forests

MDPI

Article

Impact of Melatonin on Antioxidant Enzymes and Soluble Metabolites in Salt–Alkali-Stressed Poplar (*Populus* spp.): A Comparative Study of Pretreatment and Post-Treatment Effects

Jiefei Nai [1], Wanpeng He [1], Tieming Ma [1], Xidong Han [2], Zhenxing Luo [2], Xinyu Li [3], Jiatong Sun [4] and Xiyang Zhao [1,*]

[1] Jilin Provincial Key Laboratory of Tree and Grass Genetics and Breeding, College of Forestry and Grassland, Jilin Agricultural University, Xincheng Street No. 2888, Changchun 130118, China; naijiefei@163.com (J.N.); 13369401916@163.com (W.H.); tiemingart@jlau.edu.cn (T.M.)
[2] Baicheng Academy of Forestry, Baicheng 137000, China; xidong@163.com (X.H.); luozhenxing@163.com (Z.L.)
[3] Institute of Information, Liaoning Academy of Agricultural Sciences, Shenyang 110161, China; lixy@163.com
[4] Liaoning Institute of Forest Management, Shenyang 110161, China; sunjtt89@163.com
* Correspondence: zhaoxyphd@163.com

Abstract

Melatonin plays a crucial role in modulating plant stress responses; however, its potential for mitigating salt–alkali stress remains incompletely understood. This study evaluates the efficacy of exogenous melatonin in alleviating moderate salt–alkali stress (120 mM) in poplar (*Populus davidiana* × *P. bolleana* 'Baicheng Shanxinyang No. 1') seedlings, investigating both pre- and post-stress treatments across a concentration range of 0–1000 μM. Physiological and morphological parameters, including chlorophyll content, antioxidant enzyme activities, and osmolyte accumulation, were analyzed to assess stress responses. Under salt–alkali stress, seedlings exhibited elevated stress markers and osmolyte levels, reflecting activated stress responses. Melatonin at concentrations of 200–400 μM was the most effective in mitigating stress, significantly enhancing antioxidant enzyme activities such as superoxide dismutase (SOD) and catalase (CAT), restoring chlorophyll content, and reducing oxidative damage markers such as malondialdehyde (MDA). It also regulated osmotic balance in leaves, indicating improved cellular stability under stress. Notably, post-stress application required slightly higher melatonin concentrations to achieve comparable recovery, highlighting the critical influence of application timing. These findings provide valuable insights for optimizing melatonin use to improve poplar growth in saline–alkali environments and support molecular breeding efforts aimed at developing salt–alkali-tolerant poplar varieties.

Keywords: melatonin; salt–alkali stress; antioxidant enzymes; soluble metabolites; poplar plant

Check for updates

Academic Editors: Janusz Zwiazek and Lazar Kesić

Received: 15 January 2026
Revised: 5 March 2026
Accepted: 9 March 2026
Published: 16 March 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Soil salinization, exacerbated by human activities and climate change, poses a critical threat to global agriculture. Recent studies indicate that over half of the world's arable land is at risk of salinization and desertification [1], with the FAO reporting more than 1.4 billion hectares affected by salinity, alkalinity, or their combined effects. These conditions generate saline–alkali stress, a synergistic phenomenon where salt and alkali stresses coexist, inflicting greater harm on plants than either stress alone. Saline–alkali stress

damages plants through four primary mechanisms: osmotic stress oxidative stress [2], osmotic stress [3], ion toxicity [4], and high pH stress [5]. Elevated sodium ion (Na^+) concentrations and high alkaline pH disrupt cellular functions, impair water uptake, and interfere with essential metabolic processes [6,7]. High salt levels increase soil osmotic pressure, inducing "physiological drought", a condition in which plants cannot absorb water despite its presence. This leads to cellular dehydration, wilting, and, in severe cases, death [8]. Moreover, saline–alkali conditions destabilize the redox balance in plant cells, triggering excessive production of reactive oxygen species (ROS). The overaccumulation of ROS damages cellular structures, exacerbates oxidative stress, and further compromises plant health. These multifaceted stressors collectively reduce agricultural productivity, underscoring the urgency of developing adaptive strategies to mitigate soil degradation and enhance crop resilience.

Melatonin (MEL) functions as a potent antioxidant in plants, effectively mitigating oxidative damage by scavenging reactive oxygen species (ROS) and maintaining cellular redox balance [9]. However, under stress conditions, the excessive accumulation of reactive oxygen species (ROS) disrupts chlorophyll (Chl) structure, impairs light absorption, and reduces photosynthetic efficiency. In addition, ROS-induced lipid peroxidation generates toxic by-products such as malondialdehyde (MDA), which further inhibit growth and physiological performance in species such as poplar [10]. To counteract these detrimental effects, plants activate a range of defense mechanisms, including the upregulation of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). Additionally, they enhance the accumulation of osmolytes, including soluble sugars (sSUG) and soluble proteins (sPRO), to mitigate oxidative and osmotic stress [11,12].

Poplar (*Populus* L.), a keystone genus in the *Salicaceae* family, is a high-value domesticated tree cultivated globally for woody biomass production, with plantations spanning ~31.4 million hectares. Comprising over 100 classified species such as *Tacamahaca* and *Aigeiros* [13], poplar is prized for its rapid growth, stress resilience, and ecological versatility, making it vital for industrial timber and reforestation [14]. However, sensitivity to soil conditions, especially salt–alkali stress, is a major limitation for agricultural and forestry productivity. This stress disrupts ion balance, damages cell membranes, and interferes with photosynthesis and metabolic processes, often resulting in stunted growth or plant death [15].

While melatonin (MEL) has been shown to enhance antioxidant enzyme activity and soluble metabolites in poplar trees under salt stress, its effectiveness in addressing the more complex and ecologically significant combined salt–alkali stress remains largely unexplored. To date, few studies have examined the effects of melatonin on the hybrid poplar *Populus cathayana* × *canadensis* 'Xinlin 1' under combined salt–alkali stress [16,17]. However, further research is essential to fully understand the effects of melatonin before and after exposure to salt and alkali stress. The objective of this study is to investigate the effects of exogenous melatonin on key physiological and biochemical parameters, including chlorophyll (Chl) content, malondialdehyde (MDA) levels, soluble sugars (sSUG), soluble proteins (sPRO), and the activity of antioxidant enzymes (SOD, POD, CAT) under salt and alkali stress. The effects of different concentrations of melatonin on the growth and physiology of plants were analyzed, and a melatonin concentration which could promote the accumulation of plant biomass, reduce the damage of cell membranes, enhance the regulation ability of antioxidant systems, and significantly alleviate the damage caused by saline alkali stress on plants was obtained. At the same time, two different application methods of melatonin were compared to confirm that the best planting method of this material in saline–alkali soil was to carry out melatonin pretreatment in advance. The results are expected to inform

strategies for cultivating poplars in saline and alkaline soils, thereby supporting sustainable timber production and environmental restoration.

2. Materials and Methods

2.1. Plant Material and Treatments

In this study, we selected *Populus davidiana* × *P. bolleana* 'Baicheng Shanxinyang No. 1' developed by the Baicheng Forestry Research Institute in Jilin Province, China.

A pot experiment was conducted as follows. First, a layer of non-woven fabric was placed at the bottom of each plastic pot. Sterilized peat soil and vermiculite were then mixed in a 3:1 ratio to prepare the potting substrate. The plastic pots measured 20 cm in both height and upper diameter, with the substrate filled to the rim. One- or two-year-old seedlings free of pests and diseases with an average diameter of 0.8–1.0 cm and a height exceeding 30 cm (average 38–40 cm) were selected for the experiment.

2.2. Experimental Design

The experiment consisted of two main groups: one received melatonin treatment prior to salt–alkali stress, while the other was exposed to salt–alkali stress before melatonin treatment. Each group was further divided into six treatments based on different melatonin concentrations. For each treatment, six pots were placed in the same tray, with these six pots serving as replicates for that treatment. Melatonin was obtained from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China) with a purity of 98%. For preparation, melatonin was first dissolved in 1 mL of 95% alcohol and then diluted with distilled water to achieve six different concentrations: 0, 200, 400, 600, 800, and 1000 μM (0 μM consisted of only 1 mL of alcohol and distilled water). In this experiment, 0 μM served as the control group (CK), and the alcohol content in all melatonin solutions was maintained below 0.1%. The six concentrations of exogenous melatonin were applied to the leaves using an agricultural lithium battery high-pressure cyclone sprayer (Dv0.5 < 500 μm) (Chaonongli Sprayer (Chaonongli (Zhejiang) Intelligent Technology Co., Ltd., Ningbo, China)). 120 mM NaCl solutions and 120 mM Na_2CO_3 solutions were prepared using distilled water (NaCl and Na_2CO_3 were sourced from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China), with a purity of 99.5%). The above two solutions were mixed at a ratio of 1:1 by volume to form a mixed salt solution (κ = 18.74 mS/cm, Ph = 10.74) (mixed salt solutions of other concentrations can also be prepared according to this method). In order to better simulate the local soil environment of Baicheng City (pH 7.5~8.5), the pH of the soil in each pot was detected. The detection site in the pot was 7 cm from the soil in the pot. Five sites were set up in each pot to be assayed. Distilled water was added to the test sites until the bottom was in a muddy state. The pH meter probe was inserted to check the soil pH in the pot, and we waited until the average pH in each pot reached 8.0 (κ = 1.87 mS/cm).

