Article

Effects of Exogenous Melatonin on the Growth and Physiological Characteristics of *Ginkgo biloba* L. under Salinity Stress Conditions

Dan Zhou [1,2,†], Meng Li [1,3,†], Xiujun Wang [1,2], Haiyan Li [1,4], Zihang Li [1,2] and Qingwei Li [1,2,*]

1   School of Landscape Architecture, Beijing Forestry University, Beijing 100083, China; zhoudan0807@126.com (D.Z.); vslemon@163.com (M.L.); wangxiujun0520@126.com (X.W.); haiyan0117@outlook.com (H.L.); lizihang0920@126.com (Z.L.)
2   State Key Laboratory of Efficient Production of Forest Resources, Beijing 100083, China
3   Henan Zhoukou Meiyuan Ecological Environment Technology Co., Ltd., Zhoukou, 466000, China
4   Guangdong Academy of Forestry, Guangzhou 510520, China
*   Correspondence: lqw6809@bjfu.edu.cn
†   These authors contributed equally to this paper.

Abstract: *Ginkgo* (*Ginkgo biloba* L.) is a cherished relic among plants, commonly planted as a street tree. However, it faces cultivation challenges due to escalating soil salinization and widespread snowmelt application. Therefore, this study used 4-year-old *Ginkgo* seedlings to investigate how exogenous melatonin at varying concentrations affects seedling growth and physiology under salinity stress. The results revealed that appropriate melatonin concentrations (0.02, 0.1 mmol·L^{-1}) significantly mitigated leaf yellowing under different NaCl stress levels. Furthermore, they increased ground diameter, current-year branch growth, relative water concentration, free proline, and soluble sugars in leaves. Melatonin also reduced electrolyte exudation rates, flavonoids, and malonic dialdehyde concentration, while enhancing peroxidase and superoxide dismutase activities. This led to reduced chlorophyll content, photosynthetic rate, stomatal conductance, and transpiration rate, stabilizing intercellular CO_2 concentration, preserving photosynthetic structures, and enhancing photosynthetic rates. Additionally, the decline in the photosynthetic electron transport rate, the effective photochemical quantum yield of PSII, and the potential efficiency of primary conversion of light energy of PSII was alleviated. Minimal fluorescence and the non-photochemical quenching coefficient also improved. However, high melatonin concentration (0.5 mmol·L^{-1}) exacerbated salinity stress. After analyzing composite scores, the 0.02 mmol·L^{-1} melatonin treatment was most effective in alleviating NaCl stress, while the 0.5 mmol·L^{-1} treatment intensified physiological stress under 200 mmol·L^{-1} NaCl stress. Principal component analysis and correlation analysis identified seven physiological indicators (photosynthetic rate, transpiration rate, photosynthetic electron transport rate, minimal fluorescence, superoxide dismutase, free proline, and chlorophyll a) and three growth indicators (ground diameter, branch length, and current-year branch thickness) as key markers for rapid salinity stress assessment in *Ginkgo*. These findings are crucial for addressing challenges associated with snowmelt's impact on roadside *Ginkgo* trees, expanding planting areas, and breeding exceptional salt-tolerant *Ginkgo* varieties.

Keywords: *Ginkgo biloba*; NaCl stress; exogenous melatonin; growth and physiological response

1. Introduction

Soil salinization covers approximately 9.51 million square kilometers, accounting for 7.23% of the world's land area [1]. This growing global concern threatens ecological balance and hinders global agriculture and forestry. *Ginkgo* (*Ginkgo biloba* L.), a member of the *Ginkgo*aceae family, is often referred to as the "golden living fossil" [2], and ranks among the top five street trees worldwide. Unfortunately, the environments in which *Ginkgo* trees

thrive are increasingly affected by salinity stress, which can impede their growth [3,4]. Excessive salinity adversely affects plant life, hindering seed germination [5–8] and overall growth and development [9,10], and disrupting photosynthesis [11]. This results in issues such as compromised dry matter accumulation [12], osmotic stress [13], ionic toxicity [14], and oxidative stress [15,16], potentially leading to metabolic disruptions or plant mortality [17–19]. Research conducted by Bernstein et al. [20] has shown that exposure to 100 mmol/L NaCl stress results in a reduction in the growth zone in sorghum (*Sorghum bicolor*) leaves and a decrease in the maximal cell growth rate within this area. Similarly, investigations by Grieve et al. [21] have found that salt stress leads to a significant shortening in the developmental period of the main stem in wheat (*Triticum aestivum*) by 18 days, along with a reduction in the frequency of leaf primordia formation and a decrease in leaf count. Additionally, studies by Parida et al. [22] have revealed that the mangrove (*Bruguiera parviflora*) species exhibits its highest growth rate under 100 mM NaCl stress, but experiences a marked decline in chlorophyll content (Chlt) at 400 mM NaCl. Furthermore, Chen's research on Ginkgo biloba suspension cells has demonstrated that within a salinity range of 5 mM to 50 mM NaCl, an increase in salt concentration enhances cell growth and flavonoid accumulation, with a peak in dry and fresh cell weight; Chlt, Chla, and Chlb content; and parameters such as Fv/Fm, ΦPSII, qP, and qN at 25 mM NaCl. However, at higher concentrations of 150–175 mM NaCl, the cellular structure is compromised due to osmotic stress caused by high levels of Na^+ and low levels of K^+ [11].

Ginkgo, a versatile and valuable plant species known for its edibility [23], medicinal properties [24], timber resources [25], environmental greening [26], and ornamental appeal [27] holds economic significance [28], traditional medicinal value [29], and ecological benefits [30]. However, as soil salinization and snow-melting agent use increase, *Ginkgo*'s role as a street tree faces threats. Consequently, there is a growing interest in understanding *Ginkgo*'s salinity tolerance mechanisms, especially regarding salinity stress. Current research, primarily on young *Ginkgo* trees, has explored aspects such as chlorophyll and chloroplast protein changes [11], osmoregulation [31], and alterations in antioxidant enzyme systems [32]. While some studies delve into *Ginkgo*'s molecular-level salinity resistance mechanisms, there is a notable scarcity of research into the potential of exogenous hormones like melatonin in alleviating salinity stress in *Ginkgo*. Notably, exogenous melatonin has proven effective in enhancing salinity tolerance in various plant species [33].

Melatonin, recognized as a potent endogenous free radical scavenger, is present in various plant parts, including seeds, roots, stems, leaves, and fruits [34–36], primarily serving as an antioxidant protector against cellular oxidative damage. Whether synthesized within the plant or applied externally, it enhances plant adaptability to diverse adverse stresses [15,37], including drought [38], low temperatures [39], and high temperatures [40]. While melatonin's potential to improve salinity tolerance in plants is well studied, most research has centered on cash crops, with limited attention given to alleviating salinity stress in ornamental plants through melatonin treatment. Furthermore, no studies have explored exogenous melatonin application to *Ginkgo* seedlings under salinity stress conditions. This experiment, conducted on 4-year-old *Ginkgo* seedlings, simulates salinity stress using NaCl and administers melatonin through spraying and watering. The objective is to investigate how different exogenous melatonin treatments impact *Ginkgo* seedling growth and physiology, elucidating the underlying mechanisms to mitigate salinity damage. Additionally, this study aims to address the impact of snow-melting agents on roadside *Ginkgo* trees and provide theoretical support for global planting initiatives.

2. Materials and Methods

2.1. Materials and Experimental Design

The experimental material consisted of uniform four-year-old *Ginkgo* (*Ginkgo biloba* L.) seedlings. The soil used for the experiment was a soil mixture composed of peat, perlite, and vermiculite in a ratio of 3:2:1, which was air-dried and passed through a 5 mm sieve. The soil's organic carbon content was 10.53 mg·g^{-1}, total nitrogen content was 0.64 mg·g^{-1},

and total phosphorus content was 0.53 mg·g^{-1}. The field water-holding capacity of this soil stood at 28.6%, and its bulk density was measured to be 1.04 g·cm^{-3}. In March 2020, *Ginkgo* seedlings with uniform specifications, featuring a ground diameter of 1.8~2 cm and a height of approximately 180 cm, were selected and transplanted into 15 L polyethylene plastic pots. The potting soil consisted of uncontaminated river sand mixed with soil at a 4:1 (*v/v*) ratio to ensure consistent soil weight across all pots. These potted seedlings were placed within a well-ventilated and light-permeable glasshouse at Beijing Forestry University's teaching practice nursery (40°01′ N, 116°35′ E), and diligently maintained for four months. The experimental design followed a completely randomized approach for stress combinations, involving 13 unique treatment combinations. These combinations included varying salinity concentrations and different melatonin concentrations, as shown in Table 1, with each treatment group consisting of five pot replicates. Commencing on 8 July 2020, the NaCl concentration was incrementally increased by 50 mmol·L^{-1} daily until reaching the desired level. Subsequently, the treatment group received saline water (0.5 L per pot) weekly, while the control group (CK) received an equivalent volume of fresh water. To minimize salinity loss, runoff was collected in trays beneath the pots. During the experimental period, soil salinity levels were regularly monitored by periodically sampling and measuring the soil's electrical conductivity to monitor and adjust the salt concentration in the soil, thereby ensuring consistent salinity levels throughout the course of the experiment. After 35 days of salinity stress exposure, melatonin was applied through foliar spraying and soil watering, in four applications at 7-day intervals. The melatonin solution was sprayed to saturation on the foliage, and each pot received 200 mL of water during each treatment. Physiological and photosynthetic measurements were taken before the initial treatment and three days after each subsequent treatment to monitor the response.

Table 1. Composition and concentration of each treatment.

Treatment	Salinity (mmol·L^{-1})	Exogenous Melatonin (mmol·L^{-1})
CK	0	0
N1	50	0
N1M1	50	0.02
N1M2	50	0.1
N1M3	50	0.5
N2	100	0
N2M1	100	0.02
N2M2	100	0.1
N2M3	100	0.5
N3	200	0
N3M1	200	0.02
N3M2	200	0.1
N3M3	200	0.5

Note: N1, N2 and N3 were treated with 50, 100, and 200 mmol·L^{-1} salinity stress, respectively; M1, M2, and M3 were treated with 0.02, 0.1, and 0.5 mmol·L^{-1} exogenous melatonin, respectively.

2.2. Growth Parameters

Before and after melatonin application, measurements were taken for the ground diameter of young *Ginkgo* trees at a position 5 cm from the tree's trunk, measured from the pot surface. The thickness and length of the branches from the current year were also assessed using a tape measure and vernier calipers, with five replications for each treatment. To assess the relative growth during the treatment period, the following formula was applied:

$$\text{Relative growth} = G_1 - G_0 \tag{1}$$

where G_1 corresponds to the measured values of the ground diameter, thickness, and length of the current year's branches at the treatment's conclusion; G_0 corresponds to the measured values of the ground diameter, thickness, and length of the current year's branches taken one day before the treatment commenced.

2.3. Physio-Biochemical Attributes

The determination of leaf malonic dialdehyde concentration (MDA) was conducted following the barbituric acid method outlined by Li [41]. Firstly, 0.2 g of leaves was ground with liquid nitrogen and homogenized using a 5% trichloroacetic acid (TCA) solution. After centrifugation, the resulting supernatant served as the assay solution. In separate test tubes, samples and a blank were prepared, and thiobarbituric acid (TBA) solution was added. Following heating in a boiling water bath and subsequent cooling, the absorbance values of the supernatant were measured at specific wavelengths (450 nm, 532 nm, and 600 nm). The MDA content was calculated according to the following formula:

$$\text{MDA concentration (mg·g}^{-1}) = ((A532 - A600) - 0.56 \times A450) \times VT/(W \times VS) \quad (2)$$

where A532, A600, and A450 represent absorbance values, VT represents the total volume of the extract (mL), VS represents the volume of enzyme liquid used (mL), and W represents the sample's mass in grams.

