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Salt Tolerance Induced by Plant Growth-Promoting Rhizobacteria Is Associated with Modulations of the Photosynthetic Characteristics, Antioxidant System, and Rhizosphere Microbial Diversity in Soybean (*Glycine max* (L.) *Merr.*)

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Abstract: Salinity stress poses a major obstacle to agricultural productivity. Employing plant growth-promoting rhizobacteria (PGPR) has attracted significant attention due to its potential to improve plant development in challenging conditions. Yet, additional investigation is essential to fully understand the potential of PGPR in mitigating salinity stress, especially in field applications. Hence, this study investigated the resistance mechanisms of soybean (*Glycine max* (L.) *Merr.*) under salt stress with PGPR application through a field experiment with four treatments: normal soybean planting (NN), normal planting + PGPR (NP), salt stress planting (SN), and salt stress planting + PGPR (SP). This research investigated how applying PGPR under salt stress influences soybean photosynthetic traits, osmotic regulation, rhizosphere microbial communities, and yield quality. The results demonstrated that salt stress enhanced leaf temperature and significantly reduced the leaf area index, SPAD value, stomatal conductance, photosynthetic rate, and transpiration rate of soybeans. Compared to SN treatment, SP treatment significantly improved the stomatal conductance, photosynthetic rate, and transpiration rate by 10.98%, 16.28%, and 35.59%, respectively. Salt stress substantially increased sodium (Na^+) concentration and Na^+/K^+ ratio in leaves, roots, and grains while reducing potassium (K^+) concentration in roots and leaves. Under salinity stress, PGPR application significantly minimized Na^+ concentration in leaves and enhanced K^+ concentration in leaves, roots, and grains by 47.05%, 25.72%, and 14.48%, respectively. PGPR application boosted carbon assimilation (starch synthesis) by enhancing the activities of sucrose synthase, fructokinase, and ADP-glucose pyrophosphorylase. It improved physiological parameters and increased soybean yield by 32.57% compared to SN treatment. Additionally, PGPR enhanced antioxidant enzyme activities, including glutathione reductase, peroxidase, ascorbate peroxidase, and monodehydroascorbate reductase, reducing oxidative damage from salt stress. Analysis of rhizosphere microbial communities revealed that PGPR application enriched beneficial bacterial phyla such as *Bacteroidetes*, *Firmicutes*, *Nitrospirae*, and *Patescibacteria* and fungal genera like *Metarhizium*. These microbial shifts likely contributed to improved nutrient cycling and plant–microbe interactions, further enhancing soybean resilience to salinity. This study demonstrates that PGPR enhances soybean growth, microbial diversity, and salt

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tolerance under salinity stress, while future efforts should optimize formulations, explore synergies, and scale up for sustainable productivity.

Keywords: antioxidant system; plant growth-promoting rhizobacteria; photosynthetic characteristics; salt stress; soybean; yield

1. Introduction

Soybean (*Glycine max* (L.) *Merr.*), a critical oilseed crop globally, is particularly vulnerable to salt stress. In the 2020/2021 season, approximately 127.842 million hectares were planted worldwide, producing 362.947 million tons of soybeans [1]. In China alone, soybean cultivation in 2023 covered 10.467 million hectares with a total production of 20.84 million tons [2]. Despite its economic importance, soybeans are highly sensitive to salinity stress, with potential yield losses of up to 40% depending on salinity levels [3,4]. Soil solutions with electrical conductivity exceeding 5 dS m^{-1} hinder soybean germination and growth [5]. Saline-alkaline conditions exacerbate ionic toxicity, osmotic pressure, and nutrient deficiencies, damaging key processes such as germination, metabolism, photosynthesis, and enzyme activity in soybeans. Toxic ions disrupt critical enzymes and cellular processes essential for plant development, ultimately reducing yield and quality [6,7]. Therefore, improving soybean production under salinity stress is crucial for sustainable agriculture.

Soil salinization is a global environmental problem posing significant agricultural output and food security risks. The area of saline-alkaline soil worldwide is expanding at an alarming rate of 1.0×10^6 to 1.5×10^6 hectares annually, significantly inhibiting crop growth and yield. Soil salinization is a worldwide challenge impacting 831 million hectares of farmland, leading to an estimated annual crop loss of around $27.3 billion [3,4]. Salinity is a significant non-living stressor that negatively impacts both the yield and quality of soybeans [8]. Salt stress disrupts plant growth through multiple interconnected mechanisms, including ionic toxicity, osmotic stress, and nutrient imbalances. An excessive buildup of sodium (Na^+) and chloride (Cl^-) ions in the soil limits plants' ability to absorb water, resulting in physiological drought and disrupted cellular processes. These ions interfere with enzymatic functions, destabilize cell structures, and hinder photosynthesis by harming the electron transport chain and decreasing CO_2 assimilation [9]. Salt stress triggers excessive reactive oxygen species (ROS) production, leading to oxidative damage to proteins, lipids, and nucleic acids, ultimately disrupting plant growth and productivity [10]. It negatively influences all stages of plant development, from germination to maturity, including vegetative and reproductive phases [11]. Salt stress leads to reduced photosynthetic efficiency [12], decreased stomatal conductance [13], and diminished water uptake capacity [14] while exacerbating ionic toxicity and ROS accumulation [9]. Elevated Na^+ levels in stomatal and chloroplast tissues alter membrane permeability, reduce water potential, induce stomatal closure, and disrupt the electron transport chain, further inhibiting photosynthesis [15]. These factors collectively result in decreased dry matter accumulation and substantial yield reductions. Present approaches for managing salt stress involve developing salt-resistant crop varieties [16], employing plant genetic engineering [17], using chemical treatments [18], and incorporating organic matter into the soil to improve conditions in affected agricultural lands [19]. Among these strategies, the use of plant growth-promoting rhizobacteria (PGPR) has shown significant potential in reducing the negative impacts of salinity on crops and improving their overall yield [20].

PGPR are useful bacteria that enhance plant growth within the rhizosphere. As a novel commercial biofertilizer product, PGPR enhance plant stress resistance while meeting

nutritional needs. It reduces chemical fertilizer usage, ensures crop yields, and supports sustainable agriculture practices [21]. For instance, *Bacillus* species are well-known PGPR that improve plant biomass by suppressing pathogens, promoting growth, reducing pest damage, and enhancing plant stress resistance [22]. Studies have shown that applying *Bacillus japonicum* alone improves soybean growth and nutrient content. Co-inoculating with *Pseudomonas putida* enhances its beneficial impacts on plant development and soil biochemical functions [23]. Through various molecular and physiological pathways, PGPR enhance plants' ability to withstand salt stress. These include synthesizing phytohormones, i.e., cytokinins, auxins, or gibberellins; improving root architecture; increasing nutrient uptake; secreting exopolysaccharides; enhancing antioxidant defense systems; promoting nutrient absorption; boosting fertilizer use efficiency; and increasing crop yields [24,25]. Several salt-tolerant bacterial genera, including *Bacillus subtilis*, *Burkholderia*, *Alcaligenes*, *Arthrobacter*, *Pseudomonas*, *Azospirillum*, and *Rhizobia*, have been documented to improve crop tolerance to salinity stress by minimizing Na^+ ion availability in roots or enhancing K^+ uptake to maintain ionic balance under saline conditions [26,27].

In recent years, the feasibility of replacing chemical fertilizers with PGPR-based microbial fertilizers has been confirmed. Research on plant–microbe interactions has increasingly demonstrated their potential to enhance host plant resistance under stressful environments. However, research on soybean–microbe interactions under field applications of PGPR in saline environments remains scarce. This research investigates how field inoculation with PGPR strains affects photosynthetic characteristics, enzyme activities, microbial communities, and soybean yield in the presence of salt stress. The goal was to uncover how PGPR enhances soybean resilience to salinity while offering theoretical insights and technical recommendations for sustainable soybean farming in saline soils. It was hypothesized that applying PGPR would improve soybean salt tolerance by enhancing physiological characteristics such as photosynthetic efficiency and enzyme activities and enriching beneficial microbial populations in the rhizosphere. The outcomes of this research are expected to provide valuable knowledge for developing effective strategies to mitigate salinity stress in soybean production systems and promote sustainable agricultural practices globally.

2. Materials and Methods

2.1. Experimental Site Overview and Design

The experiment was conducted at the Gongzhuling Field Experimental Station of the Chinese Academy of Sciences (125°02′ E, 43°26′ N). These seeds were then planted in both standard soil (pH 7.6, electrical conductivity of 1.02 dS m^{-1}, and Na content under 0.5 g kg^{-1}) and saline-alkaline soil (pH 8.2, EC 3.5 dS m^{-1}, and Na content of 2.35 g kg^{-1}), representing the normal and salinity treatments, respectively. The region experiences an average annual temperature of 5.9 °C, approximately 2743 h of sunshine per year, annual precipitation of 450–600 mm, and a frost-free period of about 140 days. The soybean variety used was 'Jilin 20', provided by the Jilin Academy of Agricultural Sciences Soybean Research Institute. Soybean seeds were coated with 300 g of PGPR (*strains acidobacteria* KBS 96, *Bryobacterales bacterium* KBS 96; Chr. Hansen, Hørsholm, Denmark). A randomized block design was employed with four treatments: normal planting (NN), normal planting with PGPR (NP), salt stress (SN), and salt stress with PGPR (SP). Each treatment was replicated four times, resulting in 16 plots. Planting occurred on 27 April 2023 and harvesting on October 15. The primary fertilizers used for soybean cultivation were urea (N $\geq$ 47%), diammonium phosphate (DAP; P $\geq$ 46% and N $\geq$ 18%), and potassium sulfate (K $\geq$ 52%), applied at rates of 70 kg ha^{-1}, 110 kg ha^{-1}, and 70 kg ha^{-1}, respectively.

