Effects of Salt Stress on Chlorophyll Fluorescence and the Antioxidant System in Ginkgo biloba L. Seedlings

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Abstract. Ginkgo biloba L. (ginkgo) is generally regarded as a tolerant species to environmental stresses. However, its tolerance mechanisms are not well understood, particularly for salt stress. To evaluate the species’ physiological responses to salt stress, 3-year-old ginkgo seedlings were exposed to a range of salinity levels (0% to 1.0% NaCl). A significant reduction in maximum ($F_{v}/F_{m}$) and actual ($\Phi_{psii}$) quantum yields of photosystem II (PSII) photochemistry and the nonphotochemical quenching ($q_{N}$) coefficient only occurred in late treatment stages at the salinity levels of 0.6% to 1.0%. As salt concentration increased, the response time and chlorophyll (Chl) fluorescence indices decreased. Overall, the activities of superoxide dismutase (SOD) and peroxidase (POD); contents of catalase (CAT), reduced glutathione (GSH), and flavonoids; and scavenging rate of free radicals enhanced under salinity stress. These data indicate that ginkgo seedlings are tolerant to low salt stress, and enzymatic and nonenzymatic antioxidant systems seem to work synergistically to reduce lipid oxidation under NaCl stress because malondialdehyde (MDA) content did not increase. Correlation and multiple regression analysis showed that water potential, Chl fluorescence, and Chl a/b ratio were associated with salinity stress. The rest of the Chl fluorescence parameters, activities of POD and SOD, contents of CAT and flavonoids, and hydroxyl (•OH) and diphenyl picrylhydrazyl (DPPH) free radical scavenging capability were sensitive to salt stress. These parameters can be used for in vitro or rapid and nondestructive monitoring of the responses of ginkgo seedlings to salinity stress. It is of significance to understand the tolerance mechanisms of ginkgo to salt stress, reduce the harm of NaCl and other snow-melting agents to ginkgo as shade trees, and develop new salt-tolerant varieties.

Ginkgo biloba L. (ginkgo), primarily endogenous to China (Gong et al., 2008; Tredici 2008), is the only extant species in the Ginkgoaceae family and occupies a unique position in the history of plant evolution as the oldest relic of gymnosperms. The species is priced for its medicinal (DeFeudis et al., 2003; Rainer et al., 2018), ecological, and ornamental values. Ginkgo nut is also a traditional source of food in China and Japan (van Beek and Montoro, 2009). Ginkgo is widely cultivated as an important ornamental and street tree in many urban areas throughout most of the United States, China, Japan, and other countries because of its features, such as a straight trunk; unique two-lobe, fan-shape leaves that turn vivid yellow in fall (Handa et al., 1997; Sebbo et al., 2005); and high resistance to pests, diseases (Guan et al., 2016), and general tolerance of inhospitable growing conditions (Dmuchowski et al., 2019; Swoczyna et al., 2015). In China, this species has become a national symbol of its botanical heritage.

Given the intensification of soil salinization resulting from climate and environmental changes, and the extensive use of snow-melting agents in urban streets, studying the physiological mechanism of salt tolerance of ginkgo and identifying salt-tolerant varieties are urgently needed. High salt levels can lead to a series of changes in morphology, physiology, biochemistry, and molecular biology of cells and tissues in many plant species (Gupta and Huang, 2014; Munns and Tester, 2008). Consequently, salt stress can hinder growth and even result in mortality (Bidalia et al., 2017). Salt inhibits the differentiation of plant tissues and organs, reduces the incidence of leaf primordia, accelerates plant development, and causes presenility or death (Grieve et al., 1994). Salt stress increases chloroplast enzyme activity and acceleration of Chl decomposition (Megdiche et al., 2008), and decreases photosynthesis (Stepień and Klobus, 2006). Furthermore, activities of phosphoenolpyruvate carboxylase and ribulose bisphosphate carboxylase are reduced (Seemann and Critchley, 1985), thereby adversely affecting carbon assimilation (Matoh and Murata, 1990). Another significant consequence of salinity stress in plants is the excessive generation of reactive oxygen species (ROS), such as $O_{2}^{•–}$, $H_{2}O_{2}$, $•OH$, and $O_{2}^{•}$ (Apel and Hirt, 2004). Salt damage can seriously disrupt normal metabolism through oxidative damage to proteins, lipid, DNA, and, ultimately, cellular structures (Apel and Hirt, 2004). The original ion balance of $K^{+}$/Na$^{+}$ in plant cells is broken when Na$^{+}$ content in the soil is extremely high, leading to osmotic stress and ionic toxicity accompanied by reduced $K^{+}$ and Ca$^{2+}$ (Maimaiti et al., 2014; Silva et al., 2010).