During the experiment, when the subjects were subjected to saline–alkali stress, each sample received 100 milliliters of a 120 mM/L NaCl and Na_2CO_3 compound stress solution and a nutrient solution. When treated with melatonin, six concentrations of exogenous melatonin solution were sprayed onto the leaves with an air atomizing nozzle to ensure that the entire leaf was covered.

During the sampling process, the second topmost leaf of each sample was collected, followed by sampling every 4 days for 5 rounds. Fresh samples were frozen in liquid nitrogen immediately after collection and then stored at −80 °C in a refrigerator until use. The specific treatment duration is shown in Figure 1.

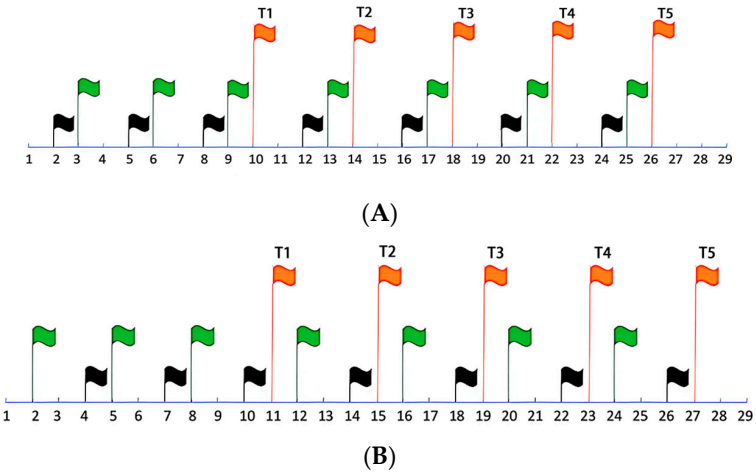

Figure 1. Schematic representation of the experimental setup: black flags indicate samples treated with melatonin (applicable to all concentrations), green flags indicate samples exposed to NaCl and Na_2CO_3, and orange flags indicate points of sample collection. T1 to T5 denote the timing and order of sample collection. (**A**) Melatonin treatment followed by saline–alkali stress; (**B**) First, saline–alkali stress and then melatonin treatment.

2.3. Index Determination

In the experiment, we utilized kits provided by Beijing BOX Biotechnology Co., Ltd. (Beijing, China) to measure the activities of SOD, POD, CAT, and the contents of MDA, Pro, soluble sugars, soluble proteins, and chlorophyll (see Appendix A for the article number and principle of the kit). The specific measurement process was as follows:

First, the samples (30 Hz, 90 s) were ground to powder using a grinder (MM400, Retsch) (Retsch, Diisseldorf, Germany). Then, 100 mg of the powder was weighed from each sample and dissolved in 1.2 mL of a 70% methanol solution. The supernatant was extracted using a centrifuge (12,000 rpm, 20 min). Then, it was pre-coated with SOD, POD, CAT, MDA, Pro, soluble proteins and soluble sugars. Firstly, the sample (30 Hz, 90 s) was ground into powder using a grinder (MM400, Retsch, Dieseldorf, Germany). Then, 100 milligrams of powder from each sample were weighed and then dissolved in 1.2 milliliters of 70% methanol solution. The supernatant was extracted using a centrifuge (12,000 rpm, 20 min). Then, the supernatant was transferred to the corresponding reagent for reaction. After the reaction was completed, the reaction solution was added to the micropores, and the absorbance (outer diameter value) of the corresponding nanometer wavelength was measured using a microplate reader (Infinite F50) (Tecan Group Ltd., Männedorf, Switzerland).

The conductivity of the samples was measured with a DDSJ-308F conductivity meter (Rex Electric Chemical, Shanghai, China).

2.4. Data Analysis

All indicators were derived from the average values of six biological replicates. Statistical analyses were performed using Microsoft Excel 2017 and SPSS 23 software. All data are presented as mean ± standard error (SE). To assess significant differences among the indicators, we applied two-way ANOVA with a significance level set at 0.05, and the analysis was conducted using ggplot2 (v3.5.0) in R [18].

3. Results

3.1. Effects of Saline–Alkali Stress on Poplar Plant Without Melatonin Treatment

The experiment investigated the effects of mixed salt–alkali stress on annual potted poplar cuttings using treatments of 0, 40, 80, 120, and 160 mM NaCl and Na_2CO_3. Plant

growth indicators were generally suppressed, as shown in Figure 2A–D. Interestingly, leaf area and plant height increased at 160 mM (Figure 2A,D), suggesting a potential reallocation of resources in response to partial mortality. However, increasing stress levels began to inhibit plant height and leaf area at 120 mM (Figure 3) while simultaneously elevating physiological parameters, including antioxidant enzyme activities, malondialdehyde (MDA), soluble sugars, soluble proteins, and conductivity in the second apical leaf, accompanied by a decrease in chlorophyll content (Figure 4A–H). Notably, antioxidant enzyme activities peaked at 120 mM before sharply declining, whereas chlorophyll content consistently decreased with rising stress. These results indicate that 120 mM represents the optimal stress threshold for poplar seedlings, beyond which growth is suppressed and physiological and biochemical defenses are negatively affected.

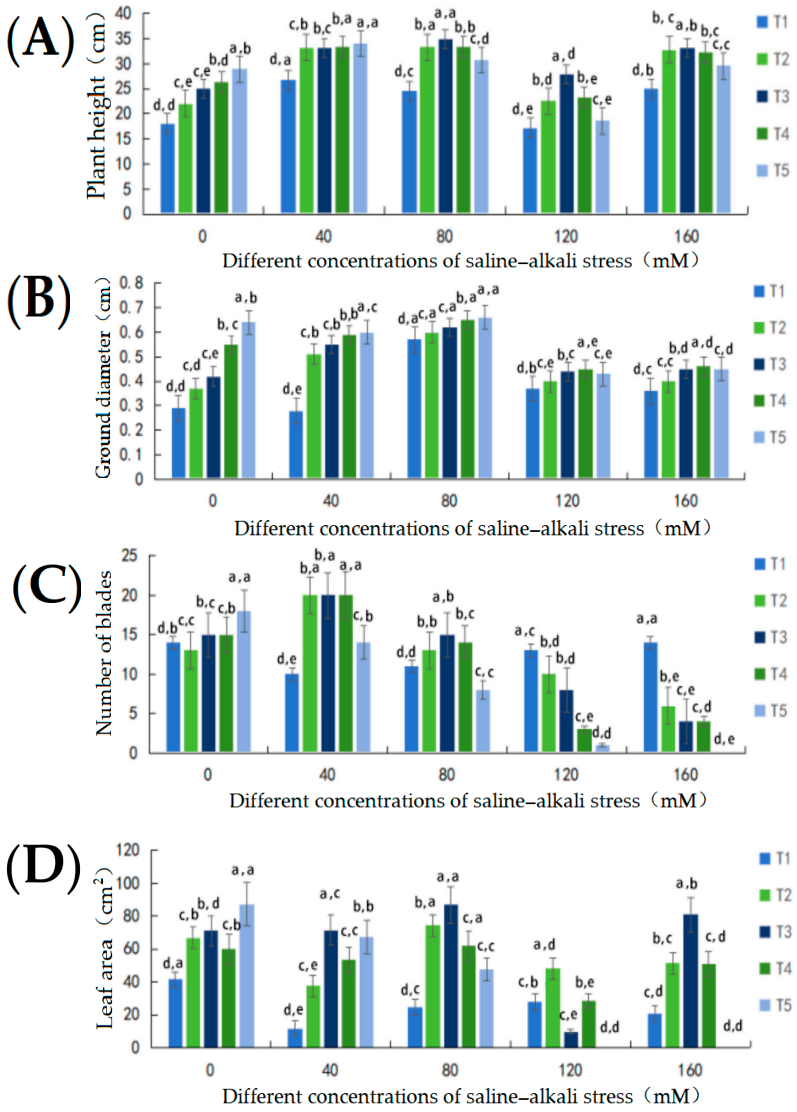

Figure 2. Effects of different salt and alkali treatments on the growth characteristics of *Populus davidiana* × *P. bolleana* 'Baicheng Shanxinyang No. 1'. (**A**) plant height; (**B**) ground diameter; (**C**) number of leaves; (**D**) leaf area. T1—5 days, T2—10 days, T3—15 days, T4—20 days, T5—25 days. All data are presented as mean ± standard error (±SE); error bars represent standard errors (n = 6, with six biological replicates set for each treatment). For two-way ANOVA, the letters on the left represent the detection phase ($p < 0.05$), and the letters on the right represent the experimental concentrations ($p < 0.05$), according to the Duncan test.

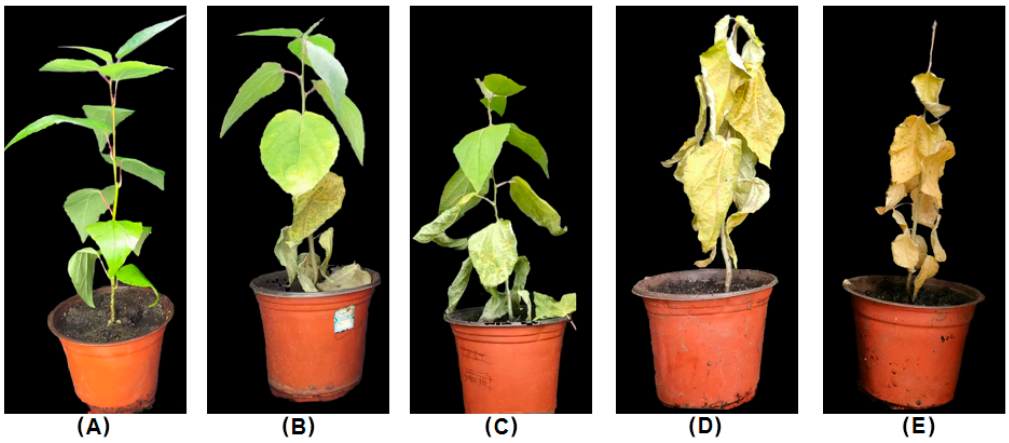

Figure 3. Effects of different salt and alkali treatments on the morphology of *Populus davidiana* × *P. bolleana* 'Baicheng Shanxinyang No. 1'. (**A**–**E**) represent 0, 40, 80, 120, and 160 Mm NaCl-Na_2CO_3 mixed salt–alkali treatment, respectively.

