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Effects of *Bacillus subtilis* HS5B5 on Maize Seed Germination and Seedling Growth under NaCl Stress Conditions

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Abstract: Salinity is one of the most important factors limiting agricultural productivity. The positive effects of an inoculation with *Bacillus subtilis* HS5B5 on maize (*Zea mays* L.) seed germination and seedling growth under saline conditions were elucidated in this study. Maize plants were treated with four NaCl concentrations (0, 100, 200, and 300 mmol·L^{-1}) under hydroponic conditions and the plants inoculated with *B. subtilis* HS5B5 were compared with the non-inoculated plants in terms of key morphophysiological leaf and root traits. Maize seed germination and seedling growth were inhibited by NaCl stress. The inoculation with *B. subtilis* HS5B5 significantly increased the germination rate, germination potential, shoot length, and root length under NaCl stress conditions. Moreover, the plant height, biomass, root to shoot weight ratio, above-ground fresh weight, and below-ground fresh weight were higher for the inoculated maize seedlings than for the non-inoculated plants under saline conditions. Additionally, *B. subtilis* HS5B5 alleviated the salt-induced damage to maize by increasing the chlorophyll content, altering the abundance of osmoregulatory substances, and increasing antioxidant enzyme activities, while decreasing the malondialdehyde content. After the NaCl treatment, the Na^+ content in the leaves and roots of maize plants inoculated with *B. subtilis* HS5B5 decreased significantly, while the K^+ content increased. Thus, the inhibitory effect of NaCl stress on maize seed germination and seedling growth was mitigated by *B. subtilis* HS5B5, suggesting the utility of this microorganism for improving crop cultivation under saline conditions.

Keywords: maize; NaCl stress; seed germination; seedling growth

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1. Introduction

Soil salinity is an important factor influencing the seed germination and growth of various crops. Physiological and biochemical properties of plants are substantially affected by abiotic stresses, including salinity [1,2]. Specifically, salt stress hinders the cultivation of crops, especially in arid and semi-arid regions where soil salinization is widespread and severe [3]. Approximately, 50% of the irrigated land and 20% of the cropland worldwide are affected by salt stress, with the associated decrease in crop growth and yield becoming one of the most important factors limiting agricultural production [4,5]. Plant responses to saline conditions include the production of reactive oxygen species (ROS), which can impair chlorophyll, DNA, protein, and membrane functions in plant cells if they accumulate to excessive levels [6,7]. Plant water loss, osmotic stress, and the overproduction of toxic ions may be induced by salt stress, which eventually leads to oxidative damage due to toxic radicals [8]. This osmotic stress may result in delayed or poor seed germination and abnormal seedling growth [9,10]. Excessive amounts of ethylene and Na^+ in crops under highly saline conditions can inhibit nutrient absorption and affect crop growth [11,12]. Plant root cell division and elongation are also restricted by salt stress, thereby affecting root growth [13]. Moreover, gas exchange, the transpiration rate, and photosynthetic activities are altered by the salt stress-induced inappropriate opening and closing of the stomata. Earlier research shows that the uptake of ions required for enzymatic and metabolic activities is modified by high salt concentrations in the soil [14].

The detrimental effects of salt stress on crop production are mitigated in several ways, including selective breeding, genetic engineering, and mutagenesis-based breeding [15], but these approaches may be limited by cost and environmental conditions. One environmental-friendly method involves the use of beneficial microorganisms that have adapted to abiotic stresses, including salinity [16]. According to recent research, certain microorganisms can enhance plant tolerance to abiotic stress through direct and indirect mechanisms [17]. Beneficial microorganisms induce resistance or tolerance mechanisms in plants, resulting in physiological and biochemical changes that alleviate salt-induced stress damage [18]. This mechanism primarily involves regulating plant hormone concentrations, the activity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and antioxidant enzyme activity [19]. They also produce osmotic-regulating substances such as proline, polysaccharides, and glycine betaine, as well as extracellular polysaccharides and volatile compounds, to ensure the normal growth of plants under salt stress conditions [20]. Additionally, some microorganisms regulate the expression of salt and alkali resistance-related genes and proteins in plants, enhancing their salt and alkali tolerance abilities [21].

Maize (*Zea mays* L.) is a major crop worldwide because of its use as food and feed as well as an important bioenergy source, but it is highly susceptible to salt stress [22]. The adverse effects of high salinity on maize growth and development are mainly reflected by weak seedling growth, poor stomatal conductance, and inefficient photosynthesis. The growth and abiotic stress tolerance of plants, such as *Arabidopsis thaliana* (L.) Heynh, can be promoted by *Bacillus subtilis*. However, the effect of *B. subtilis* on maize responses to different salt concentrations is unknown. Therefore, we investigated the effect of salinity on the biochemical activities and growth of maize plants inoculated with *B. subtilis* under controlled conditions. The objectives of this study were to clarify the effects of *B. subtilis* HS5B5 on maize seed germination under saline conditions and to determine how *B. subtilis* HS5B5 influences the growth, chlorophyll content, abundance of osmoregulatory substances, malondialdehyde (MDA) content, antioxidant enzyme activities, and Na^+ and K^+ contents of maize seedlings treated with different NaCl concentrations. The results of this study may provide useful insights into the mechanism mediating the regulatory effects of *B. subtilis* HS5B5 on the growth and development of salt-stressed maize seedlings.

2. Materials and Methods

2.1. Bacterial Strains

Bacillus subtilis HS5B5 (CGMCC No. 6088) was provided by the Special Biological Resources Development and Utilization Laboratory, College of Agriculture, Henan University of Science and Technology, China, and stored at 4 °C. *B. subtilis* HS5B5 had excellent potential for salt tolerance and growth-promoting effects. The strain was cultured in LB medium, and a bacterial suspension was prepared by culturing it in a shaker at 28 °C for 18 h. The bacterial suspension was centrifuged at 4000 $r \cdot min^{-1}$ for 10 min. The bacterial pellet was rinsed with sterile water, collected, and diluted with sterile water to prepare the stock solution (1×10^9 $CFU \cdot mL^{-1}$) for further use in the experiment [23].

2.2. Maize Seed Germination

Maize seeds were disinfected by soaking in a 10% sodium hypochlorite (NaClO) solution for 2 min, rinsed 3–5 times with distilled water, and placed in Petri dishes (diameter: 15 cm) containing two layers of filter paper (30 seeds per dish) prior to an 8 h incubation at room temperature. The Petri dishes were maintained at 25 ± 1 °C in darkness in a constant-temperature incubator for each of the following treatments: four NaCl concentrations (0, 100, 200, and 300 $mmol \cdot L^{-1}$ prepared in water) with and without the inoculation with *B. subtilis* HS5B5 (1×10^7 $CFU \cdot mL^{-1}$). A 10-mL aliquot of each treatment solution was added to individual Petri dishes (ensuring the filter paper remained moist) during the seed germination period, with three replicates or 90 seeds per treatment. Seeds were considered germinated when the germ length exceeded half the seed length and the radicle length was

equal to the seed length. The number of germinated seeds was recorded daily for 7 days, after which the germination rate was calculated.

2.3. Seed Germination Index Determination

After the seeds were treated with NaCl, the number of germinated seeds in each Petri dish was recorded daily after germination was first detected (i.e., radicle broke through the seed coat). The average germination rate was calculated for each time-point until all of the seeds had germinated or the seeds were no longer germinating [24]. The germination rate (%) was calculated using the following formula:

$$\text{Germination rate} = \text{number of germinated seeds on day 7/total number of seeds} \times 100 \quad (1)$$

The germination potential was determined as an indicator of field emergence [25]. It was calculated as follows:

$$\text{Germination potential} = \text{number of seeds germinated on day 4/total number of seeds} \times 100 \quad (2)$$

There were 3 replications for each treatment and 15 seedlings per replication. Five maize seedlings were randomly selected for an analysis of their shoot and root lengths (mm) and then average values were calculated [26].

2.4. Maize Seedling Trials

Maize seeds with a relatively full appearance were selected, surface-sterilized in a 10% NaClO solution for 2 min, rinsed repeatedly with distilled water, and immersed in water for 8 h. The seeds were transferred to a constant-temperature incubator set at 25 ± 1 °C. After a 48 h incubation, the uniformly germinated seeds were transferred to a foam board containing charcoal soil and vermiculite (1:1). The foam board was placed in a pot containing 2 L of improved Hoagland's nutrient solution (pH 6.0 ± 0.1). The seedlings were inoculated with *B. subtilis* HS5B5 (we took 20 mL of bacterial suspension with a concentration of 1×10^9 CFU·mL^{-1} and inoculated it into 2 L of nutrient solution, so that the bacterial concentration in the nutrient solution was 1×10^7 CFU·mL^{-1}) when they developed a center leaf and a functional leaf and were then treated with different NaCl concentrations 24 h later. We added 11.67 g, 23.38 g, and 35.06 g of NaCl, respectively, to 2 L of nutrient solution, and the final NaCl concentration in the nutrient solution was 100, 200, and 300 mmol·L^{-1}, respectively, and the NaCl concentration of the blank control was 0 mmol·L^{-1}. Each treatment was repeated three times. Samples were collected 15 days after initiating the NaCl treatment and analyzed.

2.5. Growth Index Determination

For each treatment, five maize seedlings were randomly selected for an analysis of plant height (mm) and then average values were calculated [26]. Additionally, the roots and leaves were collected 15 days after NaCl stress and weighed using an electronic balance (fresh weight). The roots and leaves were placed in an electric blast drying oven at 120 °C for 15 min. The oven temperature was adjusted to 75 °C and the samples were dried to a constant weight [26].