Currently, three salt tolerance mechanisms have been revealed: a salt-secreted mechanism that removes salinity through saline glands in leaves and stems, a salt-rejected mechanism that prevents salt ions from entering the plants and redistributes salt ions to safety sites, and a salt-diluted mechanism that transports salt ions to plant vacuoles (Breckle, 1995), thereby reducing the toxicity of salt ions to important organelles and enzymes in cytoplasm (Greenway and Munns, 1980). Salt tolerance is associated with osmotic and ionic stress tolerance, reactive oxygen scavenging, and changes in metabolic pathways and tissue morphology. At the molecular level, salt stress is related to signal transduction of mitogen-activated protein kinase (Apel and Hirt, 2004), salt hypersensitive signal transduction, abscisic acid signal transduction pathway (Zhu, 2002), calcineurin signal transduction (Pardo et al., 1998), and signal transduction pathways involving other protein kinases.

Compared with studies of other species, studies of salt response and tolerance mechanisms on ginkgo are limited, although ginkgo is generally considered to have a
strong overall adaptability and recovery capability. According to its genome sequencing results, ginkgo has undergone genome-wide replication and has a large number of long terminal repeat insertion events. Gene families that provide various defense mechanisms are widely amplified as a result of its long-term evolution. For instance, a dual defense mechanism against insect attacks has been found (Guan et al., 2016). Currently available stress studies in ginkgo focus mainly on drought, waterlogging, heavy metal contamination, suboptimal light conditions, and ozone (Chen et al., 2014; Liang and Sun, 2002). Chen et al. (2014) reported that cell growth and flavonoid accumulation were stimulated at low salt doses (5–50 mM) in suspension-cultured ginkgo cells. As salt concentration increased, the following parameters also increased: dry/fresh weight of suspended cells, Chl $a$, Chl $b$, Chl $(a + b)$, $F_{m}/F_{m}'$, qN, $\Phi_{P_{ST}}$, and the photochemical quenching coefficient (qP). However, at greater salinity levels (150–175 mM), the cell structure was destroyed, antioxidant enzyme activity decreased, and flavonoids were not induced to cope with greater accumulation levels of $H_2O_2$ (Chen et al., 2014).

Our previous study showed that although a 64-d treatment with 0.2% NaCl decreased water potential in 3-year-old plants, height growth and ground diameter were not adversely affected until the NaCl concentration reached 0.6% and 0.8%, respectively (Zhao et al., 2018). Observation with scanning electron microscopy (SEM) also showed that salt crystal clusters accumulated in stem parenchymal cells but not in the roots and leaves (Zhao et al., 2018). In another previous study, we found that soluble protein, soluble sugar, and proline were mainly accumulated and used for osmotic adjustment in ginkgo under NaCl stress, and the synergistic effect of $\Delta'_\gamma$-pyrroline-5-carboxylate synthetase, ornithine-$\delta$-aminotransferase, and proline dehydrogenase promoted the accumulation of proline (Sun et al., 2017). In the current study, we investigated the physiological responses of ginkgo seedlings to different doses of NaCl and evaluated their salt tolerance by tracking changes in Chl fluorescence and antioxidant and nonenzymatic systems. Our goal was to understand the response mechanisms of ginkgo to salt stress. Because the species is currently facing the challenge of snow-melting agents and soil salinization, the results of our study will provide a theoretical basis for developing new salt-tolerant varieties and guidelines for the use of ice-melting chemicals. It may also help expand ginkgo planting areas in saline areas.