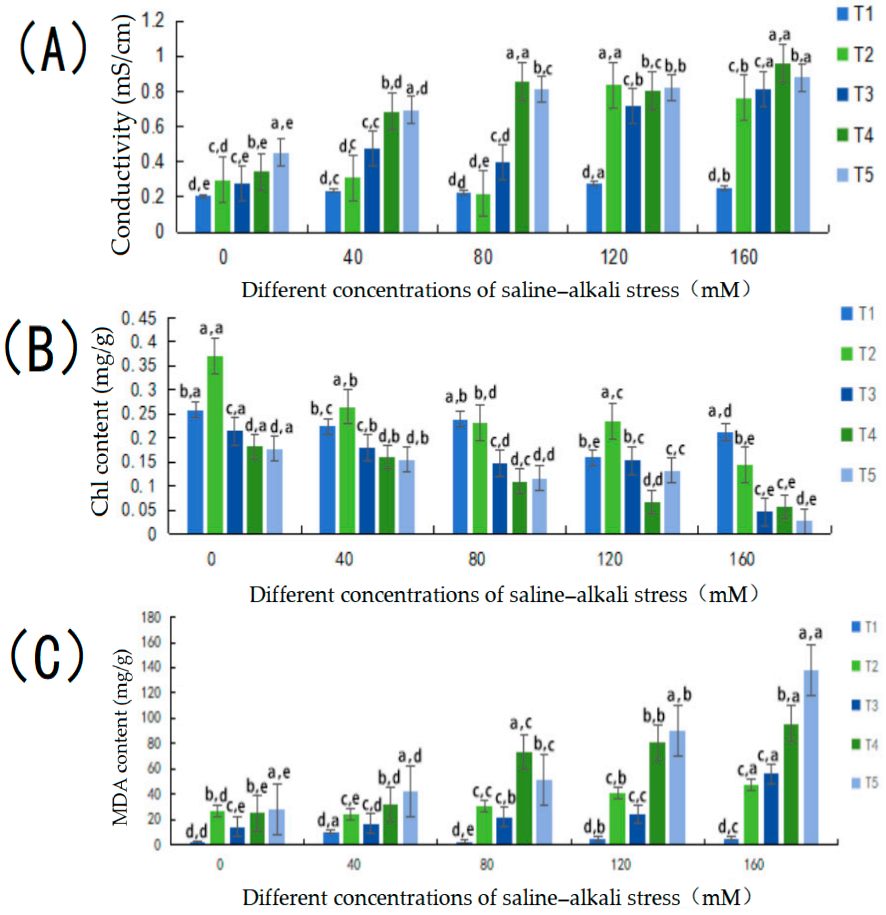

Figure 4. *Cont.*

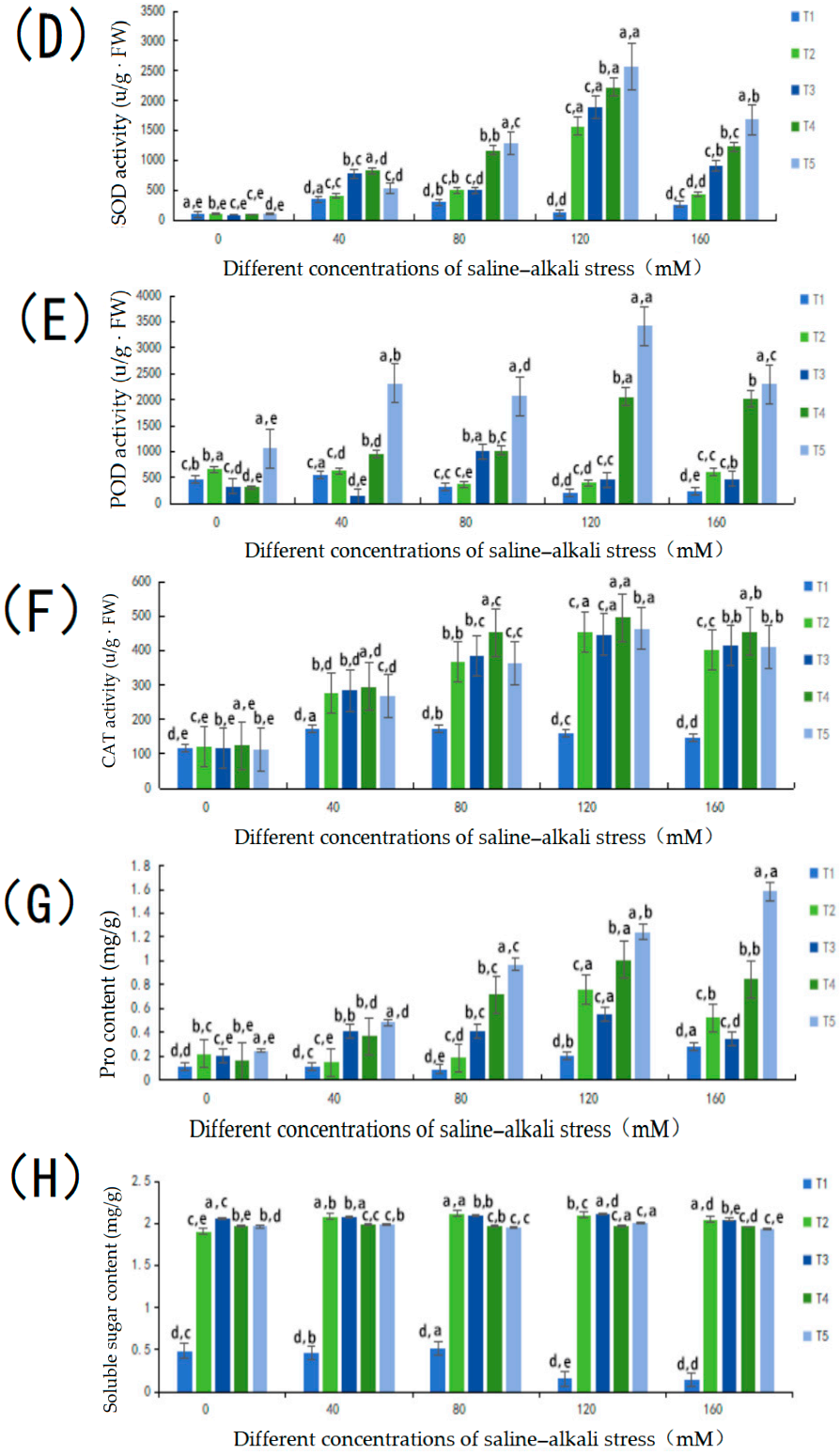

Figure 4. Effects of salt–alkali treatment with different concentrations on physiological indexes of *Populus davidiana* × *P. bolleana* 'Baicheng Shanxinyang No. 1': (**A**) electrical conductivity; (**B**) chlorophyll content; (**C**) malondialdehyde content; (**D**) superoxide dismutase activity; (**E**) peroxidase activity; (**F**) catalase activity; (**G**) soluble protein content; (**H**) soluble sugar content. T1—5 days, T2—10 days, T3—15 days, T4—20 days, T5—25 days. All data are presented as mean ± standard error (±SE), error bars represent standard errors (n = 6, with six biological replicates set for each treatment). For two-way ANOVA, the letters on the left represent the detection phase ($p < 0.05$), and the letters on the right represent the experimental concentrations ($p < 0.05$), according to the Duncan test.

3.2. Effect of Melatonin Pretreatment on Saline–Alkali Stressed Poplar Plant

Populus davidiana × *P. bolleana* 'Baicheng Shanxinyang No. 1' plants were pretreated with melatonin at concentrations of 0, 200, 400, 600, 800, or 1000 μM for 24 h, followed by exposure to 120 mM mixed saline–alkali stress. Morphological and physiological parameters were measured every two days starting at one day post-stress induction. As shown in Figures 5 and 6, melatonin application significantly increased leaf number under stress compared to the stress-only control, with the most obvious effect observed at 400 μM during the T1 and T2 stages (10 and 14 days). This concentration optimally alleviated stress impacts, while efficacy declined at higher doses (1000 μM). In contrast, melatonin pretreatment did not statistically alter ground diameter, plant height, or leaf area. These findings suggest that low melatonin concentrations enhance saline–alkali stress resistance in poplar primarily through modulating leaf development rather than overall growth metrics.