2.2. Measurement of Plant Physiological Indicators

The net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate were evaluated at the pod-setting stage using an LI-6400 portable photosynthesis system (Li-Cor Biosciences, Lincoln, NE, USA). These assessments were conducted on the central leaflet of the third fully expanded trifoliate leaf from the top. Conditions during measurements included photosynthetically active radiation of 1200 µmol m^{-2} s^{-1}, a flow rate of 500 mol s^{-1}, a leaf chamber temperature of 25 °C, and a CO_2 concentration of 400 µmol mol^{-1}. Chlorophyll fluorescence (OIJP curves) was analyzed using a FluorPen FP110 (Photon System Instruments, Drasov, Czech Republic) after 30 min of dark adaptation facilitated by leaf clips. Chlorophyll levels were quantified using a SPAD meter (Konica Minolta, Tokyo, Japan). Six leaves were selected for each treatment, and four readings were taken at different leaf positions to compute the average value. The leaf area index was determined with a canopy analyzer (AccuPAR LP-80, METER, Pullman, WA, USA). Additionally, infrared thermal imaging with a WIRIS Pro camera captured thermal images of fully developed mature leaves from plants exhibiting comparable growth.

2.3. Measurement of Leaf Water Potential and Ion Concentrations

Fully expanded leaves were sealed in plastic bags and kept in dark for 30 min before measuring leaf water potential using a WP4C dewpoint water potential meter (METER, USA). Sodium (Na^+) and potassium (K^+) ion concentrations in dried soybean leaves, roots, and grains were analyzed via flame atomic spectrophotometry following the protocol described by [28]. For this analysis, 0.1 g of finely ground plant material was placed into digestion tubes, digested with concentrated sulfuric acid, diluted to 50 mL using volumetric flasks, and filtered before measurement with a spectrophotometer. The absorbance of K^+ and Na^+ ions was measured at wavelengths of 766.4 nm and 589.0 nm, respectively, and standard curves were generated for quantification.

2.4. Key Carbon Metabolism Enzyme Activities

The activities of key enzymes involved in glycolysis and carbohydrate metabolism were assessed based on the protocol outlined by Jammer et al. [29]. Fresh leaf tissue was pulverized in liquid nitrogen, and 0.3 g of the resulting powder was transferred to a 2 mL centrifuge tube containing 0.6 mL of an extraction buffer (H_2O, 1 M pH 7.6 Tris-HCl, 1 M $MgCl_2$, 250 mM EDTA, 100 mM $C_7H_8N_2$, 14.34 M C_2H_6OS, 10 mM NADP, and 100 mM PMSF). The mixture was incubated for 30 min and centrifuged at 13,200× *g* for 30 min at 4 °C. The resulting supernatant was utilized to determine the activities of enzymes including aldolase (Ald), glucose-6-phosphate dehydrogenase (G6PDH), phosphofructokinase (PFK), phosphoglucomutase (PGM), phosphoglucoisomerase (PGI), UDP-glucose pyrophosphorylase (UGPase), and ADP-glucose pyrophosphorylase (AGPase). For fructokinase (FK), hexokinase (HXK), and sucrose synthase (SuSy) activity assays, a portion of the supernatant was subjected to overnight dialysis at 4 °C on a temperature-controlled shaker. Enzyme activities were quantified using a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) set to a wavelength of 340 nm at 30 °C. Each treatment was performed in quadruplicate to ensure data reliability.

2.5. Antioxidant Enzyme Activities

Antioxidant enzyme activities were evaluated using the protocol described by Fimognari et al. [30]. Fresh leaf tissues were pulverized in liquid nitrogen, and 0.3 g of the powdered material was transferred to a 2 mL centrifuge tube containing 0.6 mL of an extraction buffer (H_2O, 1 M pH 7.6 Tris-HCl, 1 M $MgCl_2$, 250 mM EDTA, 100 mM $C_7H_8N_2$, 14.34 M C_2H_6OS, 10 mM NADP, and 100 mM PMSF). The mixture was thoroughly ho-

mogenized, incubated for 30 min, and then centrifuged at 13,200× *g* for 30 min at 4 °C. The supernatant was collected, placed into dialysis bags, and dialyzed overnight at 4 °C. The dialyzed extract was subsequently used to assess the activities of various antioxidant enzymes, including ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), monodehydroascorbate reductase (MDHAR), glutathione S-transferase (GST), dehydroascorbate reductase (DHAR), and peroxidase (POX). The pellet was rinsed three times for cell wall peroxidase (cwPOD) activity, resuspended in 0.6 mL of a high-salt buffer, and agitated overnight. The suspension was centrifuged at 20,600× *g* for 30 min at 4 °C, and the supernatant was dialyzed overnight at 4 °C. The dialyzed extract was then used to measure cwPOD activity. Each treatment was conducted in quadruplicate to ensure experimental consistency.

2.6. Bacterial Community and Fungal Community Activity

At soybean maturity, rhizosphere soil samples were collected using a five-point sampling method around the root zone. The samples were stored at 4 °C and sent to Nanjing Personal Gene Technology Co., Ltd. (Nanjing, China) for high-throughput sequencing to analyze bacterial and fungal community structures. Total genomic DNA was extracted using a microbial DNA extraction kit (Omega Bio-Tek, Norcross, GA, USA). DNA quality and quantity were evaluated using NanoDrop ND-2000 spectrophotometry and agarose gel electrophoresis. The V3-V4 region of the 16S rRNA gene was amplified for bacterial community analysis using universal primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). For fungal community analysis, the ITS1 region was amplified using primers ITS5F (5′-GTGCCAGCMGCCGCGGTAA-3′) and ITS2R (5′-CCGTCAATTCCTTTGAGTTT-3′). PCR products were extracted from 2% agarose gels, purified using the AxyPrep DNA gel extraction kit, (Axygen, Hangzhou, China) and eluted with a Tris-HCl buffer (Boke Biotechnology, Shanghai, China). Quantification of bacterial and fungal DNA was performed using the QuantiFluor™-ST system. Paired-end sequencing (2 × 300 bp) was conducted on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) following the standard operating procedures of Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw sequencing reads were quality controlled using Trimmomatic software (version 0.39) and then merged using FLASH software (https://ccb.jhu.edu/software/FLASH/index.shtml; 21 December 2023). OTUs were clustered using UPARSE (version 7.1) with a similarity cutoff of 97%. Taxonomic classification of gene sequences was performed using the RDP Classifier Bayesian algorithm (http://rdp.cme.msu.edu/ (21 December, 2023) in conjunction with the Silva and UNITE bacterial and fungal reference gene databases. Subsequently, OTUs related to chloroplasts and mitochondria were removed from the bacterial and fungal OTU tables.

2.7. Soybean Yield Components and Quality

Following harvest, yield components, i.e., seeds per pod, pods per plant, hundred-seed weight, and grain yield, were evaluated using a diagonal five-point sampling technique. At each sampling point, three sub-samples were selected, and ten plants exhibiting uniform growth were randomly chosen to analyze these parameters. Starch content was quantified using the anthrone colorimetric method detailed by Li et al. [31]. Leaf residue (0.5 g), previously extracted with 80% ethanol and dried at 80 °C, was treated with perchloric acid to extract starch. The resulting mixture was heated in a boiling water bath for 15 min and cooled, and the absorbance was recorded at 620 nm. Soluble protein content was determined using the Coomassie Brilliant Blue G-250 staining procedure outlined by Li et al. [31]. Fresh leaf samples (0.5 g) were homogenized in 5 mL of HEPES buffer, and the extract was centrifuged at 1120× *g* for 10 min. A 0.1 mL aliquot of the supernatant was

mixed with 5 mL of Coomassie Brilliant Blue reagent, incubated at 25 °C for 30 min, and the absorbance was measured at 595 nm. All treatments were conducted in quadruplicate to ensure accuracy and reproducibility.

2.8. Statistical Analysis

Data analysis was performed using R and Origin 2021. Physiological indicators, enzyme activity, ion concentrations, yield components, and microbial relative abundance data were subjected to two-way ANOVA in R (version 4.4.1) to determine the interaction effects between salt stress conditions and PGPR addition. Additionally, Duncan's multiple range test ($p < 0.05$) was used for comparison. Microbial diversity indices, PCoA, ANOSIM, and RDA analyses were computed using the Vegan package and visualized using the ggplot2 package. Correlation analysis and principal component analysis were conducted using the Principal Components Analysis and Correlation Plot APP in Origin 2021. Results are presented as means with standard deviations (SD), and significant differences are indicated by different letters ($p < 0.05$).

3. Results

3.1. Effects of PGPR on Soybean Yield Components and Quality

Table 1 summarizes the effects of salt stress and PGPR application on grain yield, yield components, and grain quality. Under normal conditions, the addition of PGPR had no significant effect on yield and protein content but significantly reduced starch content. Compared to normal soil conditions (NN), salt stress (SN) significantly reduced soybean yield and starch content. However, under salt stress, PGPR application increased soybean yield by 32.57% and grain starch content by 15.89%.

Table 1. Effects of applying plant growth-promoting rhizobacteria (PGPR) on yield components and grain quality of soybeans grown under salt stress.

Treatments		Yield Components			Grain Quality	
Salt	**PGPR**	**Pod Number per Plant**	**100-Grain Weight (g)**	**Yield (kg ha^{-1})**	**Protein (%)**	**Starch (%)**
Normal	−PGPR	51 ± 2.51 a	25.04 ± 1.23 a	3352.51 ± 238.12 a	34.64 ± 2.87 a	3.60 ± 0.29 a
	+PGPR	54 ± 4.86 a	23.15 ± 0.46 bc	3605.14 ± 109.36 a	33.67 ± 3.18 a	3.15 ± 0.09 b
Salinity	−PGPR	39 ± 4.55 c	24.37 ± 1.82 ab	1975.99 ± 323.63 c	35.08 ± 2.63 a	3.06 ± 0.05 b
	+PGPR	44 ± 4.17 b	22.35 ± 1.32 c	2619.64 ± 185.83 b	35.43 ± 2.49 a	3.55 ± 0.07 a
Salinity		**	ns	**	ns	ns
PGPR		**	**	**	ns	ns
Salinity × PGPR		ns	ns	**	ns	**

Values with distinct letters represent significant differences among treatments as determined by Tukey's test (**, $p < 0.01$; ns, non-significant; n = 4).

