

Article

Physiological Responses of *Anoectochilus roxburghii* to Salt Stress

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Abstract

Salt stress is a significant environmental factor influencing plant growth and development. *Anoectochilus roxburghii* is a valuable medicinal plant, but it is still unclear how it responds to salinity. In this study, *A. roxburghii* was used as experimental material to investigate its physiological mechanisms underlying salt stress resistance. Seedlings were subjected to various NaCl concentrations (0, 50, 100, 150, and 200 mmol/L), and changes in key physiological parameters were subsequently analyzed. The results indicated that under NaCl-induced salt stress, the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), as well as soluble protein content, initially increased and then decreased, with peak levels observed between 100 and 150 mmol/L. Malondialdehyde (MDA) content exhibited a steady increase with rising salt concentration. Total chlorophyll content declined progressively, while anthocyanin content increased initially but decreased significantly when NaCl concentration exceeded 100 mmol/L. Additionally, the contents of total flavonoids and total phenolics decreased markedly at salt concentrations above 100 mmol/L. These findings suggest that *A. roxburghii* can tolerate salt stress up to 100 mmol/L for 24 h without exhibiting substantial physiological or morphological damage. This study provides a theoretical basis for analyzing the salt tolerance mechanism of *A. roxburghii*.



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Keywords: *Anoectochilus roxburghii*; NaCl stress; salt tolerance; enzyme activity

1. Introduction

Anoectochilus roxburghii (Wall.) Lindl., commonly known as “Jinxianlian”, is a rare perennial medicinal herb belonging to the genus *Anoectochilus* in the family Orchidaceae [1]. The entire plant of *A. roxburghii* is used medicinally and possesses various pharmacological properties, including clearing heat and cooling blood, dispelling wind, promoting diuresis, detoxifying, reducing swelling, protecting the liver, lowering blood sugar, and anti-inflammatory, antibacterial, antioxidant, sedative, analgesic, and tumor-inhibiting effects [2]. It is highly valued as the “divine medicine” and “king of medicine” in many Asian countries [1]. This species typically grows in the understory of evergreen broad-leaved forests or on shaded slopes and cliffs in deep mountain valleys at higher altitudes. It thrives in fertile, well-drained soils and exhibits optimal growth under strongly acidic conditions [3].

According to data from the Food and Agriculture Organization of the United Nations, saline-alkali land covers approximately 1.38 billion hectares globally, accounting for 10.7%

of the world's total land area [4]. In China, about 100 million hectares are affected by salinization [5]. With increasing soil salinization, salt stress has become a major constraint on plant growth and poses a significant challenge to agricultural productivity. Numerous studies have shown that salt stress not only reduces plant growth rates and crop yields but also negatively affects crop quality and nutritional value [6]. When exposed to salt stress, plants initiate complex physiological and biochemical responses to adapt to adverse conditions. One key consequence of salt stress is the accumulation of reactive oxygen species (ROS), leading to oxidative damage. To counteract this, plants activate both enzymatic antioxidants—such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX)—and non-enzymatic antioxidant systems [7]. In recent years, extensive research has been conducted on the physiological responses of various plants to salt stress [8–11]. Many species exhibit varying degrees of salt tolerance. For example, African nightshade (*Solanum scabrum* Mill.) can be cultivated in soils with NaCl concentrations up to 75 mM [8], while certain cultivars of water dropwort (*Oenanthe javanica*) demonstrate efficient tolerance to 100 mM NaCl [9]. Similarly, in cucumber, exposure to 100 mM NaCl reduces water potential and impairs water uptake, thereby inhibiting seed germination [10]. Among pitaya varieties—red, white, and hybrid—red pitaya exhibited the strongest salt tolerance following treatment with 150 mM NaCl [11]. Flavonoids are widely present in plants and belong to the secondary metabolites of polyphenols. They play a key role in various physiological processes of plants, such as flower color formation, pollination, and reproductive development [12]. In *A. roxburghii*, flavonoids are regarded as the main active ingredients that exert liver-protective effects [13]. A certain degree of salt stress can promote the accumulation of total flavonoids and help plants resist various environmental stresses in their leaves [14].

Although plant salt tolerance has been widely studied, the effects of salt stress on *A. roxburghii* physiology have been little studied. Given the unique value of *A. roxburghii* as a rare and endangered medicinal plant, as well as the cultivation challenge of increasingly salinized soil, and in order to promote the accumulation of its bioactive components, this study selected *A. roxburghii* as the experimental material and employed NaCl to simulate salt stress. The effects of varying NaCl concentrations on multiple physiological parameters in *A. roxburghii* seedlings were systematically examined, revealing the salt tolerance threshold and providing a theoretical foundation for understanding the salt tolerance mechanisms in this species.