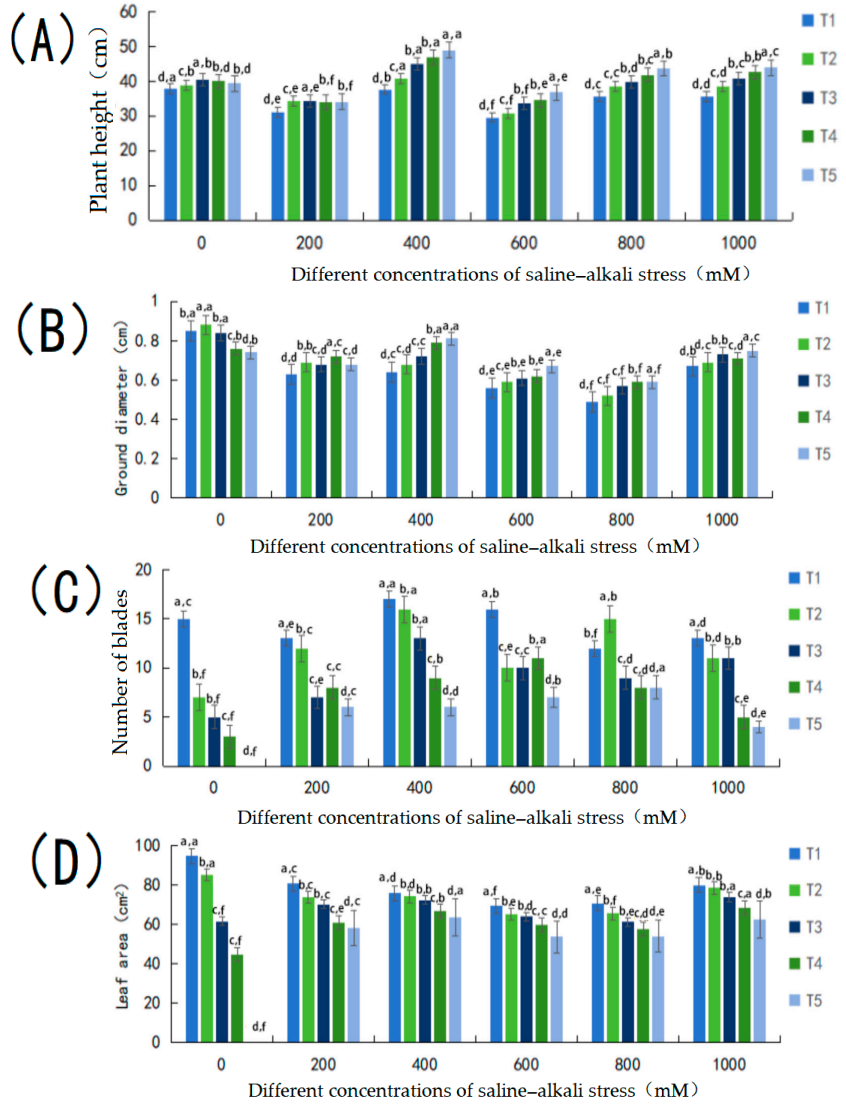

Figure 5. Effects of melatonin pretreatment on morphological indexes of *Populus davidiana* × *P. bolleana* 'Baicheng Shanxinyang No. 1' under saline–alkali stress (**A**) plant height; (**B**) ground diameter; (**C**) number of leaves; (**D**) leaf area. T1—10 days, T2—14 days, T3—18 days, T4—22 days, T5—26 days. All data are presented as mean ± standard error (±SE), error bars represent standard errors (n = 6, with six biological replicates set for each treatment). For two-way ANOVA, the letters on the left represent the detection phase ($p < 0.05$), and the letters on the right represent the experimental concentrations ($p < 0.05$).

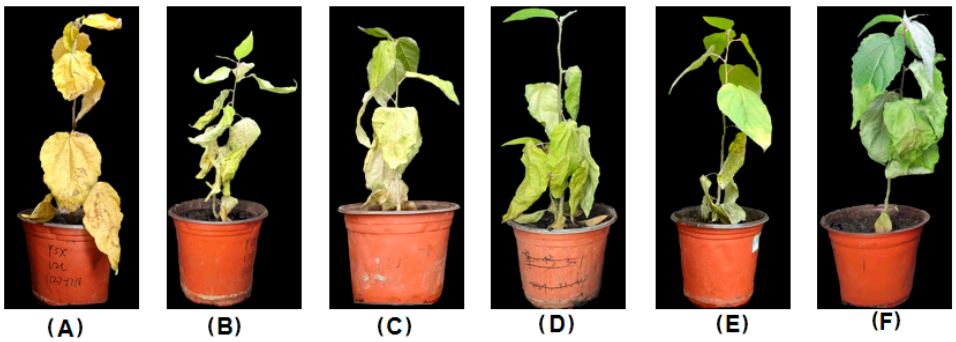

Figure 6. Effects of different melatonin pretreatments on the morphology traits of *Populus davidiana* × *P. bolleana* 'Baicheng Shanxinyang No. 1' under saline–alkali stress. (**A**–**F**) represent 0, 200, 400, 600, 800 and 1000 μM melatonin pretreatment, respectively.

Notably, as illustrated in Figure 7D,E, the activities of SOD and CAT exhibited a biphasic response to melatonin concentration. These activities increased at lower concentrations, reached a peak at 200 μM, and then decreased at higher concentrations (≥400 μM). In contrast, POD activity initially decreased at melatonin concentrations below 200 μM but increased at higher concentrations (Figure 7C), potentially reflecting its dual role in stress adaptation and alternative signaling pathways.

Furthermore, the dynamic changes in soluble metabolites, including sugars and proteins, across the experimental groups at different time points are depicted in Figure 7F,G. Melatonin's influence on osmolyte synthesis was found to be concentration-dependent, exhibiting a biphasic effect: lower concentrations (200 μM) suppressed the accumulation of soluble sugars and proteins, while higher concentrations (≥400 μM) significantly enhanced their production. This suggests that melatonin's role in osmoregulation is dose-specific, likely mediated by its dual function as both a signaling molecule and a stress modulator. The 200 μm melatonin pretreatment was proved to be the best concentration to alleviate plant stress symptoms.

To further evaluate these effects, we systematically analyzed MDA and chlorophyll dynamics across the experimental groups, as shown in Figure 7B. Specifically, MDA levels decreased progressively with increasing melatonin dosage, indicating that exogenous melatonin effectively preserved membrane integrity by reducing ROS-induced lipid degradation. In addition, chlorophyll content, an essential indicator of photosynthetic capacity, also displayed a biphasic response. While no statistically significant changes in chlorophyll levels were observed during pretreatment (Figure 7A), the concentration of 200 μM was noted for its optimal stress-alleviating effect. However, at concentrations exceeding 200 μM, chlorophyll levels declined, indicating potential phytotoxicity or resource reallocation resulting from excessively high melatonin doses.

