Effects of GA3 on Physiological Metabolism and Ion Uptake in 'Paulownia 1201' Seedlings under NaCl Stress: Postprint

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Abstract

To investigate the alleviating effect and physiological mechanism of gibberellin (GA_3) on the growth of 'Paulownia 1201' seedlings under NaCl stress, this study used 'Paulownia 1201' seedlings as experimental material, establishing different concentrations of NaCl and GA_3, and measured and analyzed the physiological indicators, photosynthetic indicators, and ion transport capacity of GA_3 in alleviating NaCl stress in Paulownia seedlings. The results showed that: (1) Under 150 $mmol \cdot L^{-1}$ NaCl stress, the growth of Paulownia seedlings decreased significantly ($P<0.05$), with a reduction exceeding 50%; appropriate concentrations of exogenous GA_3 significantly increased the plant height, root length, and biomass of Paulownia seedlings, and the dry weight under 400 $mg \cdot L^{-1}$ GA_3 treatment increased by 69.71% compared with A_0. (2) With increasing GA_3 concentration, the activities of three antioxidant enzymes (SOD, POD, CAT) increased significantly; MDA content decreased significantly; chlorophyll content and gas exchange parameters (Pn, Tr, Ci, Gs) increased, and photosynthetic efficiency improved; the contents of various ions (K^+, Ca^{2+}, Mg^{2+}) all exhibited a trend of initially increasing and then decreasing, and the Na^+ content in leaves and roots of the 400 $mg \cdot L^{-1}$ GA_3 treatment group decreased by 23.59% and 11.92% respectively compared with the A_0 group. (3) Correlation analysis and PCA analysis revealed that there were correlations among various indicators, and the differences in seedlings treated with different concentrations of GA_3 were significant, with the best alleviating effect when GA_3 concentration was 400 $mg \cdot L^{-1}$. In summary, foliar application of GA_3 can enhance the antioxidant capacity of 'Paulownia 1201' seedlings under salt stress, alleviate membrane lipid peroxidation resulting from excessive reactive oxygen species under salt stress, promote photosynthesis and biomass accumulation, reduce Na^+ uptake and promote the accumulation of nutrient ions, with the addition

of 400 mg • L^{-1} GA_3 being more effective in enhancing the resistance of Paulownia seedlings. This study provides a foundation for in-depth understanding of the salt tolerance mechanism of Paulownia and also provides a theoretical basis for the development and utilization of Paulownia in saline-alkali lands.

Full Text

Effects of GA3 on Physiological Metabolism and Ion Absorption of 'Paulownia 1201' Seedlings Under NaCl Stress

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Abstract

To investigate the alleviating effects and physiological mechanisms of gibberellin (GA_3) on the growth of 'Paulownia 1201' seedlings under NaCl stress, we examined physiological indices, photosynthetic parameters, and ion transport capacity in seedlings treated with various concentrations of NaCl and GA_3. The results showed: (1) Under 150 mmol • L^{-1} NaCl stress, seedling growth decreased significantly ($P<0.05$) by over 50%; appropriate concentrations of exogenous GA_3 significantly increased plant height, root length, and biomass, with dry weight increasing by 69.71% at 400 mg • L^{-1} GA_3 compared to the A_0 treatment. (2) As GA_3 concentration increased, the activities of three antioxidant enzymes (SOD, POD, CAT) improved significantly while MDA content decreased substantially; chlorophyll content and gas exchange parameters (Pn, Tr, Ci, Gs) increased, enhancing photosynthetic efficiency; all ion contents (K^+, Ca^{2+}, Mg^{2+}) showed a trend of initial increase followed by decrease, with Na^+ content in leaves and roots decreasing by 23.59% and 11.92%, respectively, at 400 mg • L^{-1} GA_3 compared to A_0. (3) Correlation and PCA analyses revealed significant relationships among indices, with clear differences among GA_3 treatments, showing optimal alleviation at 400 mg • L^{-1} GA_3. In conclusion, foliar GA_3 application enhances antioxidant capacity, alleviates membrane lipid peroxidation caused by excessive reactive oxygen species under salt stress, promotes photosynthesis and biomass accumulation, reduces Na^+ uptake while facilitating nutrient ion accumulation, with 400 mg • L^{-1} GA_3 being most effective for improving salt resistance. This study provides a foundation for understanding Paulownia salt tolerance mechanisms and theoretical support for utilizing Paulownia in saline soils.