2. Materials and Methods

2.1. Experimental Materials

All experimental plants were 5-month-old tissue-cultured seedlings of *A. roxburghii*, variety “Sharp-leaf”, obtained from a production base in Zhangzhou City, Fujian Province, China. They were selected as experimental materials for seedlings with uniform growth, with 6 plants per replicate. All analyses were performed in three biological replicates. The seedlings were thoroughly rinsed and transferred to distilled water in an artificial climate chamber (Topper Instrument Co., Ltd. Hangzhou, China) with light at 25 ± 0.5 °C and a relative humidity of 75% for two weeks of acclimation. The light intensity for all treatments was set at 3500 lx, and the photoperiod was 12 h/12 h (day/night).

2.2. Experimental Design

Uniformly healthy seedlings exhibiting consistent growth and free from mechanical injury or pest infestation were selected and individually placed in 250 mL culture bottles. Each bottle was filled with 150 mL of half-strength Hoagland nutrient solution, including Hoagland reagent and calcium salt reagent (Phygene Life Sciences Company, Fuzhou,

China), and the nutrient solution was replaced daily. After 7 days, five NaCl salt stress treatments were set up at concentrations of 0, 50, 100, 150, and 200 mmol/L, labeled as CK, T1, T2, T3, and T4, respectively. Each treatment was based on Hoagland solution and had three replicates. After 24 h of NaCl solution treatment, fresh seedlings should be taken immediately to measure the physiological indicators of their leaves and stems. All analyses were conducted with three biological replicates, and the results are expressed as mean $\pm$ standard deviation.

2.3. Measurement of Stem Diameter and Leaf Thickness

Healthy seedlings without mechanical damage or pests and with consistent growth were selected. The stem diameter was measured with a vernier caliper. The second leaf from the middle of each plant was selected to measure the leaf thickness with a vernier caliper. The measurements were repeated three times, and the data were recorded.

2.4. Measurement of Physiological Indicators

Fresh leaves and stems from the same position of the treated seedlings were used to measure physiological indicators. Superoxide dismutase (SOD) activity was measured with the nitrogen blue tetrazole (NBT) photoreduction method of Giannopolitis [15]. Peroxidase (POD) and catalase (CAT) were assessed, following the protocol established by Abdel [16]. Ascorbate peroxidase (APX) activity was estimated following the protocol of Cakmak and Marschner [17]. The soluble protein content (SPC) was measured according to Bradford [18] using bovine serum albumin as the standard, and the result was presented as milligrams of bovine serum albumin (mg of BSA) per gram of fresh weight (FW) in samples. Malondialdehyde (MDA) content was determined by the thiobarbituric reaction via spectrophotometric (Allsheng Instruments Co., Ltd., Hangzhou, China) analysis, and the result was presented as micromoles of malondialdehyde per gram ($\mu\text{mol/g}$) of fresh weight (FW) in samples [19]. Chlorophyll content (CHC) was measured according to the methods by Jiang [20], and the result was presented as milligrams of chlorophyll (mg/g) per gram of fresh weight (FW) in samples.

2.5. Measurement of Quality Indicators

The anthocyanin contents (ANC) were analyzed following the method of Li [21] with minor modifications. Briefly, fresh tissues were cut into small pieces, and 0.1 g of sample was accurately weighed and transferred to a test tube. Then, 5 mL of acidic methanol solution (1% HCl in methanol, $v/v = 1:99$) was added for extraction. The mixture was incubated in the dark at 4 °C for 24 h. After centrifugation, the supernatant was collected, and absorbance was measured at 530 nm for anthocyanin contents with cyanidin-3-glucoside as a standard, and the result was presented as nanomoles of cyanidin-3-glucoside (nmol/g) per gram of fresh weight (FW) in samples. The supernatant was subsequently filtered through qualitative filter paper and used for determination of total phenolic (TPC) and flavonoid contents (TFC) according to the referenced protocols by Li [21]. The total phenolic content (TPC) was determined using the Folin–Ciocalteu method, and the result was presented as milligrams of gallic acid equivalent (mg of GAE) per gram of fresh weight (FW) in samples. The flavonoid contents were determined using the colorimetric method with rutin equivalents (RE) as a standard, and the result was presented as milligrams of rutin equivalents (mg of RE) per gram of fresh weight (FW) in samples.