The root to shoot weight ratio was calculated using the following formula:

$$\text{Root to Shoot Weight Ratio} = G1/G2 \quad (3)$$

where G1 represents the below-ground fresh weight (g) and G2 represents the above-ground fresh weight (g).

2.6. Chlorophyll Content Determination

The chlorophyll content of selected maize seedlings from each pot was estimated using the third leaf from the tip. Fresh leaf disks were ground in 10 mL 80% acetone and centrifuged at 2500 r·min^{-1} for 10 min at 4 °C. The absorbance of the supernatant was measured at 645, 663, and 653 nm, respectively, using a spectrophotometer. The chlorophyll

contents were calculated using the following formulae as previously described by Lorenzen (1967) [27]:

$$\text{Chlorophyll } a = 12.7 \times (A_{663}) - 2.69 \times (A_{645}) \times V \times W \quad (4)$$

$$\text{Chlorophyll } b = 20.9 \times (A_{645}) - 4.68 \times (A_{663}) \times V \times W \quad (5)$$

where V represents the final extract volume and W represents the leaf fresh weight.

2.7. Osmoregulatory Substances and Lipid Peroxidation

The Coomassie Brilliant Blue G250 method was used to determine the soluble protein content, whereas the soluble sugar content was determined using the anthrone–sulfuric acid method and the proline (Pro) content was determined after an extraction at room temperature using 3% 5-sulfosalicylic acid. Additionally, the lipid peroxidation level was determined by measuring the MDA content [28].

2.8. Antioxidant Assays

The activities of the antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in fresh maize leaves were determined as described by Lum [29] and Bianco et al. [30].

2.9. Macronutrient Analysis

The accurately weighed samples were placed in digestion vessels and then 5 mL nitric acid was added to each vessel. After sealing the vessels with safety valves, they were placed in a microwave digestion system until the digestion was complete. The solutions were cooled and diluted with distilled water for a final volume of 50 mL. The resulting solutions were analyzed using an ICP system (Agilent 5110VDV, Santa Clara, CA, USA) to determine the sodium (Na^+) and potassium (K^+) concentrations in individual organs as well as in the whole plant. All macronutrients were analyzed using the ICP system [31].

2.10. Statistical Analyses

All experimental data underwent an analysis of variance (ANOVA) using the SPSS Statistics 22.0 software. The mean values for each treatment were compared using Duncan's multiple range test to determine significant differences (at the 0.05 level). Data are presented herein as the mean and their standard error.

3. Results

3.1. Maize Seed Germination

The maize seed germination indices tended to decrease as the concentration of the NaCl treatment increased (Table 1). In the absence of NaCl stress, the inoculation with *B. subtilis* HS5B5 did not substantially affect maize seed germination. Following the 100 mmol·L^{-1} NaCl treatment, the roots of the plants inoculated with *B. subtilis* were 24.81% longer than the roots of the non-inoculated plants ($p < 0.05$). After the 200 mmol·L^{-1} NaCl treatment, the germination rate, germination potential, shoot length, and root length were, respectively, 41.68%, 47.61%, 81.17%, and 82.20% greater for the inoculated plants than for the non-inoculated plants ($p < 0.05$). Among the samples treated with 300 mmol·L^{-1} NaCl, the germination potential was 26.68% higher for the inoculated samples than for the non-inoculated samples ($p < 0.05$).

Table 1. Effect of *B. subtilis* HS5B5 on the germination of maize seeds treated with NaCl.

Processing Group		Germination Rate	Germination Potential	Shoot Length (cm)	Root Length (cm)
Non-Inoculated	0 mmol·L^{-1} NaCl	0.94 ± 0.02 a	0.92 ± 0.22 a	11.83 ± 0.13 a	15.08 ± 0.23 a
	100 mmol·L^{-1} NaCl	0.92 ± 0.02 a	0.91 ± 0.02 a	10.98 ± 0.40 a	11.23 ± 0.38 c
	200 mmol·L^{-1} NaCl	0.53 ± 0.03 c	0.46 ± 0.03 c	3.75 ± 1.11 c	5.37 ± 0.54 e
	300 mmol·L^{-1} NaCl	0.44 ± 0.02 c	0.33 ± 0.00 d	2.10 ± 0.95 c	3.07 ± 0.43 f
Inoculated	0 mmol·L^{-1} NaCl	0.97 ± 0.02 a	0.97 ± 0.02 a	12.89 ± 0.40 a	16.79 ± 0.495 a
	100 mmol·L^{-1} NaCl	0.99 ± 0.01 a	0.99 ± 0.011 a	12.38 ± 0.26 a	14.02 ± 0.32 b
	200 mmol·L^{-1} NaCl	0.75 ± 0.04 b	0.68 ± 0.04 b	6.80 ± 0.93 b	9.79 ± 1.32 d
	300 mmol·L^{-1} NaCl	0.52 ± 0.01 c	0.42 ± 0.01 c	4.15 ± 3.16 bc	4.69 ± 0.57 f

Note: values with different letters in the same column differ significantly at the 0.05 level.

3.2. Plant Growth and Macronutrients

The effects of NaCl and the inoculation with *B. subtilis* HS5B5 on maize plant height are shown in Figure 1 and Appendix A, Figure A1. The plant height gradually decreased with increasing NaCl concentrations. In the absence of NaCl stress, the inoculation with *B. subtilis* HS5B5 significantly increased the maize plant height by 11.19% ($p < 0.05$). When the NaCl concentration was relatively low (100 mmol·L^{-1}), the inoculation with *B. subtilis* significantly increased the plant height by 18.83% ($p < 0.05$) (relative to the height of the non-inoculated plants).

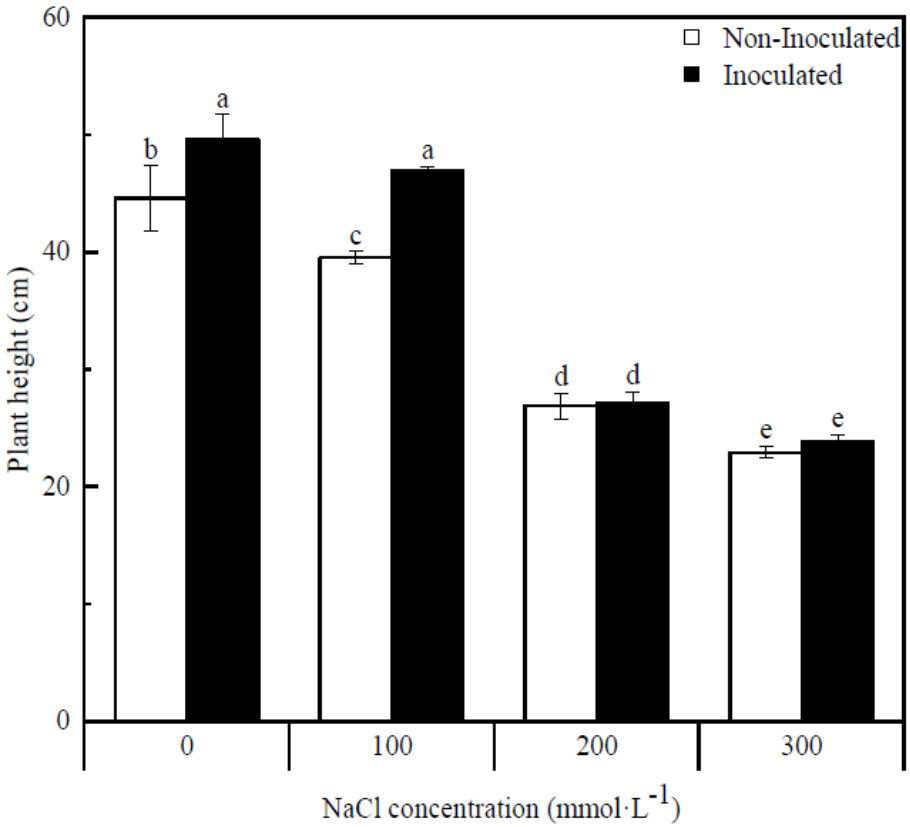


Figure 1. Effect of *B. subtilis* on the height of maize plants treated with NaCl. Note: different letters above the bars indicate significant differences ($p < 0.05$).

The biomass of the maize seedlings gradually decreased as the concentration of the NaCl treatment increased (Figure 2). The inoculation with *B. subtilis* HS5B5 significantly increased the maize seedling biomass by 18.12% ($p < 0.05$) in the absence of NaCl stress. Under NaCl stress conditions, the biomass was significantly higher for the inoculated plants than for the non-inoculated plants, with the largest difference (26.76%) observed for the 300 mmol·L^{-1} NaCl treatment ($p < 0.05$).