The electrolyte extravasation rate (EL) was assessed using the conductivity meter method as outlined by Li [41]. *Ginkgo* leaves from the same location were selected and thoroughly rinsed with distilled water. Then, 0.1 g of the leaves was accurately weighed, before being cut into small pieces and placed into a centrifuge tube containing 20 mL of ultrapure water, ensuring the material was completely submerged. The initial conductivity (C_1) was measured using a conductivity meter after allowing the mixture to rest for 3 h. The centrifuge tube was then placed in a boiling water bath and heated for 15 min. After cooling, the final conductivity (C_2) was determined. Then, the relative conductivity was calculated according to the following equation:

$$\text{EL (\%)} = (C_1 - C_0)/(C_2 - C_0) \quad (3)$$

where C_1 represents the initial conductivity (%), C_2 represents the final conductivity (%), and C_0 represents the conductivity of ultrapure water.

The leaf relative water content (RWC) was determined using the drying and weighing method described by Li [41]. Following each stress treatment, the RWC of the leaves from all four plants was measured. The procedure involved removing the leaves from the seedlings in each treatment, thoroughly washing them with deionized water, and then recording their fresh weight (FW). Fresh samples collected were immersed in distilled water for 24 h to achieve full saturation, reaching a turgid state. Subsequently, the samples were removed, superficial moisture was gently blotted off using absorbent paper, and their turgid weight (TW) was measured. This process is critical for assessing the water status of plant samples in physiological studies. Subsequently, the leaves were placed in an oven set to 105 °C for 15 min, after which the oven temperature was adjusted to a consistent 85 °C for complete drying until a constant weight was achieved. Once cool, the leaves were weighed again to obtain their dry weight (DW). The RWC of the plant leaves was calculated using the following formula:

$$\text{RWC(\%)} = (FW - DW)/(TW - DW) \times 100\% \quad (4)$$

where FW represents the fresh weight of the leaves, and DW represents the dry weight of the leaves.

The leaf flavonoid (Fla) concentration was determined using the method described by Li [41]. First, 0.2 g of Ginkgo leaf samples was weighed and ground into a fine powder. Next, the powdered samples were extracted using a suitable solvent like ethanol to extract Fla. Standard solutions of Fla were prepared to create a standard curve. Using a spectrophotometer, the absorbance of both the sample extracts and standard solutions was measured at a specific wavelength. The Fla content in the samples was then calculated based on the

standard curve, taking into account the absorbance values, concentration of fla, and the sample's mass. The Fla of the plant leaves was calculated using the following formula:

$$\text{Fla (mg·g}^{-1}) = (A - B) \times C \times D/W \tag{5}$$

where A represents the absorbance of the sample or standard solution, B represents the absorbance of the blank cuvette, C represents the concentration of Fla from the standard curve (mg·mL^{-1}), D represents the multiplication factor for the total volume, and W represents the mass of the sample (g).

The determination of leaf superoxide dismutase (SOD) antioxidant enzyme activity followed the nitrogen blue tetrazolium photochemical reduction method described by Gao [42]. First, 0.2 g of *Ginkgo* was weighed in a mortar, followed by the addition of 5 mL of 0.05 mol/L phosphate-buffered solution (PBS) with a pH of 7.8. The mixture was ground into a homogenate and then transferred into a centrifuge tube and centrifuged at $10,000 \times g$ for 12 min. The resulting supernatant was used as the enzyme extract for further analysis. After thorough mixing, one control tube was placed in the dark, while the other control tube and a test tube from the measurement group were placed in an artificial climatic chamber with a light intensity of 4000 lx and a temperature of 25 °C for 10 min. Subsequently, the test tubes were removed from the chamber and were immediately covered with a black cloth to terminate the reaction. The control tube kept in the dark served as a blank control, and the absorbance value was measured at 560 nm for each treatment, with each treatment being repeated three times. The change in SOD activity was calculated using the following equation:

$$\text{SOD activity (U·g}^{-1}\text{·min}^{-1}) = (ACK - AE) \times VT/(0.5 \times ACK \times W \times VS) \tag{6}$$

where ACK represents the absorbance value of the illuminated control tube, AE represents the absorbance value of the assay tube, VT represents the total volume of the extract (mL), VS represents the volume of enzyme solution used (mL), and W represents the fresh weight of the leaf (g).

Leaf POD activity was determined following the guaiacol method as described by Li [41]. In a beaker, 50 mL of 0.1 mol/L PBS with a pH of 6.0 was added, along with 28 μL of guaiacol and 19 μL of 30% H_2O_2. The mixture was stirred well to create the reaction preparation solution. Next, 3 mL of the reaction preparation solution was pipetted into a 190 cuvette, immediately followed by the addition of 0.1 mL of the enzyme solution, and mixed. PBS was used as a control. The absorbance value was measured at 470 nm every 30 s for 3 min, and the Δ470 slope value was recorded. This process was repeated three times for each treatment. The change in POD activity per minute was calculated using the following equation:

$$\text{POD activity (μmol·g}^{-1}) = (\Delta470 \times VT)/(W \times VS \times t \times 0.01) \tag{7}$$

where ΔA470 represents the change in absorbance value over 3 min, VT represents the total volume of the extract (mL), VS represents the volume of enzyme solution used (mL), t represents the reaction time (min), and W represents the fresh weight of the sample (g).

The determination of leaf free proline (Pro) followed the acidic ninhydrin colorimetry method described by Li [41]. First, 0.2 g of *Ginkgo* leaves was weighed and placed in a test tube; 5 mL of 3% sulfosalicylic acid solution was then added to the test tube. The tube was placed in a boiling water bath for 30 min. After heating, the solution was cooled and 2 mL of the extract was transferred to another test tube. Subsequently, 2 mL of glacial acetic acid was added to the test tube with the extract, followed by 2 mL of acid ninhydrin. The mixture was thoroughly shaken and the tube was placed back in a boiling water bath for an additional 30 min. After cooling, 4 mL of toluene was added to the test tube, which was then shaken to facilitate extraction. The mixture was allowed to settle, whereby the upper layer of the toluene solution turned red due to the presence of Pro. This upper

layer was aspirated and transferred to a cuvette; toluene was used as a blank control and its absorbance value was measured at 520 nm. The Pro content was calculated using the following formula:

$$\text{Pro concentration (mg·g}^{-1}) = (C \times VT)/(VS \times W \times 1000) \tag{8}$$

where VT represents the total volume of the extract (mL), VS represents the volume of enzyme liquid used (mL), W represents the fresh weight of the sample (g), and C represents the proline content obtained from a standardized curve (μg).

Leaf soluble sugar (SS) was determined using the anthrone colorimetric method as described by Li [41]. A volume of 0.1 mL of the enzyme solution was placed into a test tube, while a control was set up using 0.1 mL of PBS. To each test tube, 5 mL of Khao Maas Brilliant Blue G-250 solution was added; the solutions were then thoroughly mixed and allowed to stand for 5 min. The absorbance value of each solution was measured at 595 nm. The SP content was calculated using the following formula:

$$\text{SS concentration (mg·g}^{-1}) = (C \times VT)/(VS \times W \times 1000) \tag{9}$$

where VT represents the total volume of the extract (mL), VS represents the volume of enzyme liquid used (mL), W represents the fresh weight of leaves (g), and C represents the SP content (μg) obtained from a standard curve.

The method used for determining leaf soluble protein (SP) was readopted from the Thomas Brilliant Blue G-250 staining method as outlined by Li [41]. A volume of 0.1 mL of the enzyme solution was placed into a test tube, while a control was set up using 0.1 mL of PBS. To each test tube, 5 mL of Khao Maas Brilliant Blue G-250 solution was added; the solutions were then thoroughly mixed and allowed to stand for 5 min. The absorbance value of each solution was measured at 595 nm. The SP content was calculated using the following formula:

$$\text{SP concentration (mg·g}^{-1}) = (C \times VT)/(W \times VS \times 1000) \tag{10}$$

where VT represents the total volume of the extract (mL), VS represents the volume of enzyme liquid used (mL), W represents the fresh weight of leaves (g), and C represents the SP content (μg) obtained from a standard curve.

2.4. Photosynthesis Attributes

The total chlorophyll concentration (Chla + Chlb) of the leaves was determined using the acetone–ethanol method as outlined by Li [41]. To start, 0.2 g of *Ginkgo* leaves was cut into test tubes, before adding 5 mL of 80% acetone and 5 mL of 95% ethanol, and sealing the test tubes. The samples were then placed in a shaded area for 24 h, allowing the leaves to leach until they turned completely white. Using the mixture as a blank control, the absorbance values were determined at wavelengths of 663 nm and 645 nm. The Chlt was calculated using the following formula:

$$\text{Chla + Chlb (mg·g}^{-1}) = (20.21A645 + 8.02A663) \times VT/1000W \tag{11}$$

where VT represents the total volume of extract/mL and W represents the fresh weight of the leaf (g).

The photosynthetic parameters of the *Ginkgo* leaves were assessed using a Li-6400 portable photosynthesizer (Li-Cor, Lincoln, NE, USA) and a fluorescent leaf chamber (6400-40). The measurements were conducted with the following parameter settings: leaf temperature (Tleaf) = 25 °C; block temperature (Tblock) = 25 °C; and photosynthetically active radiation (PAR) = 1000 umol·m^{-2}·s^{-1}. The photosynthetic parameters, including the net photosynthetic rate (Pn), intercellular CO_2 concentration (Ci), stomatal conductance (Gs), and transpiration rate (Tr), were determined on days 0, 10, 20, 30, and 40, from 8:00 to 12:00. Each measurement was replicated nine times for each treatment.

Chlorophyll-fluorescence-related parameters, such as minimal fluorescence (Fo) and maximum fluorescence (Fm), were assessed using a German PAM2500 chlorophyll fluorometer. Measurements were taken on days 0, 10, 20, 30, and 40, from 14:00 to 19:00, respectively. Fully functional leaves located at the tips of plant branches were measured. Prior to measurement, a dark treatment with leaf clips was applied for 20 min. The results were averaged, with each measurement replicated three times for each plant.

2.5. Statistical Analysis

Data processing utilized Microsoft Excel 2016, and plot creation was accomplished with Origin 2018 software. One-way ANOVA and canonical correlation analysis (CCA) were conducted using SPSS 26 software, while multiple comparisons were performed using Duncan's complex polar deviation method. All graphs display data as mean $\pm$ standard deviation (mean $\pm$ SD) from five replicates. Adobe Illustrator 2021 was used for graph enhancement and integration.

3. Results

3.1. Effect of Exogenous Melatonin on the Growth of Ginkgo Seedlings under Salinity Stress

Relative to CK, as the NaCl concentration increased, the extent of leaf damage became more pronounced. When compared to the effects of NaCl stress alone, low and medium concentrations of melatonin significantly alleviated the symptoms of leaf yellowing induced by NaCl stress. However, the treatment with a high concentration of melatonin exacerbated leaf damage in the presence of medium and high NaCl concentrations, leading to symptoms such as leaf yellowing and apical dryness (Figure 1).
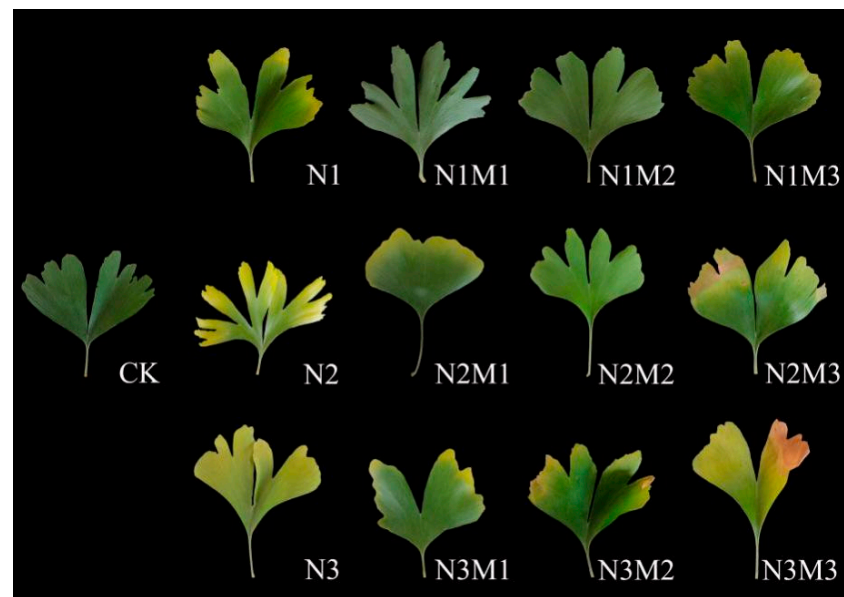

Figure 1. Comparative analysis of *Ginkgo* leaf characteristics following 28 days of treatment. Note: N1, N2, and N3 were treated with 50, 100, and 200 mmol·L^{-1} salinity stress, respectively; M1, M2, and M3 were treated with 0.02, 0.1, and 0.5 mmol·L^{-1} melatonin, respectively.