Keywords: 'Paulownia 1201' , NaCl stress, gibberellin, ion uptake, PCA analysis

Introduction

Soil salinization poses an increasingly severe challenge to agricultural production and ecological conservation worldwide, affecting over 397 million hectares globally (Liang et al., 2018; Zhang et al., 2018). Salt stress impairs normal plant growth by causing oxidative damage from excessive reactive oxygen species accumulation, inhibiting photosynthesis and ion absorption, and leading to metabolic disorders and even plant death (Shiraku et al., 2022; Balusamy et al., 2022). In China, saline and secondary salinized soils continue to expand (Li et al., 2022), with projections indicating that 50% of global arable land will be affected by soil salinization by 2050 (Nan et al., 2022). Therefore, developing effective strategies to control and utilize salinized land has become urgent (Wei et al., 2022).

Paulownia (*Paulownia fortunei*), a fast-growing deciduous tree in the Scrophulariaceae family, offers excellent wood quality, broad utility, strong adaptability, and notable salt-alkali tolerance, making it an important species for saline soil development in China (Zhu et al., 2021). Previous studies indicate that Paulownia seeds exhibit highest germination rates and strongest salt tolerance at 5% salt concentration (Wang et al., 2019). Wang et al. (2017) and Zhao et al. (2019) found that tetraploid Paulownia shows greater salt tolerance than diploid varieties through differential gene expression analysis under salt stress. While progress has been made in breeding salt-tolerant Paulownia, research on improving salt tolerance through exogenous substances remains limited.

Gibberellin (GA_3) is a crucial plant growth hormone that regulates seed germination, root and stem growth, and flower and fruit development (Wang et al., 2017). Recent studies demonstrate that appropriate GA_3 concentrations can promote plant growth under salt stress by regulating osmotic substance accumulation, aquaporin expression, plasma membrane stability, Na^+ reduction, and nutrient ion absorption, thereby enhancing salt tolerance (Liu & Xue, 2017; Sarkaria & Chinna, 2021). Guo et al. (2022) reported that gibberellin breaks physiological dormancy in pea seeds under salt stress, promoting coleoptile and sub-crown internode elongation. Gao et al. (2019) showed that optimal GA_3 concentrations regulate antioxidant enzyme activities in maize, reduce ROS accumulation, and alleviate photosynthetic organ damage while promoting photosynthetic pigment accumulation. Shang et al. (2017) found that 160 $mg \cdot L^{-1}$ GA_3 pretreatment maintained higher dry matter content and improved fruit quality in tomatoes. Li et al. (2022) observed that exogenous GA_3 enhanced chlorophyll content and photosynthetic rates in *Quercus nuttallii* seedlings, reducing Na^+ toxicity.

However, the physiological mechanisms of exogenous GA_3 in Paulownia under salt stress remain poorly understood. To address this knowledge gap, we investigated the effects of different GA_3 concentrations on physiological-biochemical characteristics and nutrient ion absorption in 'Paulownia 1201' seedlings, using correlation and PCA analyses to comprehensively evaluate multiple indices. Our objectives were to: (1) determine how different GA_3 concentrations affect

Paulownia growth and physiological metabolism under salt stress; (2) elucidate how exogenous GA_3 regulates ion absorption and tolerance relationships; and (3) identify the optimal GA_3 concentration for Paulownia seedlings under salt stress. This research provides theoretical foundations for understanding Paulownia salt tolerance mechanisms and scientific support for utilizing Paulownia in saline soil improvement.

Materials and Methods

1.1 Experimental Materials

The experiment was conducted from October 3, 2021, to January 4, 2022, at the Forest Tree Genetics and Breeding Laboratory of Henan Agricultural University. Seeds of 'Paulownia 1201' were collected from the university' s science and education park (34°86 N, 113°5 E). Analytical-grade NaCl and GA_3 were purchased from Sinopharm Group. Uniform, plump seeds were surface-sterilized in 1% NaClO solution for 30 seconds, rinsed 4-5 times with distilled water, and germinated in petri dishes under controlled conditions: 16 h light/8 h dark photoperiod, light intensity 10,000 lx, day/night temperatures 23°C/18°C, and relative humidity (50$±10)±$0.5) in hydroponic containers for greenhouse cultivation, with solution changes every 3 days. After 15 days, seedlings were transferred to full Hoagland solution and cultured in black glass bottles secured with planting cotton until the 4-leaf stage, when treatments began.