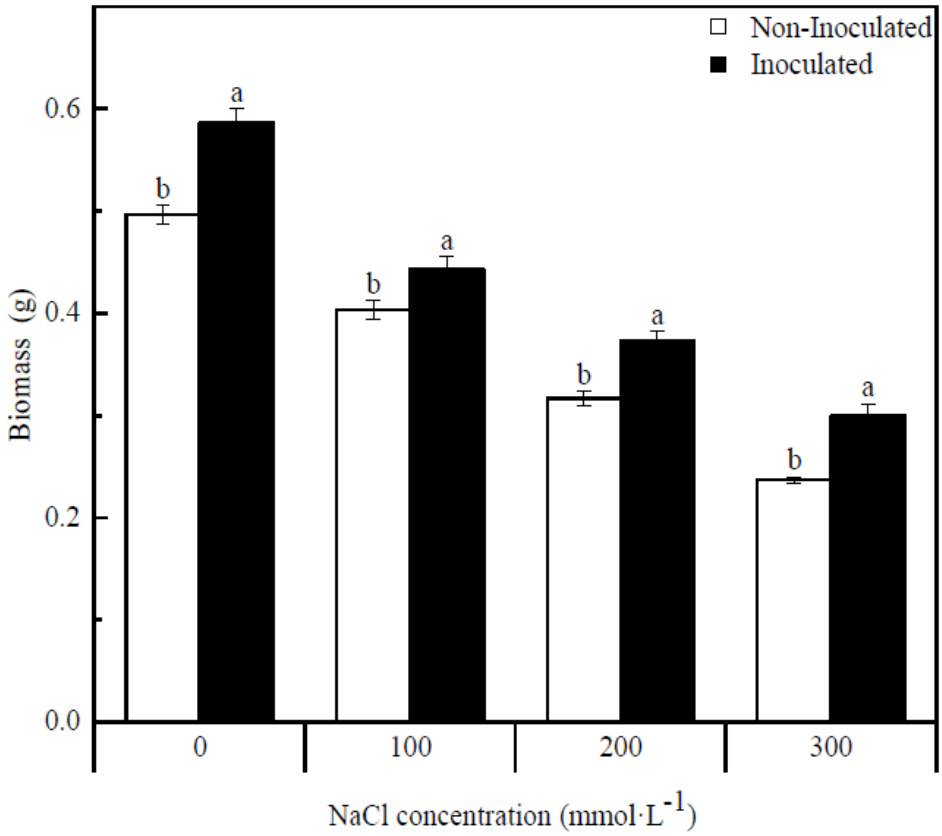


Figure 2. Effect of *B. subtilis* on the biomass of maize plants treated with NaCl. Note: different letters above the bars indicate significant differences ($p < 0.05$).

The root to shoot weight ratio of the NaCl-treated maize seedlings increased as the NaCl concentration increased (Figure 3). At 200 mmol·L^{-1} NaCl, the root to shoot weight ratio was 25.53% higher for the *B. subtilis*-inoculated plants than for the non-inoculated plants ($p < 0.05$). In contrast, at 300 mmol·L^{-1} NaCl, the root to shoot weight ratio was 8.33% higher for the *B. subtilis*-inoculated plants than for the non-inoculated plants ($p < 0.05$).

The above-ground and below-ground fresh weights of the maize plants gradually decreased as the NaCl concentration of the salt treatment increased (Figure 4). In the absence of NaCl stress, the inoculation with *B. subtilis* HS5B5 significantly increased the above-ground and below-ground fresh weights of the maize plants by 13.75% and 34.42%, respectively ($p < 0.05$). In response to the 100 mmol·L^{-1} NaCl treatment, the above-ground fresh weight was 18.10% greater for the plants inoculated with *B. subtilis* HS5B5 than for the non-inoculated plants ($p < 0.05$). Following the NaCl treatments, the below-ground fresh weight was significantly greater for the inoculated plants than for the non-inoculated plants, with the largest difference (27.78%) detected for the 200 mmol·L^{-1} NaCl treatment ($p < 0.05$).

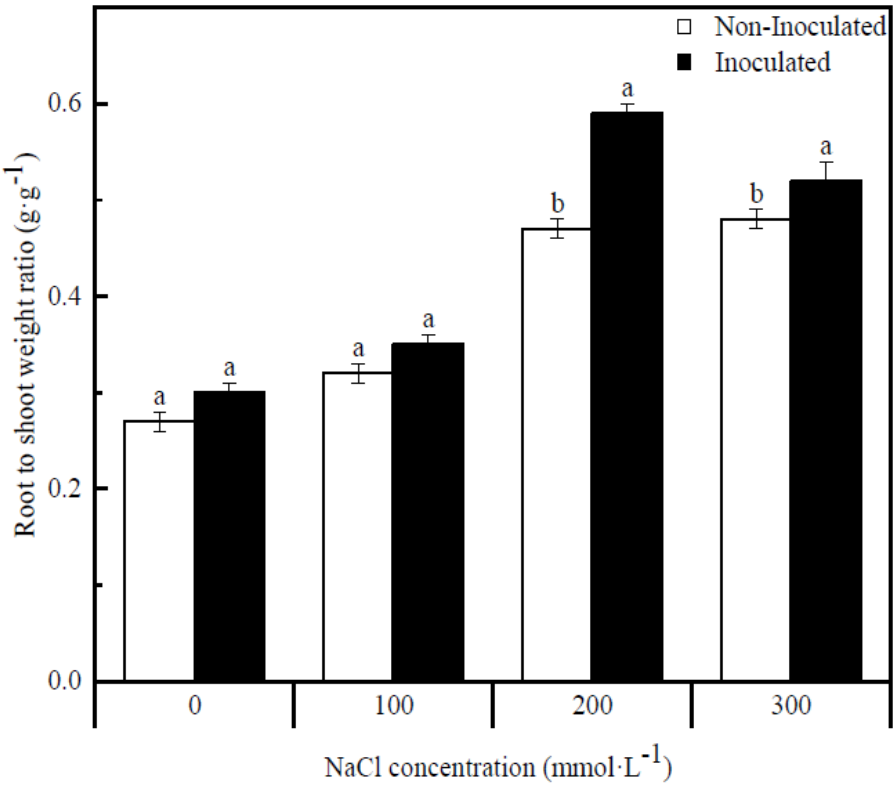


Figure 3. Effect of *B. subtilis* on the root to shoot weight ratio of maize plants treated with NaCl. Note: different letters above the bars indicate significant differences ($p < 0.05$).

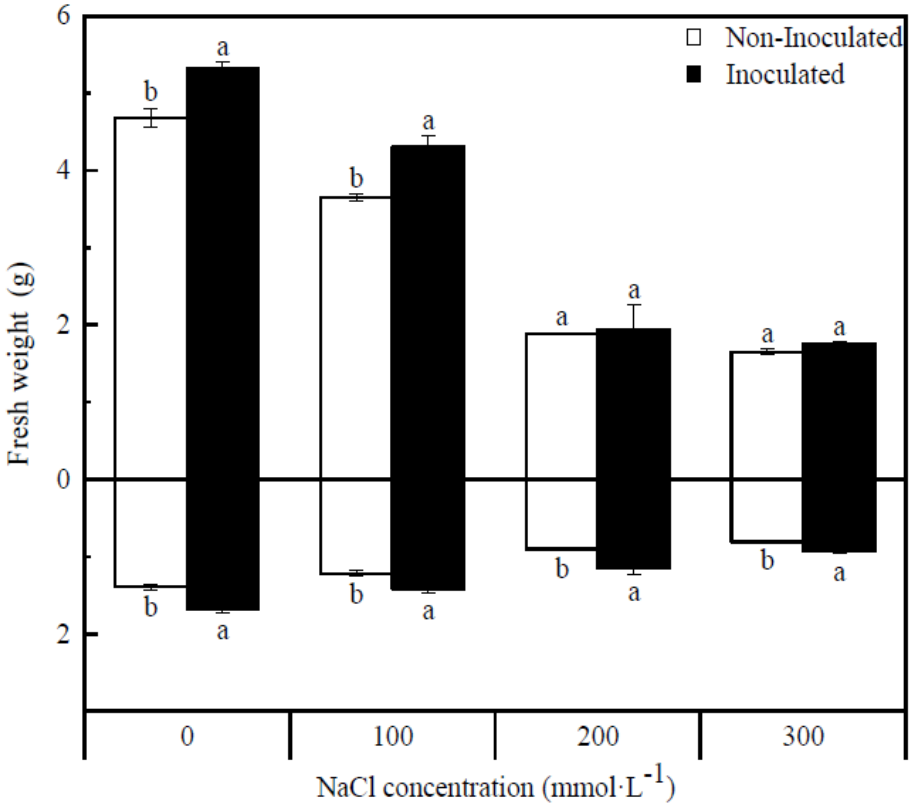


Figure 4. Effect of *B. subtilis* on the fresh weight of maize treated with NaCl. The data above and below the x-axis are for the leaves and roots, respectively. Note: different letters above the bars indicate significant differences ($p < 0.05$).

3.3. Chlorophyll Contents

Under NaCl stress conditions, the chlorophyll *a* and *b* contents of the maize leaves gradually decreased as the NaCl concentration increased (Figure 5). In the absence of NaCl stress, the inoculation with *B. subtilis* HS5B5 significantly increased the chlorophyll *b* content in maize leaves, but the increase in the chlorophyll *a* content was not significant. For the treatments involving low and moderate NaCl stress (100 and 200 mmol·L^{-1}), the inoculation with *B. subtilis* HS5B5 significantly increased the chlorophyll *a* and *b* contents of maize, with the greatest increases (46.47% and 36.11% in the chlorophyll *a* and *b* contents, respectively) observed for the inoculated plants treated with 200 mmol·L^{-1} NaCl ($p < 0.05$).