Varying NaCl stress levels inhibited *Ginkgo* seedling growth, leading to reduced ground diameter, shorter current-year branches, and thinner branches. External melatonin application positively influenced growth parameters, increasing ground diameter, length of current-year branches, and branch coarseness under NaCl stress (Table 2). However, melatonin's overall mitigating effect decreased with higher NaCl stress concentrations, with M1 treatment being the most effective at alleviating these effects.

Table 2. Effects of exogenous melatonin on the growth of *Ginkgo* seedlings under salinity stress.

Level of Stress	Treatments	Soil Salinity Content (mmol·L^{-1})	Ground Diameter (cm)	Branch Length (cm)	Branch Thickness (cm)
Low	CK	(13.587 ± 0.277)	(0.094 ± 0.029)	(0.424 ± 0.153)	(0.028 ± 0.012)
	N1	(59.742 ± 6.525)	(0.069 ± 0.012)	(0.302 ± 0.100)	(0.024 ± 0.004)
	N1M1	(57.336 ± 2.322)	(0.168 ± 0.048)	(0.900 ± 0.094)	(0.067 ± 0.020)
	N1M2	(66.216 ± 10.378)	(0.121 ± 0.027)	(0.792 ± 0.036)	(0.059 ± 0.006)
	N1M3	(47.131 ± 0.714)	(0.078 ± 0.031)	(0.398 ± 0.047)	(0.024 ± 0.008)
Medium	CK	(13.587 ± 0.277)	(0.094 ± 0.029)	(0.424 ± 0.153)	(0.028 ± 0.012)
	N2	(103.479 ± 1.459)	(0.040 ± 0.016)	(0.212 ± 0.076)	(0.016 ± 0.003)
	N2M1	(110.275 ± 3.896)	(0.076 ± 0.018)	(0.886 ± 0.159)	(0.044 ± 0.011)
	N2M2	(89.000 ± 2.031)	(0.080 ± 0.016)	(0.516 ± 0.073)	(0.042 ± 0.006)
	N2M3	(81.385 ± 3.502)	(0.053 ± 0.007)	(0.432 ± 0.099)	(0.024 ± 0.009)
High	CK	(13.587 ± 0.277)	(0.094 ± 0.029)	(0.424 ± 0.153)	(0.028 ± 0.012)
	N3	(159.577 ± 6.661)	(0.028 ± 0.013)	(0.268 ± 0.078)	(0.021 ± 0.004)
	N3M1	(205.015 ± 1.679)	(0.050 ± 0.025)	(0.748 ± 0.119)	(0.045 ± 0.010)
	N3M2	(168.673 ± 8.82)	(0.058 ± 0.028)	(0.568 ± 0.073)	(0.035 ± 0.007)
	N3M3	(134.034 ± 4.653)	(0.018 ± 0.008)	(0.216 ± 0.050)	(0.012 ± 0.003)

Note: N1, N2, and N3 were treated with 50, 100, and 200 mmol·L^{-1} salinity stress, respectively; M1, M2, and M3 were treated with 0.02, 0.1, and 0.5 mmol·L^{-1} melatonin, respectively.

3.2. Effects of Exogenous Melatonin on the Physiological and Biochemical Characteristics of Ginkgo under Salinity Stress

NaCl treatment significantly increased MDA concentration in *Ginkgo* compared to CK. In the CK group, MDA concentration initially increased and then decreased during treatment. However, in both the NaCl stress treatment and NaCl + melatonin treatment groups, there was an initial decrease in MDA concentration, followed by an increase. Under different NaCl stress concentrations, M2 significantly reduced MDA accumulation compared to N1 on the 28th day of treatment, indicating the efficiency of M2 in mitigating MDA accumulation. Conversely, M3 treatment increased MDA accumulation and exacerbated membrane lipid peroxidation, particularly under high NaCl concentrations (Figure 2a).

The EL exhibited an upward trend with increasing salinity stress concentration and followed a pattern of initial decrease and subsequent increase after melatonin treatment. When applying melatonin under low NaCl stress concentration, represented by M1, M2, and M3, a significant reduction in EL was observed. Specifically, under medium NaCl stress concentration, M1 significantly reduced the rate of electrolyte exudation. In the case of high NaCl stress concentration, at the 28th day of treatment, N3 experienced a 23.76% reduction in electrolyte leakage, while M1, M2, and M3 exhibited reductions of 18.55%, 12.52%, and 7.03%, respectively (Figure 2b).

The RWC of leaves in NaCl stress treatments showed a consistent decline with the duration of treatment. Under low and medium NaCl stress concentrations, M1 treatment resulted in leaf RWC values that were 6.22% and 6.23% higher than CK and 4.97% and 4.23% higher than N1 at 21 days of treatment, respectively. Conversely, under high NaCl stress concentration, N3 experienced an 11.33% decrease in leaf RWC at the 28th day of treatment, while the reductions in leaf RWC for the M1, M2, and M3 treatments were 8.27%, 3.16%, and 3.62%, respectively, which were significantly lower than the decrease observed in N3 (Figure 2c).

The Fla concentration in the leaves exhibited a pattern of initial increase and subsequent decrease over the course of treatment, with NaCl treatment promoting the accumulation of Fla in the leaves. Under low NaCl stress concentration, both the M1 and M2 treatments resulted in significantly lower Fla concentration compared to NaCl stress alone ($p < 0.05$). In the case of medium NaCl stress concentration, N2 treatment showed a 14.11% increase in Fla concentration at 28 days of treatment, with only M2 treatment exhibiting a lower increase of 8.58% compared to N2 treatment. Under high NaCl stress concentration, N3 treatment demonstrated a 2.28% increase in Fla concentration at the 28th

day of treatment, whereas the M1, M2, and M3 treatments exhibited substantial increases of 75.18%, 12.99%, and 70.03%, respectively. These increases were significantly higher than the increase in the Fla concentration of N3 (Figure 2d).
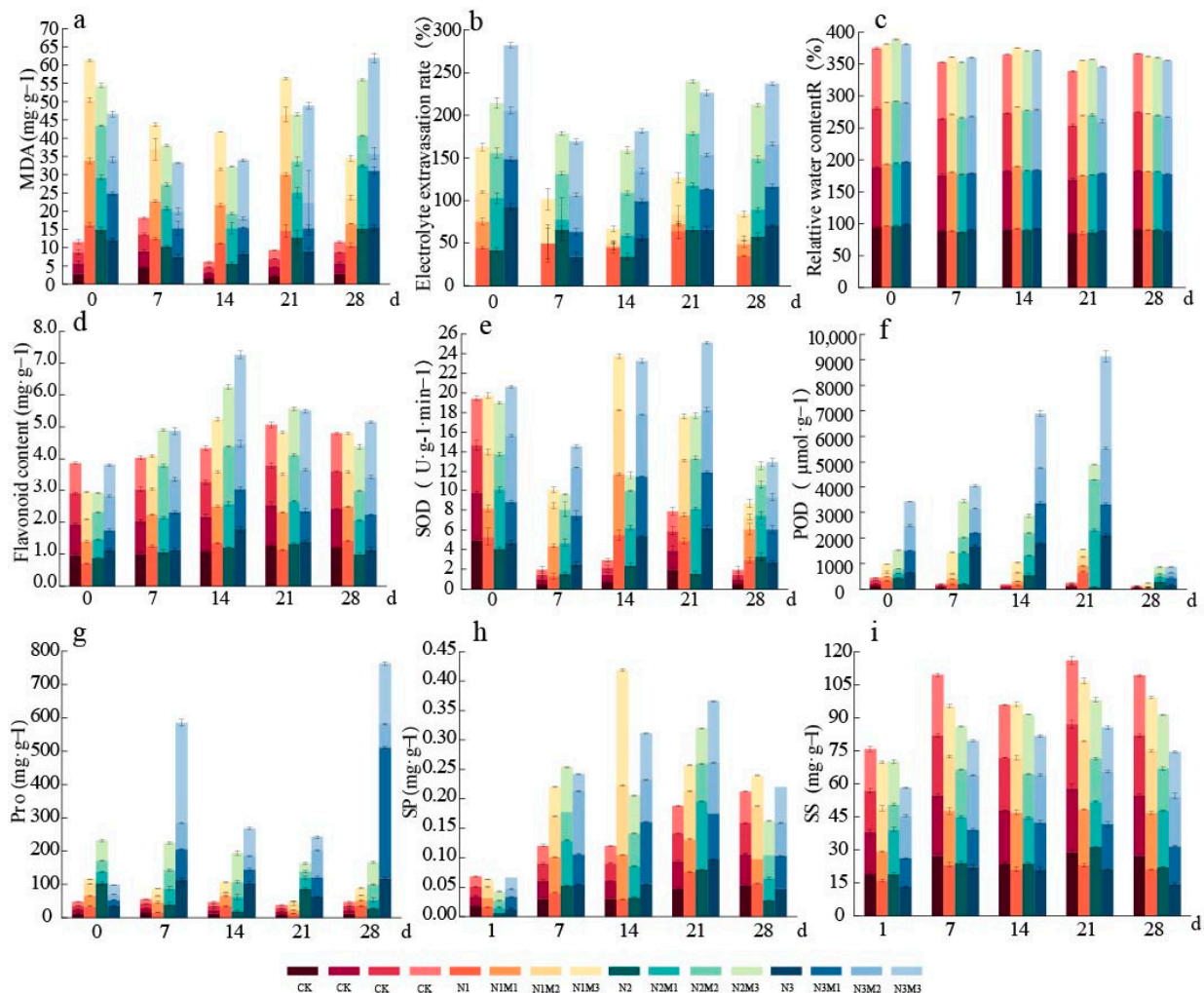

Figure 2. Effects of exogenous melatonin on the physiological and biochemical characteristics of Ginkgo under salinity stress: (**a**) malonic dialdehyde (MDA); (**b**) electrolyte leakage (EL); (**c**) relative water concentration (RWC); (**d**) flavonoid concentration (Fla); (**e**) peroxidase activity (POD); (**f**) superoxide dismutase activity (SOD); (**g**) free proline (Pro); (**h**) soluble protein concentration (SP); (**i**) soluble sugar concentration (SS). Values represent mean ± SD (n = 3). Note: N1, N2, and N3 were treated with 50, 100, and 200 mmol·L^{-1} salinity stress, respectively; M1, M2, and M3 were treated with 0.02, 0.1, and 0.5 mmol·L^{-1} melatonin, respectively.

Exogenous melatonin significantly stimulated the initial increase in SOD activity, which showed a stage-wise increase followed by a decrease as the treatment duration extended. Under low NaCl stress, at the 28th day of treatment, the SOD activities of the N1, M2, and M3 treatments decreased by 44.56%, 78.06%, and 76.71%, respectively, while the SOD activity of the M1 treatment increased by 8.01%. The decreases in the SOD activity of the M2 and M3 treatments were notably greater than that of the N1 treatment. The M1 treatment promoted the elevation of SOD activities under low NaCl stress, and the promoting effects of M2 and M3 significantly weakened as the treatment duration extended. Under medium NaCl stress, the SOD activities of the M1 and M2 treatments were significantly higher than that of N2 ($p < 0.05$) from day 7 to day 21, peaking at day 21 with elevations of 10.15% and 40.46%, respectively, whereas the SOD activities of the N2 and M3 treatments decreased by 61.89% and 16.67%, respectively. For high NaCl stress,

M1 and M2 effectively promoted SOD activity during the pre-treatment period, while M3 effectively mitigated the decrease in SOD activity during the later treatment period (Figure 2e).