3.2. Effects of PGPR on Soybean Physiological Indicators Under Salt Stress

Figure 1 depicts the effects of PGPR application on soybean physiological parameters under salt stress conditions. Under normal conditions, the addition of PGPR had varying effects on the physiological indicators of soybean leaves, with no significant impact on Gs, Tr, and Ci. The findings revealed that salt stress increased leaf temperature while significantly decreasing the leaf area index, SPAD readings, Pn, Gs, and Tr. However, PGPR application effectively reduced leaf temperature (Figure 1A) and significantly improved transpiration rate, with the SP treatment increasing by 35.59% compared to the SN treatment (Figure 1C). Stomatal conductance and photosynthetic rate in the SP treatment were significantly higher than in the SN treatment by 10.98% and 16.28%, respectively. Additionally, the performance index based on absorbed light energy (PIABS) increased by 45.90% with PGPR application under salt stress (Figure 1B).

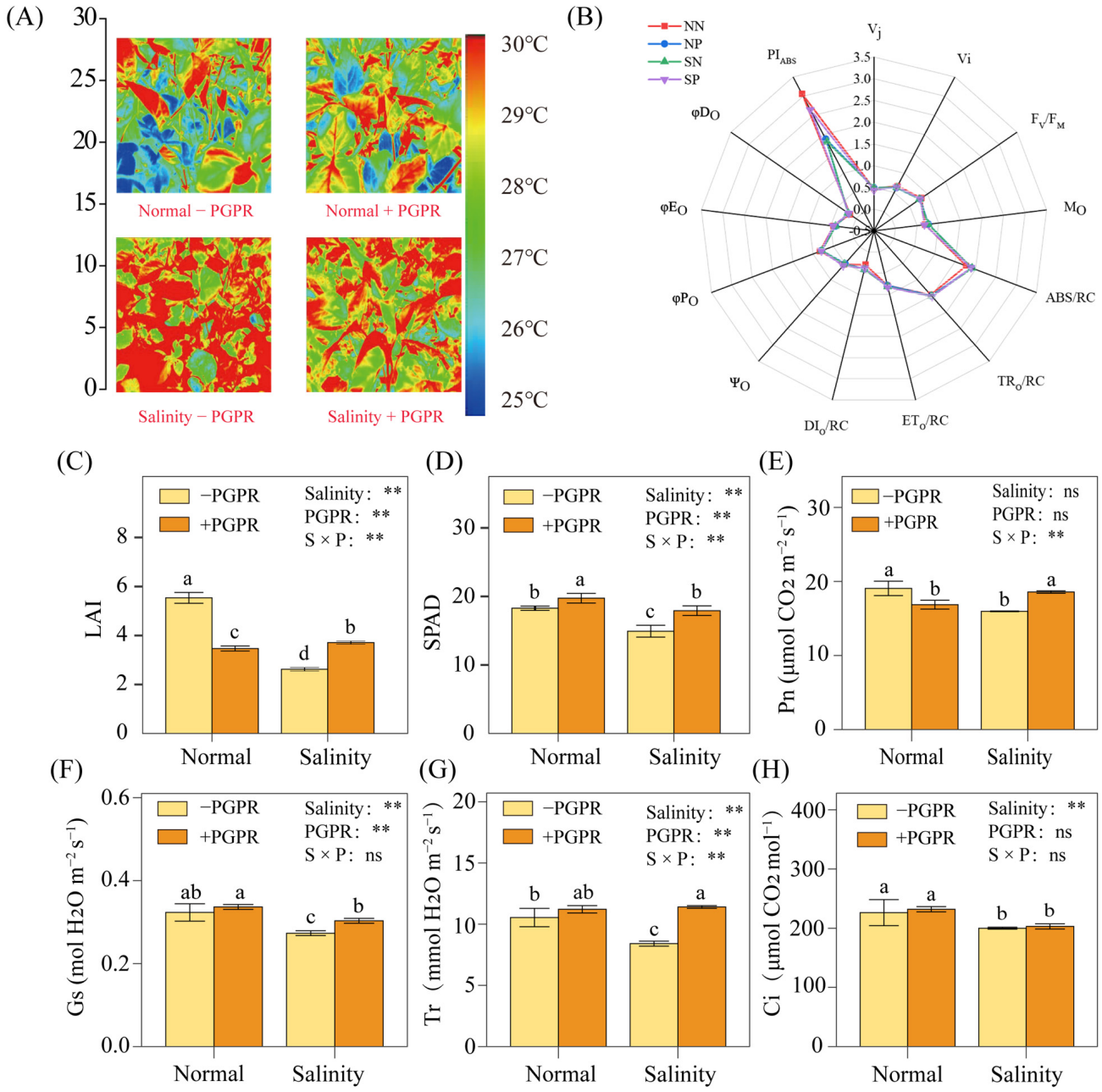


Figure 1. Effects of applying plant growth-promoting rhizobacteria (PGPR) on photosynthetic and physiological indicators of soybeans grown under salt stress. (**A**) Infrared thermography; (**B**) chlorophyll fluorescence parameters; (**C**) leaf area index; (**D**) SPAD; (**E**) net photosynthetic rate; (**F**) stomatal conductance; (**G**) transpiration rate; and (**H**) intercellular CO_2 concentration. Note: Values followed by different letters indicate significant differences among treatments (** $p < 0.01$, ns, non-significant; n = 4), the same below.

3.3. Effects of PGPR on Na^+ and K^+ Concentrations, Na^+/K^+ Ratio, and Leaf Water Potential

As shown in Figure 2, under normal conditions, the addition of PGPR had no significant effect on leaf water potential, Na^+ and K^+ concentrations, or the Na^+/K^+ ratio in roots, stems, and leaves. Salt stress significantly reduced leaf water potential, with SN treatment showing a 61.80% decrease compared to NN treatment. PGPR application mitigated this damage, increasing leaf water potential by 43.30% compared to SN treatment. Figure 2 shows the effects of PGPR on Na^+ and K^+ ion concentrations in different parts of soybean under salt stress. Salt stress significantly increased Na^+ concentration and Na^+/K^+ ratio in leaves, roots, and grains while reducing K^+ concentration in roots and leaves.

However, PGPR application under salt stress significantly reduced Na^+ concentration in leaves and increased K^+ concentrations in leaves, roots, and grains by 47.05%, 25.72%, and 14.48%, respectively.

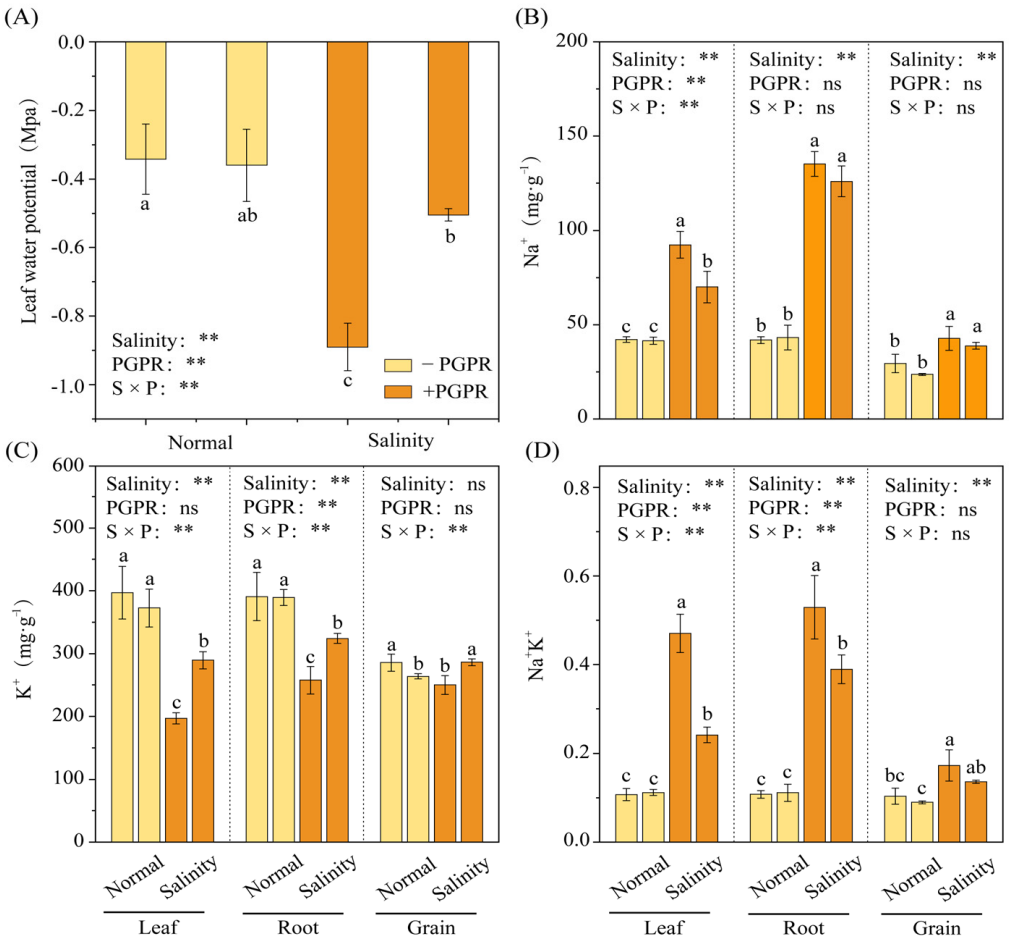


Figure 2. Effects of applying plant growth-promoting rhizobacteria (PGPR) on sodium (Na^+) and potassium (K^+) concentrations, Na^+/K^+ ratio, and leaf water potential of soybeans grown under salt stress. (**A**) Leaf water potential; (**B**) sodium; (**C**) potassium; and (**D**) sodium/potassium ratio. Note: Values with distinct letters represent significant differences among treatments as determined by Tukey's test (** $p < 0.01$, ns, non-significant; n = 4), the same below.