Materials and Methods

Plant materials and growth conditions. Three-year-old healthy ginkgo seedlings (diameter, 1.0–1.3 cm; height, 80–100 cm) were collected from a ginkgo nursery in Xuzhou, Jiangsu Province, China. Seedlings were transplanted into 10-L polyethylene pots on 18 Mar. 2015. The potting mix comprised an equal combination of local soil and peat (v/v). All seedlings were placed in a well-ventilated plastic shed at a nursery at Beijing Forestry University (Lat. 40°01’ N, long. 116°35’ E) for 3 months before the salt stress treatment was applied. The daytime/nighttime temperatures were 29 ± 4°C/20 ± 3°C, the averaged relative humidity was 65%, and photosynthetically active radiation (PAR) was 500 μmol·m$^{-2}$·s$^{-1}$, with an average of 217 h sunlight per month during the experiment period.

Salt treatment. A total of 30 healthy seedlings of similar height were selected and divided randomly into six groups (five plants per group). NaCl solutions (500 mL each) were prepared in concentrations of 0.2%, 0.4%, 0.6%, 0.8%, or 1.0% with tap water and were applied to the pots once per week. The control seedlings were treated with an equal volume of tap water (0% NaCl). Healthy leaf samples were collected at various time points from similar locations among individual plants, frozen rapidly in liquid nitrogen, and stored at −80°C for measurement of physiological indexes, unless otherwise indicated. No treatment, all groups were irrigated with tap water. After 66 d of treatment, the soil salt concentrations were calculated as described in Zhao et al. (2018) and were determined to be 0.22%, 0.44%, 0.57%, 0.84%, 0.95%, and 1.11% for the 0%, 0.2%, 0.4%, 0.6%, 0.8%, and 1.0% NaCl treatments, respectively. After 66 d of treatment, all groups were irrigated with tap water.

Determination of pigmentation content. Leaves (0.2 g, fresh weight) from each treatment time point of 8, 15, 36, and 64 d after salt treatment were ground individually and were applied to the pots once per week. After 66 d of treatment, the soil salt concentrations were calculated as described in Zhao et al. (2018) and were determined to be 0.22%, 0.44%, 0.57%, 0.84%, 0.95%, and 1.11% for the 0%, 0.2%, 0.4%, 0.6%, 0.8%, and 1.0% NaCl treatments, respectively. After 66 d of treatment, all groups were irrigated with tap water.

Analysis of Chl fluorescence parameters. Chl fluorescence parameters were monitored with a portable Chl fluorescence device (Junior-PAM; Walz, Germany) at 1000 HR (duration, 800 ms; $F_{m}/F_{m}'$, $F_{m}'$) to determine minimal fluorescence ($F_{o}$). Then, the maximal fluorescence ($F_{m}$) was measured using a saturation pulse (5000 μmol·m$^{-2}$·s$^{-1}$ PAR; duration, 800 ms). $F_{m}/F_{m}'$ was calculated as $F_{m}' / F_{m}$. After actinic light was switched on for 1 min (100 μmol·m$^{-2}$·s$^{-1}$ PAR), 10 saturation pulses were triggered to measure maximal Chl fluorescence in the light-adapted state ($F_{m}'$). $\Phi_{P_{ST}}$ was calculated as ($F_{m}' - F_{o}$)/$F_{m}'$ according to Genty et al. (1989). $q_{P}$ and $q_{N}$ were calculated according to Havaux et al. (1991) as follows: $q_{P} = (F_{m}' - F_{o})/(F_{m}' - F_{m})$, $q_{N} = (F_{m} - F_{m}')/(F_{m} - F_{m})$.