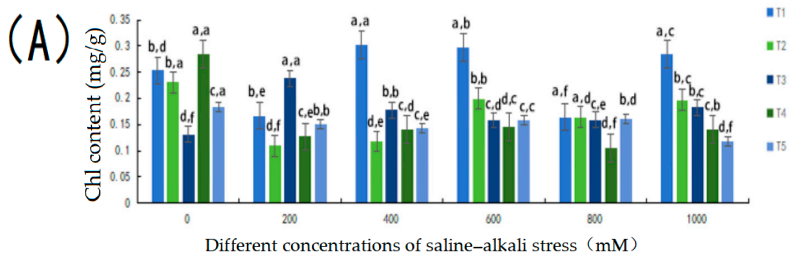

Figure 7. *Cont.*

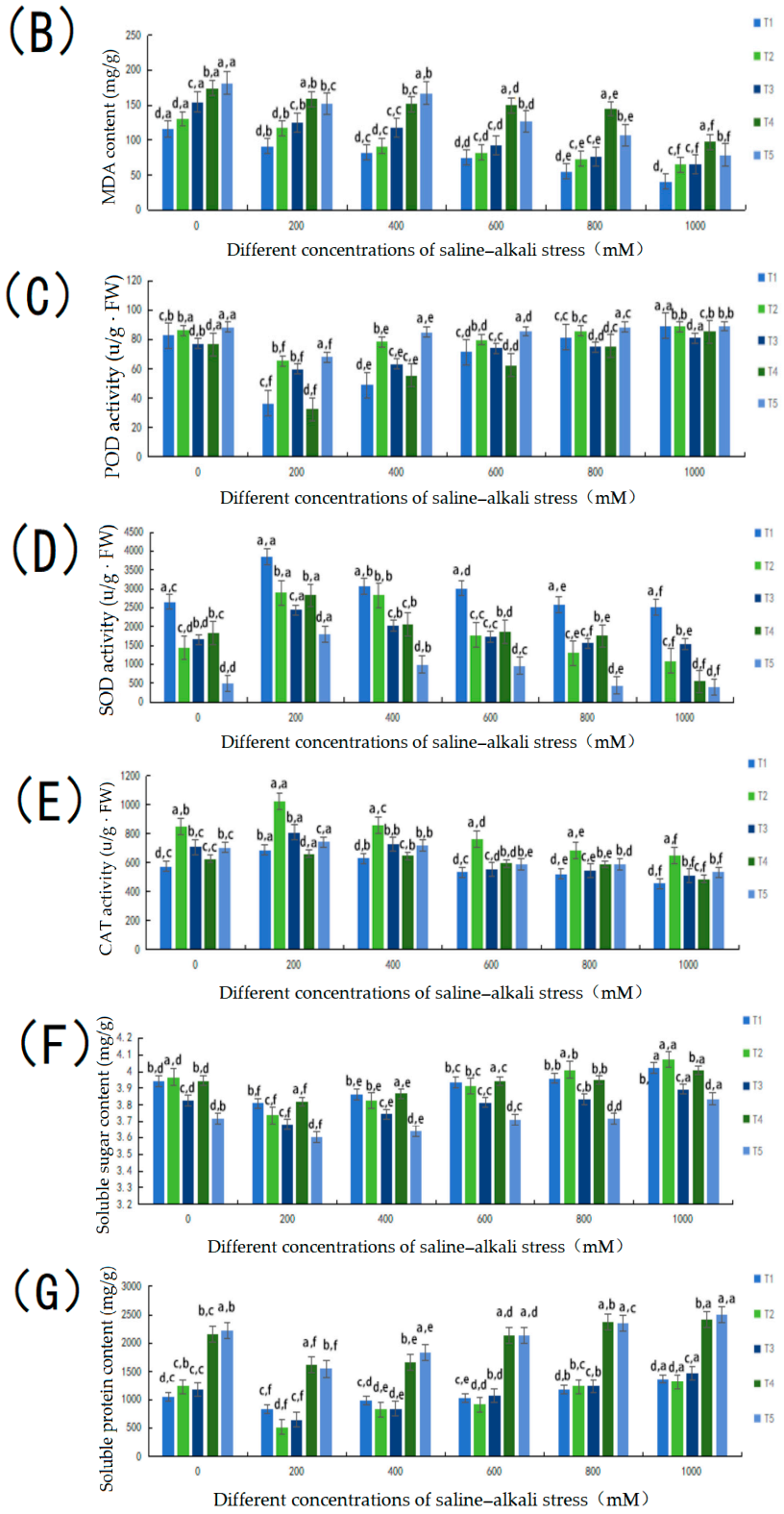

Figure 7. Effects of different concentrations of melatonin pretreatment on physiological characteristics of *Populus davidiana* × *P. bolleana* 'Baicheng Shanxinyang No. 1'. (**A**) chlorophyll content (**B**) malondialdehyde content (**C**) peroxidase activity (**D**) superoxide dismutase activity (**E**) catalase activity (**F**) soluble sugar content. (**G**) soluble protein content. T1—10 days, T2—14 days, T3—18 days, T4—22 days, T5—26 days. All data are presented as mean ± standard error (±SE), error bars represent standard errors (n = 6, with six biological replicates set for each treatment). For two-way ANOVA, the letters on the left represent the detection phase ($p < 0.05$), and the letters on the right represent the experimental concentrations ($p < 0.05$), according to the Duncan test.

3.3. Effect of Melatonin Post-Treatment on Saline–Alkali Stressed Poplar Plant

Following exposure to 120 mM mixed saline–alkali stress, poplar seedlings were treated with melatonin at concentrations of 0, 200, 400, 600, 800, and 1000 μM, and morphological and physiological parameters were assessed. Phenotypic analyses revealed pronounced stress-induced reductions in leaf number and leaf area (Figures 8 and 9), indicative of growth inhibition. Melatonin treatment counteracted these effects in a dose-dependent manner. Seedlings treated with 400–1000 μM melatonin showed significant increases in leaf number and expanded leaf area compared to untreated controls, with consistent stress-mitigating effects across this concentration range. Interestingly, 200 μM melatonin reduced leaf number relative to controls despite promoting an increase in leaf area, highlighting a concentration-dependent variation in morphological responses.

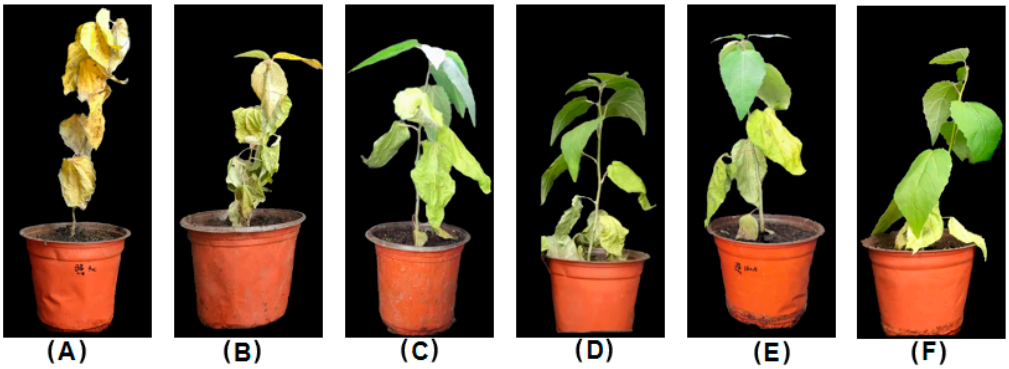

Figure 8. Effects of different concentrations of melatonin on the morphology of *Populus davidiana* × *P. bolleana* 'Baicheng Shanxinyang No. 1' under saline–alkali stress. (**A**–**F**) represent 0, 200, 400, 600, 800 and 1000 μM melatonin treatment, respectively.

Notably, Figure 10 illustrates the temporal variations in antioxidant enzyme activities (SOD, CAT, and POD) across experimental groups under different melatonin treatments. Over the treatment period, SOD and CAT activities exhibited a progressive decline in all groups. However, melatonin application at 200 μM significantly attenuated this reduction, with SOD and CAT levels remaining markedly higher than those in the untreated control group. Specifically, SOD activity peaked during the T1 phase (10 days), while CAT activity reached its maximum value of 1034.18 U/g in the T2 phase (14 days) under the 200 μM treatment (Figure 10C,E). In contrast to SOD and CAT, peroxidase (POD) in the 200 μM melatonin group displayed comparatively lower POD levels than other melatonin-treated groups. Meanwhile, higher melatonin concentrations (600–1000 μM) showed no statistically significant differences in POD activity relative to the control group (Figure 10D).

We also analyzed temporal changes in MDA and chlorophyll content across experimental groups, as shown in Figure 10A,B. MDA levels, a biomarker of oxidative lipid damage, exhibited a biphasic trend in all groups, initially rising before declining post-treatment. Notably, the 1000 μM melatonin group demonstrated the most pronounced stress-alleviating effect, with MDA accumulation reduced to the lowest observed levels at 200 μM. In parallel, chlorophyll content exhibited a distinct dose-dependent pattern. Under 200 μM melatonin treatment, chlorophyll concentrations reached their minimum values; however, a progressive recovery was noted at higher doses (400–1000 μM). This recovery was particularly evident during the T1 phase (10 days), where chlorophyll levels rebounded significantly.

Furthermore, we analyzed osmotic regulators; Figure 10F,G illustrates the temporal dynamics of osmotic regulator content (soluble sugars and proteins) across experimental groups exposed to varying concentrations of melatonin. Soluble sugar levels reached their lowest point on day 25 across all melatonin-treated groups, with the 200 μM group record-

ing the minimum value (3.61 mg g^{-1}). Similarly, under the 200 μM melatonin treatment, soluble protein content dropped to minimal levels of 821.5 μg g^{-1} and 658.2 μg g^{-1} on days 14 and 18, respectively. Importantly, melatonin concentration had a dose-dependent effect on osmotic regulator accumulation. The 200 μM and 400 μM groups consistently showed significantly lower average soluble sugar and slightly reduced protein levels compared to the control group. Conversely, higher melatonin doses (800–1000 μM) resulted in moderately increased osmotic regulator content relative to the control, although these differences were less obvious.

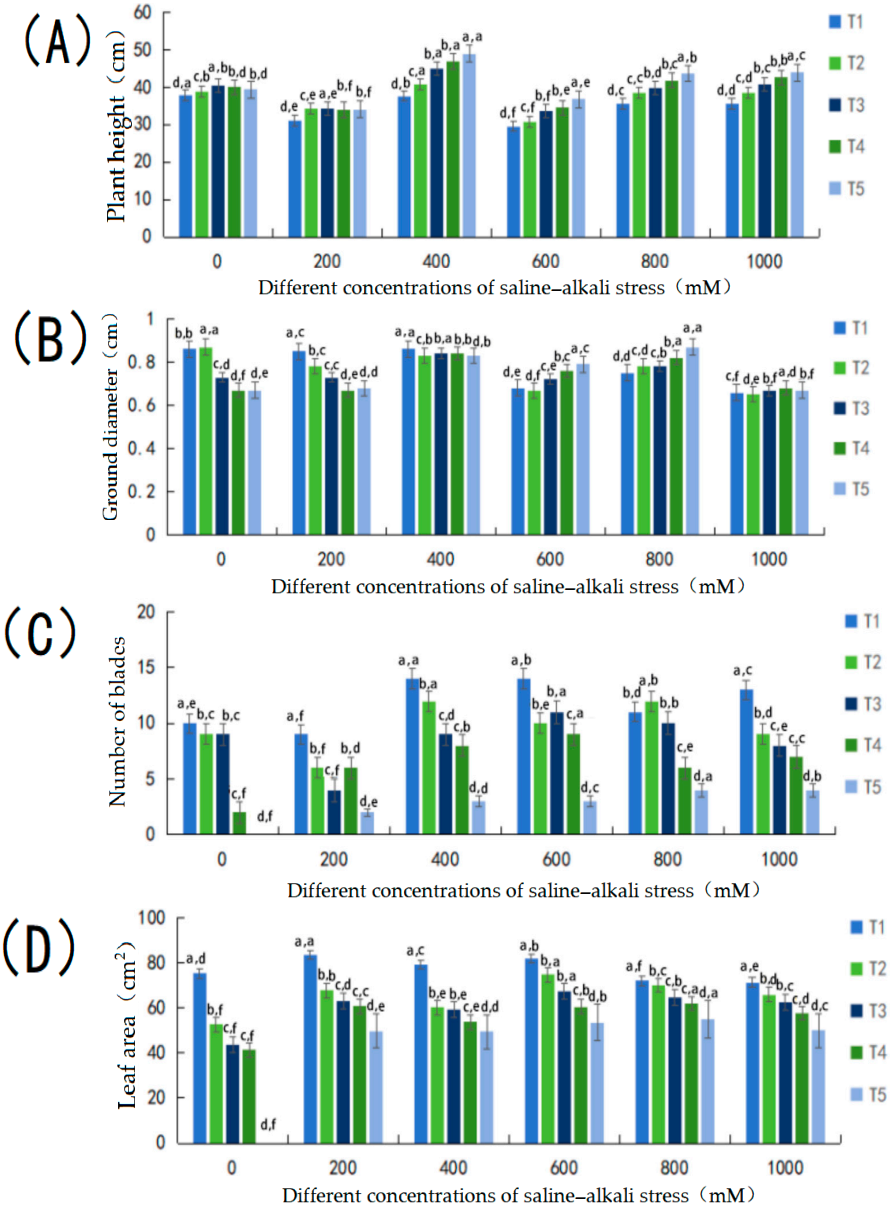

Figure 9. Effects of different concentrations of melatonin on the growth characteristics of *Populus davidiana* × *P. bolleana* 'Baicheng Shanxinyang No. 1' after salt and alkali stress. (**A**). plant height (**B**). ground diameter (**C**). number of leaves (**D**). leaf area. T1—11 days, T2—15 days, T3—19 days, T4—23 days, T5—27 days. All data are presented as mean ± standard error (±SE), error bars represent standard errors (n = 6, with six biological replicates set for each treatment). For two-way ANOVA, the letters on the left represent the detection phase ($p < 0.05$), and the letters on the right represent the experimental concentrations ($p < 0.05$), according to the Duncan test.