2.6. Data Statistical Analysis

The data were organized and graphed by Microsoft Excel 2010. All data were analyzed using SPSS 20.0 (IBM, Armonk, NY, USA) for analysis of variance (ANOVA), and Duncan's multiple comparison method was used for significant difference analysis ($p < 0.05$). The

heatmap of Pearson's correlation was generated by an online website (<https://mypdftools.cn/heatmap> (accessed on 15 September 2025)). All results were expressed as mean $\pm$ SD, with three replicates for each treatment ($n = 3$).

3. Results

3.1. Effects of Salt Stress on Leaf Thickness and Stem Diameter of *A. roxburghii*

Following salt stress treatment, visible morphological changes were observed in the stems of *A. roxburghii* seedlings (as shown in Figure 1). As the NaCl concentration increased, the degree of stem bending intensified. Exposure to increasing concentrations of NaCl solution resulted in a general reduction in both leaf thickness and stem diameter of *A. roxburghii* seedlings (as shown in Figure 2). Specifically, as illustrated in Figure 2A, leaf thickness decreased significantly under NaCl treatment, with values following the order $T1 > T2 > T3 > T4$, reaching the lowest measurement at T4 (200 mmol/L). Similarly, as shown in Figure 2B, stem diameter progressively declined across the treatment groups from T1 to T4, with all treated groups exhibiting smaller diameters than the control (CK), and the most pronounced reduction observed at the highest salt concentration. Taken together, these results indicate that NaCl stress exerts a significant inhibitory effect on the growth of *A. roxburghii* seedlings, inducing clear physiological stress responses.



Figure 1. Morphological changes of *A. roxburghii* seedlings under different NaCl concentrations (CK–T4: 0 mmol/L, 50 mmol/L, 100 mmol/L, 150 mmol/L, 200 mmol/L).

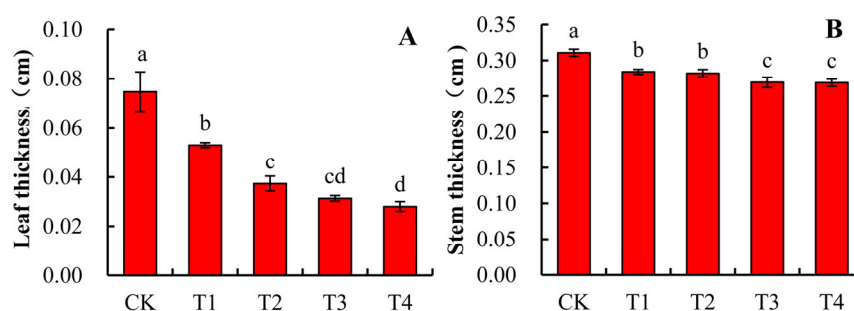


Figure 2. The effects of NaCl on leaf thickness (A) and stem thickness (B) in *A. roxburghii* seedlings. (CK–T4: 0 mmol/L, 50 mmol/L, 100 mmol/L, 150 mmol/L, 200 mmol/L). Different lowercase letters in this figure indicate significant differences ($p < 0.05$). Data are expressed as mean $\pm$ SD ($n = 3$).

3.2. Effects of Salt Stress on Antioxidant Enzyme Activities in *A. roxburghii*

As shown in Figure 3, under different concentrations of NaCl stress, the activities of SOD, CAT, POD, and APX activity in the leaves and stems of *A. roxburghii* all showed an initial increase followed by a decrease, indicating that salt treatment caused oxidative stress to the plant. Among them, the SOD activity in both leaves and stems of all treatment groups was significantly higher than that of the control group (CK), with the T2 group (100 mmol/L) having the highest activity (Figure 3A). The CAT activity in leaves of T1–T4

groups was significantly higher than CK, reaching the highest in the T3 group (150 mmol/L); in stems, only the T2 and T3 groups were significantly higher than CK, with the peak also appearing in the T3 group (Figure 3B). The POD activity in leaves was the highest in the T1 and T2 groups; in stems, the T1–T3 groups were significantly higher than CK, with the T1 group (50 mmol/L) having the highest activity (Figure 3C). The APX activity in leaves was significantly higher than CK in the T1–T3 groups, with the T3 group having the highest; in stems, only the T1 and T2 groups were significantly higher than CK, with the T2 group reaching the peak (Figure 3D). These results suggest that an appropriate concentration of NaCl stress (50–150 mmol/L) can significantly activate the antioxidant enzyme system of *A. roxburghii*, enhancing the ability to scavenge reactive oxygen species, thereby alleviating membrane damage and improving stress resistance. However, when salt concentration exceeds the plant's tolerance threshold, enzyme activity declines, indicating that severe salt stress may cause irreversible physiological damage.