POD activity showed an increase with the rising NaCl stress concentration, followed by a decrease as the stress duration extended. Under low and medium NaCl stress, externally applied melatonin had a modest promotional effect on POD activity, although this effect significantly diminished over time. Under high NaCl stress, neither the M1 or M2 treatments had a substantial alleviating effect on the decline in POD activity. Only the M3 treatment significantly boosted POD activity at the 21st day of treatment, resulting in a 2.81-fold increase, slightly higher than the 2.24-fold increase observed in the N3 treatment compared to the initial POD (Figure 2f).

Pro concentration exhibited an increase corresponding to the rising NaCl stress concentration. When exposed to low NaCl stress levels, the Pro concentration for both the N1 and M3 treatments increased over time, while the M1 and M2 treatments resulted in decreased Pro concentration, with no significant difference ($p < 0.05$) compared to the CK treatment at 28 days. Meanwhile, under medium NaCl stress, the Pro concentration of the N2 and M1 treatments decreased by 73.32% and 26.97%, respectively, while that of the M2 and M3 treatments increased by 38.95% and 11.38%, respectively, by the 28th day of treatment. At high NaCl stress levels, Pro concentration initially increased, then decreased, and ultimately spiked. By the 28th day of treatment, the Pro concentration for the N3, M1, M2, and M3 treatments had increased by 2.35-fold, 20.92-fold, 3.05-fold, and 5.38-fold, respectively (Figure 3a), with M1 displaying the most significant effect on Pro increase under high NaCl stress levels (Figure 2g).
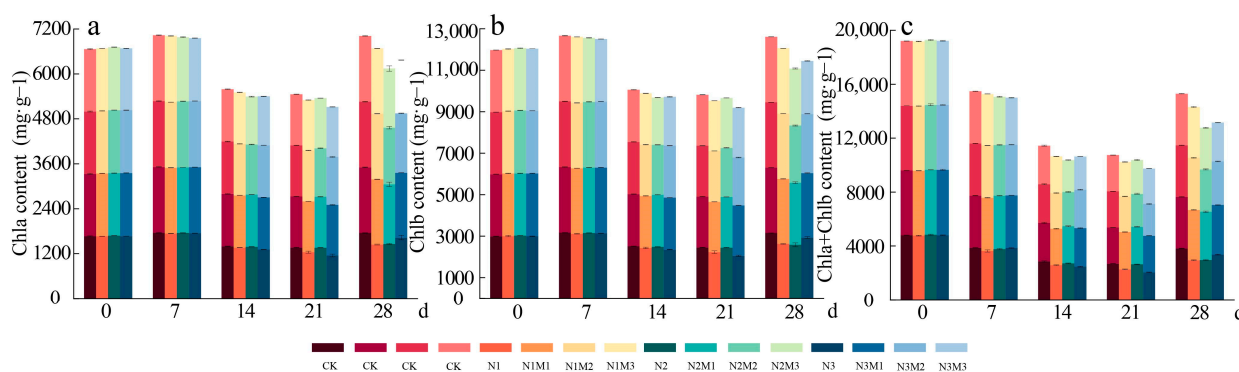

Figure 3. Effects of exogenous melatonin on chlorophyll concentration of *Ginkgo* under salinity stress: (**a**) chlorophyll-a concentration (Chla); (**b**) chlorophyll-b concentration (Chlb); (**c**) total chlorophyll concentration (Chla + Chlb). Values represent mean $\pm$ SD (n = 3). Note: N1, N2, and N3 were treated with 50, 100, and 200 mmol·L^{-1} salinity stress, respectively; M1, M2, and M3 were treated with 0.02, 0.1, and 0.5 mmol·L^{-1} melatonin, respectively.

Relative to CK, NaCl treatment increased SP concentration initially but decreased with prolonged stress. Under low NaCl stress, at day 28, the N1, M1, M2, and M3 treatments increased SP concentration 2.51, 1.76, 3.61, and 3.09 times, respectively. Similarly, under medium NaCl stress, the N2, M1, M2, and M3 treatments increased SP concentration by 3.59-fold, 3.30-fold, 2.43-fold, and 2.76-fold, respectively, by day 28. Exogenous melatonin mitigated the SP increase induced by medium-concentration NaCl stress. Under high NaCl stress, melatonin had no significant impact on SS concentration until day 28 when the N3 treatment increased it by 9.53%, while the M1, M2, and M3 treatments increased it by 32.82%, 17.53%, and 57.94%, respectively, significantly higher than the N3 treatment (Figure 2h).

Compared to CK, the SS concentration decreased in all NaCl concentrations, showing an initial increase followed by a decrease with higher stress levels and longer duration. Under low NaCl stress, both the M1 and M2 treatments significantly increased SS con-

centration, whereas the M3 treatment did not. Under medium NaCl stress, melatonin treatment suppressed the initial rise in SS concentration in the early stages and promoted its increase in the later stages. Conversely, under high NaCl stress, exogenous melatonin did not significantly increase SS concentration until the 28th day of treatment, mitigating the decline observed In the N3 treatment during this period (Figure 2i).

3.3. Effect of Exogenous Melatonin on Photosynthesis of Ginkgo Leaves under Salinity Stress

Chla, Chlb, and Chla + Chlb initially decreased and then increased during treatment, with no significant effect of NaCl concentration on Chl. Under low NaCl stress, the N1 treatment reduced Chla, Chlb, and Chla + Chlb at day 28, while the M1, M2, and M3 treatments increased these parameters and mitigated the decrease in Chla + Chlb. Medium NaCl stress saw melatonin significantly boost Chlt in later treatment stages, with the M1 treatment having the most pronounced effect, particularly on Chla. However, at high NaCl stress levels, exogenous melatonin inhibited the reduction in Chla, Chlb, and Chla + Chlb starting at day 14, with the most significant effect at day 21 (Figure 3).

Under low NaCl stress, the N1 treatment showed significant decreases in Pn, Tr, Gs, and Ci with prolonged treatment time. The M1 and M2 treatments effectively alleviated these reductions in each photosynthetic parameter. Exogenous melatonin reduced the decline in Pn, Tr, and Ci at 21 days of treatment, with Gs being notably enhanced at 14 days of treatment. Ci and Gs changes paralleled those of Pn and Tr, with exogenous melatonin application significantly curtailing the decrease in Ci, increasing Gs values, and mitigating the reduction in Pn and Tr. With medium NaCl stress, during the pre-treatment period, the trend in Tr over time mirrored that of Ci and Gs, while Pn continued to decline. In the later treatment phase, Ci and Gs began to rise, but Pn continued to decrease. Exogenous melatonin's capacity to alleviate the decrease in Gs was effective only in the first 21 days of treatment, beyond which it accelerated the decrease in Gs. Compared to N2, exogenous melatonin significantly mitigated the reduction in Pn, with Pn values for N2, M1, M2, and M3, decreasing by 56.46%, 39.25%, 39.33%, and 74.38% at 28 days of treatment, respectively. Under high NaCl stress, Pn, Tr, and Gs decreased over time, while Ci initially decreased and then increased. Within a specific timeframe, the M1 and M2 treatments significantly reduced the decrease in Pn and Tr values, whereas M3 exacerbated the reduction in Tr values, causing Pn to reach zero earlier. At 28 days, Ci values for N3, M1, M2, and M3 increased by 4.21%, 11.24%, 1.98%, and 27.93%, respectively. M1 and M3 significantly amplified the increase in Ci values, although Pn and Tr values reached zero (Figure 4).

At low and medium NaCl stress levels, there was a modest increase in the photosynthetic electron transport rate (ETR), the effective photochemical quantum yield of PSII (Y(II)), the photochemical quenching coefficient (qP), and the potential efficiency of primary conversion of light energy of PSII (Fv/Fm) during the pre-treatment period. However, at high NaCl stress levels, these parameters declined, while Fo and the non-photochemical quenching coefficient (NPQ) increased. Under low NaCl stress, both the M1 and M2 treatments significantly boosted ETR, Y(II), qP, and Fv/Fm, and mitigated the increase in Fo and NPQ to some extent. Meanwhile, the M3 treatment did not show significant alleviation effects. Under medium NaCl stress, the M1 and M2 treatments significantly increased Y(II), qP, and Fv/Fm, curbing the rise in ETR to some extent, and reducing NPQ. However, the M3 treatment notably decreased ETR and Fv/Fm while increasing NPQ and Fo. In the presence of high NaCl stress, the M1 and M2 treatments significantly attenuated the decline in Y(II) and Fv/Fm, lowered ETR, and decreased Fo. They also somewhat mitigated the increase in NPQ and the decline in qP, while the M3 treatment significantly counteracted the decline in ETR, Y(II), qP, and Fv/Fm, but significantly increased NPQ (Figure 5).
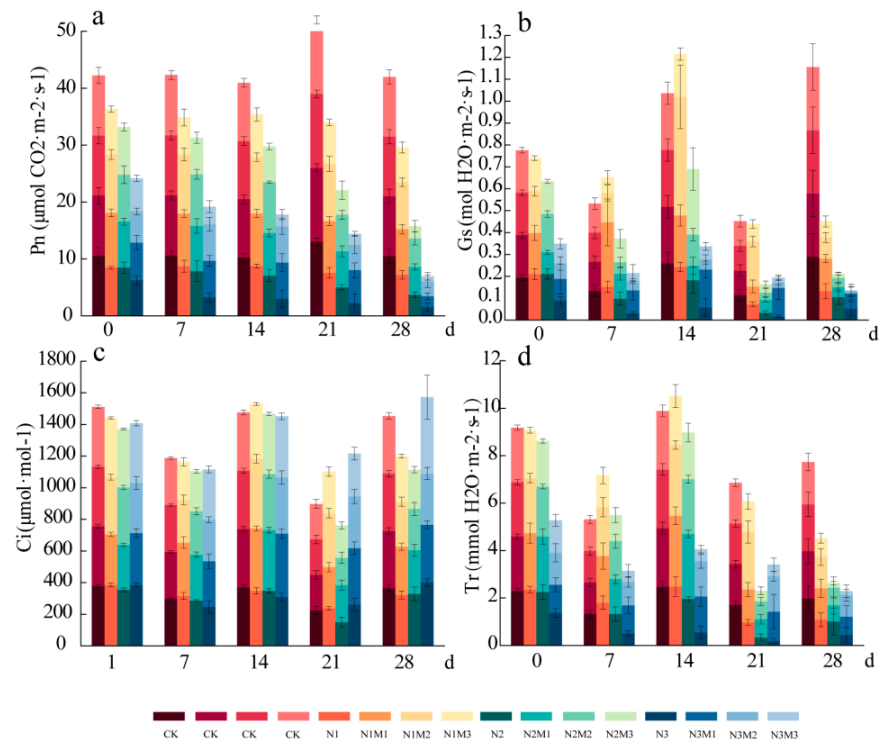
Figure 4. Effects of exogenous melatonin on photosynthetic parameters of *Ginkgo* under salinity stress: (**a**) photosynthetic rate (Pn); (**b**) transpiration rate (Tr); (**c**) stomatal conductance (Gs); (**d**) intercellular CO_2 concentration (Ci). Values represent mean $\pm$ SD (n = 3). Note: N1, N2, and N3 were treated with 50, 100, and 200 mmol·L^{-1} salinity stress, respectively; M1, M2, and M3 were treated with 0.02, 0.1, and 0.5 mmol·L^{-1} melatonin, respectively.
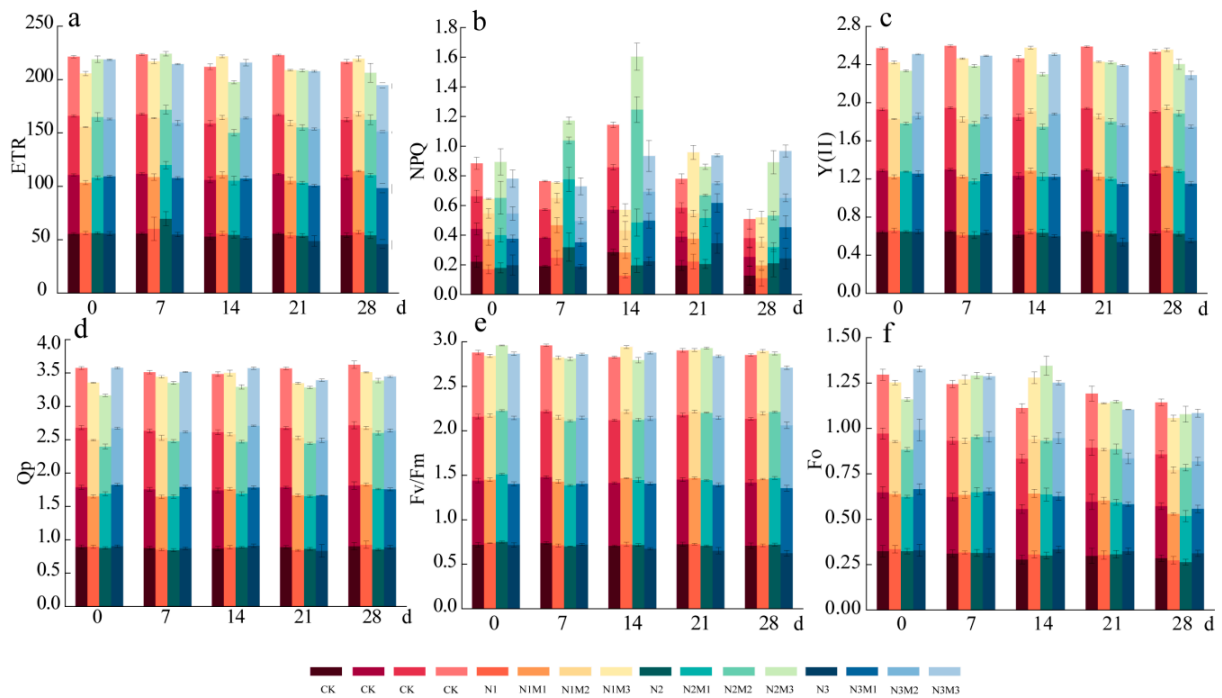