3.4. Effects of PGPR on Carbohydrate Metabolism

Under normal conditions, the NP treatment significantly increased the activities of ADP-glucose pyrophosphorylase (AGPase) and hexokinase (HXK) compared to the NN treatment, with increases of 103.28% and 27.23%, respectively. Salt stress significantly reduced the activities of HXK, phosphoglucoisomerase (PGI), and sucrose synthase (SuSy). However, PGPR application significantly increased the activities of AGPase, fructokinase (FK), and SuSy under salt stress while reducing the activities of UDP-glucose pyrophosphorylase (UGPase) and phosphoglucomutase (PGM). Compared to SN treatment, SP treatment increased AGPase, FK, and SuSy activities by 63.51%, 21.61%, and 62.66%, respectively, while reducing UGPase and PGM activities by 11.21% and 31.40%.

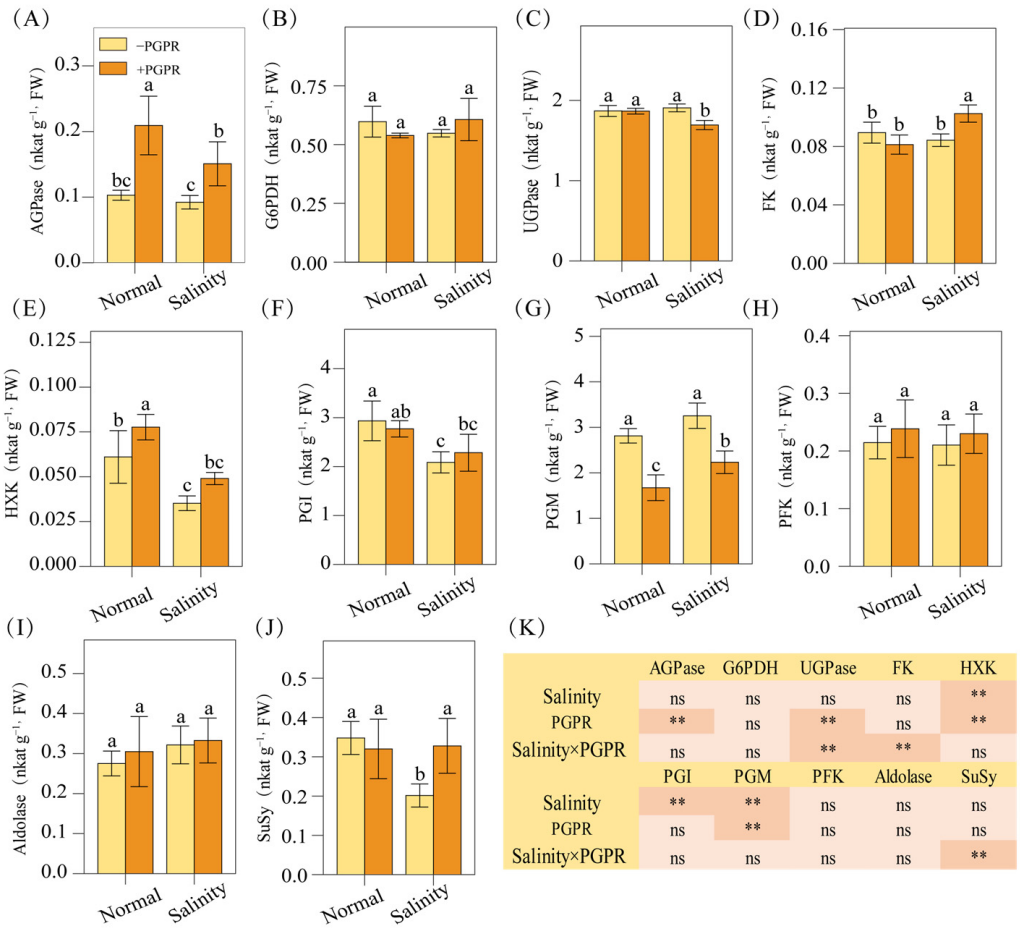


Figure 3. Effects of applying plant growth-promoting rhizobacteria (PGPR) on carbohydrate metabolism of soybeans grown under salt stress. (**A**) ADP-glucose pyrophosphorylase (AGPase); (**B**) glucose-6-phosphate dehydrogenase (G6PDH); (**C**) UDP-glucose pyrophosphorylase (UGPase); (**D**) fructokinase (FK); (**E**) hexokinase (HXK); (**F**) phosphoglucoisomerase (PGI); (**G**) phosphoglucomutase (PGM); (**H**) phosphofructokinase; (**I**) fructose-bisphosphate aldolase (Aldolase); (**J**) sucrose synthase (SuSy); and (**K**) Two-way analysis of variance of carbon metabolism. Note: Values with distinct letters represent significant differences among treatments as determined by Tukey's test (** $p < 0.01$, ns, non-significant; n = 4), the same below.

3.5. Effects of PGPR on Antioxidant Systems

Figure 4 demonstrates PGPR's effects on soybean's antioxidant enzyme activities under salt stress. Under normal conditions, the addition of PGPR had varying effects on different enzyme activities, significantly increasing the activity of GR. Salt stress significantly reduced POX activity, with slight declines in catalase (CAT) and superoxide dismutase (SOD) activities. However, SP treatment significantly increased APX, GR, MDHAR, and POX activities by 63.27%, 65.97%, 56.94%, and 69.41%, respectively.

3.6. Effects of PGPR on Rhizosphere Microbial Communities

As shown in Figure 5A, the top ten bacterial phyla across all treatments were *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, *Gemmatimonadetes*, *Bacteroidetes*, *Rokubacteria*, *Firmicutes*, *Nitrospirae*, and *Patescibacteria*. PGPR application under salt stress significantly increased the relative abundances of *Bacteroidetes*, *Firmicutes*, *Nitrospirae*, and *Patescibacteria*. Principal coordinate analysis (PCoA) revealed a clear separation between treatments along the PC1 and PC2 axes, with ANOSIM analysis confirming significant differences in microbial communities among treatments (Figure 5C). Linear discriminant analysis identified *Flavobacterium* as the bacterial genus with the highest LDA score under PGPR treatment

in salt-stressed conditions (Figure 5D). Similarly, fungal phyla such as *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, and others were identified across treatments, with *Metarhizium* being the fungal genus with the highest LDA score under PGPR treatment during salt stress (Figure 5H).

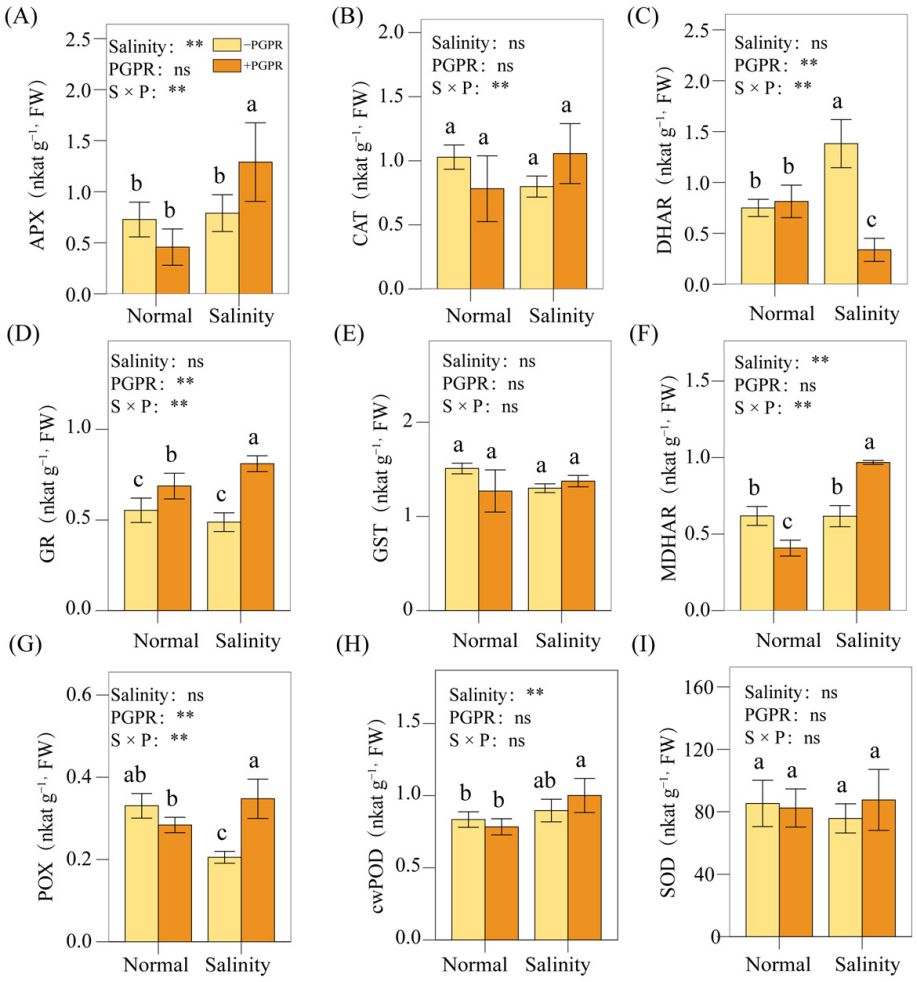


Figure 4. Effects of applying plant growth-promoting rhizobacteria (PGPR) on osmotic regulation and redox homeostasis of soybeans grown under salt stress. (**A**) Ascorbate peroxidase (APX); (**B**) catalase (CAT); (**C**) dehydroascorbate reductase (DHAR); (**D**) glutathione reductase (GR); (**E**) glutathione S-transferase (GST); (**F**) monodehydroascorbate reductase (MDHAR); (**G**) peroxidase (POX); (**H**) cell wall peroxidase (cwPOD); and (**I**) superoxide dismutase (SOD). Note: Values with distinct letters represent significant differences among treatments as determined by Tukey's test (** $p < 0.01$, ns, non-significant; n = 4), the same below.