Measurement of cell damage induced by salt treatment. To estimate cell damage induced by salt treatment, relative electrical conductivity (REC) was measured with a LaMotte Conductivity Meter (LaMotte CON6, USA) to assess cell membrane permeability, according to the method of Sun et al. (2010). Specifically, 10 leaf disks from an individual plant were collected using a 0.6-cm-diameter hole punch; these were placed immediately in a tube with 10 mL distilled water. The air in the tube was removed with a vacuum pump for 20 min and the tube was allowed to stand for 40 min at room temperature before the electrical conductivity ($S_{1}$, μS·cm$^{-1}$) of the solution was recorded. The samples were then boiled for 15 min and the electrical conductivity ($S_{2}$, μS·cm$^{-1}$) was recorded after cooling to room temperature. The REC of the samples was calculated as ($S_{1}$/$S_{2}$) × 100%.

The MDA level in the leaves was measured using the thiobarbituric acid (TBA) method (Heath and Packer, 1965). Briefly, 0.2 g fresh leaves from each plant was homogenized with 5.0 mL 10% trichloroacetic acid solution and silica sand, followed by centrifugation at 2000 rpm for 10 min. The supernatant (2.0 mL) was collected and mixed with 2.0 mL 0.6% TBA. Absorption was recorded at 450, 532, and 600 nm.

Evaluation of the antioxidant system. Leaves (0.2 g, fresh weight) without midribs were homogenized in an ice bath mortar with 0.05 mol·L$^{-1}$ phosphate-buffered saline (PBS; pH 7.0) and placed in a tube with 5 mL PBS. The homogenates were centrifuged at 10,000 rpm for 15 min at 4°C to obtain supernatants for further analyses. The activities of SOD, CAT, and POD were estimated using nitroblue tetrazolium (Stewart and Bewley, 1980), the guaiacol test (Polle et al., 1994), and the method of Chance and Maehly (Aebi, 1984), respectively. GSH and flavonoid levels were determined using colorimetric methods described previously (Guri, 1983; Jia et al., 1999). DPPH and ·OH radical scavenging activities were assessed according to methods described by Hsu et al. (2008) and Mathew and Abraham (2006), respectively.

Data analyses. At least three technical repeats were performed for each assay, and the data were processed using SPSS version 23.0 (SPSS Inc., Chicago, IL) for statistical and principle component analyses. One-way analysis of variance was performed to identify statistically significant differences among treatments, followed by Duncan’s multiple range test at $P < 0.05$. Pearson’s correlation coefficient was used for correlation analysis. Raw data for water potential and plant growth included in correlation and principle component analyses were acquired from our previous report of ginkgo plants using the same experimental design (Zhao et al., 2018). Charts were made using Origin 8.0 software.
Results

Changes in pigment content under salinity stress. As shown in Fig. 1, leaf wilting was observed in the 0.8% and 1.0% NaCl treatment groups 64 d after treatment. Pigment content largely decreased significantly as NaCl concentration and treatment duration increased (Fig. 2). For instance, 1.0% NaCl significantly reduced all types of pigments, including Chl \((a + b)\), Chl \(a\), Chl \(b\), and Car, regardless of treatment period, compared with the control. Given that all leaves of seedlings in the 1.0% treatment group withered off by 64 d, no data were available for this time point. There were some exceptions in the treatments with a lower NaCl concentration. No significant difference was found between the 0.6% and 0.8% treatment groups at 64 d for all types of pigments, among 0.2% to 0.6% treatments at 8 d for Chl \(b\), and

Fig. 1. Leaves and stems of 3-year-old ginkgo seedlings grown under various NaCl concentrations (0%, 0.2%, 0.4%, 0.6%, 0.8%, 1.0%) after 64 d.

Fig. 2. (A–D) Effect of NaCl on the contents of chlorophyll (Chl) and carotenoid (Car) in leaves of 3-year-old ginkgo seedlings treated for 8, 15, 36, and 64 d. Data are mean ± se, \(n = 5\). Within the same time point, different letters indicate a significant difference by Duncan’s multiple range test \((P < 0.05)\).
among all less than 1.0%-treated groups at 64 d for Car.