Figure 5. Effects of exogenous melatonin on chlorophyll fluorescence parameters of *Ginkgo* under salinity stress: (**a**) photosynthetic electron transport rate (ETR); (**b**) non-photochemical quenching coefficient (NPQ); (**c**) effective photochemical quantum yield of PSII (Y(II)); (**d**) photochemical

quenching coefficient (qP); (**e**) potential efficiency of primary conversion of light energy of PSII (Fv/Fm); (**f**) minimal fluorescence (Fo). Values represent mean ± SD (n = 3). Note: N1, N2, and N3 were treated with 50, 100, and 200 mmol·L^{-1} salinity stress, respectively; M1, M2, and M3 were treated with 0.02, 0.1, and 0.5 mmol·L^{-1} melatonin, respectively.

3.4. Comprehensive Analysis

The CCA results revealed significant ($p < 0.05$) or highly significant positive correlations ($p < 0.01$) between antioxidant activity, osmoregulatory substances, and photosynthetic parameters across low- concentration, mid-concentration, and high-concentration stresses (Figure 6a–c). However, notably, Tr showed a highly significant positive correlation ($p < 0.001$) with Pn, Ci, Gs, SOD, and RWC. Similar correlations were observed between ERT, YII, and qP, as well as SP and Fla, under low concentrations of salinity stress. Conversely, highly significant negative correlations ($p < 0.01$) were detected between NPQ and YII, Fv/Fm, and EL; EL and RWC, SOD, Chla, and Chlb; and Fla and Chla + Chlb (Figure 6d). Under medium-concentration salinity stress, Tr exhibited significant positive correlations with Pn, Ci, and Gs ($p < 0.01$), while YII and qP displayed consistent correlations as observed under low-concentration stress. However, the correlations of Tr with SOD and RWC decreased. SS demonstrated significant ($p < 0.05$) or highly significant negative ($p < 0.01$) correlations with Pn and Tr, SP with Tr and Ci, NPQ with YII and Fv/Fm, and Fv/Fm with Fla. Notably, the negative correlation of SOD with Chla and Chlb decreased, but the highly significant negative correlation between Fla and Chla + Chlb persisted ($p < 0.001$) (Figure 6e). Under high salinity stress, Pn exhibited a highly significant positive correlation ($p < 0.001$) with Tr, and ETR with YII, Chla and Chlb. Tr displayed significant positive correlations ($p < 0.01$) with Gs, ETR, YII, and Fv/Fm. Intriguingly, the negative correlation of SOD with Chla and Chlb was consistent with that observed under low-concentration stress, and POD, SS, and SP also showed significant negative correlations with SOD ($p < 0.01$). A significant negative correlation ($p < 0.05$) persisted between Fla and Chla + Chlb (Figure 6f).
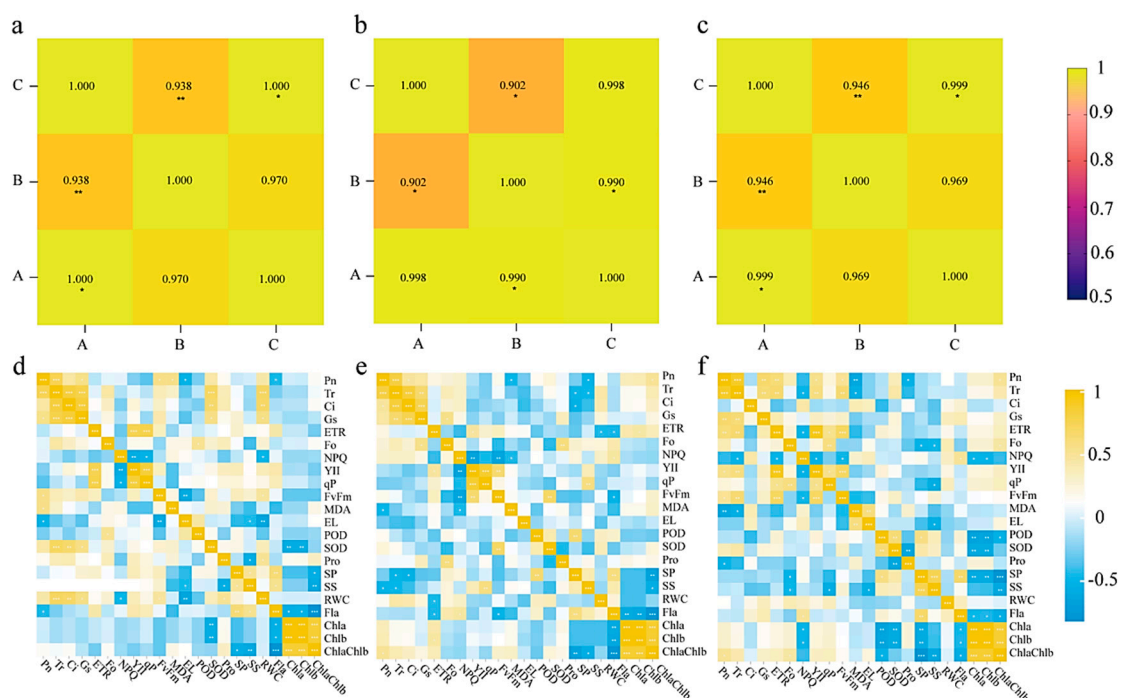

Figure 6. The a-c represent the typical correlation analysis among oxidative, osmotic, and photosynthetic indicators at low (**a**),medium (**b**), and high (**c**) concentrations. The (**d–f**), on the other hand, depict the correlation analysis among various indicators at low (**d**), medium (**e**), and high

(**f**) concentrations. In these figures, A–C respectively denote oxidative, osmotic, and photosynthetic indicators. An asterisk (*) denotes statistically significant differences at $p < 0.05$. Two asterisks (**) denotes statistically significant differences at $p < 0.01$. Three asterisks (***) denotes statistically significant differences at $p < 0.001$. MDA = malonic dialdehyde; EL = electrolyte leakage; RWC = relative water concentration; Fla = flavonoid concentration; SOD = superoxide dismutase; POD = peroxidase; Pro = free proline; SP = soluble protein concentration; SS = soluble sugar concentration; Chla = chlorophyll-a concentration; Chlb = chlorophyll-b concentration; Chla + Chlb = total chlorophyll concentration; ETR = photosynthetic electron transport rate; NPQ = non-photochemical quenching coefficient; Y(II) = effective photochemical quantum yield of PSII; qP = photochemical quenching coefficient; Fv/Fm = maximum quantum yield of photosystem II (PSII) photochemistry; Fo = minimal fluorescence; Pn = photosynthetic rate; Tr = transpiration rate; Gs = stomatal conductance; Ci = intercellular CO_2 concentration.

Principal component analysis (PCA) results indicated that the eigenvalues of the first six principal components were all greater than 1 under low, medium, and high salinity stress conditions, with cumulative contribution rates of 83.77%, 83.30%, and 86.68%, respectively. This suggests that these initial six principal components effectively capture the fundamental characteristics of growth and physiological parameters influenced by exogenous melatonin under varying salinity stress levels. Under low salinity stress, the first principal component, contributing 24.27%, primarily reflected the impact of exogenous melatonin on the photosynthetic parameters and the antioxidant system of *Ginkgo* leaves. It was characterized by larger absolute values of the eigenvectors associated with Tr, RWC, Ci, Gs, and SOD. In contrast, the second principal component (21.33%) under low salinity stress was marked by higher absolute eigenvector values of Chla + Chlb, Pn, Chla, and Chlb, signifying its focus on the influence of exogenous melatonin on Chlt and photosynthetic parameters in *Ginkgo* leaves (Table S1). Under medium and high salinity stress conditions, the first principal component made contributions of 24.49% and 28.44%, respectively. In both cases, it emphasized the effects of exogenous melatonin on Chlt and photosynthetic parameters, as evidenced by larger absolute eigenvector values of Chla + Chlb, Chlb, Chla, Pn, and ETR. The second principal component, contributing 21.22% and 22.01%, under medium and high NaCl stress, respectively, was characterized by larger absolute eigenvector values of Pn and Tr. Additionally, at high salinity concentrations, POD and SOD exhibited notable contributions to this component (Tables S2 and S3). Combining the correlation analysis results, seven physiological indicators (Pn, Tr, ETR, Fo, SOD, Pro, and Chla) and three growth indicators (ground diameter, length of current year's branches, and coarseness) were identified as key parameters for assessing the impact of exogenous melatonin on the growth and physiology of *Ginkgo* seedlings under NaCl stress.

4. Discussion

4.1. Relationship between Exogenous Melatonin and Growth of Ginkgo Plants under Salinity Stress

Salt stress is a significant abiotic stressor that impedes the normal growth of plant seedlings, while exogenous melatonin can substantially alleviate the inhibition of plant biomass accumulation caused by salt stress [19,43]. In this study, the application of NaCl stress alone significantly reduced the ground diameter and length of current-year branches of *Ginkgo biloba* seedlings. Exogenous application of melatonin (MT) at low (0.02 mmol·L^{-1}) and medium (0.1 mmol·L^{-1}) concentrations resulted in improved growth of *Ginkgo biloba* seedlings, with the combination of 50 mmol·L^{-1} NaCl and 0.02 mmol·L^{-1} MT exhibiting the most pronounced alleviating effect. This indicates that MT treatment can effectively mitigate the inhibitory impact of salt stress on the growth of *Ginkgo* seedlings. The underlying mechanism may involve MT-mediated modulation of cell wall composition, promoting cell wall extensibility under salt stress, thereby stimulating root system development. As the rhizospheric environment improves, the enhanced water absorption capacity of the roots further facilitates overall plant growth and development [44].However, exogenous applica-

tion of a high concentration of melatonin (0.5 mmol·L^{-1}) notably reduced the growth of *Ginkgo biloba* seedlings under 200 mmol·L^{-1} NaCl stress, indicating that high-concentration MT treatment exacerbates the inhibitory effects of salt stress, suggesting a dosage effect similar to findings in maize seedlings [45].