The correlation analysis among 21 soybean indicators (Figure 6A) revealed a strong positive relationship between grain yield and SPAD values, stomatal conductance (Gs), leaf K^+ concentration, and leaf water potential. Conversely, grain yield exhibited a significant negative correlation with leaf Na^+ concentration and the Na^+/K^+ ratio. Starch content was positively associated with the photosynthetic rate. The principal component analysis (PCA) of all variables (Figure 6B) indicated that the first two principal components (PC1 and PC2) accounted for 51.3% and 24.1% of the total variance, respectively. The top contributors to PC1 included the Na^+/K^+ ratio, leaf water potential, SPAD values, transpiration rate (Tr), and leaf K^+ concentration, while MDHAR, APX, FK, UGPase, and grain yield predominantly influenced PC2.

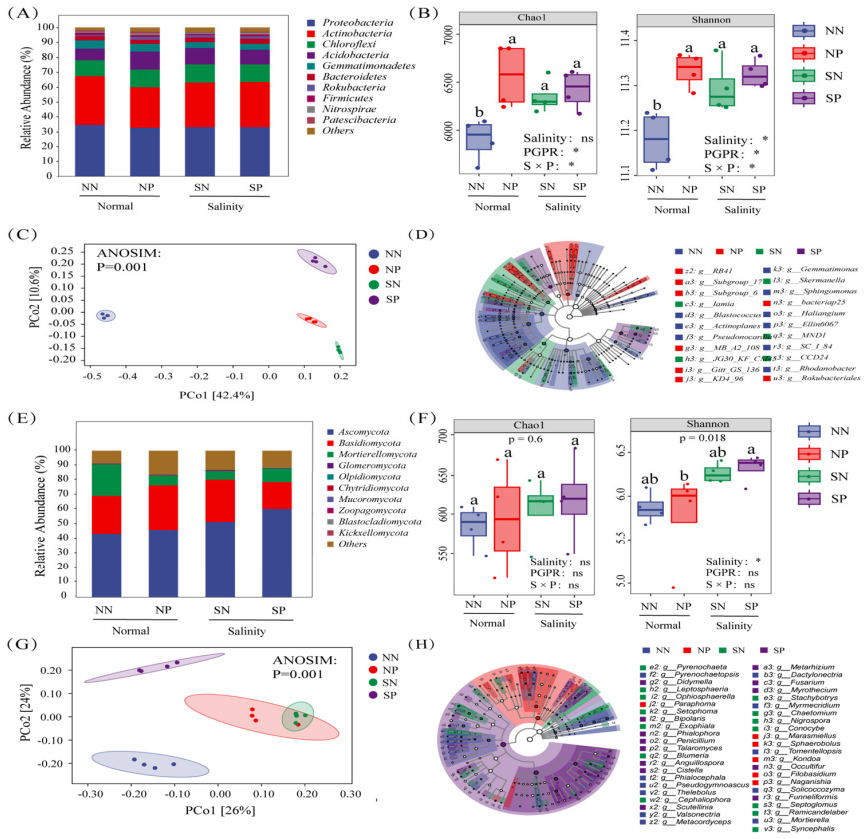


Figure 5. Effects of applying plant growth-promoting rhizobacteria (PGPR) on the rhizosphere microbial community of soybeans grown under salt stress. Note: (**A**) Dominant bacterial phyla; (**B**) bacterial Shannon index and Chao1 index; (**C**) principal coordinate analysis (PCoA) of bacteria based on Bray–Curtis distance; (**D**) LDA score chart of bacterial genera; (**E**) dominant fungal phyla; (**F**) fungal Chao1 index and Shannon index; (**G**) principal coordinate analysis (PCoA) of fungi based on Bray–Curtis distance; and (**H**) LDA score chart of fungal genera. Note: Values with distinct letters represent significant differences among treatments as determined by Tukey's test (* $0.01 < p < 0.05$, ns, non-significant; n = 4), the same below.3.7. Correlation Analysis and Principal Component Analysis.

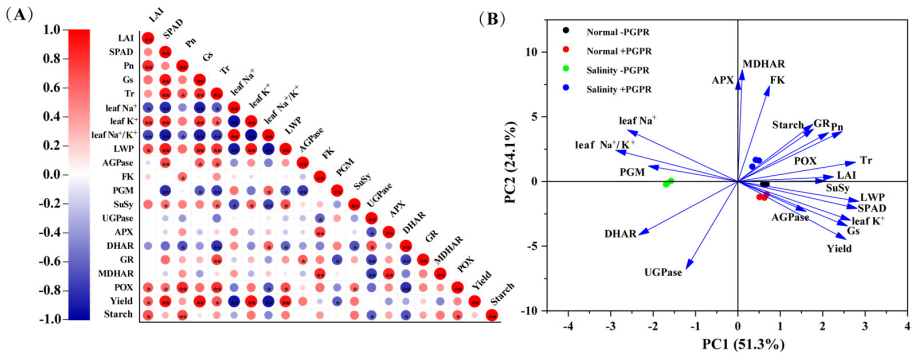


Figure 6. (**A**) Correlation analysis and (**B**) principal component analysis of soybean traits under salt stress conditions with PGPR application. Note: Distinct letters denote significant differences among treatments as determined by Tukey's test (* $0.01 < p < 0.05$, ** $p < 0.01$; n = 4).

4. Discussion

This research investigates the hypothesis that applying PGPR enhances soybean's ability to tolerate salt by improving physiological traits, including photosynthesis rates and enzyme activities, while enriching beneficial microbial communities within the rhizosphere. The results support the idea that PGPR application mitigates the adverse effects of salt stress through various physiological, biochemical, and microbiological mechanisms,

ultimately promoting soybean growth and yield in saline conditions. Salt stress disrupts plant ion homeostasis, causing nutrient imbalances, lowered photosynthetic efficiency, and interference with carbon assimilation processes, collectively suppressing crop productivity [32]. Photosynthesis, a crucial and sensitive process in plants, can be significantly affected by environmental conditions like salinity, heat, drought, and intense visible light. In this study, salt stress substantially reduced the transpiration and photosynthetic rates of soybean plants. However, PGPR application reduced leaf temperature while increasing transpiration and photosynthetic rates by 16.28% and 35.59%, respectively. This aligns with previous findings by Mohamed [10] and El-Esawi [33], where inoculation with *Bacillus* (SW5) enhanced photosynthetic rates and gas exchange parameters in soybeans under salt stress. This phenomenon could be attributed to the ability of PGPR to boost nutrient uptake and pigment synthesis. Increased chlorophyll content results in elevated photosynthetic rates and starch production, which supports plant growth under challenging saline and drought conditions [34].

In this study, salt stress considerably reduced the LAI, chlorophyll content, and stomatal conductance, indicating that salt stress causes water deficiency in plants, leading to stomatal closure, chlorophyll and chloroplast degradation, and consequently, reduced photosynthesis [35]. However, the application of PGPR significantly increased stomatal conductance and chlorophyll content, suggesting that under salt stress, PGPR enhances the photosynthetic characteristics of soybeans, possibly by altering the chloroplast structure and increasing chlorophyll content. With soil salinization worsening yearly, it has caused a significant decline in crop yields. In this study, salt stress significantly reduced soybean yield, mainly due to a reduction in the number of grains per plant. However, PGPR application significantly increased soybean yield by 32.57% (Table 1), which was closely associated with improved photosynthetic efficiency. The increased photosynthetic rate allows plants to accumulate more photosynthetic products, thereby boosting crop yield. This indicates that PGPR plays a critical role in improving soybean yield. The photosystem II (PSII) reaction center is one of the most vulnerable components of the plant photosynthesis system. Salt stress increases intracellular NaCl concentration, deactivates ATP synthase, reduces ATP levels, and disrupts protein synthesis [36]. This study's PI_{ABS} (performance index based on absorbed light energy) significantly declined under salt stress, indicating severe inhibition of PSII structure and function (Figure 1). However, PGPR application increased PI_{ABS} under salt stress, suggesting that PGPR promotes the PSII reaction center, electron transport chain, and oxygen-evolving complex, thereby enhancing the formation of photosynthetic products [37].

Transpiration is strongly linked to Na^+/K^+ exchange processes. In this study, salt stress reduced transpiration rates in soybean leaves (Figure 2) primarily due to water imbalance and ion toxicity [38]. In salt stress conditions, increased Na^+ and Na^+/K^+ ratios were detected in the leaves, roots, and grains, whereas K^+ concentrations declined in both roots and leaves. These observations align with the findings of Anwar-ul-Haq et al. [39], which indicated that salt stress raises osmotic pressure in the roots, reduces water absorption, and leads to elevated Na^+ accumulation in plant tissues. The K^+/Na^+ ratio is a critical salt tolerance determinant, as higher K^+ levels counteract Na^+ toxicity. In the present study, PGPR application significantly lowered Na^+ concentrations and Na^+/K^+ ratios while enhancing K^+ levels under saline conditions. This suggests that PGPR facilitates Na^+ exclusion and K^+ absorption, thereby improving the K^+/Na^+ ratio [40,41]. Similarly, studies have shown that *Bacillus* strains can expel excess Na^+ from plant shoots and increase root K^+ uptake through the coordinated action of ion transporters like FaSOS1, FaHKT1, and FaHAK1 [42,43]. PGPR may achieve this by upregulating aquaporin (AQP) gene expression to maintain ion homeostasis and reduce H_2O_2 accumulation under saline conditions. Salt stress lowers external water potential, causing osmotic stress that triggers physiological

drought in soybean plants [5]. In this study, leaf water potential was positively correlated with K^+ concentration. Salt stress significantly reduced leaf water potential; however, PGPR application effectively increased it under salt conditions, alleviating toxic effects and promoting subsequent growth and yield [44]. Similar findings were reported for white clover (*Trifolium repens*), where GB03 application significantly reduced leaf osmotic potential under severe salt stress [45].