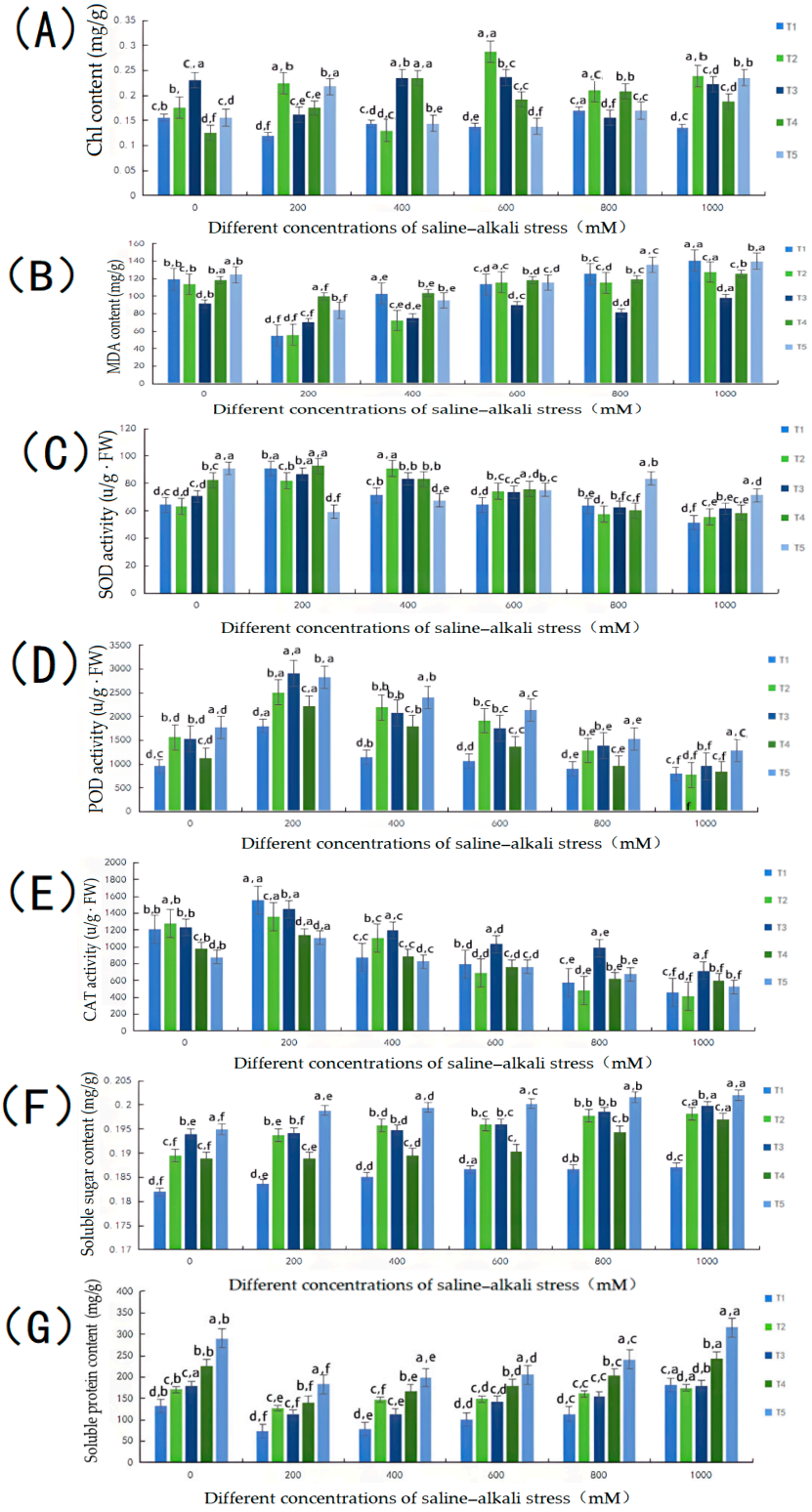

Figure 10. Effects of different concentrations of melatonin treatment on physiological characteristics of *Populus davidiana* × *P. bolleana* 'Baicheng Shanxinyang No. 1' after salt and alkali stress (**A**). chlorophyll content; (**B**). malondialdehyde content; (**C**). peroxidase activity; (**D**). superoxide dismutase activity; (**E**). catalase activity; (**F**). soluble sugar content; (**G**). soluble protein content. T1—11 days, T2—15 days, T3—19 days, T4—23 days, T5—27 days. All data are presented as mean ± standard error (±SE), error bars represent standard errors (n = 6, with six biological replicates set for each treatment). For two-way ANOVA, the letters on the left represent the detection phase ($p < 0.05$), and the letters on the right represent the experimental concentrations ($p < 0.05$), according to the Duncan test.

4. Discussion

4.1. Background, Purpose and Significance of the Study

Among numerous abiotic stresses, salt–alkali stress is one of the most common environmental factors restricting plant growth and development. Long-term exposure to salt–alkali stress can adversely affect plant physiological functions. Melatonin is a multifunctional signaling molecule with the dual capacity to regulate growth dynamics and enhance resilience to abiotic stress [19].

This study systematically investigates the role of melatonin in alleviating salt–alkali stress in poplar (*Populus* spp.), with a focus on its regulatory effects on antioxidant enzyme activity and soluble metabolite profiles. To assess melatonin's stress-mitigating potential, a two-phase experimental design was employed: (1) an initial screening of salt–alkali stress intensity using a range of concentrations (0, 40, 80, 120, 160 mM), and (2) the subsequent application of melatonin at varying doses (0, 200, 400, 600, 800, 1000 μM) under both pre- and post-treatment schedules. The initial stress assessment identified 120 mM as the threshold concentration causing significant physiological disruption (Figure 4), which was subsequently adopted as the baseline stress condition, consistent with previous studies [17]. This concentration was subsequently applied to assess melatonin's efficacy in alleviating stress when administered either before or after exposure to stress. The dual experimental approach comparing stress-only conditions with melatonin-augmented interventions provides a comprehensive understanding of how melatonin influences stress adaptation in poplar.

4.2. Regulation of Melatonin on Antioxidant Enzyme Activity in Poplar

Saline–alkali stress primarily damages plants by disrupting cell membrane integrity and promoting reactive oxygen species (ROS) accumulation. Plants mitigate these effects through antioxidant enzymes such as SOD, CAT, and POD. In this study, melatonin pretreatment enhanced salt–alkali tolerance in poplar seedlings by modulating antioxidant enzyme activity. Under stress, SOD and CAT activities increased (Figure 4D,F) and were further elevated following melatonin application.

Enzyme responses showed a concentration-dependent biphasic pattern. The activities of SOD and CAT peaked at 200 μM melatonin (Figure 7D,E), reflecting enhanced ROS scavenging and a higher upregulation of antioxidant defenses. At higher concentrations ($\geq$400 μM), osmotic imbalance and ROS rebound dominated, and enzyme upregulation was insufficient to prevent membrane and metabolic damage, leading to reduced activity. POD activity initially decreased at 200 μM and rebounded at higher concentrations (Figure 7C), highlighting its complex role in stress signaling. These findings align with previous studies demonstrating that exogenous melatonin can enhance SOD and CAT activity in stressed poplar and apple plants, reducing oxidative damage and stabilizing cell membranes [17,18]. Notably, while post-treatment melatonin slightly increased CAT and POD activities, SOD activity remained unchanged at lower doses, and all enzymes declined at higher concentrations, emphasizing the critical role of dosage optimization. Similarly, managing melatonin as a pretreatment notably improves the salinity tolerance of poplar seedlings. This enhancement is achieved through efficiently scavenging of ROS and improvement of cellular membrane stability, thereby effectively reducing oxidative damage caused by salt [17]. Interestingly, a recent study on the transcriptome and metabolome shown of poplar seedlings has demonstrated that melatonin affects genes and metabolites associated with stress tolerance, with findings indicating that lower concentrations (100 μM) are supported by recent multi-omics studies [16,20]. Furthermore, prior research has shown that melatonin treatment significantly enhances the salt and drought tolerance of rice plants. This effect is realized by strengthening antioxidant defense mechanisms and

upregulating stress-responsive genes, including *OsSOS*, *OsNHX*, *OsHSF*, and *OsDREB*, in rice [21]. Notably, we strongly suggest prioritizing the identification of regulatory genes underlying stress response pathways in poplar plants subjected to abiotic stressors. Similarly, our results align with earlier research indicating that low levels of exogenous melatonin can significantly enhance antioxidant enzyme activity in *Triticum aestivum* and *Pennisetum glaucum* when exposed to salt or drought stress [19,22]. These findings collectively highlight melatonin's potential as a priming agent to enhance stress tolerance, though its concentration-specific effects require further mechanistic investigation.