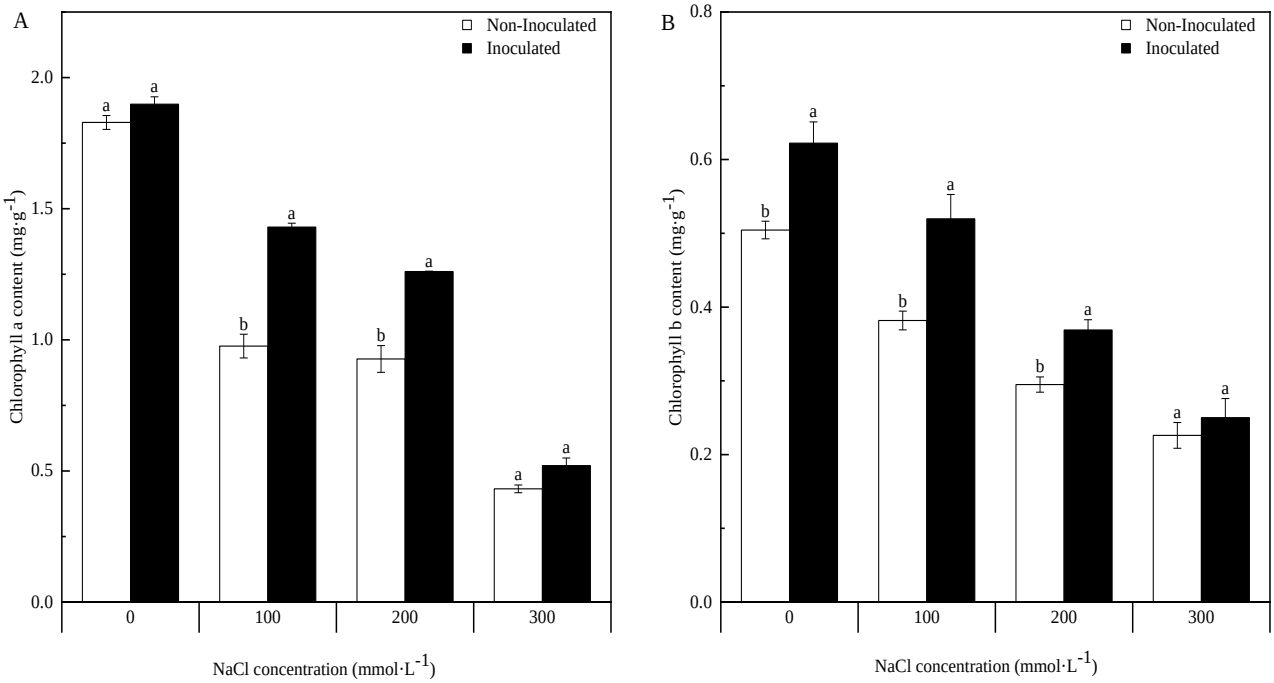


Figure 5. Effect of *B. subtilis* on the chlorophyll *a* and *b* contents in the maize leaves treated with NaCl. (**A**) Chlorophyll *a* content. (**B**) Chlorophyll *b* content. Note: different letters above the bars indicate significant differences ($p < 0.05$).

3.4. Osmoregulatory Substances and MDA Content

When maize seedlings were treated with different NaCl concentrations, the soluble protein content of the leaves and roots tended to increase and then decrease as the NaCl concentration increased (Figure 6). In the absence of NaCl stress, the inoculation with *B. subtilis* HS5B5 had no significant effect on the soluble protein content in maize leaves and roots. Compared with the non-inoculated plants, the maize leaf soluble protein content was 5.89%, and 11.35% higher in the plants inoculated with *B. subtilis* HS5B5 following the treatments with 100 and 200 mmol·L^{-1}, respectively; these differences were significant ($p < 0.05$). The soluble protein content in maize leaves was not significantly affected by 300 mmol·L^{-1} NaCl. Under saline conditions, the soluble protein content in maize roots was significantly higher for the plants inoculated with *B. subtilis* HS5B5 than for the non-inoculated plants, with the greatest difference (16.01%) observed for the 200 mmol·L^{-1} NaCl treatment ($p < 0.05$).

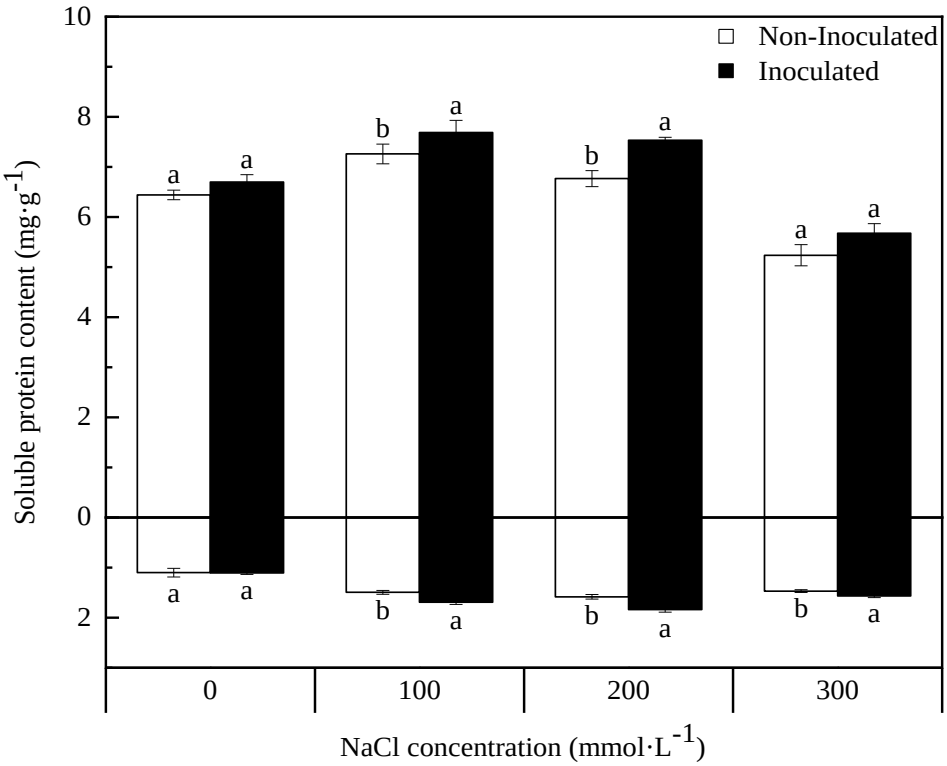


Figure 6. Effect of *B. subtilis* on the soluble protein content in maize leaves and roots treated with NaCl. The data above and below the x-axis are for the leaves and roots, respectively. Note: different letters above the bars indicate significant differences ($p < 0.05$).

There was an overall increase and after 200 mmol L^{-1} NaCl decrease in the soluble sugar content in maize leaves and roots as the concentration of the NaCl treatment increased (Figure 7). In the absence of NaCl stress, the inoculation with *B. subtilis* HS5B5 increased the soluble sugar content in maize leaves by 11.26% ($p < 0.05$). Under medium to low concentrations of NaCl stress (100 mmol·L^{-1}, 200 mmol·L^{-1}), compared with the uninoculated plants, inoculation with *B. subtilis* HS5B5 significantly increased the soluble sugar content in maize leaves, increasing by 17.00% and 31.70%, respectively, with significant differences ($p < 0.05$). There was no significant increase in the soluble sugar content in maize leaves after the 300 mmol·L^{-1} treatment. Under salt stress conditions, the soluble sugar content in maize roots was significantly higher in the plants inoculated with *B. subtilis* HS5B5 than in the non-inoculated plants, with the largest difference (12.10%) detected for the 300 mmol·L^{-1} NaCl treatment ($p < 0.05$).

The Pro content in maize leaves and roots gradually increased with increasing NaCl concentrations (Figure 8). In the absence of NaCl stress, the Pro content in maize leaves and roots was not significantly affected by the inoculation with *B. subtilis* HS5B5. Under saline conditions, the Pro content in maize leaves was significantly lower in the plants inoculated with *B. subtilis* HS5B5 than in the non-inoculated plants, with the largest difference (40.16%) detected for the 100 mmol·L^{-1} NaCl treatment ($p < 0.05$). Under medium to low concentrations of NaCl stress (100 mmol·L^{-1}, 200 mmol·L^{-1}), compared with the uninoculated plants, inoculation with *B. subtilis* HS5B5 significantly increased the Pro content in maize roots, increasing by 27.68% and 26.63%, respectively, with significant differences ($p < 0.05$). The 300 mmol·L^{-1} NaCl treatment did not significantly increase the Pro content in maize roots.

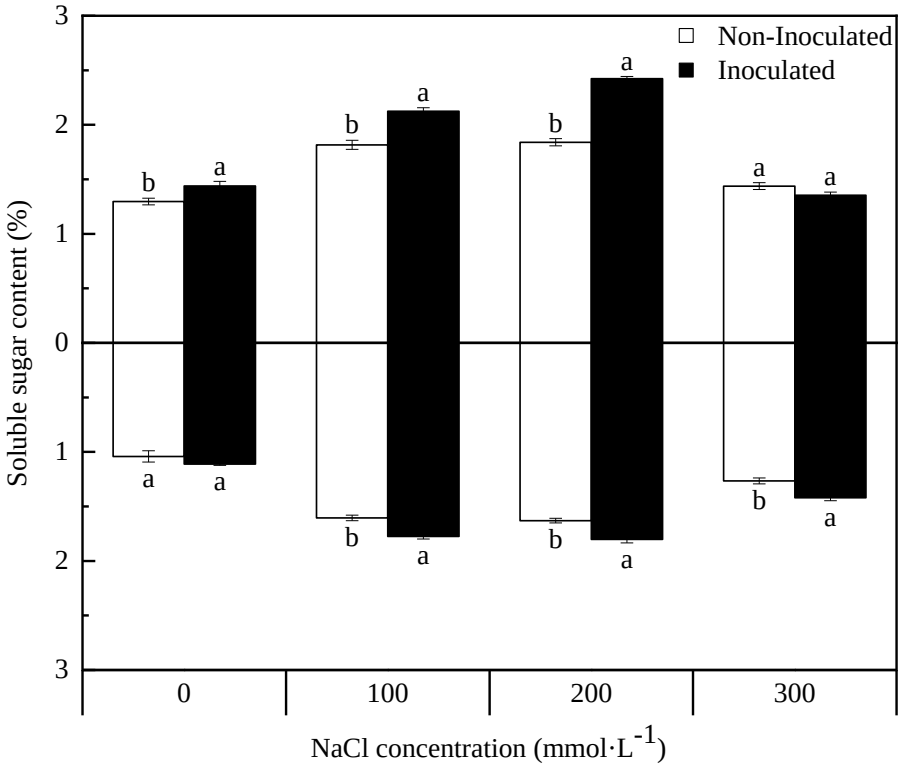


Figure 7. Effect of *B. subtilis* on the soluble sugar content in maize leaves and roots treated with NaCl. The data above and below the x-axis are for the leaves and roots, respectively. Note: different letters above the bars indicate significant differences ($p < 0.05$).