Changes in Chl fluorescence parameters under salinity stress. Fluorescence parameters (Fig. 3) can be used to monitor the growth, physiological response, and PSII changes in plants under stress. No significant differences in Chl fluorescence parameters were observed during the early stage of stress, and the 0.2% treatment did not have an effect in all observed periods, with one exception ($F_{V}/F_{M}$ at 36 d). For the 1.0%, 0.8%, and 0.6% NaCl treatments, a significant reduction in $F_{V}/F_{M}$ was observed at 29, 36, and 50 d of treatment, respectively (Fig. 3A), whereas $F_{PSII}$ significantly decreased after 22, 36, and 64 d of treatment (Fig. 3B). For the 0.4% concentration, $F_{V}/F_{M}$ and $F_{PSII}$ were unaffected, except at 44 d, when a significant decrease occurred.

No significant differences in $q_{N}$ existed among different salinity groups before 36 d of treatment, whereas a strong decline in $q_{N}$ of seedlings exposed to 0.8% and 1.0% NaCl was observed compared with controls after 44 to 57 d of treatment. In the 0.4% and 0.6% groups, a significant reduction was found at 44 d. For $q_{P}$, significant differences were detected in salt-treated seedlings at 3, 44, and 57 d.

Cell damage induced by salt treatment. Changes in REC are commonly used as indicators of cell membrane permeability induced by cell damage. As shown in Fig. 4A, REC largely increased as NaCl concentration and treatment day increased. Although the 0.2% treatment did not have a significant effect at 8 and 36 d, REC increased significantly at 15 and 64 d for 0.2% NaCl. No significant difference was found between the 0.6% and 0.8% treatments, regardless of treatment day. The greatest REC value was found in the 36-d 1.0% treatment group, which more than tripled the control value.

Effects on antioxidant systems under salinity stress. When common stress-related markers were examined (Fig. 4B–F), their activities or contents largely showed an increasing trend, with the exception of MDA, which decreased, compared with the 0% NaCl control (Fig. 4B). During the early stages of salt stress, no significant differences in GSH levels were observed. As salt treatment concentration and duration increased, GSH level increased significantly (Fig. 4C). Flavonoid content was significantly high in all NaCl treatments and time points (Fig. 4D). For CAT, none of the treatments had an effect at 8 and 64 d (Fig. 4E). SOD activity increased in all NaCl concentrations and time points, except for 0.2% at 8 d (Fig. 3F). POD increased in all NaCl concentrations and time points with two exceptions—0.2% NaCl at 8 d and 0.8% at 15 d, which were similar to the control (Fig. 4G). At 15 d, 0.2% to 0.6% NaCl decreased the scavenging rate on DPPH free radicals, whereas greater concentrations did not have an effect (Fig. 4H). In comparison, 0.2% and 0.4% NaCl decreased the scavenging rate on the hydroxyl radical in 64 d, but 0.6% and 0.8% NaCl showed results similar to the control (Fig. 4I).

Correlation and principle component analyses. NaCl treatment had a significant correlation relationship with all traits being studied in at least one of the time points (Fig. 5). In particular, NaCl had a positive correlation with REC and a negative correlation with $F_{V}/F_{M}$, Chl ($a + b$), and Chl $a$ on all treatment days. At 64 d, the correlation of NaCl with plant and soil water potentials, plant height, and ground diameter was significantly negative. NaCl generally exhibited a significantly positive correlation with
stress-related markers and a negative correlation with Chl fluorescence indexes.

As expected, a positive relationship existed between soil and plant water potential. Plant height and stem ground diameter had a positive relationship with Chl, Car, $F_v/F_m$, and $F_{PSII}$, with the exception of plant height with Car. Enzymes and activities related to ROS scavenging mostly had a positive correlation among them and had a negative correlation with Chl fluorescence indices. Interestingly, a negative relationship was found between REC and MDA.