4.3. Effects of Melatonin on Morphological Indexes of Poplar

Melatonin pretreatment induced distinct morphological responses in poplar seedlings exposed to salt–alkali stress. Plants treated with 400 μM melatonin exhibited significant increases in leaf number and expanded leaf area compared to untreated controls, demonstrating consistent stress-mitigating effects at this concentration (Figure 2C,D). Interestingly, pretreatment with 200 μM melatonin resulted in a reduction in leaf number relative to controls, despite an increase in leaf area, indicating a dose-dependent variation in physiological responses. Furthermore, recent studies have reported that low concentrations of melatonin pretreatment (100 μM) can promote plant growth and alleviate the adverse effects of saline–alkali stress in tomato plants [23]. Furthermore, an early study on cotton (*Gossypium hirsutum*) demonstrated that the combined stress of salt and drought results in a significant reduction in plant growth and chlorophyll content [24].

In contrast, the post-stress application of melatonin produced distinct effects. Under salt–alkali stress, leaf number increased significantly compared to controls exposed only to stress, with the greatest alleviation observed at 400 μM across different growth stages. Higher concentrations, such as 1000 μM, were less effective. Notably, melatonin pretreatment did not significantly affect stem diameter, plant height, or overall leaf area, although subtle similarities between pre- and post-treatment responses were observed. Seedlings exposed to saline–alkali stress showed increases in plant height, stem diameter, leaf number, and leaf area, suggesting that higher melatonin doses may be required post-stress to fully mitigate damage, as stress conditions elevate physiological demands.

These findings highlight the nuanced role of melatonin dosage and timing in modulating morphological adaptations. Optimal stress alleviation was achieved at intermediate concentrations (400 μM), whereas excessive doses reduced beneficial effects. Overall, the results indicate that melatonin's effectiveness in enhancing salt–alkali tolerance depends on balancing its regulatory influence on growth with its capacity to counteract stress-induced oxidative and ionic imbalances.

4.4. Introduction to Research and Discussion

This study revealed the dose-dependent effects of melatonin on osmoregulation in poplar seedlings under salt–alkali stress. Lower melatonin concentrations (200 μM) suppressed the accumulation of soluble sugars and proteins during pretreatment, whereas higher concentrations (≥400 μM) significantly enhanced their production, highlighting melatonin's dual role as both a signaling molecule and a stress modulator in osmoregulation. Notably, the 200 μM pretreatment emerged as the optimal dose for improving stress resilience despite its inhibitory effect on osmolyte levels. A similar pattern was observed following salt–alkali stress: seedlings treated with 200 μM or 400 μM melatonin exhibited consistently lower soluble sugar levels and slightly reduced protein content compared to untreated controls. These findings are consistent with previous work by Song et al. (2022) [17], which demonstrated that low concentrations of exogenous melatonin can significantly influence osmoregulatory activity in plants.

Saline–alkaline stress triggers initial root-derived stress signals that disrupt aboveground plant growth by degrading photosynthetic pigments, accelerating leaf senescence, and reducing photosynthetic capacity. Exogenous melatonin application mitigates these effects by slowing chlorophyll decline and preserving membrane integrity in poplar plants [19]. In this study, we confirmed that melatonin pretreatment significantly reduced MDA levels, a marker of oxidative membrane damage, with the lowest MDA observed at 200 μM. A recent study has demonstrated that pretreatment with melatonin reduces MDA content in rice plants under salt stress [25]. Conversely, post-stress melatonin supplementation showed concentration-dependent efficacy: MDA levels decreased progressively with higher doses, with the 1000 μM treatment group showing the most significant alleviation of oxidative stress. In parallel, pretreatment with melatonin did not statistically alter chlorophyll levels; however, post-treatment application under stress revealed a dose-dependent recovery. At 200 μM, chlorophyll concentrations dropped to minimal levels, yet higher doses (400–1000 μM) induced progressive recovery, particularly during the T2 phase (14 days), where chlorophyll rebounded significantly. This suggests that post-stress conditions require higher melatonin concentrations to counteract severe oxidative damage as stress intensifies physiological demands. Notably, melatonin's ability to stabilize membranes and scavenge free radicals aligns with previous findings [16,17,23], where lower concentrations (100 μM) alleviated stress by reducing cellular damage. Our results highlight melatonin's dual role in osmoregulation and antioxidant defense, demonstrating optimal efficacy at 400–1000 μM post-stress. This concentration enhances the synthesis of photosynthetic pigments and enhances resilience by counteracting chlorophyll degradation and oxidative damage [26]. These findings emphasize the significance of dosage and timing in melatonin's protective function, indicating its potential as a targeted intervention for enhancing plant tolerance under saline–alkaline stress.

5. Conclusions

Overall, we evaluated two different melatonin application strategies: pretreatment before exposure to saline–alkali conditions and post-treatment after planting in saline–alkali soil. The aim was to identify an effective growth approach for poplar trees in saline–alkali environments and reduce the challenges associated with soil amelioration. Our findings demonstrate that exogenous melatonin is a promising strategy for enhancing salt–alkali stress tolerance in poplar seedlings. It notably improves plant growth, antioxidant enzyme activity (SOD, CAT), and osmoregulatory capacity, collectively enhancing ROS scavenging and restoring the growth and development of salt–alkali-stressed seedlings by strengthening physiological and morphological resistance. While pretreatment offers a cost-effective approach to bolster stress resistance, post-treatment supplementation also alleviates stress effects, further enhancing tolerance and reducing potential agricultural losses. These dual-phase benefits underscore melatonin's versatility as a protective agent, providing both preventive and reparative mechanisms to mitigate stress-induced damage, as illustrated in the proposed model (Figure 11).

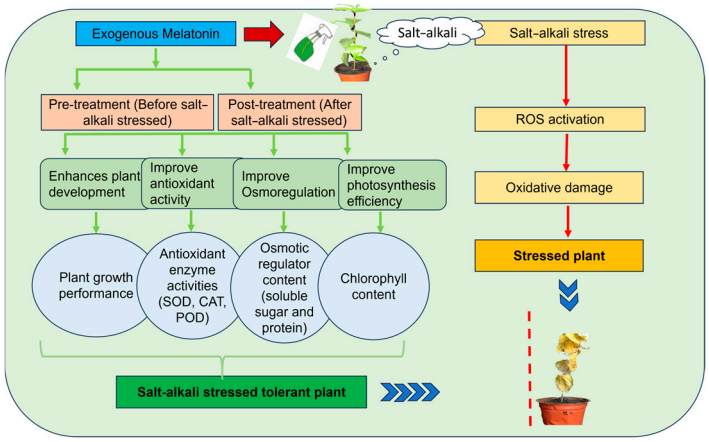

Figure 11. Proposed model for the response of *Populus davidiana* × *P. bolleana* 'Baicheng Shanxinyang No. 1' to melatonin under salt–alkali stress.

Author Contributions: Conceptualization, J.N. and X.Z.; methodology, J.N. and X.Z.; validation, W.H. and X.Z.; formal analysis, J.N., X.Z., W.H., T.M., X.H., Z.L., X.L. and J.S.; data curation, J.N. and X.Z.; writing original draft preparation, J.N., X.H., X.L. and J.S.; writing review and editing, J.N., W.H., T.M., Z.L., X.L. and J.S.; visualization, X.Z.; project administration, X.Z.; funding acquisition X.Z. All authors have read and agreed to the published version of the manuscript.

Funding: We are grateful to the researchers who are contributing to this field. This paper was funded by the Scientific Research Project of the Education Department of Jilin Province (JJKH20250597KJ).

Data Availability Statement: Research data are available upon request from the corresponding author.

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

Appendix A

Name	Catalog Number	Principle
Superoxide dismutase (SOD) kits	AKAO001M-50S	The reaction system of xanthine and xanthine oxidase produces superoxide anion (O_2^-), which reduces nitroblue tetrazolium to form formazan. The product has a characteristic absorption peak at 560 nm. SOD activity is characterized by changes in absorbance.
Peroxidase (POD) kits	AKAO005M	Peroxidase catalyzes the oxidation of guaiacol by H_2O_2 to produce brown 4-hydroxyphenol. The product has a characteristic absorption peak at 470 nm. POD activity is characterized by changes in absorbance.
Catalase (CAT) kits	AKAO003-2M	H_2O_2 oxidizes MoO_4^{2-} to MoO_5^{2-}, which accepts electrons from hydroxide ions, dehydrates and condenses to form a stable yellow complex $(H_2MoO_4 \cdot \times H_2O)_n$. The product has a characteristic absorption peak at 405 nm. CAT activity is characterized by changes in absorbance.
Malondialdehyde (MDA) kits	AKFA013M	Under acidic and high-temperature conditions, malondialdehyde reacts with thiobarbituric acid (TBA) to form a red product. MDA content is quantified by absorbance values at 532 nm, 450 nm, and 600 nm.