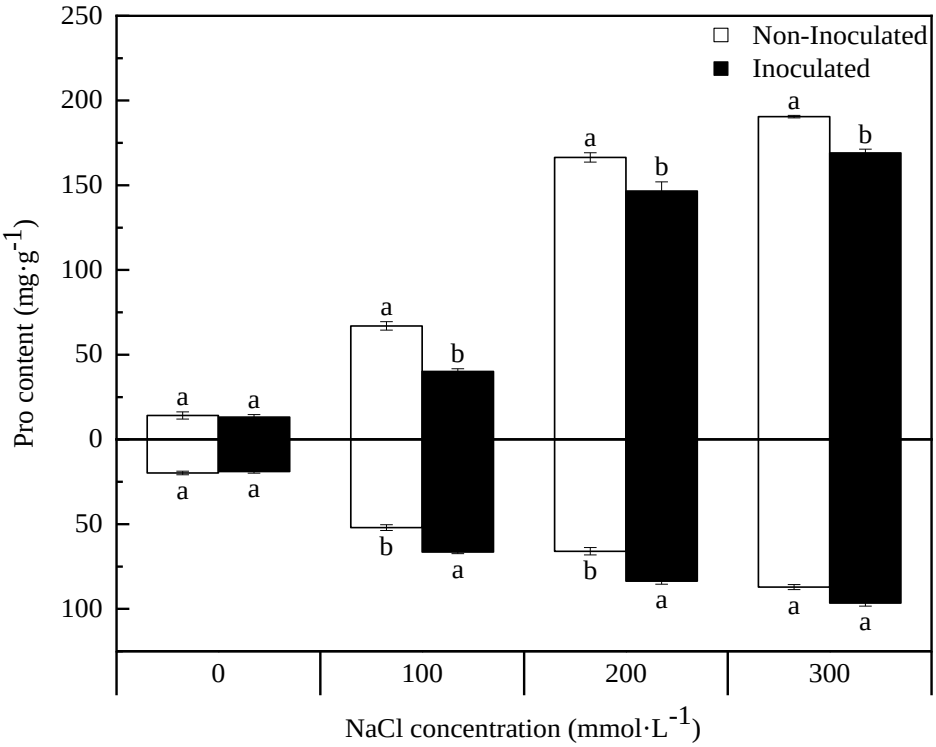


Figure 8. Effect of *B. subtilis* on the Pro content in maize leaves and roots treated with NaCl. The data above and below the x-axis are for the leaves and roots, respectively. Note: different letters above the bars indicate significant differences ($p < 0.05$).

The MDA content of maize leaves and roots gradually increased as the concentration of the NaCl treatment increased (Figure 9). In the absence of NaCl stress, the MDA content in maize leaves and roots was not significantly affected by the inoculation with *B. subtilis* HS5B5. During the exposure to salt stress, the MDA content in maize leaves was significantly lower in the plants inoculated with *B. subtilis* HS5B5 than in the non-inoculated plants, with the greatest difference (34.99%) observed following the 200 mmol·L^{-1} NaCl treatment ($p < 0.05$). Under medium to low concentrations of NaCl stress (100 mmol·L^{-1}, 200 mmol·L^{-1}), compared with the uninoculated plants, inoculation with *B. subtilis* HS5B5 significantly reduced the MDA content in maize roots by 13.26% and 11.21%, respectively, with significant differences ($p < 0.05$).

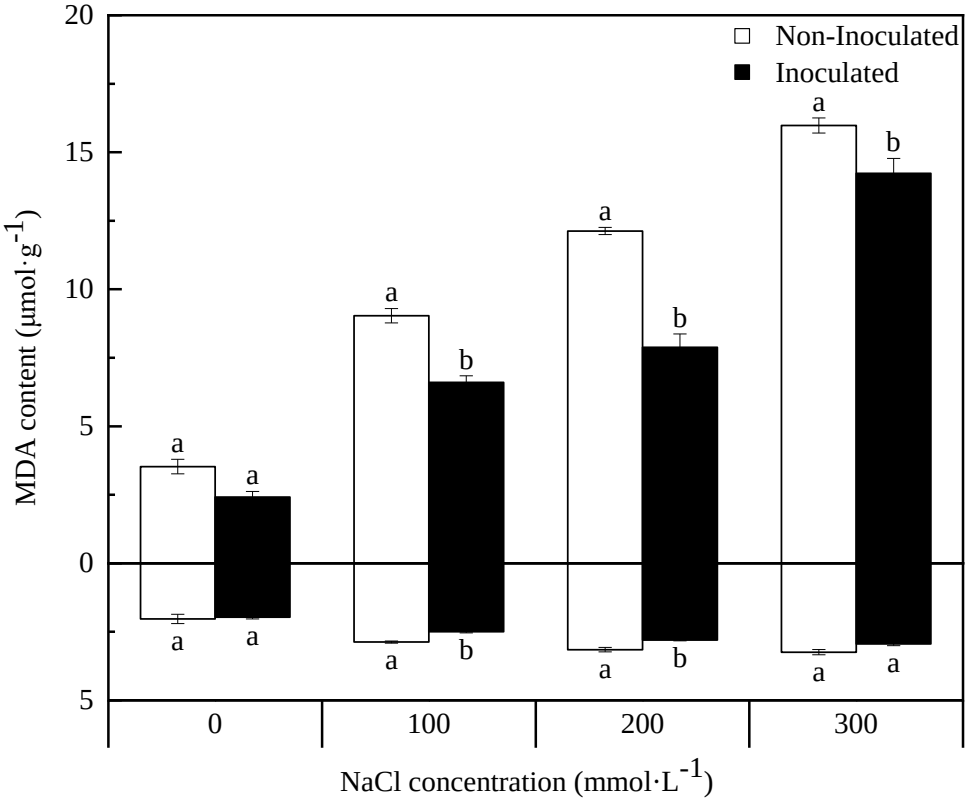


Figure 9. Effect of *B. subtilis* on the MDA content in maize leaves and roots treated with NaCl. The data above and below the x-axis are for the leaves and roots, respectively. Note: different letters above the bars indicate significant differences ($p < 0.05$).

3.5. Antioxidant Enzyme Activities

The SOD activity in the NaCl-treated maize seedlings increased and then decreased as the NaCl concentration increased (Figure 10). Additionally, the SOD activity was higher in maize leaves than in the root system. In the absence of NaCl stress, the inoculation with *B. subtilis* HS5B5 increased the SOD activity in maize leaves and roots by 37.40% and 20.43%, respectively ($p < 0.05$). Under NaCl stress conditions, the SOD activity of maize leaves was significantly higher in the plants inoculated with *B. subtilis* HS5B5 than in the non-inoculated plants, with the greatest difference (25.33%) observed for the 300 mmol·L^{-1} NaCl treatment ($p < 0.05$). For the 100 mmol·L^{-1} NaCl treatment, the SOD activity of the roots was 13.38% higher for the plants inoculated with *B. subtilis* HS5B5 than for the non-inoculated plants ($p < 0.05$).

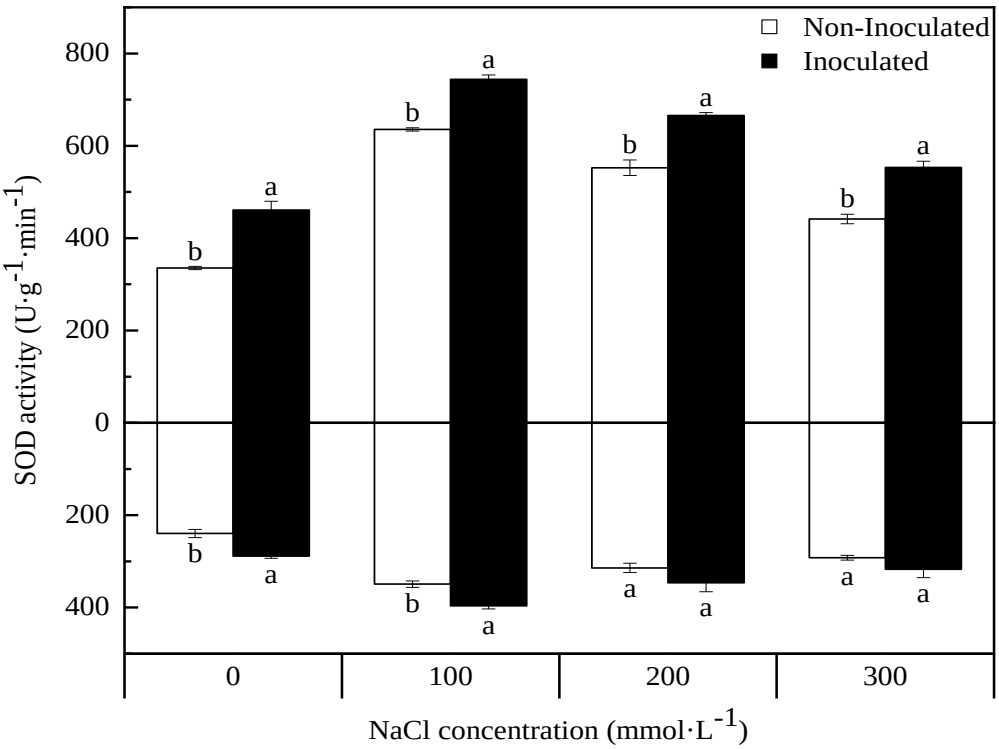


Figure 10. Effect of *B. subtilis* on the SOD activity in maize leaves and roots treated with NaCl. The data above and below the x-axis are for the leaves and roots, respectively. Note: different letters above the bars indicate significant differences ($p < 0.05$).