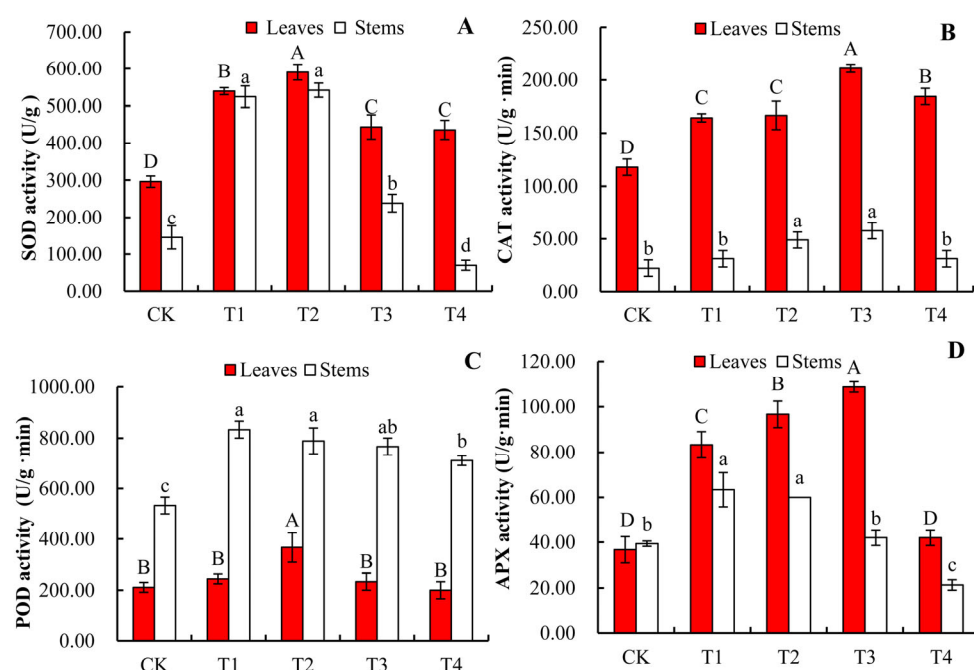


Figure 3. The effects of NaCl on leaf and stem of antioxidant enzyme activities ((A): SOD activity; (B): CAT activity; (C): POD activity; (D): APX activity) in *A. roxburghii*. (CK–T4: 0 mmol/L, 50 mmol/L, 100 mmol/L, 150 mmol/L, 200 mmol/L). Data are expressed as mean $\pm$ SD ($n = 3$). Different uppercase letters indicate significant differences in different salt concentrations of leaves ($p < 0.05$), and different lowercase letters indicate significant differences in salt concentration of stems ($p < 0.05$). Mean values in the column marked by different letters are significantly different within the same group ($p < 0.05$; ANOVA and Duncan test).

3.3. Effects of Salt Stress on Membrane Lipid Peroxidation and Osmotic Adjustment Substances in *A. roxburghii*

As shown in Figure 4, the contents of soluble protein and malondialdehyde (MDA) in leaves and stems of *A. roxburghii* responded differently under varying NaCl concentrations. The content of soluble protein in the leaves first increased and then decreased, with the T4 group being the highest and significantly higher than the others; in the stems, it continuously increased with the increase of salt concentration, and the T2–T4 groups were significantly higher than the others (Figure 4A). This indicates that moderate salt stress (T2–T3) can induce the synthesis and accumulation of soluble protein in *A. roxburghii*, thereby maintaining cellular osmotic balance and enhancing salt tolerance. At the same time, the content of MDA in the leaves and stems continuously increased with the increase

of salt concentration, reaching the maximum in the T4 group, and all treatment groups were significantly higher than CK (Figure 4B). This result indicates that salt stress induces the accumulation of reactive oxygen species and membrane lipid peroxidation, causing damage to the cell membrane system, and the degree of damage increases with the increase of salt concentration. High salt stress (T4) causes severe damage to the stability of the plasma membrane.