4.2. Relationship between Exogenous Melatonin and Osmoregulation in Ginkgo under Salinity Stress

Salinity stress initially induces osmotic stress, which subsequently leads to ionic toxicity and oxidative stress [46,47]. Contrastingly, melatonin alleviates osmotic stress primarily by regulating cellular osmotic substances like Pro, SS, and SP [48,49]. Existing research has demonstrated that melatonin treatment can regulate the osmotic protection of proline homeostasis at both enzymatic (P5CS) and gene (*P5CS*, *P5CR*) expression levels, thereby enhancing the plant's tolerance to stress [50]. In this study, Pro and SP concentrations increased with higher NaCl treatment concentrations, while SS concentration firstly increased before decreasing with rising treatment concentration, in line with previous findings [51]. Following the exogenous application of melatonin, the impact of externally applied melatonin on SS, SP, and Pro concentrations in *Ginkgo* seedlings under NaCl stress depended on both melatonin and NaCl concentrations. Specifically, low (0.02 mmol·L^{-1}) and medium (0.1 mmol·L^{-1}) melatonin treatments promoted SS concentration under various NaCl treatments, while high-concentration (0.5 mmol·L^{-1}) melatonin did not enhance SS concentration under low (50 mmol·L^{-1}) NaCl stress. Additionally, all three melatonin concentrations significantly increased SS concentration under low (50 mmol·L^{-1}) NaCl treatment, with a stronger effect at higher melatonin concentrations, but the effect diminished with prolonged treatment time. Conversely, melatonin did not promote SP concentration in medium (100 mmol·L^{-1}) and high (200 mmol·L^{-1}) NaCl treatments. Low (0.02 mmol·L^{-1}) melatonin significantly increased Pro concentration under high (200 mmol·L^{-1}) NaCl treatment, while low and medium (0.1 mmol·L^{-1}) melatonin decreased Pro concentration under low (50 mmol·L^{-1}) NaCl treatment, approximating control levels. This concentration-dependent pattern aligns with previous research on SP concentration by Bano [52], and on SS concentration and Pro concentration by Cheng [53] in camphor pine. This may be due to melatonin's role as a signaling molecule influencing the synthesis of SP and SS [54], which is affected by NaCl stress concentration. Conversely, *Ginkgo* synthesizes substantial Pro only when NaCl stress reaches a certain level to enhance osmotic tolerance [55]. In this study, low melatonin treatment partly alleviated osmotic stress from low NaCl stress in *Ginkgo* seedlings, negating the need to increase Pro concentration.

4.3. Relationship between Exogenous Melatonin and Oxidative Stress and Antioxidant System of Ginkgo under NaCl Stress

Under normal conditions, plants maintain a dynamic equilibrium between the production and scavenging of reactive oxygen species (ROS). However, salt stress disrupts this balance. In response, plants activate their ROS scavenging mechanisms, enhancing the activity of antioxidant enzymes, thereby neutralizing excess ROS and mitigating the oxidative damage caused by salt stress to the plants [56]. Recent research has made significant progress in elucidating how melatonin enhances plants' resistance to oxidative stress. Studies have shown that exogenous melatonin can reduce electrolyte exudation rates either by directly affecting plant cell osmoregulation or by employing its antioxidant capacity to eliminate endogenous ROS, thereby impeding membrane lipid peroxidation [57]. Research conducted by Tan et al. [58] has shown that melatonin serves as the primary line of defense against external environmental stressors. Melatonin not only directly interacts with ROS but also enhances the activity of endogenous antioxidant enzymes in plants [59]. In this study, applying an appropriate melatonin concentration under NaCl stress alleviated oxidative stress but was concentration-sensitive. Specifically, high-dose (0.5 mmol·L^{-1}) melatonin treatment exacerbated membrane lipid peroxidation induced by NaCl stress. Moreover, under a high NaCl concentration (200 mmol·L^{-1}), melatonin application no longer enhanced POD and SOD activities and increased electrolyte exudation, disrupting cell membrane in-

tegrity. This phenomenon is consistent with the findings in grape studies [60]. Melatonin's mechanism of action involves inducing antioxidant enzyme synthesis, such as POD, SOD, and non-enzymatic antioxidants including ASA and GSH, and up-regulating resistance genes [61]. However, high melatonin and NaCl concentrations hinder oxidative stress signaling, delaying the response to saline and alkaline stress [60]. Consequently, selecting the appropriate melatonin concentration is crucial for different crops facing varying adversity conditions [62]. Additionally, melatonin treatment's impact on NaCl stress was time-sensitive. During the initial 7 days, exogenous melatonin minimally affected MDA accumulation but altered electrolyte exudation by directly modifying plant cell osmoregulation. In later stages, melatonin primarily hindered membrane lipid peroxidation using its antioxidant capacity. However, melatonin's promotion of POD and SOD activities weakened with prolonged treatment. After 21 days, only low-concentration (0.02 mmol·L^{-1}) melatonin treatment continued to enhance POD activity under low (50 mmol·L^{-1}) and medium (100 mmol·L^{-1}) NaCl stress, aligning with the temporal protective effect observed in *Brassica napus* seedlings [63].

4.4. Relationship between Exogenous Melatonin and Photosynthesis in Ginkgo under Salinity Stress

Photosynthetic pigments are fundamental substances in plant photosynthesis, with their concentration directly affecting the plant's capability to capture, transport, and convert light energy during the photosynthetic process. Salinity stress reduces Chlt in plants through two main pathways: by decreasing chlorophyll synthase activity and inhibiting chlorophyll synthesis, and by promoting chlorophyll decomposition through plasma peroxidation-induced membrane structure damage [64]. In this study, an appropriate melatonin concentration significantly mitigated Chlt reduction under NaCl stress, with the effect being dose-dependent. Specifically, a low melatonin concentration (0.02 mmol·L^{-1}) effectively alleviated Chlt reduction caused by NaCl stress, especially under low NaCl stress. In contrast, medium (0.1 mmol·L^{-1}) and high (0.5 mmol·L^{-1}) melatonin concentrations minimized the decline in Chlt primarily under high NaCl stress conditions (200 mmol·L^{-1}). This suggests that high melatonin concentrations could exacerbate photosynthetic damage in *Ginkgo* seedlings under high NaCl stress. The reason behind this might be that low melatonin concentrations alleviate chlorophyll synthesis inhibition by enhancing chlorophyll synthase activity under low and medium NaCl stress, while high melatonin concentrations exacerbate chlorophyll decomposition by increasing membrane lipid peroxidation under high NaCl stress [65]. However, the precise mechanisms underlying these effects require further investigation.

The reduction in photosynthetic rate in plant leaves results from stomatal limitation, caused by partial stomatal, and non-stomatal limitation, due to decreased photosynthetic activity in chloroplasts. Stomatal limitation leads to a decrease in the Ci value, while non-stomatal limitation increases it. The change in Ci depends on the predominant factor when both factors coexist [66,67]. In this study, stomatal limitation was observed under low NaCl concentration (50 mmol·L^{-1}) stress. For the medium NaCl concentration (100 mmol·L^{-1}) stress treatment, stomatal limitation was observed in the early stage, while non-stomatal limitation was observed in the later stage. High NaCl concentration (200 mmol·L^{-1}) stress resulted in non-stomatal limitation, consistent with the findings by Zhao et al. [68]. The external application of all three melatonin concentrations significantly mitigated Ci reduction under low NaCl concentration (50 mmol·L^{-1}) stress. This effect enhanced reactant supply, increased Gs values, alleviated stomatal limitation, inhibited Pn and Tr reduction, and improved leaf photosynthesis. This aligns with results from Wang [38] and Ahmad [54]. Melatonin's ability to alleviate the photoinhibition of PSI and PSII under low salinity stress and promote stomatal opening likely contributed to this improvement. Furthermore, the enhancement of leaf photosynthetic performance under medium (100 mmol·L^{-1}) and high (200 mmol·L^{-1}) NaCl stress by externally applied low (0.02 mmol·L^{-1}) and medium (0.1 mmol·L^{-1}) melatonin treatments exhibited some time sensitivity, with the mitigation

effect significantly weakening after treatments exceeded 21 days, similar to observations in wheat [69].

Chlorophyll fluorescence parameters serve as sensitive indicators reflecting various aspects of light energy absorption, transformation, transfer, and distribution in plants. Also, they can unveil the underlying reasons for changes in gas exchange parameters [70]. In this study, *Ginkgo* seedlings exhibited elevated levels of ETR, Y(II), qP, Fv/Fm, Fo, and NPQ under NaCl treatment, which aligns with previous findings in *Lycium ruthenicum* [71] and okra [72], indicating that NaCl stress hampers the photosynthetic electron transfer process in *Ginkgo* leaves. It reduces the efficiency of photosynthetic pigments in converting light energy into chemical energy, causing damage to photosynthetic organs due to excess light energy. Ultimately, this inhibition results in reduced photosynthesis in the leaves [73]. External application of melatonin under NaCl stress mitigated the decrease in ETR, Y(II), qP, and Fv/Fm in *Ginkgo* seedling leaves. This favored the conversion of captured light energy into chemical energy by photosynthetic pigments, maintained higher photochemical activity of PSII, supplied more energy for carbon assimilation, and effectively alleviated the inhibitory effect of NaCl stress on *Ginkgo* seedling photosynthesis. These findings are consistent with Xie et al.'s study [74]. However, the effectiveness of melatonin treatment is dependent on its concentration, the NaCl treatment concentration, and treatment duration. Notably, the most significant improvement in ETR, Y(II), qP, and Fv/Fm with externally applied melatonin was observed in response to 200 mmol·L^{-1} NaCl treatment, with better alleviation in the late treatment stage compared to the pre-treatment period. This late-stage enhancement may result from melatonin accumulation, leading to increased concentration and enhanced alleviating capabilities.

4.5. Correlation Analysis among Physiological Indicators of Young Ginkgo Trees

Salinity tolerance in young *Ginkgo* trees results from various mechanisms, including tissue morphology, osmoregulation, antioxidant systems, and anatomical adaptations. Chlorophyll parameters and photosynthetic efficiency have been identified as reliable indicators of salinity tolerance in previous research, such as Tsai et al.'s analysis of eight rice (*Oryza sativa*) cultivars [75]. Likewise, He et al. [76] found a strong correlation between photosynthetic rate and salinity tolerance in soybeans (*Glycine max*). In the current study, a significant positive correlation was observed between the oxidative and photosynthetic systems, whereby SOD showed highly significant negative correlations with Chla and Chlb, while Fla exhibited a similar relationship with Chla + Chlb under both low and high salinity stress conditions, consistent with Chavez et al.'s findings [77]. This suggests that salinity stress may indirectly affect *Ginkgo*'s photosynthetic capacity by inducing oxidative stress. Furthermore, among the photosynthetic parameters, Pn directly reflects a plant's photosynthetic capacity and serves as an effective physiological indicator for evaluating its response to salinity stress and characterizing salinity tolerance [78]. In this study, Tr exhibited a significant positive correlation with Pn and Gs, aligning with the results of Harris et al. [79]. This implies that transpiration rate can also serve as a physiological indicator for assessing *Ginkgo*'s salinity tolerance in saline environments.

5. Conclusions

In summary, NaCl stress significantly inhibits the normal growth and development of *Ginkgo biloba* seedlings, while the exogenous application of melatonin at suitable concentrations can alleviate the growth inhibition caused by salt stress. Exogenous melatonin treatment increased leaf water concentration, raised Pro and SS levels to maintain osmotic balance, boosted SOD and POD enzyme activity for better antioxidant defense, reduced cellular oxidative stress to maintain cell membrane integrity, protected the photosynthetic structure, and enhanced PSII's photochemical activity, ultimately increasing photosynthesis rates, and ultimately enhancing the salt tolerance of Ginkgo biloba seedlings. This study indicated that 0.02 mmol·L^{-1} melatonin is most effective in mitigating the damage to Ginkgo biloba seedlings under NaCl stress, whereas a concentration of 0.5 mmol·L^{-1} melatonin

exacerbated physiological damage under 200 mmol·L^{-1} NaCl stress. Key indicators for rapidly assessing salinity stress in *Ginkgo* include Pn, Tr, ETR, Fo, SOD, Pro, and Chla. This research provided a theoretical basis for the role of melatonin in enhancing plant salt tolerance and offered technical support for addressing the impact of de-icing agents on the growth of *Ginkgo biloba* street trees and for expanding the planting area of *Ginkgo biloba*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10010089/s1, Table S1: Result of principal component analysis among studied traits in Ginkgo seedlings under low NaCl stress; Table S2: Result of principal component analysis among studied traits in Ginkgo seedlings under medium NaCl stress; Table S3: Result of principal component analysis among studied traits in Ginkgo seedlings under high NaCl stress.