The POD activity in maize seedlings increased and then decreased as the NaCl concentration increased (Figure 11). Moreover, the POD activity was higher in the maize root system than in the leaves. In the absence of NaCl stress, the inoculation with *B. subtilis* HS5B5 increased the POD activity in maize leaves and roots by 9.66% and 9.82%, respectively ($p < 0.05$). Under medium to low concentrations of NaCl stress (100 mmol·L^{-1}, 200 mmol·L^{-1}), compared with uninoculated plants, inoculation with *B. subtilis* HS5B5 significantly increased the POD activity in maize leaves by 7.14% and 26.27%, respectively, with significant differences ($p < 0.05$). In response to salt stress, the POD activity in maize roots was significantly higher in the plants inoculated with *B. subtilis* HS5B5 than in the non-inoculated plants, with the greatest difference (17.66%) detected for the 100 mmol·L^{-1} NaCl treatment ($p < 0.05$).

The CAT activity in maize seedlings increased and after 200 mmol L^{-1} NaCl decreased as the concentration of the NaCl treatment increased (Figure 12), with a higher CAT activity in the roots than in the leaves. In the absence of NaCl stress, the inoculation with *B. subtilis* HS5B5 increased the CAT activity in maize leaves by 84.01% ($p < 0.05$). Under salt stress conditions, the CAT activity in maize leaves was significantly higher in the plants inoculated with *B. subtilis* HS5B5 than in the non-inoculated plants, with the largest difference (53.55%) observed for the 100 mmol·L^{-1} NaCl treatment ($p < 0.05$). Following the 100 mmol·L^{-1} NaCl treatment, the CAT activity in maize roots was 18.92% higher in the plants inoculated with *B. subtilis* HS5B5 than in the non-inoculated plants ($p < 0.05$).

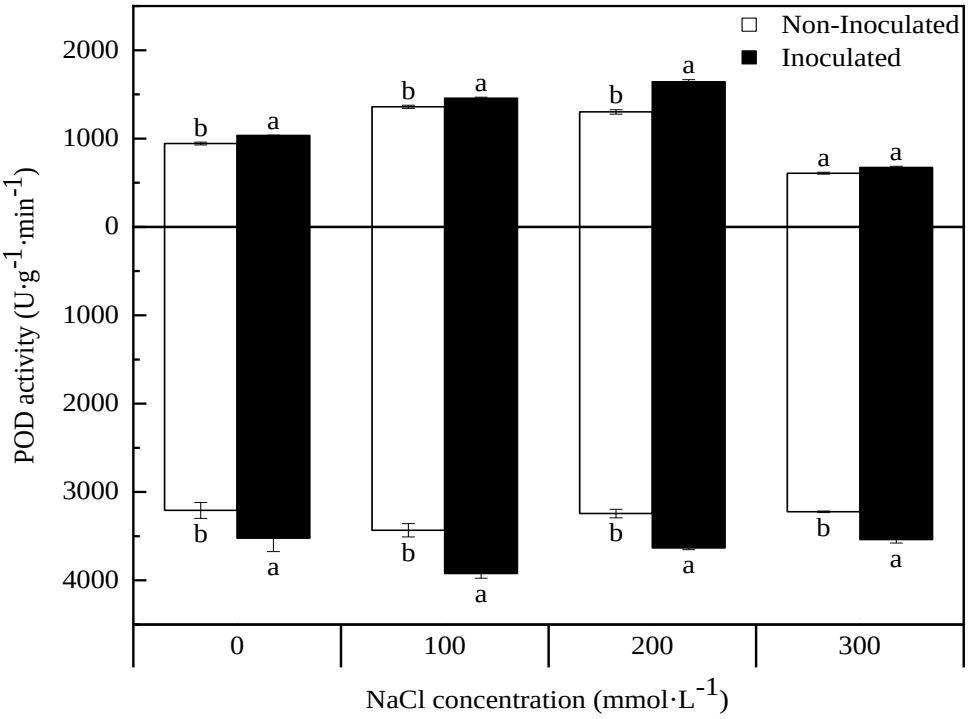


Figure 11. Effect of *B. subtilis* on the POD activity in maize leaves and roots treated with NaCl. The data above and below the x-axis are for the leaves and roots, respectively. Note: different letters above the bars indicate significant differences ($p < 0.05$).

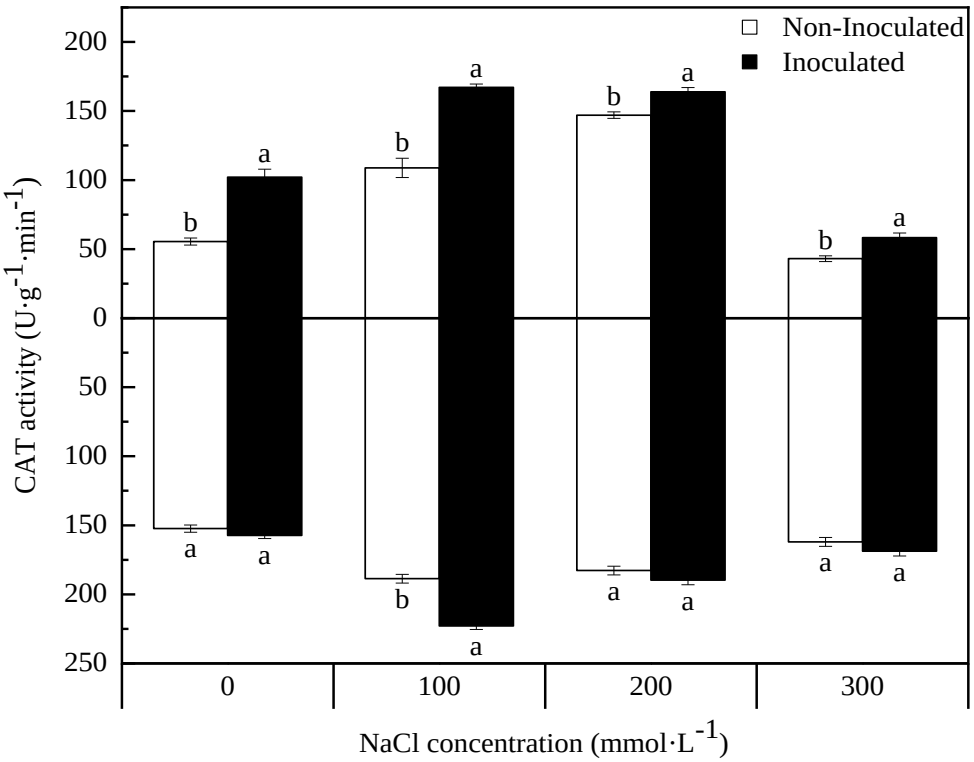


Figure 12. Effect of *B. subtilis* on the CAT activity in maize leaves and roots treated with NaCl. The data above and below the x-axis are for the leaves and roots, respectively. Note: different letters above the bars indicate significant differences ($p < 0.05$).

3.6. Na^+ and K^+ Contents

The Na^+ content in maize leaves and roots increased with increases in the NaCl concentration (Figure 13A). In the absence of NaCl stress, the Na^+ content was relatively low in maize roots and leaves. In addition, the inoculation with *B. subtilis* HS5B5 had no significant effect on the Na^+ content in maize. Under saline conditions, the Na^+ content in maize leaves was significantly lower in the plants inoculated with *B. subtilis* HS5B5 than in the non-inoculated plants, with the largest difference (28.15%) observed for the 100 mmol·L^{-1} NaCl treatment ($p < 0.05$). Under medium to high concentrations of NaCl stress (200 mmol·L^{-1}, 300 mmol·L^{-1}), inoculation with *B. subtilis* HS5B5 significantly reduced the Na^+ content in maize leaves compared to uninoculated plants, reducing by 43.71% and 50.25%, respectively, with significant differences ($p < 0.05$).