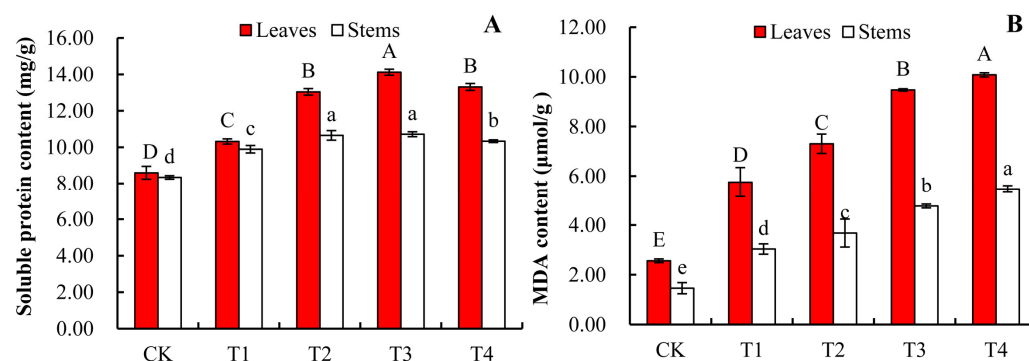


Figure 4. The effects of soluble protein content (A) and MDA content (B) in leaves and stems of *A. roxburghii* under different NaCl concentrations. (CK–T4: 0 mmol/L, 50 mmol/L, 100 mmol/L, 150 mmol/L, 200 mmol/L). Different uppercase letters indicate significant differences in different salt concentrations of leaves ($p < 0.05$), and different lowercase letters indicate significant differences in salt concentration of stems ($p < 0.05$). Mean values in the column marked by different letters are significantly different within the same group ($p < 0.05$; ANOVA and Duncan test).

3.4. Effects of Salt Stress on Pigment Content in *A. roxburghii*

As shown in Figure 5A, with the increase of NaCl concentration, the chlorophyll content in the leaves of *A. roxburghii* showed a decreasing trend, and each salt treatment group was significantly lower than the control group (CK). The chlorophyll content in the stem tissue was extremely low, and no significant changes were observed among the treatment groups. This indicates that as the salt concentration increases, salt stress significantly inhibits the biosynthesis of chlorophyll in the leaves, thereby affecting the photosynthetic capacity of the plant. As shown in Figure 5B, after treatment with different concentrations of NaCl, the anthocyanin accumulation in the leaves reached its peak at the T1 treatment, while in the stems, the anthocyanin content was the highest in the T3 group and significantly higher than that in other treatment groups. This response suggests that *A. roxburghii* enhances its antioxidant capacity by promoting the accumulation of secondary metabolites such as anthocyanins to respond to salt stress environments.

3.5. Effects of Salt Stress on Contents of Flavonoids and Total Phenols in *A. roxburghii*

As shown in Figure 6A, the total flavonoid content of *A. roxburghii* decreased overall with the increase of NaCl treatment concentration. In leaves, the T1 treatment group had the highest total flavonoid content, which was significantly higher than that of the other groups; in stems, except for the T2 group having the lowest content, there was no significant difference among the other treatment groups. Generally speaking, the increase of salt concentration had a relatively small impact on the total flavonoid content in stems. As can be seen from Figure 6B, the total phenol content in leaves and stems of *A. roxburghii* under NaCl stress showed different change patterns with the increase of salt concentration. In leaves, the total phenol content in the T1 group was the lowest, and there was no significant difference among the other groups; in stems, the total phenol content in the T3 and T4 groups was significantly lower than that of the other treatment groups. In conclusion, with the increase of salt concentration, salt stress led to a decrease in the total flavonoid and total

phenol content in the leaves of *A. roxburghii*, while having a relatively small impact on the content of these two substances in stems.

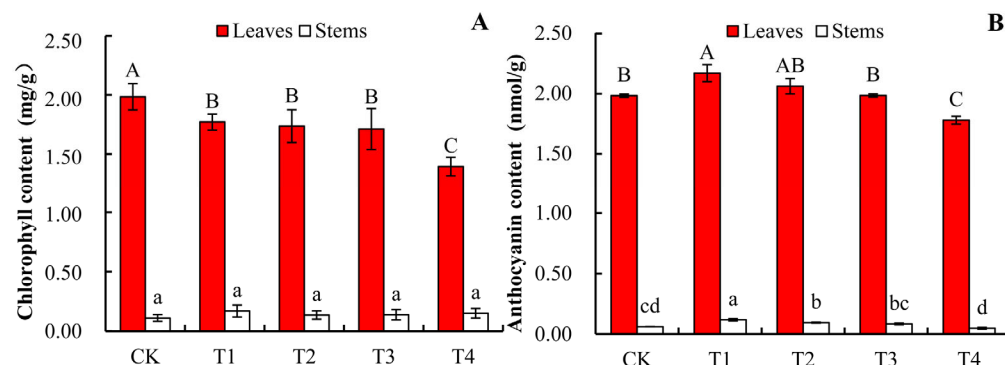


Figure 5. Effects of the contents of chlorophyll (A) and anthocyanin (B) in *A. roxburghii* under different NaCl concentrations. (CK–T4: 0 mmol/L, 50 mmol/L, 100 mmol/L, 150 mmol/L, 200 mmol/L). Different uppercase letters indicate significant differences in different salt concentrations of leaves ($p < 0.05$), and different lowercase letters indicate significant differences in salt concentration of stems ($p < 0.05$). Mean values in the column marked by different letters are significantly different within the same group ($p < 0.05$; ANOVA and Duncan test).