Salt stress typically begins at the rhizosphere before spreading to internal cellular structures. It increases osmotic pressure, causes stomatal closure, reduces CO_2 availability, lowers photosynthetic rates, and disrupts carbon fixation processes [46]. In this study, salt stress significantly reduced the activities of hexokinase (HXK), phosphoglucoisomerase (PGI), and sucrose synthase (SuSy), likely due to excessive Na^+ accumulation causing stomatal closure and enzyme structural damage (Figure 3). This restricted CO_2 assimilation during photosynthesis, severely reducing dry matter accumulation [47,48]. However, PGPR application significantly increased AGPase, fructokinase (FK), and SuSy activities while reducing UGPase and PGM under salt conditions. The overall glucose-to-starch pathway showed an upward trend with increased starch content (Table 1). This indicates that PGPR influences output by modulating enzyme activity during enzymatic reactions. Similar results were observed when *Burkholderia phytofirmans* (PsJN) was applied to grapevines (*Vitis vinifera*), altering carbohydrate metabolism to increase starch levels [49].

Under salt stress, decreased carbon assimilation and reduced electron transport rates in photosynthesis lower the CO_2 supply for the Calvin cycle. This depletes $NADP^+$ levels while diverting electrons to O_2 to form superoxide radicals (O_2^-) [50,51]. These ROS, including hydrogen peroxide (H_2O_2), hydroxyl radicals (-OH), and superoxide anions (O_2^-), damage biomolecules like carbohydrates, lipids, proteins, and nucleic acids, causing oxidative damage, photo-inhibition intensification, and membrane disruption [52]. Salt stress exacerbates ROS formation by reducing leaf water potential, causing stomatal closure and impairing cell development, ultimately lowering photosynthesis rates [53,54]. Plants activate antioxidant defense systems under stress to scavenge ROS effectively. This enhances adaptability and survival rates [31]. In this study, salt stress significantly reduced POX activity while slightly decreasing SOD and CAT activities (Figure 4). However, PGPR application significantly increased APX, GR, MDHAR, and POX activities. By producing these antioxidants, PGPR boosts antioxidant levels within plants to resist oxidative damage and enhance salt tolerance capacity [55]. Similar findings were reported for *Bacillus megaterium* (UPMR2) and *Enterobacter* (UPMR18) strains with elevated APX and CAT activities compared to controls under saline conditions [56]. This indicates that PGPR improves salt tolerance by modulating osmolyte concentrations, enhancing the activity of antioxidant enzymes, and influencing the expression of genes related to stress response [57].

SOD acts as the primary defense mechanism against ROS by neutralizing superoxide radicals. CAT, APX, and POD act as secondary defenses by removing excess H_2O_2 to maintain normal cellular metabolism for improved plant resilience against stresses [58]. The AsA-GSH cycle also plays a critical role in ROS scavenging. In this study, MDHAR activity increased under salt stress, potentially boosting ascorbate (AsA) accumulation. AsA reacts with H_2O_2 via APX activity to produce monodehydroascorbate (MDHA) or dehydroascorbate (DHA). GSH participates in the AsA-GSH cycle and reacts directly with O_2^- radicals by converting reduced GSH into oxidized glutathione disulfide (GSSG), aiding plant survival under saline conditions [59].

Soil microorganisms play a vital role in plant growth and metabolism. Soil salinity is a key factor affecting bacterial and fungal abundance/diversity levels [60]. PGPR application under salt stress significantly increased the relative abundances of *Bacteroidetes*, *Firmicutes*, *Nitrospirae*, and *Patescibacteria*. Bacterial ANOSIM analysis indicated significant differences

among treatments (Figure 5). LEfSe analysis identified *Flavobacterium* as having the highest LDA score under PGPR treatment during salt stress. Studies have shown that inoculating wheat with the *Pseudomonas strain* improves photosynthesis under salinity conditions by influencing rhizosphere bacterial diversity/structure [35].

This study similarly found that PGPR application improved soybean photosynthetic rate/transpiration rate under salt stress, indicating its ability to enhance crop photosynthesis processes. Additionally, PGPR inoculation may alter specific rhizosphere microbial subgroups' physiological traits to improve crop resistance against adverse environments [61]. Salt stress significantly reduced pod number per plant/yield in soybeans; however, PGPR application markedly improved crop yield, aligning with previous findings where PGPR alleviated salinity effects while enhancing wheat growth/yield under similar conditions [62]. The schematic diagram in Figure 7 illustrates how PGPR mitigates salinity stress in soybean production. Despite its significant findings, this study has limitations, including its focus on field conditions in a specific region, which may limit applicability to other environments with different soils or climates. This study did not evaluate the long-term impacts of repeated PGPR applications on soil health. Future investigations should prioritize the creation of region-specific PGPR formulations tailored to particular crops, soils, and climates for maximum effectiveness. Additionally, exploring synergistic methods that combine PGPR with other biostimulants or genetic techniques could enhance plant resilience. Long-term studies assessing the cumulative effects of PGPR on soil health and microbial diversity are also necessary. Molecular studies may uncover critical genes or proteins involved in plant–PGPR interactions through transcriptomic or proteomic analyses. Furthermore, scaling up PGPR applications should consider the economic viability across various agricultural systems. This research provides valuable insights for addressing salinity stress in soybean production and promoting sustainable agricultural practices. Solutions based on PGPR are essential for ensuring food security in challenging environments.

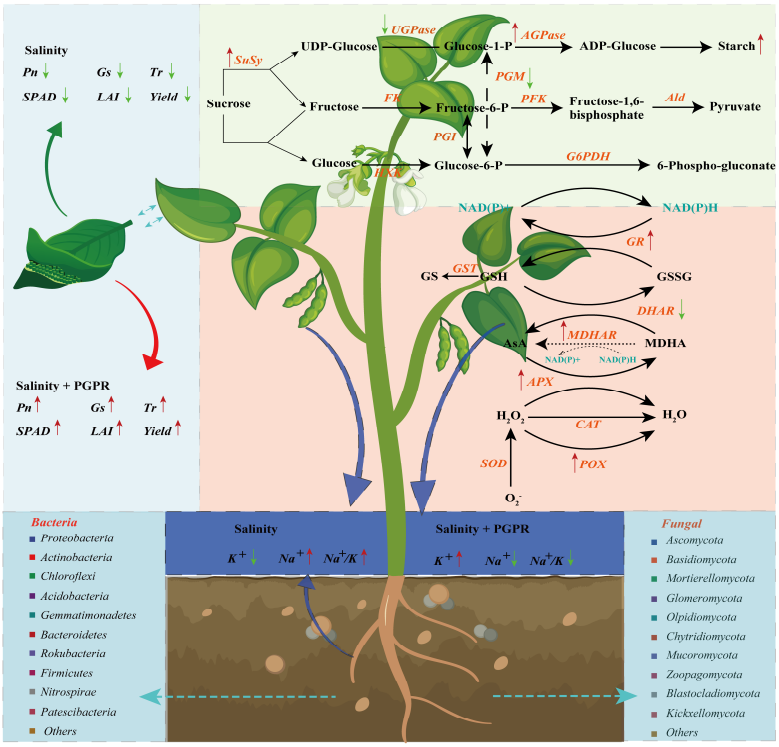


Figure 7. Effects of applying plant growth-promoting rhizobacteria (PGPR) on photosynthetic characteristics, osmotic regulation, rhizosphere microbial communities, and yield quality of soybeans grown under salt stress. Note: Red arrows indicate significant increases with PGPR application under salt stress; green arrows indicate significant decreases.

5. Conclusions

This study demonstrated that adding PGPR under salt stress significantly enhanced soybean salt tolerance through physiological, biochemical, and microbial mechanisms. Under salt stress, PGPR reduced Na^+ and K^- concentrations in leaves, improved ion balance and the Na^+/K^- ratio, alleviated osmotic stress, and mitigated ion toxicity. PGPR application significantly increased photosynthetic efficiency, stomatal conductance, transpiration rate, and PIABS under salt stress, while promoting the activity of key carbon metabolism enzymes, i.e., AGPase, HXK, and SuSy, which facilitated starch synthesis and dry matter accumulation. Additionally, PGPR enhanced antioxidant defense by enhancing the activity of enzymes, i.e., APX, GR, MDHAR, and POX, effectively reducing ROS-related oxidative damage. These combined effects under salt stress led to a 32.57% increase in soybean yield, indicating that PGPR is an effective strategy for improving crop yield and quality under salt stress conditions. In sustainable agriculture, PGPR can alter the rhizosphere microenvironment, reduce chemical fertilizers and pesticides, ensure soybean growth and development, enhance salt tolerance, and maintain growth and yield in saline soils, ensuring a stable supply. Future studies should involve long-term field trials to investigate the long-term impacts of PGPR on soil ecosystems and biodiversity in large-scale applications, ensuring its safety and sustainability. Future prospects include developing region-specific PGPR formulations optimized for specific crops, soil types, and climatic conditions to maximize effectiveness. Combining PGPR with other biostimulants or genetic engineering techniques could enhance plant resilience under stress conditions. Yet, extended research is essential to assess the cumulative impacts of repeated PGPR applications on soil health, microbial diversity, and crop productivity over time. Additionally, transcriptomic and proteomic analyses could help identify key genes or proteins involved in plant–PGPR interactions under salinity stress. Finally, assessing large-scale PGPR applications' economic feasibility and scalability across diverse agricultural systems is essential for broader adoption. This study provides valuable insights into mitigating soybean salt stress while advancing global sustainable agricultural practices and ensuring food security.