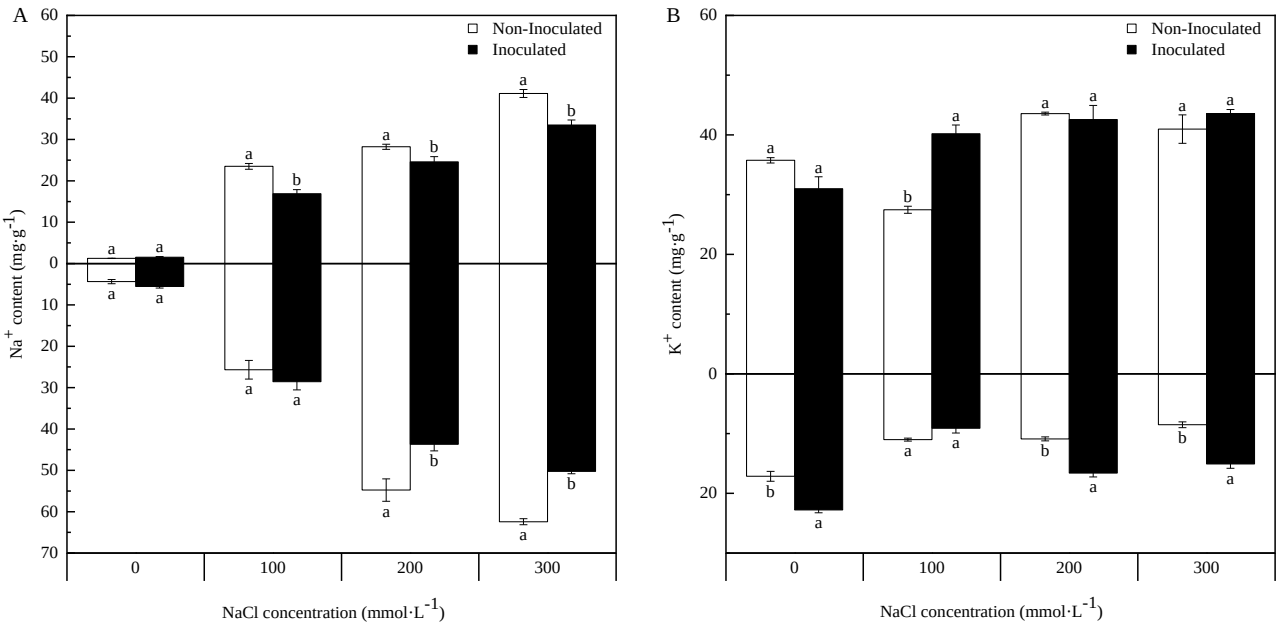


Figure 13. Effect of *B. subtilis* on the Na^+ and K^+ contents in maize leaves and roots treated with NaCl. The data above and below the x-axis are for the leaves and roots, respectively. (**A**) Sodium ion concentration. (**B**) Potassium ion concentration. Note: different letters above the bars indicate significant differences ($p < 0.05$).

The K^+ content in maize leaves and roots increased as the NaCl concentration increased (Figure 13B). Under salt stress conditions, the K^+ content in maize leaves was significantly higher in the plants inoculated with *B. subtilis* HS5B5 than in the non-inoculated plants, with the greatest difference (46.27%) detected for the 100 mmol·L^{-1} NaCl treatment ($p < 0.05$). Under medium to high concentrations of NaCl stress (200 mmol·L^{-1}, 300 mmol·L^{-1}), inoculation with *B. subtilis* HS5B5 significantly increased the K^+ content in maize leaves compared to uninoculated plants, increasing by 16.60% and 15.09%, respectively, with significant differences ($p < 0.05$).

4. Discussion

A high germination rate is critical for a superior crop yield. An exposure of seeds to high NaCl concentrations generally leads to excessive seed osmolarity, thereby inhibiting the uptake of water by seeds. The toxic effects of salt stress may impose changes in the activity of enzymes involved in nucleic acid and protein metabolism as well as decrease the use of seed nutrient reserves [32]. In the current study, the maize seed germination potential, germination rate, shoot length, and root length decreased significantly in response to NaCl stress. These changes were likely associated with the inhibited maize seed germination due to the excessive accumulation of Na^+ during germination [33], which is consistent

with the findings of an earlier study by Tariq et al. [34]. Previous research demonstrated that seed viability may be significantly enhanced by *B. subtilis*, while root cell division is promoted, crop shoot and root lengths increase, and seeds are protected from stress-related injuries [35]. In this study, under a NaCl concentration of 200 mmol·L^{-1}, significant enhancements were observed in the germination potential, germination rate, shoot length, and root length of maize seeds upon inoculation with *B. subtilis* HS5B5. These results are in accordance with the reported beneficial effects of microorganisms on the growth and development of various plant species under abiotic stress conditions [36].

The results of this study suggest *B. subtilis* HS5B5 may have plant growth-promoting effects. Specifically, the maize plant height, biomass, and fresh weight (above-ground and below-ground parts) decreased following the NaCl treatment, possibly because of the salt-induced cellular osmotic imbalance in maize plants and the subsequent modulations to the cellular uptake of nutrient elements and related enzyme activities [37]. These changes can ultimately decrease the maize biomass and fresh weight [38]. The maize plant height, above-ground and below-ground fresh weights, and biomass increased significantly after the inoculation with *B. subtilis* HS5B5, which also significantly increased maize tolerance to salinity stress. Ferreira et al. [39] observed that *B. subtilis* may increase maize stem and root dry weights. Barra et al. [40] revealed that salt-tolerant bacteria can promote crop root nutrient uptake under saline conditions and increase the maize plant height, root length, above-ground fresh weight, and below-ground fresh weight, while also limiting the toxic effects of salt stress on maize seedlings. These findings are consistent with the results of the present study. The root-to-crown ratio is a useful indicator of good seedling root development. We observed that the root-to-crown ratio of maize increased to some degree in response to the NaCl treatment, the salt stress suppressed the growth of the above-ground plant parts more than the growth of the roots, which is consistent with the research findings of Sechenbater and Wu [41]. Under saline conditions, the root-to-crown ratio and below-ground fresh weight were significantly higher for the maize plants inoculated with *B. subtilis* HS5B5 than for the non-inoculated plants, likely because *B. subtilis* HS5B5 can induce maize root cell division. Earlier research showed that inoculation with *B. subtilis* HS5B5 positively affects maize root growth more than the growth of the above-ground plant parts [42].

Salt stress can inhibit leaf photosynthetic activities, possibly because of structural modifications to the chloroplast thylakoid membrane due to the accumulation of Na^+ and Cl^- in chloroplasts. These changes modulate electron transport, enzyme activities, and protein synthesis, ultimately altering photosynthetic processes, especially photosynthesis-related phosphorylation and carbon metabolism [43]. Chlorophyll *a* and *b* contents reportedly decrease in salt-stressed alfalfa, but this decrease may be mitigated by the inoculation with *Pseudomonas* spp. [44]. Zarei et al. [45] determined that the chlorophyll content of sweet corn may increase following an inoculation with fluorescent *Pseudomonas* strains. AlKahtani et al. [46] observed that the chlorophyll content of sweet pepper plants increases after a treatment with plant growth-promoting rhizobacteria. In the current study, the chlorophyll *a* and *b* contents in maize leaves treated with NaCl increased for the plants inoculated with *B. subtilis* HS5B5. This is consistent with the results of previous studies. This effect of *B. subtilis* HS5B5 may involve the ability of the microorganism to control the expression of plant genes encoding regulators of photosynthesis [47]. In addition, *B. subtilis* can promote the uptake of iron by plants, resulting in increased chlorophyll contents and photosynthetic rates [48]. Metabolites of *B. subtilis* can enhance photosynthetic activities by increasing the vein density and stomatal conductance, with an appropriately organized vein placement increasing the photosynthetic capacity at the leaf surface [49].

Plant cell osmoregulation contributes to plant adaptations to the external environment and increased stress resistance [50]. In the present study, the soluble sugar and soluble protein contents increased in the leaves and roots of salt-stressed maize plants inoculated with *B. subtilis* HS5B5. Qu et al. [51] reported that the production of soluble proteins and sugars in plants in high-salt environments is promoted by the inoculation with rhi-

zobacteria, which enables the plants to withstand oxidative and osmotic stresses, which is in agreement with the findings of the present study. In addition, the inoculation with *B. subtilis* HS5B5 increased the Pro content in maize roots, which was in contrast to the decrease in the Pro content in maize leaves. According to Rai et al. [52], the accumulation of Pro and the compatible solutes consumes energy along with other metabolic activities. Nadeem et al. [53] detected an increase in the leaf Pro concentration in response to an increasing NaCl concentration, whereas the Pro content decreases in NaCl-treated plants inoculated with certain bacteria. These results are similar to those of an earlier study by Han et al. [54] in which the Pro content of soybean grown under NaCl stress conditions decreased significantly when plants were inoculated with bacteria. Malondialdehyde is an indicator of membrane damages. Several previous investigations showed that the MDA content increases in salt-stressed plants [55–57]. In the present study, we observed that the MDA content in maize leaves and roots gradually increased as the NaCl concentrations increased, but the inoculation with *B. subtilis* HS5B5 decreased the MDA content in maize leaves and roots, implying this bacterial strain may restrict cell membrane peroxidation. El-Esawi et al. [58] reported that MDA levels decrease significantly in maize plants inoculated with microorganisms under NaCl stress conditions. According to Abadi and Sepehri [59], beneficial plant inoculants can enhance antioxidant enzyme activities, limit oxidative damage, and improve plant cell membrane integrity. These findings are similar to those of earlier studies in which MDA levels decreased in maize and white clover plants inoculated with microorganisms under saline conditions [60,61].

