by their host plants, as reported by Dimkpa *et al.* [38].

The present study also revealed that PGPR inoculation enhanced the chlorophyll content and LRWC of pepper leaves. Similar results were reported by Mayak *et al.* [28], who observed that the fresh and dry weights of tomato were improved by inoculation with *Achromobacter piechaudii*. Another study showed that inoculation with PGPR, including *Bacillus subtilis*, *Bacillus atrophaeus*, *Bacillus sphaericus*, *Staphylococcus kloosii*, and *Kocuria erythromyxa*, increased both the shoot and root weights (fresh and dry) of strawberry plants growing under high-salinity conditions [39]. Han and Lee [26] also reported that PGPR, such as *Serratia* and *Rhizobium* species, enhance the growth, nutrient uptake, and chlorophyll content of lettuce grown under different levels of soil salinity.

Salt stress induces the formation of reactive oxygen species (ROS), which can cause severe oxidative damage to plants. Antioxidant systems play an important role in protecting plants from oxidative stress [26, 40] and involve a variety of antioxidant enzymes, including superoxide dismutase (SOD), dehydroascorbate reductase (DHAR), glutathione reductase (GR), APX, CAT, and GPX [41, 42]. In plant systems, enzymes and redox metabolites act in synergy to detoxify ROS. For example, both APX and GPX catalyze the conversion of H_2O_2 to water, and CAT converts H_2O_2 to oxygen and water. In the present study, the

activities of antioxidant enzymes (APX, CAT, and GPX) in the leaf extracts of PGPR-inoculated pepper plants were significantly greater than those observed for the non-inoculated control plants, regardless of growing conditions (*i.e.*, normal or saline). Our results support those of Gururari *et al.* [13], who also reported that the activities of ROS-scavenging enzymes, such as APX, CAT, DHAR, GR, and SOD, were enhanced in PGPR-inoculated potato plants exposed to various stressors (salt, drought, and heavy metals) [13]. Moreover, increased SOD, APX, and CAT activities were also observed in salt-stressed okra plants treated with the PGPR *Enterobacter* sp. UPMR18 [14].

Proline accumulation is an adaptive response by plants to both general stress and salinity, since it mediates osmotic adjustment at the cellular level, thereby protecting intracellular macromolecules from dehydration, and also because it serves as a hydroxyl radical scavenger [13, 31]. Increased TSS content is another important defense strategy for plants facing salinity stress [22, 43], and in the present study, we found that the contents of both proline and TSS were enhanced in the PGPR-inoculated pepper plants under saline conditions. Therefore, it is likely that the PGPR strains promoted plant growth under salinity stress by enhancing metabolic defense strategies.

The present study demonstrates that PGPR strains (*e.g.*, *M. oleivorans* KNUC7074, *B. iodinum* KNUC7183, and *R. massiliae* KNUC7586) can improve the growth and development of plants under salt stress by solubilizing phosphate and by producing ACC deaminase, siderophore, and IAA. The tolerance of pepper seedlings to salt stress was also correlated with increased levels of ROS-scavenging enzymes (APX, CAT, and GPX), as well as the accumulation of proline and TSS, which function as osmoregulants. The pot experiments in this study were just a preliminary study to identify efficient strains isolated from the rhizosphere of pepper greenhouses that could enhance the growth of pepper under non-stressed and salt-stressed conditions. Therefore, the present study suggests that PGPR can alleviate the deleterious effects of salt stress on plants, possibly by functioning as elicitors that enhance plant tolerance to various abiotic stresses. Future research should focus on determining the mRNA expression profiles associated with such tolerance mechanisms in pepper.

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