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References

1. Esmaeeli, M.; Roozbahani, A.; Daneshian, J. Combined effects chitosan and genotype on agronomic, physiologic, and biochemical characteristics of soybean under drought stress conditions. *South Afr. J. Bot.* **2024**, *174*, 678–685. [CrossRef]
2. Lv, D.; Xing, Q.; Wang, T.; Song, J.; Duan, R.; Hao, X.; Zong, Y.; Zhang, D.; Shi, X.; Zhao, Z.; et al. Elevated CO_2 concentration enhances plant growth, photosynthesis, and ion homeostasis of soybean under salt-alkaline stress. *Environ. Exp. Bot.* **2024**, *228*, 106000. [CrossRef]
3. Hasanuzzaman, M.; Raihan, M.R.; Nowroz, F.; Fujita, M. Insight into the mechanism of salt-induced oxidative stress tolerance in soybean by the application of *Bacillus Subtilis*: Coordinated actions of osmoregulation, ion homeostasis, antioxidant defense, and methylglyoxal detoxification. *Antioxidants* **2022**, *11*, 1856. [CrossRef]

4. El-Esawi, M.A.; Ali, H.M.; Hatamleh, A.A.; Al-Dosary, M.A.; El-Ballat, E.M. Multi-functional PGPR serratia liquefaciens confers enhanced resistance to lead stress and bacterial blight in soybean (*Glycine max* L.). *Current Plant Biology* **2024**, *40*, 100403. [CrossRef]
5. Phang, T.H.; Shao, G.; Lam, H.M. Salt tolerance in soybean. *J. Integr. Plant Biol.* **2008**, *50*, 1196–1212. [CrossRef]
6. Adhikari, B.; Dhungana, S.K.; Kim, I.; Shin, D. Effect of foliar application of potassium fertilizers on soybean plants under salinity stress. *J. Saudi Soc. Agric. Sci.* **2020**, *19*, 261–269. [CrossRef]
7. Yang, Y.; Ren, R.; Karthikeyan, A.; Yin, J.; Jin, T.; Fang, F.; Cai, H.; Liu, M.; Wang, D.; Zhi, H.; et al. The soybean GmPUB21-interacting protein GmDi19-5 responds to drought and salinity stresses via an ABA-dependent pathway. *Crop J.* **2023**, *11*, 1152–1162. [CrossRef]
8. Wang, J.; Yao, L.; Li, B.; Meng, Y.; Ma, X. Comparative Proteomic Analysis of Cultured Suspension Cells of the Halophyte Halogeton glomeratus by iTRAQ Provides Insights into Response Mechanisms to Salt Stress. *Front. Plant Sci.* **2016**, *7*, 110. [CrossRef]
9. Omar, S.; Salim, H.; Eldenary, M.; Nosov, A.V.; Allakhverdiev, S.I.; Alfiky, A. Ameliorating effect of nanoparticles and seeds' heat pre-treatment on soybean plants exposed to sea water salinity. *Heliyon* **2023**, *9*, e21446. [CrossRef]
10. Mohamed, H.; El-Sayed, A.; Rady, M.; Caruso, G.; Sekara, A.; Abdelhamid, M. Coupling effects of phosphorus fertilization source and rate on growth and ion accumulation of common bean under salinity stress. *PeerJ* **2021**, *9*, e11463. [CrossRef]
11. Läuchli, A.; James, R.A.; Huang, C.X.; McCully, M.; Munns, R. Cell-specific localization of Na SUP/SUP in roots of durum wheat and possible control points for salt exclusion. *Plant Cell Environ.* **2008**, *31*, 1565–1574. [CrossRef]
12. Rojas-Tapias, D.; Moreno-Galván, A.; Pardo-Díaz, S.; Obando, M.; Rivera, D.; Bonilla, R. Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (*Zea mays*). *Appl. Soil Ecol.* **2012**, *61*, 264–272. [CrossRef]
13. Moez, H.; Chantal, E.; Mariama, N.; Laurent, L.; Khaled, M. New insights on plant salt tolerance mechanisms and their potential use for breeding. *Front. Plant Sci.* **2016**, *7*, 1787. [CrossRef]
14. Papiernik, S.K.; Grieve, C.M.; Lesch, S.M.; Yates, S.R. Effects of salinity, imazethapyr, and chlorimuron application on soybean growth and yield. *Commun. Soil Sci. Plant Anal.* **2005**, *36*, 951–967. [CrossRef]
15. Assaha, D.V.; Ueda, A.; Saneoka, H.; Al-Yahyai, R.; Yaish, M.W. The role of Na^+ and K^+ transporters in salt stress adaptation in glycophytes. *Front. Physiol.* **2017**, *8*, 509. [CrossRef]
16. Aycan, M.; Baslam, M.; Asiloglu, R.; Mitsui, T.; Yildiz, M. Development of new high-salt tolerant bread wheat (*Triticum aestivum* L.) genotypes and insight into the tolerance mechanisms. *Plant Physiol. Biochem.* **2021**, *166*, 314–327. [CrossRef]
17. Shabala, S.; Cuin, T.A. Potassium transport and plant salt tolerance. *Physiol. Plant* **2008**, *133*, 651–669. [CrossRef]
18. Fatma, M.; Asgher, M.; Masood, A.; Khan, N.A. Excess sulfur supplementation improves photosynthesis and growth in mustard under salt stress through increased production of glutathione. *Environ. Exp. Bot.* **2014**, *107*, 55–63. [CrossRef]
19. Lakhdar, A.; Hafsi, C.; Debez, A.; Montemurro, F.; Abdelly, C. Assessing solid waste compost application as a practical approach for salt-affected soil reclamation. *Acta Agric. Scand.* **2011**, *61*, 284–288. [CrossRef]
20. Bhattacharyya, P.; Jha, D. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World J. Microbiol. Biotechnol.* **2012**, *28*, 1327–1350. [CrossRef]
21. Pérez-Montaño, F.; Alías-Villegas, C.; Bellogín, R.A.; Cerro, P.D.; Espuny, M.R.; Jiménez-Guerrero, I.; López-Baena, F.J.; Ollero, F.J.; Cubo, T. Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. *Microbiol. Res.* **2014**, *169*, 325–336. [CrossRef]
22. Yin, Z.Q.; Wang, X.; Hu, Y.J.; Zhang, J.K.; Li, H.; Cui, Y.R.; Zhao, D.Y.; Dong, X.S.; Zhang, X.H.; Liu, K.; et al. *Metabacillus dongyingensis* sp. nov. is represented by the plant growth-promoting bacterium by2g20 isolated from saline-alkaline soil and enhances the growth of *Zea mays* L. under salt stress. *Msystems* **2022**, *7*, e01426-21. [CrossRef]
23. Jabborova, D.; Wirth, S.; Kannepalli, A.; Narimanov, A.; Desouky, S.; Davranov, K.; Sayyed, R.Z.; El Enshasy, H.; Abd Malek, R.; Syed, A.; et al. Co-inoculation of rhizobacteria and biochar application improves growth and nutrientsin soybean and enriches soil nutrients and enzymes. *Agronomy* **2020**, *10*, 1142. [CrossRef]
24. Kumari, S.; Vaishnav, A.; Jain, S.; Varma, A.; Choudhary, D.K. Bacterial-mediated induction of systemic tolerance to salinity with expression of stress alleviating enzymes in soybean (*Glycine max* L. *Merrill*). *J. Plant Growth Regul.* **2015**, *34*, 558–573. [CrossRef]
25. Lu, L.; Liu, N.; Fan, Z.H.; Liu, M.H.; Zhang, X.X.; Tian, J.; Yu, Y.J.; Lin, H.H.; Huang, Y.; Kong, Z.S. A novel PGPR strain, streptomyces lasalocidi JCM 3373, alleviates salt stress and shapes root architecture in soybean by secreting indole-3-carboxaldehyde. *Plant Cell Environ.* **2024**, *47*, 1941–1956. [CrossRef]
26. Arora, N.K.; Fatima, T.; Mishra, J.; Mishra, I.; Bharti, C. Halo-tolerant plant growth promoting rhizobacteria for improving productivity and remediation of saline soils. *J. Adv. Res.* **2020**, *26*, 69–82. [CrossRef]
27. Upadhyay, S.K.; Singh, J.S.; Saxena, A.K.; Singh, D.P. Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions. *Plant Biol.* **2012**, *14*, 605–611. [CrossRef]
28. Allen, S.E.; Grimshaw, H.M.; Rowland, A.P. *Chemical analysis. Methods in Plant Ecology*, 2nd ed.; Blackwell: Oxford, UK, 1986; pp. 285–344.