1.2 Experimental Design

1.2.1 NaCl Stress Treatment Uniform, healthy seedlings were subjected to NaCl stress at concentrations of 0, 50, 100, 150, and 200 $mmol \cdot L^{-1}$, with 5 seedlings per treatment. After 15 days of continuous treatment, morphological and biomass indices were measured.

1.2.2 GA_3 Alleviation Experiment Based on preliminary results, 150 $mmol \cdot L^{-1}$ NaCl significantly inhibited growth while maintaining seedling survival, so this concentration was selected for salt stress treatment. Uniform seedlings were assigned to six treatments with five replicates each. Different GA_3 solutions (0, 100, 200, 400, 600 $mg \cdot L^{-1}$) were sprayed evenly on both leaf surfaces every 5 days, with distilled water spray as control (CK). Treatment details are shown in Table 1 . After 15 days, all indices were measured.

Table 1 Experimental treatments

Treatment	Method
0 $mmol \cdot L^{-1}$ NaCl + 0 $mg \cdot L^{-1}$ GA_3	Control
150 $mmol \cdot L^{-1}$ NaCl + 0 $mg \cdot L^{-1}$ GA_3	A_0
150 $mmol \cdot L^{-1}$ NaCl + 100 $mg \cdot L^{-1}$ GA_3	A_1
150 $mmol \cdot L^{-1}$ NaCl + 200 $mg \cdot L^{-1}$ GA_3	A_2

Treatment	Method
150 mmol · L^{-1} NaCl + 400 mg · L^{-1} GA_3	A_3
150 mmol · L^{-1} NaCl + 600 mg · L^{-1} GA_3	A_4

1.3 Measurement Indices

1.3.1 Physiological and Biochemical Indices **Growth indices**: After 15 days, seedlings were harvested, roots rinsed 2-4 times with distilled water, surface moisture absorbed with filter paper, and plant height and root length measured with a ruler. Fresh weight was determined using an analytical balance, then samples were oven-dried at 105°C for 30 minutes and at 75°C to constant weight for dry weight measurement.

Physiological indices: Fresh leaf and root tissues (0.1 g) were sampled for physiological measurements. Superoxide dismutase (SOD) activity was determined by the nitroblue tetrazolium (NBT) method, peroxidase (POD) by the guaiacol method, catalase (CAT) by the hydrogen peroxide method, and malondialdehyde (MDA) by the thiobarbituric acid method (Yin et al., 2019; Zhang et al., 2020). Chlorophyll content was measured by acetone extraction (Yin et al., 2019). Photosynthetic parameters were measured 15 days after treatment on 3 randomly selected seedlings per treatment between 8:00-11:00 AM using a portable photosynthesis system (Li-6400XT). Net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) were measured at ambient CO_2 concentration (Ca) of 400 mol · mol^{-1}. Water use efficiency (WUE = Pn/Tr) and stomatal limitation value (Ls = 1 - Ci/Ca) were calculated.

1.3.2 Ion Content Measurement Following Li et al. (2020), leaf and root samples were collected, rinsed with deionized water, killed at 105°C for 30 minutes, dried at 75°C to constant weight, ground, sieved (30 mesh), and digested with HNO_3-$HClO_4$. Na^+, K^+, Ca^{2+}, and Mg^{2+} contents were determined using a FAAS-M6 atomic absorption spectrometer.

1.4 Data Analysis

Data were organized using Excel 2019. One-way ANOVA was performed with SPSS 25.0. Correlation analysis graphs were generated with Origin 2021, and principal component analysis (PCA) graphs with Canoco 5 software.

Results

2.1 Seedling Growth Under NaCl Stress

Morphological indices directly reflect stress severity. NaCl effects on Paulownia growth showed low-concentration promotion and high-concentration inhibition (Table 2). At 50 mmol·L^{-1} NaCl, main root length, fresh weight, and dry weight reached maximum values, significantly exceeding CK (P<0.05). Compared with CK, 100, 150, and 200 mmol$\cdot$L^{-1} NaCl treatments reduced plant height by 20.61%, 50.97%, and 53.84%; fresh weight by 4.79%, 55.33%, and 68.46%; and dry weight by 14.48%, 52.36%, and 56.79%, respectively. At 150 mmol$\cdot$L^{-1} NaCl, main root length decreased significantly with reductions in plant height, fresh weight, and dry weight all exceeding 50%, establishing this concentration as the semi-lethal dose for subsequent experiments.