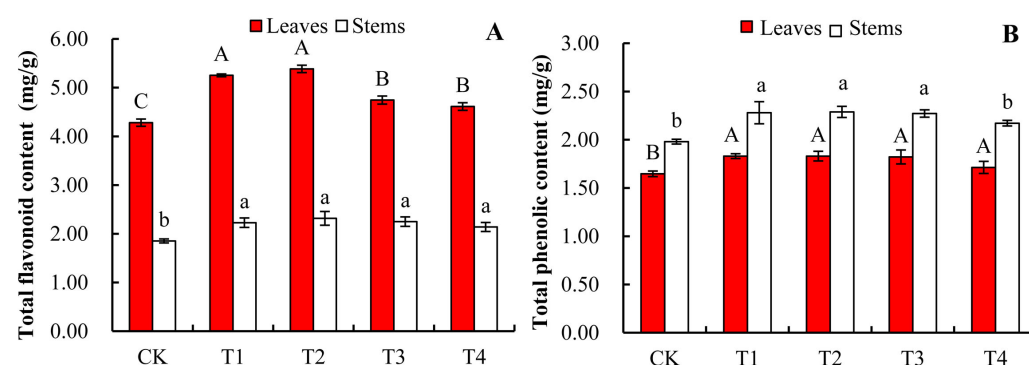


Figure 6. Effects of contents of total flavonoids (A) and total phenols (B) in leaves and stems of *A. roxburghii* under different NaCl concentrations. (CK–T4: 0 mmol/L, 50 mmol/L, 100 mmol/L, 150 mmol/L, 200 mmol/L). Different uppercase letters indicate significant differences in different salt concentrations of leaves ($p < 0.05$), and different lowercase letters indicate significant differences in salt concentration of stems ($p < 0.05$). Mean values in the column marked by different letters are significantly different within the same group ($p < 0.05$; ANOVA and Duncan test).

3.6. Correlation Analysis of Different NaCl Concentrations in Leaves and Stems of *A. roxburghii*

The Pearson correlation heatmap illustrates complex interactions among physiological and biochemical indices for salt stress in *A. roxburghii* (Figure 7). All the observed variables were divided in this study, namely osmoregulatory substances, ROS and antioxidant enzyme systems, pigment parameters, and growth and quality indicators in leaves (Figure 7A) and stems (Figure 7B) of *A. roxburghii*. The results revealed significant correlations among various parameters ($p < 0.01$ or $p < 0.05$). In leaves, leaf thickness (LT) was found to have a highly significant negative correlation with CAT, SPC, and MDA. Among them, the correlation with MDA was the strongest ($r = -0.960$), while it exhibited a significant positive correlation with CHC. The activities of four antioxidant enzymes, namely SOD, POD, CAT, and APX, were all significantly positively correlated with each other ($r > 0.6$). Among these, the correlation between SOD and POD was the highest ($r = 0.677$). As a marker of membrane lipid peroxidation, MDA was strongly correlated with CAT ($r = 0.910$). SPC was significantly positively correlated with CAT and APX ($r = 0.550$ – 0.886). Anthocyanin was positively correlated with chlorophyll and TFC ($r = 0.569$), and TFC was significantly

positively correlated with SOD, POD, and APX, and the correlation with SOD was the strongest ($r = 0.888$). TPC was significantly positively correlated with SOD, CAT, APX, and TFC ($r > 0.53$). In addition, in stems, stem thickness (ST) was significantly negatively correlated with CAT, POD, soluble protein, MDA, and TPC. The strongest correlation was observed with MDA ($r = -0.909$). SOD, POD, CAT, and APX maintained significant positive correlations with each other (the highest correlation was between SOD and APX, $r = 0.933$). SPC was significantly positively correlated with CAT, POD, MDA, and TPC ($r > 0.717$). ANC was positively correlated with SOD, POD, and APX ($r > 0.626$), and chlorophyll was significantly positively correlated with TFC.