Name	Catalog Number	Principle
Proline (Pro) kits	AKAM003M	Proline is extracted by sulfosalicylic acid and reacts with acidic ninhydrin to form a red substance. After extraction with toluene, proline content is quantified by absorbance at 520 nm.
Soluble sugar kits	AKPL008M	Under sulfuric acid, sugars dehydrate to form furfural or hydroxymethylfurfural, which condenses with anthrone to form a blue-green derivative. The product has a characteristic absorption peak at 620 nm. Soluble sugar content is quantified by changes in absorbance.
Soluble protein kits	AKPR001M	Proteins form a purple complex with biuret reagent in a strong alkaline solution. The product has a characteristic absorption peak at 540 nm. Soluble protein content is quantified by changes in absorbance.
Chlorophyll kits	AKPL003M	Chlorophyll a and chlorophyll b have characteristic absorption peaks at 663 nm and 645 nm, respectively. Contents of chlorophyll a, chlorophyll b, and total chlorophyll are calculated using empirical formulas.

References

1. Tarolli, P.; Luo, J.; Park, E.; Barcaccia, G.; Masin, R. Soil salinization in agriculture: Mitigation and adaptation strategies combining nature-based solutions and bioengineering. *iScience* **2024**, *27*, 108830. [CrossRef]
2. Xu, Z.; Wang, F.; Ma, Y.; Dang, H.; Hu, X. Transcription Factor SlAREB1 Is Involved in the Antioxidant Regulation under Saline-Alkaline Stress in Tomato. *Antioxidants* **2022**, *11*, 1673. [CrossRef]
3. Hongna, C.; Junmei, S.; Leyuan, T.; Xiaori, H.; Guolin, L.; Xianguo, C. Exogenous Spermidine Priming Mitigates the Osmotic Damage in Germinating Seeds of *Leymus chinensis* Under Salt-Alkali Stress. *Front. Plant Sci.* **2020**, *12*, 701538. [CrossRef]
4. Fan, Y.; Shen, W.-Y.; Vanessa, P.; Cheng, F.-Q. Synergistic effect of Si and K in improving the growth, ion distribution and partitioning of *Lolium perenne* L. under saline-alkali stress. *J. Integr. Agric.* **2021**, *20*, 1660–1673. [CrossRef]
5. Wang, Y.; Wang, J.; Guo, D.; Zhang, H.; Che, Y.; Li, Y.; Tian, B.; Wang, Z.; Sun, G.; Zhang, H. Physiological and comparative transcriptome analysis of leaf response and physiological adaption to saline alkali stress across pH values in alfalfa (*Medicago sativa*). *Plant Physiol. Biochem.* **2021**, *167*, 140–152. [CrossRef] [PubMed]
6. Zhang, H.; Yu, F.; Xie, P.; Sun, S.; Qiao, X.; Tang, S.; Chen, C.; Yang, S.; Mei, C.; Yang, D. A *Gγ* protein regulates alkaline sensitivity in crops. *Science* **2023**, *379*, eade8416. [CrossRef] [PubMed]
7. Zhao, C.; Zhang, H.; Song, C.; Zhu, J.K.; Shabala, S. Mechanisms of Plant Responses and Adaptation to Soil Salinity. *Innovation* **2020**, *1*, 100017. [CrossRef] [PubMed]
8. Wei, T.-J.; Li, G.; Cui, Y.-R.; Xie, J.; Gao, X.-A.; Teng, X.; Zhao, X.-Y.; Guan, F.-C.; Liang, Z.-W. Variation Characteristics of Root Traits of Different Alfalfa Cultivars under Saline-Alkaline Stress and their Relationship with Soil Environmental Factors. *Phyton* **2024**, *93*, 29–43. [CrossRef]
9. Khan, T.A.; Saleem, M.; Fariduddin, Q. Recent advances and mechanistic insights on melatonin-mediated salt stress signaling in plants. *Plant Physiol. Biochem.* **2022**, *188*, 97–107. [CrossRef]
10. Harfouche, A.; Meilan, R.; Altman, A. Molecular and physiological responses to abiotic stress in forest trees and their relevance to tree improvement. *Tree Physiol.* **2014**, *34*, 1181–1198. [CrossRef]
11. Qi, W.; Wang, F.; Ma, L.; Qi, Z.; Liu, S.; Chen, C.; Wu, J.; Wang, P.; Yang, C.; Wu, Y. Physiological and biochemical mechanisms and cytology of cold tolerance in *Brassica napus*. *Front. Plant Sci.* **2020**, *11*, 1241. [CrossRef]
12. Zhao, L.; Yang, T.; Xing, C.; Dong, H.; Qi, K.; Gao, J.; Tao, S.; Wu, J.; Wu, J.; Zhang, S.; et al. The β-amylase PbrBAM3 from pear (*Pyrus betulaefolia*) regulates soluble sugar accumulation and ROS homeostasis in response to cold stress. *Plant Sci.* **2019**, *287*, 110184. [CrossRef]
13. Müller, N.A.; Kersten, B.; Leite Montalvão, A.P.; Mähler, N.; Bernhardsson, C.; Bräutigam, K.; Carracedo Lorenzo, Z.; Hoenicka, H.; Kumar, V.; Mader, M.; et al. A single gene underlies the dynamic evolution of poplar sex determination. *Nat. Plants* **2020**, *6*, 630–637. [CrossRef] [PubMed]
14. Guo, Y.; Wang, S.; Yu, K.; Wang, H.L.; Xu, H.; Song, C.; Zhao, Y.; Wen, J.; Fu, C.; Li, Y.; et al. Manipulating microRNA miR408 enhances both biomass yield and saccharification efficiency in poplar. *Nat Commun.* **2023**, *14*, 4285. [CrossRef]

15. Mei, X.; Dai, T.; Shen, Y. Adaptive strategy of *Nitraria sibirica* to transient salt, alkali and osmotic stresses via the alteration of Na+/K+ fluxes around root tips. *J. For. Res.* **2023**, *34*, 425–432. [CrossRef]
16. Li, Y.; Song, R.; Cai, K.; Pang, Z.; Qian, C.; Xu, S.; Zhang, Y.; Bai, H.; Zhan, W.; Xiao, R. Exploring the molecular mechanisms of melatonin-induced tolerance to salt-alkali stress in *Populus cathayana* × *canadansis* 'Xinlin 1'. *Ind. Crops Prod.* **2024**, *215*, 118638. [CrossRef]
17. Song, R.; Ritonga, F.N.; Yu, H.; Ding, C.; Zhao, X. Effects of exogenous antioxidant melatonin on physiological and biochemical characteristics of *Populus cathayana* × *canadansis* 'xin lin 1'under salt and alkaline stress. *Forests* **2022**, *13*, 1283. [CrossRef]
18. Zhang, Z.; Liu, L.; Li, H.; Zhang, S.; Fu, X.; Zhai, X.; Yang, N.; Shen, J.; Li, R.; Li, D. Exogenous melatonin promotes the salt tolerance by removing active oxygen and maintaining ion balance in wheat (*Triticum aestivum* L.). *Front. Plant Sci.* **2022**, *12*, 787062. [CrossRef]
19. Mc Carthy, D.J.; Campbell, K.R.; Lun, A.T.; Wills, Q.E. Scater: Pre-processing quality control, normalization and visualization of single-cell RNA-seq data in R. *Bioinformatics* **2017**, *33*, 1179–1186. [CrossRef]
20. Xian, X.; Zhang, Z.; Wang, S.; Cheng, J.; Gao, Y.; Ma, N.; Li, C.; Wang, Y. Exogenous melatonin strengthens saline-alkali stress tolerance in apple rootstock M9-T337 seedlings by initiating a variety of physiological and biochemical pathways. *Chem. Biol. Technol. Agric.* **2024**, *11*, 58. [CrossRef]
21. Duan, W.; Lu, B.; Liu, L.; Meng, Y.; Ma, X.; Li, J.; Zhang, K.; Sun, H.; Zhang, Y.; Dong, H. Effects of exogenous melatonin on root physiology, transcriptome and metabolome of cotton seedlings under salt stress. *Int. J. Mol. Sci.* **2022**, *23*, 9456. [CrossRef]
22. Khan, Z.; Jan, R.; Asif, S.; Farooq, M.; Jang, Y.-H.; Kim, E.-G.; Kim, N.; Kim, K.-M. Exogenous melatonin induces salt and drought stress tolerance in rice by promoting plant growth and defense system. *Sci. Rep.* **2024**, *14*, 1214. [CrossRef] [PubMed]
23. Awan, S.A.; Khan, I.; Rizwan, M.; Brestic, M.; Wang, X.; Zhang, X.; Huang, L. Exogenous melatonin regulates the expression pattern of antioxidant-responsive genes, antioxidant enzyme activities, and physio-chemical traits in pearl millet under drought stress. *J. Plant Growth Regul.* **2024**, *43*, 1061–1075. [CrossRef]
24. Dou, J.; Tang, Z.; Yu, J.; Wang, G.; An, W.; Zhang, Y.; Yang, Q. Effects of exogenous melatonin on the growth and photosynthetic characteristics of tomato seedlings under saline-alkali stress. *Sci. Rep.* **2025**, *15*, 5172. [CrossRef] [PubMed]
25. Ibrahim, W.; Qiu, C.W.; Zhang, C.; Cao, F.; Shuijin, Z.; Wu, F. Comparative physiological analysis in the tolerance to salinity and drought individual and combination in two cotton genotypes with contrasting salt tolerance. *Physiol. Plant.* **2019**, *165*, 155–168. [CrossRef]
26. Ubaidillah, M.; Farooq, M.; Kim, K.-M. Enhancing salt tolerance in rice genotypes through exogenous melatonin application by modulating growth patterns and antistress agents. *Sci. Rep.* **2024**, *14*, 25217. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.