Table 2 Growth of 'Paulownia 1201' seedlings under salt stress

NaCl concentration (mmol$\cdot$L^{-1})	Plant height	Root length	Fresh weight	Dry weight
0 (CK)	11.121$±0.176*b*	10.104±0.101*c*	3.427±0.048*b*	1.354±0.051*b*
50	12.183±0.153*a*	17.1		

Note: Different lowercase letters indicate significant differences at P<0.05 (n=5). The same below.

2.2 Effects of Exogenous GA_3 on Seedlings Under NaCl Stress

2.2.1 Morphological Indices and Biomass NaCl stress significantly inhibited Paulownia growth (P<0.05), while exogenous GA_3 effectively alleviated this inhibition, with effects peaking then declining as GA_3 concentration increased (Table 3). Compared with CK, the A_0 treatment (150 mmol$\cdot$L^{-1} NaCl alone) significantly reduced all growth indices, with main root length showing the greatest inhibition (61.16% reduction). Relative to A_0, dry weight increased by 30.96%, 59.38%, 69.71%, and 42.23% under A_1-A_4 treatments, respectively, with maximum enhancement at A_3 (400 mg$\cdot$L^{-1} GA_3). Plant height, main root length, and fresh weight showed similar trends. Leaf length and width peaked at A_2 treatment (200 mg$\cdot$L^{-1} GA_3), increasing by 47.03% and 51.54% over A_0, respectively. These results suggest GA_3 promotes dry matter accumulation by enhancing photosynthetic rates, thereby improving seedling salt resistance.

Table 3 Effects of GA_3 on morphological indices and biomasses of 'Paulownia 1201' seedlings under salt stress

Treatment	Leaf length	Leaf width	Plant height	Root length	Fresh weight	Dry weight
CK	4.801$±0.05[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

2.2.2 Antioxidant Enzyme Activities and MDA Content NaCl stress weakened Paulownia' s antioxidant capacity, while exogenous GA_3 alleviated damage to the enzymatic system (Figure 1 [Figure 1: see original paper]A-C). Compared with CK, A_0 treatment significantly reduced SOD, POD, and CAT activities in both leaves and roots (P<0.05). GA_3 application (A_1-A_4) enhanced all three antioxidant enzyme activities. SOD and CAT peaked at A_3 treatment, increasing by 92.69% and 82.81% in leaves and 82.10% and 70.54% in roots over A_0, respectively. POD activity peaked at A_2 treatment, increasing by 69.99% in leaves and 47.52% in roots. Notably, leaf POD and CAT activities exceeded those in roots, while SOD activity showed the opposite pattern, likely because roots first contact salt and suffer greater damage. Foliar GA_3 application significantly mitigated NaCl-induced inhibition of antioxidant enzymes, maintaining dynamic equilibrium between ROS production and scavenging.

MDA content reflects membrane system damage under stress. As shown in Figure 1 [Figure 1: see original paper]D, A_0 treatment significantly increased MDA content in leaves and roots by 102.96% and 64.16% compared with CK ($P<0.05$). MDA content decreased progressively with increasing GA_3 concentration, with maximum reduction (33.21%) in leaves at A_3 treatment. Root MDA content remained higher than in leaves, indicating that exogenous GA_3 reduced lipid peroxidation products and alleviated salt-induced membrane damage, thereby enhancing salt tolerance.

Figure 1 Effects of GA_3 on antioxidant enzyme activities and MDA content of 'Paulownia 1201' seedlings under NaCl stress

2.2.3 Photosynthetic Pigment Content Leaf pigment content correlates closely with photosynthetic rate and nutritional status. As shown in Figure 2 [Figure 2: see original paper], A_0 treatment significantly reduced chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll [Chl(a+b)], chlorophyll a/b ratio [Chl(a/b)], and carotenoid content compared with CK ($P<0.05$), indicating that high salt stress accelerated pigment degradation and inhibited chlorophyll synthesis. Under A_1-A_4 treatments, Chla, Chl(a+b), Chl(a/b), and carotenoid content increased initially then decreased, peaking at A_3 treatment with increases of 112.50%, 90.83%, 44.00%, and 56.00% over A_0, respectively. This suggests optimal GA_3 concentration promotes pigment accumulation, maintains high chlorophyll levels, provides nutritional support, and improves photosynthetic performance. Chlb showed smaller variations among treatments but remained slightly higher than A_0, indicating low GA_3 concentrations promote Chlb synthesis while high concentrations may cause stress.