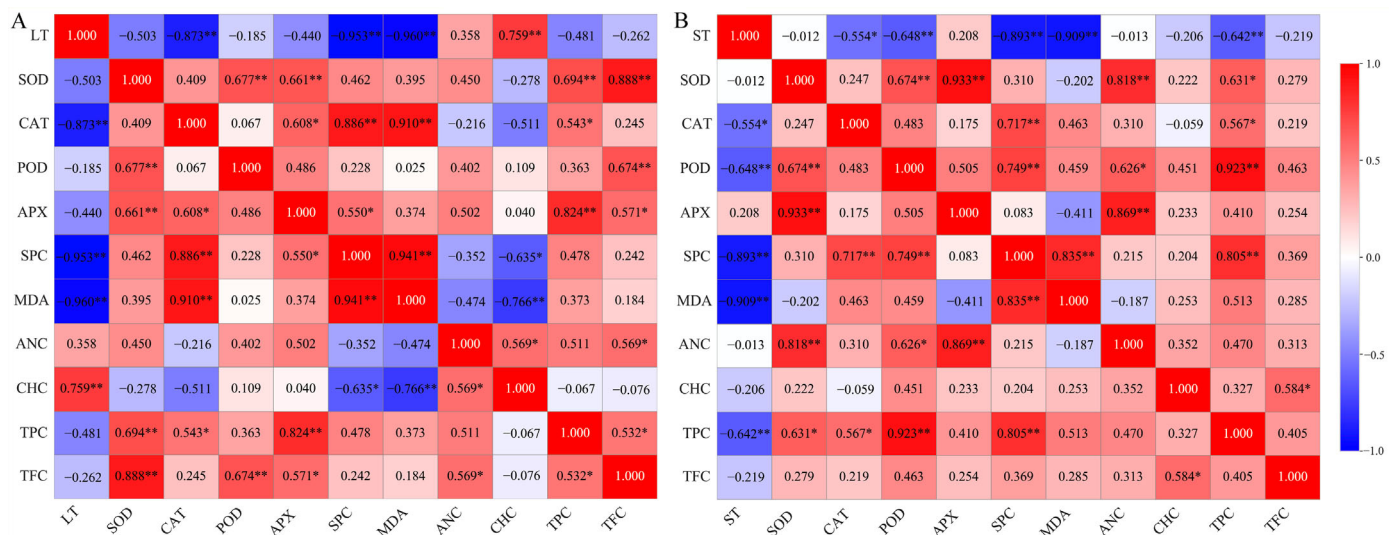


Figure 7. Correlation analysis of different NaCl concentrations in leaves (A) and stems (B) of *A. roxburghii*. An asterisk (*) denotes statistically significant differences at $p < 0.05$. Two asterisks (**) denote statistically significant differences at $p < 0.01$. LT = stem thickness; ST = stem thickness; CAT = catalase activity; SOD = superoxide dismutase activity; POD = peroxidase activity; APX = Ascorbate peroxidase activity; SPC = soluble protein content; MDA = malondialdehyde content; ANC = Anthocyanin content; CHC = chlorophyll content; TPC = total phenols content; TFC = total flavonoids content.

4. Discussion

Salt stress represents a critical environmental factor that constrains plant growth and agricultural productivity on a global scale [22]. It exerts adverse effects on plant development through multiple physiological and biochemical mechanisms, including osmotic stress, ion toxicity, and nutrient imbalance, ultimately leading to a reduction in crop yield and quality [23]. In this study, it was observed that salt stress significantly inhibits the morphological development of *A. roxburghii* seedlings, as indicated by the decrease in leaf and stem thickness (Figure 2), with the most pronounced inhibitory effect occurring under 200 mmol/L NaCl treatment. This morphological alteration is a typical adaptive response of plants to saline conditions. High salinity reduces soil water potential, inducing osmotic stress that impedes water uptake and results in physiological drought [24]. Concurrently, salt stress diminishes root hydraulic conductivity, thereby impairing water transport to the aerial parts of the plant, which may directly hinder cell expansion and division [25], thereby causing the stem thickness of the *A. roxburghii* to decrease and the leaves to become thinner.

In this study, it was found that under salt stress, the activities of antioxidant enzymes, including SOD, CAT, POD, and APX, in the leaves and stems of *A. roxburghii* increased significantly (Figure 3). Previous studies have shown that salt stress with appropriate concentration could reduce seedling growth but increase antioxidant enzyme activity and