Principle component analysis identified five components that contributed to 90% of the response variations in NaCl treatment (Table 1). The major indices (absolute coefficient, $>0.7$) in each component were the scavenging rate of the hydroxyl radical, Chl b, Chl (a + b), $F_v/F_m$, and Car; plant water potential, GSH, and soil water potential; MDA, flavonoid, and POD; $q_N$ and $q_P$; and CAT (Table 2). Among these indices, the scavenging rate of the hydroxyl radical, Car, GSH, and MDA had a negative coefficient, whereas the rest had a positive coefficient.

**Discussion**

Ginkgo, also known as the maidenhair tree, is generally regarded as tolerant to environmental stresses. We reported previously that the height and ground diameter of 3-year-old ginkgo seedlings were not affected adversely until the NaCl concentration reached 0.6% and 0.8%, respectively (Zhao et al., 2018). This condition is comparable to that for 6-month-old poplar cuttings ($Populus euphratica$), in which the $0.88\%$ NaCl treatment for 2 months reduced diameter and height growth (Neko et al., 2018). In contrast, the growth rate of silver maple ($Acer saccharinum$ L.), a less-tolerant species, was affected by $0.3\%$ NaCl (Patykowski et al., 2018). When comparing salt tolerance among several 1-year-old tree species, Sun et al. (2009) reported that a $0.2\%$ NaCl treatment led to growth inhibition of stem height and diameter in $Platanus occidentalis$. For $Gleditsia triacanthos$, $Quercus virginiana$, $Myrica cerifera$, $Fraxinus pennsylvanica$, and $Salix matsudana$, the stem-diameter/growth-inhibiting NaCl concentration was $0.4\%$. For stem height, $G. triacanthos$ and $F. pennsylvanica$ were affected negatively by $0.4\%$ NaCl, whereas $Q. virginiana$ and $M. cerifera$ were affected negatively by $0.6\%$ NaCl. In our previous study, SEM revealed a large number of salt crystal clusters in the stem parenchyma cells of salt-treated ginkgo seedlings (Zhao et al., 2018). Thus, compartmentation may be a mechanism used in ginkgo for salt tolerance.

Chl is an important component of pigment–protein complexes on the thylakoid membrane, which reflects plant photosynthetic capacity. This component plays an important role in capturing and transmitting light energy in photosynthesis and is one of the most important indicators of salt tolerance. In the current study, Chl a and Chl b contents decreased gradually as NaCl treatment concentration and duration increased, which agreed with the results seen in other species, such as apple (Jia et al., 2019) and
Table 1. Results of principal component analysis of 19 indexes of 3-year-old ginkgo seedlings under NaCl stress.

| Principal component | Initial eigenvalue | Contribution rate (%) | Cumulative contribution rate (%) |
|---------------------|--------------------|------------------------|----------------------------------|
| 1                   | 5.179              | 27.260                 | 27.260                           |
| 2                   | 4.454              | 23.440                 | 50.700                           |
| 3                   | 3.836              | 20.190                 | 70.890                           |
| 4                   | 1.953              | 10.281                 | 81.171                           |
| 5                   | 1.680              | 8.844                  | 90.015                           |

Fig. 5. Pearson correlation coefficients among studied traits in 3-year-old ginkgo seedlings. An asterisk indicates significant differences at \( P < 0.05 \). REC, relative electrical conductivity; MDA, malondialdehyde; SOD, superoxide dismutase; POD peroxidase; CAT, catalase; GSII, reduced glutathione; Chl, chlorophyll; Car, carotenoids; \( F_v/F_m \), maximum quantum yield of photosystem II (PSII) photochemistry; \( \Phi_{PSII} \), actual quantum yield of PSII photochemistry; \( q_N \), nonphotochemical quenching coefficient; \( q_P \), photochemical quenching coefficient; DPPH, diphenyl picrylhydrazyl; \( \cdot \)OH, hydroxyl radical; WP, water potential.