Figure 2 Effects of GA_3 on pigment mass fraction in 'Paulownia 1201' leaves under NaCl stress

2.2.4 Photosynthetic Gas Exchange Parameters Photosynthetic gas parameters reflect leaf photosynthetic processes. As shown in Table 4 , A_0 treatment reduced Pn, Tr, Ci, Gs, and WUE by 33.91%, 32.99%, 40.96%, 62.50%, and 1.34%, respectively, while increasing Ls by 300.00% ($P<0.05$), indicating that Na^+ inhibition of Paulownia photosynthesis was primarily stomatal limitation. GA_3 application (A_1-A_4) caused initial increases then decreases in most parameters, with Pn, Ci, Gs, and WUE peaking at A_3 treatment (increases of 60.93%, 63.40%, 133.33%, and 14.92% over A_0, respectively). Ls showed the opposite trend, decreasing initially then increasing, with minimum value at A_3 treatment (69.17% reduction from A_0). These results demonstrate that GA_3 spraying significantly reduced salt damage to photosynthesis, with optimal alleviation at A_3.

Table 4 Effects of GA_3 on photosynthesis gas parameters of 'Paulownia 1201' seedlings under NaCl stress

Treatment	Pn (mol · m^{-2} · s^{-1})	Tr (mmol · m^{-2} · s^{-1})	Ci (mol · mol^{-1})	Gs (mmol · m^{-2} · s^{-1})	WUE (mol · mol^{-1})	Ls
CK	14.33 ± 1.27a	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

2.3 Ion Absorption Capacity

Ion contents (Na^+, K^+, Ca^{2+}, Mg^{2+}) in leaves and roots differed significantly among treatments ($P<0.05$). As shown in Table 5 , A_0 treatment significantly increased Na^+ content while decreasing K^+, Ca^{2+}, and Mg^{2+} in both tissues compared with CK, indicating that Na^+ accumulation reduced nutrient ion absorption. GA_3 application caused Na^+ content to decrease initially then increase, reaching minimum values at A_3 treatment (23.59% and 11.92% lower than A_0 in leaves and roots, respectively). K^+, Ca^{2+}, and Mg^{2+} contents showed the opposite trend, with leaf K^+ and Ca^{2+} accumulation exceeding that in roots. This suggests GA_3 reduces Na^+ accumulation and transport to shoots while promoting K^+ and Ca^{2+} absorption. K^+/Na^+, Ca^{2+}/Na^+, and Mg^{2+}/Na^+ ratios decreased significantly under NaCl stress but increased initially then decreased with GA_3 concentration, peaking at A_3 treatment. These findings demonstrate that GA_3 improves ion balance and reduces Na^+ uptake, enhancing salt tolerance.

Table 5 Effects of GA_3 on contents and ratios of 'Paulownia 1201' ions under NaCl stress

Organ	Treatment	Ion content (mg · g^{-1})	Ion content ratio
		Na^+	K^+
Leaf	CK	2.475 ± 0.071e, 4.479 ± 0.201b	[illegible]

2.4 Correlation Analysis Among Leaf Indices

Correlation analysis revealed significant relationships among physiological indices in Paulownia leaves (Figure 3 [Figure 3: see original paper]). SOD activity showed extremely significant positive correlations with CAT, Tr, Ci, and Gs ($P<0.01$). CAT activity was extremely significantly positively correlated with Chl(a+b), Pn, Ci, and Gs, and significantly correlated with Chl(a+b) and POD. Pn was extremely significantly positively correlated with Gs and

K^+/Na^+. Mg^{2+}/Na^+ was extremely significantly positively correlated with Gs and significantly correlated with SOD, CAT, and Ci. MDA was significantly negatively correlated with Tr and Mg^{2+}/Na^+, and extremely significantly negatively correlated with SOD, CAT, Chl(a+b), Pn, Ci, and K^+/Na^+. These results demonstrate that GA_3 effects on physiological characteristics are multifaceted, requiring comprehensive evaluation rather than single indices.