29. Jammer, A.; Gasperl, A.; Luschin-Ebengreuth, N.; Heyneke, E.; Chu, H.; Cantero-Navarro, E.; Großkinsky, D.K.; Albacete, A.A.; Stabentheiner, E.; Franzaring, J. Simple and robust determination of the activity signature of key carbohydrate metabolism enzymes for physiological phenotyping in model and crop plants. *J. Exp. Bot.* **2015**, *66*, 5531–5542. [CrossRef]
30. Fimognari, L.; Dlker, R.; Kaselyte, G.; Jensen, C.N.G.; Akhtar, S.S.; Grokinsky, D.K.; Roitsch, T. Simple semi-high throughput determination of activity signatures of key antioxidant enzymes for physiological phenotyping. *Plant Methods* **2020**, *16*, 42. [CrossRef]
31. Li, H.; Liu, S.Q.; Guo, J.H.; Liu, F.L.; Song, F.B.; Li, X.N. Effect of the transgenerational exposure to elevated CO_2 on low temperature tolerance of winter wheat: Chloroplast ultrastructure and carbohydrate metabolism. *J. Agron. Crop Sci.* **2020**, *206*, 773–783. [CrossRef]
32. Pan, J.; Peng, F.; Xue, X.; You, Q.G.; Zhang, W.J.; Wang, T.; Huang, C.H. The growth promotion of two salt-tolerant plant groups with pgpr inoculation: A Meta-analysis. *Sustainability* **2019**, *11*, 378. [CrossRef]
33. El-Esawi, M.A.; Alaraidh, I.A.; Alsahli, A.A.; Alamri, S.A.; Ali, H.M.; Alayafi, A.A. Bacillus firmus (SW5) augments salt tolerance in soybean (*Glycine max* L.) by modulating root system architecture, antioxidant defense systems and stress-responsive genes expression. *Plant Physiol. Biochem.* **2018**, *132*, 375–384. [CrossRef] [PubMed]
34. Kang, S.M.; Radhakrishnan, R.; Khan, A.L.; Kim, M.J.; Park, J.M.; Kim, B.R.; Shin, D.H.; Lee, I.J. Gibberellin secreting rhizobacterium, pseudomonas putida H-2-3 modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions. *Plant Physiol. Biochem.* **2014**, *84*, 115–124. [CrossRef]
35. Ahmadi-Nouraldinvand, F.; Sharifi, R.S.; Siadat, S.A.; Khalilzadeh, R. Fascinating Role of Silicon dioxide Nanoparticles and Co-inoculation of Mycorrhiza and Rhizobacteria to Combat NaCl Stress: Changes in Physiological Characteristics, Uptake of Nutrient Elements, and Enhancing Photosystem II Activities in Wheat. *J. Soil Sci. Plant Nutr.* **2024**, *24*, 277–294. [CrossRef]
36. Murata, N.; Takahashi, S.; Nishiyama, Y.; Allakhverdiev, S.I. Photoinhibition of photosystem II under environmental stress. *Biochim. Et Biophys. Acta (BBA)—Bioenerg.* **2007**, *1767*, 414–421. [CrossRef]
37. Xie, X.; Gan, L.Z.; Wang, C.Y.; He, T.X. Salt-tolerant plant growth-promoting bacteria as a versatile tool for combating salt stress in crop plants. *Arch. Microbiol.* **2024**, *206*, 341. [CrossRef]
38. Sui, X.N.; Xu, Z.C.; Zheng, Y.F.; Li, Y.Q.; Zhang, C.S.; Meng, C. Transcriptomic and comprehensive analysis of salt stress-alleviating—Alleviating mechanisms by *Ensifer sesbaniae* DY22 in soybean. *Environ. Exp. Bot.* **2024**, *226*, 105908. [CrossRef]
39. Anwar-ul-Haq, M.; Iftikhar, I.; Akhtar, J.; Maqsood, M. Role of exogenous osmolyte supplementation in ameliorating osmotic and oxidative stress and promoting growth in salinity-stressed soybean genotypes. *J. Soil Sci. Plant Nutr.* **2023**, *23*, 3682–3694. [CrossRef]
40. Khan, M.A.; Asaf, S.; Khan, A.L.; Jan, R.; Kang, S.M.; Kim, K.M.; Lee, I.J. Rhizobacteria AK1 remediates the toxic effects of salinity stress via regulation of endogenous phytohormones and gene expression in soybean. *Biochem. J.* **2019**, *476*, 2393–2409. [CrossRef] [PubMed]
41. Li, Y.Y.; You, X.K.; Tang, Z.; Zhu, T.Q.; Liu, B.W.; Chen, M.X.; Xu, Y.F.; Liu, T.Y. Isolation and identification of plant growth-promoting rhizobacteria from tall fescue rhizosphere and their functions under salt stress. *Physiol. Plant.* **2022**, *174*, e13817. [CrossRef]
42. Hu, W.; Yuan, Q.Q.; Wang, Y.; Cai, R.; Deng, X.M.; Wang, J.; Zhou, S.Y.; Chen, M.J.; Chen, L.H.; Huang, C.; et al. Overexpression of a wheat aquaporin gene, taaqp8, enhances salt stress tolerance in transgenic tobacco. *Plant Cell Physiol.* **2012**, *53*, 2127–2141. [CrossRef] [PubMed]
43. Marulanda, A.; Azcón, R.; Chaumont, F.; Ruiz-Lozano, J.M.; Aroca, R. Regulation of plasma membrane aquaporins by inoculation with a Bacillus megaterium strain in maize (*Zea mays* L.) plants under unstressed and salt-stressed conditions. *Planta* **2010**, *232*, 533–543. [CrossRef]
44. Ilangumaran, G.; Schwinghamer, T.D.; Smith, D.L. Rhizobacteria from root nodules of an indigenous legume enhance salinity stress tolerance in soybean. *Front. Sustain. Food Syst.* **2021**, *4*, 617978. [CrossRef]
45. Han, Q.Q.; Lu, X.P.; Bai, J.P.; Qiao, Y.; Paré, P.W.; Wang, S.M.; Zhang, J.L.; Wu, Y.N.; Pang, X.P.; Xu, W.B.; et al. Beneficial soil bacterium *Bacillus subtilis* (GB03) augments salt tolerance of white clover. *Front. Plant Sci.* **2014**, *5*, 525. [CrossRef]
46. Wang, X.M.; Chen, Z.T.; Sui, N. Sensitivity and responses of chloroplasts to salt stress in plants. *Front. Plant Sci.* **2024**, *15*, 1374086. [CrossRef]
47. Delgado-González, C.R.; Madariaga-Navarrete, A.; Rodríguez-Laguna, R.; Capulín-Grande, J.; Sharma, A.; Islas-Pelcastre, M. Microorganism rhizosphere interactions and their impact on the bioremediation of saline soils: A review. *Int. J. Environ. Sci. Technol.* **2022**, *19*, 12775–12790. [CrossRef]
48. Souza, R.P.; Machado, E.C.; Silva, J.A.B.; Lagôa, A.; Silveira, J.A.G. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. *Environ. Exp. Bot.* **2004**, *51*, 45–56. [CrossRef]
49. Fernandez, O.; Theocharis, A.; Bordiec, S.; Feil, R.; Jacquens, L.; Clément, C.; Fontaine, F.; Barka, E.A. *Burkholderia phytofirmans* psjn acclimates grapevine to cold by modulating carbohydrate metabolism. *Mol. Plant-Microbe Interact.* **2012**, *25*, 496–504. [CrossRef]

50. Lu, C.X.; Li, L.Y.; Liu, X.L.; Chen, M.; Wan, S.B.; Li, G.W. Salt stress inhibits photosynthesis and destroys chloroplast structure by downregulating chloroplast development-related genes in robinia pseudoacacia seedlings. *Plants* **2023**, *12*, 1283. [CrossRef]
51. Srivastava, S.; Yadav, S. A positive role of polyunsaturated fatty acids on sustainable crop production against salt stress: An overview. *Biologia* **2024**, *79*, 1599–1610. [CrossRef]
52. Meloni, D.A.; Oliva, M.A.; Martinez, C.A.; Cambraia, J. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ. Exp. Bot.* **2003**, *49*, 69–76. [CrossRef]
53. Cerqueira, J.V.A.; Silveira, J.A.G.; Carvalho, F.E.L.; Cunha, J.R.; Neto, M.C.L. The regulation of P700 is an important photoprotective mechanism to NaCl-salinity in Jatropha curcas. *Physiol. Plant.* **2019**, *167*, 404–417. [CrossRef] [PubMed]
54. Islam, F.; Ali, B.; Wang, J.; Farooq, M.A.; Gill, R.A.; Ali, S.; Wang, D.Y.; Zhou, W.J. Combined herbicide and saline stress differentially modulates hormonal regulation and antioxidant defense system in *Oryza sativa* cultivars. *Plant Physiol. Biochem.* **2016**, *107*, 82–95. [CrossRef] [PubMed]
55. Hashem, A.; Abd Allah, E.F.; Alqarawi, A.A.; Al-Huqail, A.A.; Shah, M.A. Induction of osmoregulation and modulation of salt stress in *Acacia gerrardii* benth. by arbuscular mycorrhizal fungi and *Bacillus subtilis* (BERA 71). *Biomed Res. Int.* **2016**, *11*, 6294098. [CrossRef]
56. Habib, S.H.; Kausar, H.; Saud, H.M. Plant growth-promoting rhizobacteria enhance salinity stress tolerance in okra through ros-scavenging enzymes. *Biomed Res. Int.* **2016**, *10*, 6284547. [CrossRef]
57. El-Esawi, M.A.; Al-Ghamdi, A.A.; Ali, H.M.; Alayafi, A.A. *Azospirillum lipoferum* FK1 confers improved salt tolerance in chickpea (*Cicer arietinum* L.) by modulating osmolytes, antioxidant machinery and stress-related genes expression. *Environ. Exp. Bot.* **2019**, *159*, 55–65. [CrossRef]
58. Abd Allah, E.F.; Alqarawi, A.A.; Hashem, A.; Radhakrishnan, R.; Al-Huqail, A.A.; Al-Otibi, F.O.N.; Malik, J.A.; Alharbi, R.I.; Egamberdieva, D. Endophytic bacterium *Bacillus subtilis* (BERA 71) improves salt tolerance in chickpea plants by regulating the plant defense mechanisms. *J. Plant Interact.* **2018**, *13*, 37–44. [CrossRef]
59. Pallavi, S.; Bhushan, J.A.; Shanker, D.R.; Mohammad, P. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.* **2012**, *2012*, 1–26. [CrossRef]
60. Jacoby, R.; Peukert, M.; Succurro, A.; Koprivova, A.; Kopriva, S. The role of soil microorganisms in plant mineral nutrition-current knowledge and future directions. *Front. Plant Sci.* **2017**, *8*, 1617. [CrossRef]
61. Kong, Z.Y.; Liu, H.G. Modification of rhizosphere microbial communities: A possible mechanism of plant growth promoting rhizobacteria enhancing plant growth and fitness. *Front. Plant Sci.* **2022**, *13*, 920813. [CrossRef]
62. Zafar-ul-Hye, M.; Bhutta, T.S.; Shaaban, M.; Hussain, S.; Qayyum, M.F.; Aslam, U.; Zahir, Z.A. Influence of plant growth promoting rhizobacterial inoculation on wheat productivity under soil salinity stress. *Phyton-Int. J. Exp. Bot.* **2019**, *88*, 119–129. [CrossRef]

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