Author Contributions: D.Z.: writing—original draft, writing—review and editing, visualization. M.L.: data curation, formal analysis, software, validation. X.W.: data curation, formal analysis, software, validation. H.L.: investigation, methodology, resources. Z.L.: writing—review and editing, validation. Q.L.: conceptualization, funding acquisition, project administration, supervision. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: Data supporting reported results can be requested by contacting the corresponding author. The data are not publicly available due to compliance with data protection regulations.

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

References

1. van Zelm, E.; Zhang, Y.; Testerink, C. Salt tolerance mechanisms of plants. *Annu. Rev. Plant Biol.* **2020**, *71*, 403–433. [CrossRef] [PubMed]
2. Shen, G.; Pang, Y.; Wu, W.; Liao, Z.; Zhao, L.; Sun, X.-f.; Tang, K. Cloning and characterization of a root-specific expressing gene encoding 3-hydroxy-3-methylglutaryl coenzyme a reductase from ginkgo biloba. *Mol. Biol. Rep.* **2006**, *33*, 117–127. [CrossRef] [PubMed]
3. Qadir, M.; Qureshi, R.H.; Ahmad, N. Amelioration of calcareous saline sodic soils through phytoremediation and chemical strategies. *Soil Use Manag.* **2002**, *18*, 381–385. [CrossRef]
4. Basak, N.; Rai, A.K.; Sundha, P.; Meena, R.L.; Bedwal, S.; Yadav, R.K.; Sharma, P.C. Assessing soil quality for rehabilitation of salt-affected agroecosystem: A comprehensive review. *Front. Environ. Sci.* **2022**, *10*, 935785. [CrossRef]
5. Misganaw, A.; Aklil, M.; Kesete, N. Review on the effects of seed priming on performance of maize seedlings. *J. Biol. Agric. Healthc.* **2020**, *10*, 31–39. [CrossRef]
6. Masteling, R.; Voorhoeve, L.; Ijsselmuiden, J.; Dini-Andreote, F.; de Boer, W.; Raaijmakers, J.M. Discount: Computer vision for automated quantification of *Striga* seed germination. *Plant Methods* **2020**, *16*, 6010. [CrossRef] [PubMed]
7. Kijowska-Oberc, J.; Staszak, A.M.; Ratajczak, E. Climate change affects seed aging? Initiation mechanism and consequences of loss of forest tree seed viability. *Trees* **2021**, *35*, 1099–1108. [CrossRef]
8. Kumar, A.; Gautam, R.D.; Kumar, A.; Singh, S.; Singh, S. Understanding the effect of different abiotic stresses on wild marigold (*Tagetes minuta* L.) and role of breeding strategies for developing tolerant lines. *Front. Plant Sci.* **2022**, *12*, 754457. [CrossRef]
9. Bidalia, A.; Hanief, M.; Rao, K.S. Tolerance of Mitragyna parvifolia (Roxb.) Korth. seedlings to NaCl salinity. *Photosynthetica* **2017**, *55*, 231–239. [CrossRef]
10. Oliveira, L.M.d.; Mendonça, V.; Moura, E.A.d.; Irineu, T.H.d.S.; Figueiredo, F.R.A.; Melo, M.F.; Celedônio, W.F.; Rêgo, A.L.B.; Mendonça, L.F.d.M.; Andrade, A.U.d. Salt stress and organic fertilization on the growth and biochemical metabolism of Hylocereus costaricensis (red pitaya) seedlings. *Braz. J. Biol. Rev. Brasileira De Biol.* **2022**, *84*, e258476–e258487. [CrossRef]
11. Chen, Y.; Lin, F.-P.; Yang, H.; Yue, L.; Hu, F.; Wang, J.; Luo, Y.; Cao, F. Effect of varying NaCl doses on flavonoid production in suspension cells of Ginkgo biloba: Relationship to chlorophyll fluorescence, ion homeostasis, antioxidant system and ultrastructure. *Acta Physiol. Plant.* **2014**, *36*, 3173–3187. [CrossRef]
12. Eker, S.; Cömertpay, G.; Konuşkan, Ö.; Ülger, A.C.; Ozturk, L.; Cakmak, I. Effect of salinity stress on dry matter production and ion accumulation in hybrid maize varieties. *Turk. J. Agric. For.* **2006**, *30*, 365–373.
13. Shahid, M.A.; Sarkhosh, A.; Khan, N.; Balal, R.M.; Ali, S.; Rossi, L.; Gómez, C.; Mattson, N.S.; Nasim, W.; García-Sánchez, F. Insights into the physiological and biochemical impacts of salt stress on plant growth and development. *Agronomy* **2020**, *10*, 938–971. [CrossRef]

14. Seemann, J.R.; Critchley, C.N.R. Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. *Planta* **1985**, *164*, 151–162. [CrossRef] [PubMed]
15. Farouk, S.; Al-Huqail, A.A. Sustainable biochar and/or melatonin improve salinity tolerance in borage plants by modulating osmotic adjustment, antioxidants, and ion homeostasis. *Plants* **2022**, *11*, 765. [CrossRef]
16. Sofy, M.R.; Elhindi, K.M.; Farouk, S.; Alotaibi, M.A. Zinc and paclobutrazol mediated regulation of growth, upregulating antioxidant aptitude and plant productivity of pea plants under salinity. *Plants* **2020**, *9*, 119710. [CrossRef]
17. Rus, A.M.; Yokoi, S.; Sharkhuu, A.; Reddy, M.P.; Lee, B.-h.; Matsumoto, T.K.; Koiwa, H.; Zhu, J.K.; Bressan, R.A.; Hasegawa, P.M. Athkt1 is a salt tolerance determinant that controls Na+ entry into plant roots. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 14150–14155. [CrossRef]
18. Blumwald, E. Engineering salt tolerance in plants. *Biotechnol. Genet. Eng. Rev.* **2002**, *20*, 261–275. [CrossRef]
19. Munns, R.; Tester, M.A. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **2008**, *59*, 651–681. [CrossRef] [PubMed]
20. Bernstein, N.; Silk, W.K.; Läuchli, A. Growth and development of sorghum leaves under conditions of NaCl stress: Possible role of some mineral elements in growth inhibition. *Planta* **1995**, *196*, 699–705. [CrossRef]
21. Grieve, C.M.; Francois, L.E.; Maas, E. Salinity affects the timing of phasic development in spring wheat. *Crop Sci.* **1994**, *34*, 1544–1549. [CrossRef]
22. Parida, A.K.; Das, A.B.; Mittra, B. Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, Bruguiera parviflora. *Trees* **2004**, *18*, 167–174. [CrossRef]
23. van Beek, T.A.; Montoro, P. Chemical analysis and quality control of Ginkgo biloba leaves, extracts, and phytopharmaceuticals. *J. Chromatogr. A* **2009**, *1216*, 2002–2032. [CrossRef] [PubMed]
24. Defeudis, F.V.; Papadopoulos, V.; Drieu, K. Ginkgo biloba extracts and cancer: A research area in its infancy. *Fundam. Clin. Pharmacol.* **2003**, *17*, 405–417. [CrossRef]
25. Spiegel, R.; Kalla, R.; Mantokoudis, G.; Maire, R.; Mueller, H.; Hoerr, R.; Ihl, R. Ginkgo biloba extract EGb 761® alleviates neurosensory symptoms in patients with dementia: A meta-analysis of treatment effects on tinnitus and dizziness in randomized, placebo-controlled trials. *Clin. Interv. Aging* **2018**, *13*, 1121–1127. [CrossRef] [PubMed]
26. Hayashi, H.; Alía; Mustárdy, L.s.; Deshnium, P.; Ida, M.; Murata, N. Transformation of Arabidopsis thaliana with the codA gene for choline oxidase; accumulation of glycinebetaine and enhanced tolerance to salt and cold stress. *Plant J. Cell Mol. Biol.* **1997**, *12*, 133–142. [CrossRef]
27. Sæbø, A.; Borzan, Ž.; Ducatillion, C.; Hatzistathis, A.; Lagerström, T.; Supuka, J.; García-Valdecantos, J.L.; Rego, F.C.; Slycken, J.v. The Selection of Plant Materials for Street Trees, Park Trees and Urban Woodland. In *Urban Forests and Trees*; Springer: Berlin, Germany, 2005; pp. 257–280. Available online: https://link.springer.com/chapter/10.1007/3-540-27684-X_11#citeas (accessed on 10 January 2024).
28. Major, R.T. The Ginkgo, the Most Ancient Living Tree. *Science* **1967**, *157*, 1270–1273. [CrossRef]
29. Curtis-Prior, P.; Vere, D.; Fray, P. Therapeutic value of ginkgo biloba in reducing symptoms of decline in mental function. *J. Pharm. Pharmacol.* **1998**, *51*, 535–541. [CrossRef]
30. Smith, J.V.; Luo, Y. Studies on molecular mechanisms of Ginkgo biloba extract. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 465–472. [CrossRef]
31. Yang, X.S.; Chen, G.X.; Wei, X.D.; Xie, K.B. Enhanced antioxidant protection at the early stages of leaf expansion in ginkgo under natural environmental conditions. *Biol. Plant.* **2011**, *56*, 181–186. [CrossRef]
32. Wei, X.; Shi, D.W.; Chen, G. Physiological, structural, and proteomic analysis of chloroplasts during natural senescence of Ginkgo leaves. *Plant Growth Regul.* **2013**, *69*, 191–201. [CrossRef]
33. Yu, Z.; Duan, X.; Luo, L.; Dai, S.; Ding, Z.; Xia, G. How plant hormones mediate salt stress responses. *Trends Plant Sci.* **2020**, *25*, 1117–1130. [CrossRef]
34. Arnao, M.B.; Hernández-Ruiz, J. Melatonin: Plant growth regulator and/or biostimulator during stress? *Trends Plant Sci.* **2014**, *19*, 789–797. [CrossRef]
35. Erland, L.A.E.; Murch, S.J.; Reiter, R.J.; Saxena, P.K. A new balancing act: The many roles of melatonin and serotonin in plant growth and development. *Plant Signal. Behav.* **2015**, *10*, e1096469. [CrossRef] [PubMed]
36. Erland, L.A.E.; Yasunaga, A.B.; Li, I.T.S.; Murch, S.J.; Saxena, P.K. Direct visualization of location and uptake of applied melatonin and serotonin in living tissues and their redistribution in plants in response to thermal stress. *J. Pineal Res.* **2018**, *66*, e12527. [CrossRef]
37. Farouk, S.; Al-Amri, S.M. Exogenous melatonin-mediated modulation of arsenic tolerance with improved accretion of secondary metabolite production, activating antioxidant capacity and improved chloroplast ultrastructure in rosemary herb. *Ecotoxicol. Environ. Saf.* **2019**, *180*, 333–347. [CrossRef] [PubMed]
38. Wang, P.; Sun, X.; Li, C.-Z.; Wei, Z.; Liang, D.; Ma, F. Long-term exogenous application of melatonin delays drought-induced leaf senescence in apple. *J. Pineal Res.* **2013**, *54*, 292–302. [CrossRef] [PubMed]
39. Bajwa, V.S.; Shukla, M.R.; Sherif, S.M.; Murch, S.J.; Saxena, P.K. Role of melatonin in alleviating cold stress in Arabidopsis thaliana. *J. Pineal Res.* **2014**, *56*, 238–245. [CrossRef] [PubMed]
40. Xu, W.; Cai, S.; Zhang, Y.; Wang, Y.; Ahammed, G.J.; Xia, X.; Shi, K.; Zhou, Y.; Yu, J.; Reiter, R.J.; et al. Melatonin enhances thermotolerance by promoting cellular protein protection in tomato plants. *J. Pineal Res.* **2016**, *61*, 457–469. [CrossRef] [PubMed]
41. LI, H. *Modern Plant Physiology*, 4th ed.; Higher Education Press: Beijing, China, 2019.