Figure 3 Correlation coefficient analysis of leaf indexes of 'Paulownia 1201' seedlings

2.5 Principal Component Analysis

PCA of different GA_3 concentrations under NaCl stress showed that principal component 1 (PC1) and principal component 2 (PC2) explained 83.55% and 10.97% of variance, respectively, with a cumulative contribution of 94.52% (Figure 4 [Figure 4: see original paper]). PC1 was highly positively correlated with Ls and negatively correlated with Pn, Ci, WUE, root SOD, and root length. PC2 was highly positively correlated with Chl(a/b) and negatively correlated with root MDA. These two components effectively reflected comprehensive effects of GA_3 treatments. A_1, A_2, and A_3 treatments clustered in quadrants II and III, indicating better alleviation effects, while A_4 distributed in quadrant I, showing poorer efficacy. Ls was extremely significantly negatively correlated with Pn, root POD, and root length, while leaf and root MDA were extremely significantly negatively correlated with Chl(a/b), leaf Ca^{2+}, and root Mg^{2+}. These relationships confirm that GA_3 application enhances growth, antioxidant enzyme activity, photosynthesis, and nutrient ion absorption while reducing MDA accumulation. The vertical axis values indicate that higher values correspond to lower stress and better GA_3 alleviation, confirming A_3 (400 $mg \cdot L^{-1}$ GA_3) as the optimal treatment.

Figure 4 Principal component analysis of environmental factors and physiological indexes of 'Paulownia 1201' seedlings

Discussion

3.1 Effects of NaCl and GA_3 on Physiological Indices

Seedling establishment is critical for plant development. Salt stress typically reduces germination rates, leaf area, and root growth (Zhang et al., 2018). In this study, 150 $mmol \cdot L^{-1}$ NaCl significantly inhibited Paulownia growth, reducing plant height and biomass by over 50%, likely due to increased osmotic potential hindering water uptake, causing osmotic imbalance and reduced nutrient absorption (Zhang et al., 2018). Exogenous GA_3 enhances nutrient absorption capacity, promoting germination and seedling growth under salt stress (Gao et al., 2019). Our results demonstrate dose-dependent effects, with GA_3 triggering gene expression, increasing hydrolytic enzyme synthesis, repairing damaged membranes, and regulating endogenous hormone balance (e.g., increasing gibberellin and decreasing abscisic acid) to improve stress resistance (Shang et al., 2017; Guo et al., 2022).

In the plant enzymatic system, SOD, POD, and CAT scavenge ROS, with their activities determining stress tolerance (Chen et al., 2021). MDA accumulation reflects cellular damage and affects membrane permeability (Ren et al., 2022). Our results showing suppressed antioxidant enzymes and elevated MDA under high salt stress align with previous studies (Jiang et al., 2013; Zhu et al., 2015), indicating damaged antioxidant systems and enhanced membrane lipid peroxidation. Exogenous GA_3 induced ROS scavenging capacity, reducing oxidative damage (Ma et al., 2020; Zadeh et al., 2015). The observed trends—peaking POD at 200 $mg \cdot L^{-1}$ GA_3 and maximum SOD/CAT with minimum MDA at 400 $mg \cdot L^{-1}$—suggest that SOD, CAT, and MDA play primary roles in the salt resistance mechanism, though coordination among the three enzymes requires further investigation.

3.2 Effects of GA_3 on Photosynthesis Under NaCl Stress

Photosynthetic pigment content directly reflects photosynthetic capacity (Zhang et al., 2022). Salt stress damages chloroplast ultrastructure, generates excessive ROS, and blocks chloroplast protein synthesis, reducing total chlorophyll content, while GA_3 application significantly increases pigment levels (Keawmanee et al., 2022). Our study showed significant reductions in Chla, Chlb, and carotenoids under high salt stress, likely due to decreased pigment synthesis enzyme activity and accelerated chlorophyll degradation (Huo et al., 2021). GA_3 spraying partially restored pigment contents, possibly by stabilizing thylakoid membranes and reducing chloroplast damage (Li et al., 2022), though irreversible salt damage prevented full recovery to CK levels within the short treatment period. These findings generally align with previous studies, though some discrepancies with Shang et al. (2017) may reflect differences in experimental materials or salt concentrations.