cucumber (Stepień and Klobus, 2006). The decrease in Chl content may be attributed to increased degradation and inhibited synthesis of the pigment. The loss of Chl is usually accompanied by inactivation of photochemical reactions, especially those mediated by PSII in plants exposed to salt stress (Sharma and Hall, 1992). Enhanced activities of enzymes, such as chlorophyllase, hydroxylase, and dioxygenase, accelerate the catabolism of Chl (Matoh and Murata, 1990), whereas the relaxation of Chl and Chl proteins induced by ion toxicity prompts the dissociation of Chl (Maimaiti et al., 2014). During the early stage (8 d), Chl b content was significantly less only in our 1.0% NaCl groups compared with the control group, whereas Chl a and Car contents were affected by lower concentrations, indicating that Chl b was more stable under salt stress. This finding is in contrast with the results of a previous study of the European searocket (Cakile maritima) (Megdiche et al., 2008). This inconsistency may be the result of the different degrees to which chloroplasts are affected by ionic toxicity during the early stages of salt stress. Different plants may also have different Chl b responses. During the later stage (64 d), Chl b content increased in the 0.2% NaCl treatment group, indicating that low NaCl concentrations can promote photosynthesis. This result is consistent with the findings from ginkgo suspension cells (Chen et al., 2014).

Car can play a role in light protection by helping dissipate excess light energy in PSI and PSII by thermal and chemical reactions under stress (Lu et al., 2003). No significant differences in Car were observed between the treatments and control groups at 64 d. This finding suggests that Car is relatively stable and exhibits some tolerance to salt stress, which may help in scavenging active oxygen and protecting the photosynthetic membrane.

We reported previously that 0.2% NaCl enhanced the net photosynthesis rate (\( P_n \)) during 1 to 35 d of treatment, but greater NaCl treatments reduced \( P_n \) from day 1 (Zhao et al., 2018). Stomatal conductance was affected negatively by salt stress. However, water use efficiency was enhanced, at least under low salinity and short exposure. In the current study, the yield of Chl fluorescence emission was investigated. Chl fluorescence provides valuable information about the quantum efficiency of photochemistry and heat dissipation, and is commonly used as a criterion for photosystem efficiency. \( F_v/F_m \) reflects the maximum light energy conversion efficiency of PSII after adaptation to darkness, indicating that photoinhibition reflects the efficiency of light energy conversion in the active center of PSII (Lichtenthaler and Burkart, 1999). \( \Phi_{PSII} \) reflects the actual photochemical efficiency when the PSII reaction center is partly shut down under light. In the current study, Chl fluorescence parameters for different salt treatments were not significantly different from those of controls during the early stage of stress, indicating that PSII was unaffected. As salt treatment concentration and duration increased, \( F_v/F_m \) and \( \Phi_{PSII} \) decreased gradually in the medium- and high-concentration treatment groups (0.6% to 1.0%), and stability of the membrane system was seriously affected at 0.6% NaCl and more, suggesting the aggravation of the PSI reaction center at greater stress levels (Lu and Zhang, 2000). It has been similarly reported that an \( F_v/F_m \) value less than 0.6 indicates serious disturbances in PSIII performance (Percival, 2005), and this corresponds with diminished photosynthesis (Kalaji et al., 2011; Percival and Sheriffs, 2002). Additional effects of salt...
may be evoked by yearly salt accumulation in perennial trees (Zhao et al., 2018) and may lead to increasing leaf injuries (Swoczyna et al., 2009). In addition, light energy conversion efficiency and use were less, and photosynthesis was inhibited. These results reflect what was observed in ginkgo suspension cells (Chen et al., 2014), and species such as Elaeagnus oxyccarpa Linn. (Maimaiti et al., 2014) and Calendula officinalis (Baniyasadi et al., 2018), upon exposure to NaCl. In ginkgo seedlings treated with low concentrations of NaCl, a significant decrease in $\Phi_{PSII}$ was detected only after 36 and 44 d of treatment.