42. Gao, J. *Experimental Supervision of Plant Physiology*; Higher Education Press: Beijing, China, 2006.

43. Jiang, C.-q.; Cui, Q.; Feng, K.; Xu, D.; Li, C.; Zheng, Q. Melatonin improves antioxidant capacity and ion homeostasis and enhances salt tolerance in maize seedlings. *Acta Physiol. Plant.* **2016**, *38*, 82. [CrossRef]

44. Raza, M.A.S.; Saleem, M.F.; Ashraf, M.Y.; Ali, A.; Asghar, H.N. Glycinebetaine applied under drought improved the physiological efficiency of wheat (*Triticum aestivum* L.) plant. *Soil Environ.* **2012**, *31*, 67–71.

45. Chen, Y.; Mao, J.-J.; Sun, L.; Huang, B.; Ding, C.; Gu, Y.; Liao, J.; Hu, C.; Zhang, Z.-w.; Yuan, S.; et al. Exogenous melatonin enhances salt stress tolerance in maize seedlings by improving antioxidant and photosynthetic capacity. *Physiol. Plant.* **2018**, *164*, 349–363. [CrossRef]

46. Chen, Z.C.; Yamaji, N.; Horie, T.; Che, J.; Li, J.; An, G.; Ma, J.F. A Magnesium Transporter OsMGT1 Plays a Critical Role in Salt Tolerance in Rice1[OPEN]. *Plant Physiol.* **2017**, *174*, 1837–1849. [CrossRef]

47. Li, J.; Yuan, F.; Liu, Y.; Zhang, M.; Liu, Y.; Zhao, Y.; Wang, B.; Chen, M. Exogenous melatonin enhances salt secretion from salt glands by upregulating the expression of ion transporter and vesicle transport genes in Limonium bicolor. *BMC Plant Biol.* **2020**, *20*, 1–11. [CrossRef] [PubMed]

48. Khan, M.N.; Zhang, J.; Luo, T.; Liu, J.; Rizwan, M.; Fahad, S.; Xu, Z.; Hu, L. Seed priming with melatonin coping drought stress in rapeseed by regulating reactive oxygen species detoxification: Antioxidant defense system, osmotic adjustment, stomatal traits and chloroplast ultrastructure perseveration. *Ind. Crops Prod.* **2019**, *140*, 111597. [CrossRef]

49. Jiang, D.; Lu, B.; Liu, L.; Duan, W.-j.; Meng, Y.; Li, J.; Zhang, K.; Sun, H.; Zhang, Y.; Dong, H.; et al. Exogenous melatonin improves the salt tolerance of cotton by removing active oxygen and protecting photosynthetic organs. *BMC Plant Biol.* **2021**, *21*, 331. [CrossRef]

50. Antoniou, C.; Chatzimichail, G.; Xenofontos, R.; Pavlou, J.J.; Panagiotou, E.; Christou, A.; Fotopoulos, V. Melatonin systemically ameliorates drought stress-induced damage in Medicago sativa plants by modulating nitro-oxidative homeostasis and proline metabolism. *J. Pineal Res.* **2017**, *62*, e12401. [CrossRef] [PubMed]

51. Rahdari, P.; Tavakoli, S.; Hosseini, S.M. Studying of salinity stress effect on germination, proline, sugar, protein, lipid and chlorophyll content in Purslane (*Portulaca oleracea* L.) leaves. *J. Stress Physiol. Biochem.* **2012**, *8*, 182–193.

52. Bano, S.; Iqbal, S.; Naqvi, B.; Abbasi, K.Z.; Siddiqui, K.; Sattar, H.; Aman, A. Antioxidant Enzymes and Germination Pattern: Upshot of High Salinity on Soluble Protein and Average Weight of Spinacia oleracea (Spinach) Seedlings. *Asian Food Sci. J.* **2021**, *20*, 112–122. [CrossRef]

53. Cheng, W.S.; Zhang, N.; Zeng, H.; Shi, X.F.; Li, Y.; Sun, Y.H. The salicylic acid effect on the contents of citrullus lanatus l. sugar, protein and proline under salinity (nacl) stress. *Adv. Mater. Res.* **2014**, *1048*, 469–474.

54. Ahmad, S.; Cui, W.; Kamran, M.; Ahmad, I.; Meng, X.; Wu, X.; Su, W.; Javed, T.; El-Serehy, H.A.; Jia, Z.-k.; et al. Exogenous application of melatonin induces tolerance to salt stress by improving the photosynthetic efficiency and antioxidant defense system of maize seedling. *J. Plant Growth Regul.* **2020**, *40*, 1270–1283. [CrossRef]

55. Sun, C.-C.; Zhao, H.-Y.; Zheng, C.-X. Effects of NaCl stress on osmolyte and proline metabolism in Ginkgo biloba seedling. *Plant Physiol. J.* **2017**, *53*, 470–476. [CrossRef]

56. Treutter, D. Significance of flavonoids in plant resistance: A review. *Environ. Chem. Lett.* **2006**, *4*, 147–157. [CrossRef]

57. Cui, G.; Zhao, X.; Liu, S.; Sun, F.; Zhang, C.; Xi, Y. Beneficial effects of melatonin in overcoming drought stress in wheat seedlings. *Plant Physiol. Biochem.* **2017**, *118*, 138–149. [CrossRef] [PubMed]

58. Tan, D.-X.; Hardeland, R.; Manchester, L.C.; Korkmaz, A.Ş.; Ma, S.; Rosales-Corral, S.A.; Reiter, R.J. Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. *J. Exp. Bot.* **2012**, *63*, 577–597. [CrossRef] [PubMed]

59. Pal, P.K.; Maitra, S.K. Response of gastrointestinal melatonin, antioxidants, and digestive enzymes to altered feeding conditions in carp (Catla catla). *Fish Physiol. Biochem.* **2018**, *44*, 1061–1073. [CrossRef]

60. Li, H.; Chang, J.; Chen, H.; Wang, Z.; Gu, X.; Wei, C.; Zhang, Y.; Ma, J.; Yang, J.; Zhang, X. Exogenous melatonin confers salt stress tolerance to watermelon by improving photosynthesis and redox homeostasis. *Front. Plant Sci.* **2017**, *8*, 295. [CrossRef] [PubMed]

61. Arora, D.; Bhatla, S.C. Melatonin and nitric oxide regulate sunflower seedling growth under salt stress accompanying differential expression of Cu/Zn SOD and Mn SOD. *Free Radical Biol. Med.* **2017**, *106*, 315–328. [CrossRef] [PubMed]

62. Agastian, P.; Kingsley, S.J.; Vivekanandan, M. Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. *Photosynthetica* **2000**, *38*, 287–290. [CrossRef]

63. Zeng, L.; Cai, J.; Li, J.; Lu, G.; Li, C.; Fu, G.-p.; Zhang, X.; Ma, H.; Liu, Q.; Xiling, Z.; et al. Exogenous application of a low concentration of melatonin enhances salt tolerance in rapeseed (*Brassica napus* L.) seedlings. *J. Integr. Agric.* **2018**, *17*, 328–335. [CrossRef]

64. Wang, L.; Liu, J.; Wang, W.X.; Sun, Y.F. Exogenous melatonin improves growth and photosynthetic capacity of cucumber under salinity-induced stress. *Photosynthetica* **2016**, *54*, 19–27. [CrossRef]

65. Hu, C.-h.; Zheng, Y.; Tong, C.-l.; Zhang, D.-j. Effects of exogenous melatonin on plant growth, root hormones and photosynthetic characteristics of trifoliate orange subjected to salt stress. *Plant Growth Regul.* **2021**, *97*, 551–558. [CrossRef]

66. Bethke, P.C.; Drew, M.C. Stomatal and nonstomal components to inhibition of photosynthesis in leaves of capsicum annuum during progressive exposure to NaCl salinity. *Plant Physiol.* **1992**, *99*, 219–226. [CrossRef] [PubMed]

67. Lovelli, S.; Rivelli, A.R.; Nardiello, I.; Perniola, M.; Tarantino, E. Growth, leaf ion concentration, stomatal behaviour and photosynthesis of bean (*Phaseolus vulgaris* L.) irrigated with saline water. *Acta Hortic.* **2000**, *537*, 679–686. [CrossRef]

68. Zhao, H.; Wei, N.; Sun, C.; Bai, Y.; Zheng, C. Effects of salt stress on anatomic structure of tissue and photosynthesis in *Ginkgo biloba* seedlings. *J. Beijing For. Univ.* **2018**, *40*, 28–41. [CrossRef]

69. Kurt-Celebi, A.; Colak, N.; Torun, H.; Dosedělová, V.; Tarkowski, P.; Ayaz, F.A. Exogenous melatonin ameliorates ionizing radiation-induced damage by modulating growth, osmotic adjustment and photosynthetic capacity in wheat seedlings. *Plant Physiol. Biochem. PPB* **2022**, *187*, 67–76. [CrossRef]

70. Zhang, Y.; Guanter, L.; Berry, J.A.; Joiner, J.; Tol, C.v.d.; Huete, A.R.; Gitelson, A.A.; Voigt, M.; Köhler, P. Estimation of vegetation photosynthetic capacity from space-based measurements of chlorophyll fluorescence for terrestrial biosphere models. *Glob. Chang. Biol.* **2014**, *20*, 3727–3742. [CrossRef] [PubMed]

71. Luo, J.; Huang, C.; Peng, F.; Xue, X.; Wang, T. Effect of salt stress on photosynthesis and related physiological characteristics of Lycium ruthenicum Murr. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **2017**, *67*, 680–692. [CrossRef]

72. Shahid, M.A.; Pervez, M.A.; Balal, R.M.; Ahmad, R.; Ayyub, C.M.; Abbas, T.; Akhtar, N. Salt stress effects on some morphological and physiological characteristics of okra (*Abelmoschus esculentus* L.). *Soil Environ.* **2011**, *30*, 66–73.

73. Liang, D.; Zhiyou, N.; Xia, H.; Xie, Y.; Lv, X.-l.; Wang, J.; Lin, L.; Deng, Q.; Xian, L. Exogenous melatonin promotes biomass accumulation and photosynthesis of kiwifruit seedlings under drought stress. *Sci. Hortic.* **2019**, *246*, 34–43. [CrossRef]

74. Xie, Q.W.; Luo, H.; Cheng, X.; Li, Z.; Lu, W.; He, Z.-q.; Zhou, X. Effects of melatonin on growth, non-photochemical quenching and related components in tomato seedlings under calcium nitrate stress. *IOP Conf. Ser. Earth Environ. Sci* **2021**, *621*, 012104. [CrossRef]

75. Tsai, Y.-C.; Chen, K.; Cheng, T.-S.; Lee, C.; Lin, S.-H.; Tung, C.-W. Chlorophyll fluorescence analysis in diverse rice varieties reveals the positive correlation between the seedlings salt tolerance and photosynthetic efficiency. *BMC Plant Biol.* **2019**, *19*, 403–420. [CrossRef] [PubMed]

76. He, Y.; Chen, Y.; Yu, C.-L.; Lu, K.X.; Jiang, Q.S.; Fu, J.; Wang, G.M.; Jiang, D.-A. Photosynthesis and yield traits in different soybean lines in response to salt stress. *Photosynthetica* **2016**, *54*, 630–635. [CrossRef]

77. Chaves, M.M.; Flexas, J.; Pinheiro, C. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Ann. Bot.* **2009**, *103*, 551–560. [CrossRef] [PubMed]

78. Mi, P.; Yuan, F.; Guo, J.; Han, G.; Wang, B. Salt glands play a pivotal role in the salt resistance of four recretohalophyte Limonium Mill. species. *Plant Biol.* **2021**, *23*, 1063–1073. [CrossRef]

79. Harris, B.N.; Sadras, V.O.; Tester, M.A. A water-centred framework to assess the effects of salinity on the growth and yield of wheat and barley. *Plant Soil* **2010**, *336*, 377–389. [CrossRef]