GA_3 application improved photosynthetic gas parameters (Pn, Tr, Gs, Ci) in salt-stressed sorghum (Nimir et al., 2017). Our study found that NaCl reduced Pn, Tr, Ci, Gs, and WUE while increasing Ls, suggesting stomatal limitation as the primary mechanism of photosynthetic inhibition. As GA_3 concentration increased, gas parameters showed initial increases then decreases, peaking at 400 $mg \cdot L^{-1}$ GA_3, while Ls showed the opposite trend. These patterns, consistent with studies on *Zelkova sinica* (Huo et al., 2021) and tomato (Keawmanee et al., 2022), indicate that salt stress triggers stomatal closure to reduce water loss, limiting CO_2 diffusion and photosynthetic substrates. Optimal GA_3 concentration alleviated stomatal limitation, enhanced substrate accumulation in mesophyll cells, and improved photosynthesis and salt tolerance.

3.3 Effects of GA_3 on Ion Absorption Under NaCl Stress

Salt ions are essential nutrients, but excess amounts inhibit cytoplasmic enzyme synthesis, causing osmotic and ionic stress that disrupts ion balance and physiological functions (Luo et al., 2022). Na^+ accumulation suppresses uptake of macronutrients (K, Ca, Mg), while plants adapt through selective Na^+ absorption/exclusion or compartmentalization to maintain cellular ion balance (Albaladejo et al., 2017; Deinlein et al., 2014). Our finding that roots accumulated more Na^+ than shoots aligns with Hao et al. (2020), indicating that Paulownia roots intercept Na^+, limiting transport to shoots and reducing leaf Na^+ accumulation as an active adaptation mechanism. Maintaining high K^+/Na^+, Ca^{2+}/Na^+, and Mg^{2+}/Na^+ ratios indicates strong salt tolerance (Luo et al., 2022). Exogenous GA_3 increased these ratios in both leaves and roots, with greater effects in leaves, likely because high-affinity transporters sequestered Na^+ in roots while promoting K^+, Ca^{2+}, and Mg^{2+} release to shoots. This demonstrates that GA_3 facilitates selective nutrient transport, improves inter-organ ion balance, and enhances salt resistance, consistent with findings in jujube (Ma et al., 2020), sorghum (Nimir et al., 2017), and spinach (Gong et al., 2022).

Salt stress in Paulownia primarily causes osmotic imbalance and ion toxicity. As the first organ contacting salt, roots experience impaired water and nutrient absorption, leading to Na^+ accumulation in leaves, photosynthesis inhibition, and reduced growth and dry matter accumulation. Exogenous GA_3 effectively mitigates these effects. Correlation analysis revealed extremely significant negative correlations between MDA and SOD, CAT, Chl(a+b), Pn, Ci, and K^+/Na^+ ($P<0.01$), suggesting that high MDA reduces antioxidant enzyme activity, impairs photosynthesis, disrupts ion balance, and inhibits seedling development. Given the complexity and concentration-dependent effects of GA_3 on seedling physiology, PCA analysis confirmed that 400 $mg \cdot L^{-1}$ GA_3 provided optimal alleviation and strongest salt tolerance. GA_3 primarily enhances resistance by promoting dry matter accumulation, chlorophyll synthesis, photosynthesis, and osmotic balance while reducing Na^+ toxicity.

Conclusion

In summary, 150 $mmol \cdot L^{-1}$ NaCl significantly inhibited Paulownia seedling growth by over 50%. Foliar GA_3 application enhanced antioxidant enzyme activity, alleviated membrane lipid peroxidation from excessive ROS, regulated pigment content and photosynthetic gas parameters to relieve photosynthetic inhibition, promoted photosynthate accumulation and biomass increase, and modulated ion transport to reduce Na^+ content. Comprehensive correlation and PCA analyses identified 400 $mg \cdot L^{-1}$ GA_3 as the optimal concentration, increasing seedling dry weight by 69.71%. This study elucidates Paulownia salt damage mechanisms and GA_3 regulatory pathways, providing a basis for Paulownia utilization in saline soils.

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Note: Figure translations are in progress. See original paper for figures.