Chl fluorescence quenching is an approach by which energy dissipates in chloroplasts. The qN value reflects the aperture opening degree, PSII reaction center activity, and conversion efficiency of the captured light quantum into chemical energy. qP estimates the rate constant for heat loss from PSII. Before 36 d, salt treatment did not affect qN and qP, regardless of NaCl concentration, reflecting the capacity of young ginkgo seedlings to dissipate heat under salt stress. Decreased qN and qP were found only in high salt concentrations after 36 d, indicating the exacerbation of damage to the plants. Although these results were different from the ones from ginkgo suspension cultures (Chen et al., 2014) and Cakile maritima (Megdiche et al., 2012), they are consistent in part with the reports for Medicago arborea (Boughalleb et al., 2009).

The membrane system is the barrier of material and information exchange between plant cells and their surroundings. Its integrity and stability are important for maintaining normal physiological activities in plants. Excessive accumulation of Na+ can shift the original balance of free radicals in cells, thus causing membrane lipid peroxidation, which reduces membrane fluidity, increases permeability and electrolyte leakage, and affects metabolic processes (Ahmad et al., 2014). In the current study, as treatment duration progressed, membrane permeability (REC value) first increased and then decreased, indicating that seedling cell permeability had been noticeably damaged. This result is consistent with reports for coastal C. maritima seedlings (Amor et al., 2010). MDA results from the lipid peroxidation of polyunsaturated fatty acids and is an indicator of free radical production and consequent tissue damage. MDA accumulation generally increases along with REC (Tounekli et al., 2011). An opposite correlation was observed in the current study. This condition may be a salinity tolerance mechanism of ginkgo seedlings, similar to tolerant ramie (Boehmeria nivea) plants. Huang et al. (2015) reported that the trends of MDA content in salt-tolerant and salt-sensitive ramie cultivars were completely disparate when exposed to salinity. Although REC increased in both cultivars, MDA increased in the sensitive cultivar but decreased in the tolerant one.

Plants have enzymatic and nonenzymatic antioxidant systems. SOD, POD, and CAT are important protective enzymes in the plant enzymatic defense system. These enzymes relieve damage caused by membrane lipid peroxides (Wu et al., 2014). The collaboration of SOD, POD, and CAT effectively prevents lipid peroxidation; reduces membrane damage caused by ROS; and improves salt tolerance. During the early stages of salt stress in the current study, SOD, POD, and CAT activities were all enhanced, consistent with observations for Malus robusta (Bai et al., 2009). GSH levels were observed after 8 d, but a significant increase was detected after 15 d, indicating that the response of GSH production to salt stress occurred at a later period. In recent decades, exogenous GSH has been applied successfully to alleviate stress damage in Malus robusta (Bai et al., 2009). Abundant flavonoids are present in ginkgo leaves, which protect cells and chromosomes from ROS damage (Agati et al., 2012). In the current study, the flavonoid content in ginkgo leaves increased significantly after salt treatment, indicating that flavonoid production and accumulation had a positive response to salt stress; this finding is analogous to those found previously in ginkgo cells (Chen et al., 2014).

In conclusion, severe salt stress decreases pigment content and activity of photosynthetic electron transport ($\Phi_{PSII}$, qN), inhibits conversion ($F_v/F_m$) of light energy, and destroys cell membrane structure (REC) in ginkgo seedlings. However, Chl fluorescence is unaffected under low salt stress, and growth is only affected under severe stress. Therefore, ginkgo seedlings are tolerant only to low salt stress. The current study suggests that active enzymic and nonenzymatic antioxidant systems work synergistically to reduce lipid oxidation under NaCl stress. The reduced accumulation of MDA under salt stress warrants further investigation because of its possible association with tolerance. Last, correlation and principal component analyses indicated that water potential, Chl fluorescence parameters, activities of POD and SOD, contents of CAT and flavonoids, and hydroxyl and DPPH free radical scavenging capability are sensitive to salt stress and can be used for in vitro or rapid and nondestructive monitoring of the responses of ginkgo seedlings to salinity stress.

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