An exposure to NaCl stress leads to the substantial accumulation of ROS in plant cells, membrane lipid peroxidation, changes to the cell membrane structure and function, and abnormal intracellular metabolism [62]. In the current study, the SOD, POD, and CAT activities in maize plants increased and then decreased as the NaCl concentration increased. Similarly, Hou [63] investigated maize physiological responses to salt stress and determined that SOD, POD, and CAT activities initially increase and then decrease during an exposure to increasing salt concentrations. These observations may be explained by the fact that plants can overcome the adverse effects of low salt concentrations through various defense responses (e.g., increased antioxidant enzyme activities to eliminate ROS); however, these plant responses are ineffective against extremely high salt concentrations, which can disrupt normal enzyme activities in plants [64]. There are several major antioxidant enzymes, including SOD, POD, and CAT. In the present study, the SOD, POD, and CAT activities increased significantly in maize seedlings under saline conditions, thereby mitigating some of the salt-related toxicity [65]. Moreover, we observed that SOD, POD, and CAT activities in leaves increased significantly in the plants inoculated with *B. subtilis* HS5B5 and treated with a low NaCl concentration. The inoculation with *B. subtilis* HS5B5 also significantly promoted the SOD, POD, and CAT activities in the roots of plants treated with a high NaCl concentration. Hence, the positive effect of *B. subtilis* HS5B5 on antioxidant enzyme activities may be greater in the roots than in the leaves. The inoculation with certain beneficial microorganisms (e.g., *Bacillus paramycoides* JYZ-SD5 and *B. subtilis* SU47) can alleviate the harmful effects of abiotic stress on plant growth by increasing antioxidant enzyme activities [66,67].

Following a NaCl treatment, Na^+ is the main toxic ion. In cells, Na^+ can compete with K^+ for K^+-binding sites associated with diverse processes, such as enzymatic reactions, protein synthesis, and ribosome activities, thereby disrupting physiological metabolism, while also more likely causing irreversible damages in plants than osmotic stress [68]. We observed that the Na^+ content in maize roots and leaves increased significantly after the NaCl treatment, but the Na^+ content was lower in maize inoculated with *B. subtilis* HS5B5 than in non-inoculated maize. These results are similar to those reported by Abdelgawad et al. [69]. In the present study, under saline conditions, Na^+ accumulated more in the roots than in the above-ground plant parts, reflecting the retention of Na^+ in the roots as the NaCl concentration increases, which may protect the shoots and leaves from the harmful effects of excessive Na^+ levels [70]. Furthermore, microorganisms can alter the

uptake of nutrients and toxic ions through the root system by changing the physiological structure of the plant, ultimately restricting the accumulation of toxic ions in the leaves and improving the nutritional status of the plant [71]. As the concentration of NaCl increased, the overall K^+ content in leaves gradually increased, which was in contrast to the decrease in the root K^+ content. These findings may be associated with the fact that NaCl stress leads to K^+ loss, while also increasing the translocation of K^+ to above-ground plant parts (at high NaCl concentrations) during the regulation of osmotic stress responses and the alleviation of salt-induced damages. However, the K^+ content in maize roots and leaves increased to varying degrees in the plants inoculated with *B. subtilis* HS5B5. Earlier research indicated that the survival of plants under saline conditions depends on the maintenance of K^+/Na^+ homeostasis via the continuous acquisition of K^+ when the external Na^+ level is high [72]. Wang et al. [73] confirmed that the inoculation with microorganisms may help salt-stressed plants maintain sufficient K^+ levels to decrease the toxic effects of Na^+. Another study revealed that K^+ levels in radish roots and leaves increase significantly if seeds are pretreated with *Pseudomonas fluorescens* and *B. subtilis* [74].

5. Conclusions

In the present study, maize seed germination and seedling growth were affected by salt stress, which altered plant physiological and biochemical characteristics. *B. subtilis* HS5B5 significantly alleviated the toxic effect of NaCl stress on maize by increasing osmoregulatory substances and regulating the antioxidant system. The inoculation also positively affected specific plant growth-related parameters (e.g., height, biomass, root-to-crown ratio, fresh weight, and chlorophyll content). Furthermore, the inoculation with *B. subtilis* HS5B5 also decreased the toxicity of Na^+ by hindering its uptake. Collectively, the findings of this study indicate that *B. subtilis* HS5B5 can significantly alleviate the detrimental effects of NaCl (up to 200 $mmol \cdot L^{-1}$) on maize plants, with a greater effect on root growth than on the growth of the above-ground plant parts. The study findings may be useful for optimizing the application of microbial strains, including *B. subtilis* HS5B5, to protect agriculturally important crops from salinity (abiotic stress). However, further studies are required to validate the utility of *B. subtilis* for improving maize stress tolerance under field conditions.

Author Contributions: P.S.: Conceptualization, Methodology, Validation, Formal analysis, Writing—original draft, Visualization, Supervision, Project administration, Funding acquisition. B.Z.: Formal analysis, Investigation, Writing—original draft. X.S.: Resources, Data curation. L.L.: Writing—review & editing. Z.W.: Resources, Data curation. C.M.: Writing—review & editing, Supervision. J.Z.: Writing—review & editing, Formal analysis. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

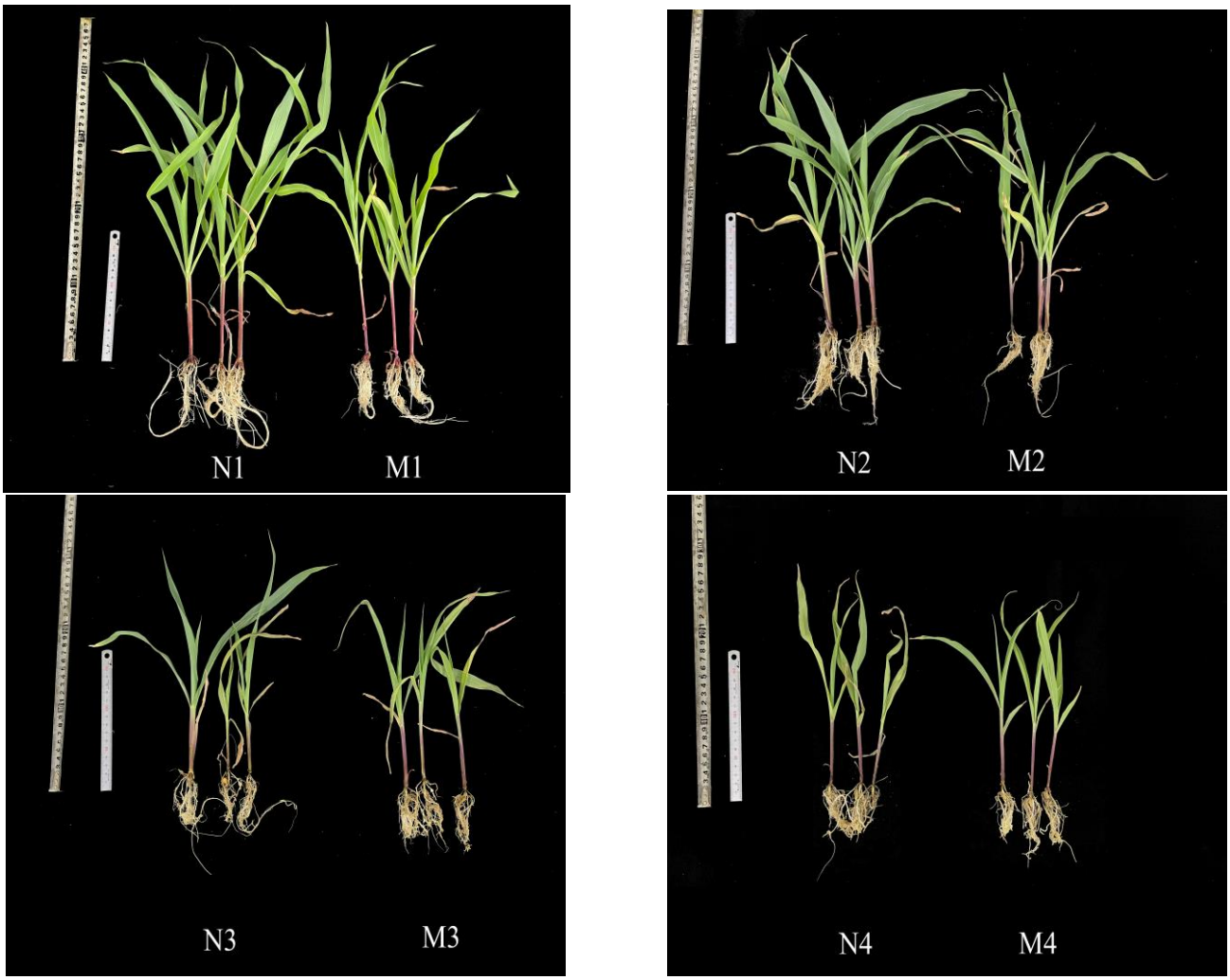


Figure A1. Effect of NaCl treatments with and without the application of the bacterial solution. N1: 0 mmol·L^{-1} NaCl + 1 × 10^{7} CFU·mL^{-1} bacterial solution. M1: 0 mmol·L^{-1} NaCl. N2: 100 mmol·L^{-1} NaCl + 1 × 10^{7} CFU·mL^{-1} bacterial solution. M2: 100 mmol·L^{-1} NaCl. N3: 200 mmol·L^{-1} NaCl + 1 × 10^{7} CFU·mL^{-1} bacterial solution. M3: 200 mmol·L^{-1} NaCl. N4: 300 mmol·L^{-1} NaCl + 1 × 10^{7} CFU·mL^{-1} bacterial solution. M4: 300 mmol·L^{-1} NaCl.

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