osmotic compound levels [26,27]. This synergistic mechanism effectively maintains the cellular redox homeostasis of ROS [28]. Notably, peak enzyme activities occurred at different salt concentrations, suggesting potential differences in their regulatory mechanisms. For instance, SOD activity peaked at 100 mmol/L NaCl, whereas CAT reached its maximum at 150 mmol/L. This sequential activation pattern may reflect a hierarchical defense strategy employed by plants against oxidative stress [29]. However, when salt concentration exceeded the plant's tolerance threshold (150 mmol/L), the activities of all antioxidant enzymes declined, indicating that the antioxidant defense system was overwhelmed. The content of soluble proteins in leaves exhibited an initial increase followed by a decline, whereas in stems, it showed a continuous upward trend (Figure 4A). This organ-specific response indicates that the plant employs distinct osmotic adjustment strategies across different tissues [30]. This observation aligns with the trend in malondialdehyde (MDA) content, which increased continuously with rising salt concentration (Figure 4B), reflecting progressive membrane lipid peroxidation. These findings are consistent with those reported in Hollyhock (*Alcea rosea* L.), suggesting that the activation and subsequent collapse of the antioxidant system represent conserved mechanisms in plant responses to salt stress [31]. In this study, it was observed that chlorophyll content decreased with increasing salt concentration, which is closely related to the damage of photosynthetic apparatus [22]. Salt stress may affect chlorophyll metabolism through multiple mechanisms: on the one hand, it directly inhibits the activity of enzymes related to chlorophyll synthesis; on the other hand, it promotes the degradation process mediated by chlorophyllase [32,33]. In contrast, anthocyanin content significantly accumulated at specific salt concentrations (50 mmol/L in leaves and 150 mmol/L in stems), which may be an adaptive response. As effective antioxidants, anthocyanins can scavenge ROS, reduce oxidative damage, and prevent photoinhibition by absorbing excess light energy [33]. Additionally, among secondary metabolites, the contents of flavonoids and total phenols first increased and then decreased with the increase of salt concentration. When plants are subjected to salt stress (50–150 mmol/L), they may recognize it as a “stress signal”. To cope with this stress, plants promote the synthesis of secondary metabolites with strong antioxidant capacity, such as flavonoids and total phenols. When the salt concentration is too high (100 mmol/L), exceeding the tolerance range of plants, physiological functions are impaired, and the synthesis capacity decreases, leading to a decrease in their contents. This finding is similar to the results in vegetable amaranth (*Amaranthus lividus*), indicating that appropriate salt stress can enhance the accumulation of high phenolic and flavonoid compounds in plant cells [34].

In the correlation analysis, the significant positive correlations among antioxidant enzymes (SOD, POD, CAT, APX) in leaves and stems confirmed the theoretical framework of multi-enzyme collaborative clearance of ROS. Among these enzymes, the high correlation between SOD and POD highlights their key roles as core components in maintaining oxidative balance [35]. Notably, the strong negative correlation between leaf thickness and MDA reveals an antagonistic relationship between morphological traits and membrane lipid peroxidation. Furthermore, the significant positive correlation between flavonoids and antioxidant enzymes supports the hypothesis that phenolic compound accumulation contributes to oxidative stress resistance and enhanced plant tolerance, which is consistent with previous findings [36].

This study demonstrates that *A. roxburghii* employs an integrated strategy involving morphological modulation, antioxidant activation, and metabolic reprogramming to cope with salt stress. However, in this experiment, the plants were subjected to salt treatment in the short term (24 h). When the salt concentration exceeded the critical threshold (100–150 mmol/L NaCl), these adaptive mechanisms were insufficient to counteract the

stress, resulting in growth inhibition and physiological dysfunction. In order to have a more comprehensive understanding of the physiological response of *A. roxburghii* to salt stress, we will focus on including biomass, root morphology, and photosynthetic performance in subsequent work to further improve the systematic analysis of the stress response mechanism. Meanwhile, future research could further investigate the molecular regulatory networks underlying the salt stress response in *A. roxburghii* using transcriptomic and proteomic approaches or explore the potential of cultivation management practices—such as exogenous substance application and water regulation—to mitigate salt stress effects.

5. Conclusions

In conclusion, excessively high NaCl concentrations significantly inhibit the growth and physiological performance of *A. roxburghii*. This study demonstrates that *A. roxburghii* exhibits moderate tolerance to salt stress, effectively withstanding NaCl concentrations up to 100 mmol/L. Within this threshold, the plant activates its antioxidant defense system, as evidenced by increased activities of SOD, POD, CAT, and APX, and accumulates osmoregulatory substances such as soluble proteins, thereby mitigating oxidative damage under saline conditions. However, beyond this concentration, these protective mechanisms become compromised, resulting in decreased antioxidant enzyme activity, reduced accumulation of beneficial metabolites, and a significant increase in oxidative stress, as indicated by elevated MDA levels. The observed declines in chlorophyll, anthocyanin, total flavonoid, and total phenolic contents further confirm the adverse effects of high salinity. These findings not only elucidate the physiological basis of salt tolerance in *A. roxburghii* but also provide a valuable theoretical foundation for the selection and breeding of salt-tolerant genotypes. Therefore, in practical cultivation, it is recommended to grow *A. roxburghii* in low-salt environments with NaCl concentrations not exceeding 100 mmol/L, along with the implementation of scientific management practices.

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Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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

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