Front. For. China (2006) 1: 82–88
DOI 10.1007/s11461-005-0019-8

RESEARCH ARTICLE

**Wang Ruigang, Chen Shaoliang, Ma Haiyuan,
Liu Liyuan, Li He, Weng Haijiao,
Hao Zhiyong, Yang Shuang**

Genotypic Differences in Antioxidative Stress and Salt Tolerance of Three Poplars Under Salt Stress

© Higher Education Press and Springer-Verlag 2006

Abstract To evaluate genotypic difference in antioxidative ability and salt tolerance in poplars, the authors investigated the effects of increasing content of soil NaCl on salt concentration in leaves, superoxide dismutase (SOD) and peroxidase (POD) activities, malondialdehyde (MDA) content, and membrane permeability (MP) in *Populus euphratica* Oliv., *P. popularis* "35–44," and *P.* × *euramericana* cv. I-214 (hereafter abbreviated as *P.* cv. I-214). Na^+ and Cl^- concentrations in leaves of *P. popularis* increased markedly over the increase of the duration of exposure to salinity, and culminated after 28 days of salt stress. SOD and POD activities declined correspondingly, followed by significant increases of MDA and MP, and leaf injury was finally observed. Compared with *P. popularis*, leaf Na^+ and Cl^- in *P.* cv. I-214 exhibited a trend similar to *P. popularis*, but a lower salt-induced increase of MDA and permeability was observed and lighter leaf necrosis occurred. In contrast to *P. popularis* and *P.* cv. I-214, SOD and POD activities in *P. euphratica* leaves increased rapidly at the beginning of salt stress with a light soil NaCl concentration of 58.5 mmol/L. Furthermore, salt ion concentration, MDA content, and MP in *P. euphratica* leaves did not increase significantly during 28 days of increasing salt stress. Therefore, the increase in MP in *P. popularis* and *P.* cv. I-214 had a close relationship with a salt buildup in leaves under increasing salt stress. Salt-induced declines of SOD and POD activities might accelerate lipid peroxide and consequently resulted in ion leakage. *P. euphratica* rapidly activated antioxidant enzymes after the onset of salt stress, which might reduce the accumulation of reactive oxygen species and the subsequent acceleration of lipid peroxide. *P. euphratica* leaves exhibited a higher capacity to exclude salt in a longer period of increasing salinity, thus limited salt-induced lipid peroxide and MP, which contributed to membrane integrity maintenance and salt tolerance of *P. euphratica*.

Keywords *Populus*, Na^+, Cl^-, superoxide dismutase (SOD), peroxidase (POD), malondialdehyde (MDA), membrane permeability, antioxidative, salt tolerance

Translated from *Journal of Beijing Forestry University*, 2005, 27(3) (in Chinese)

Wang Ruigang, Chen Shaoliang (✉), Ma Haiyuan,
Liu Liyuan, Li He, Weng Haijiao,
Hao Zhiyong, Yang Shuang
College of Biological Sciences and Biotechnology,
Beijing Forestry University,
Beijing 100083, China
E-mail: lschen@bjfu.edu.cn

1 Introduction

Normally, there is a balance between the production of reactive oxygen species (ROS) and its removal in plants, but this balance will be destroyed by salt stress and will result in an accumulation of ROS. Excessive ROS may initiate membrane lipid peroxidation, weaken membrane lipid unsaturation, trigger membrane protein polymerization, and result in an increase in membrane permeability (MP) [1]. ROS can also cause dysfunction of proteins, attack nucleic acids, lead to DNA destruction and mutation [2], disrupt DNA copy and transcription [3], and finally cause cell death and formation of abnormal proteins like heat shock proteins. Therefore, plants have developed a complex antioxidant system to repair the damage caused by ROS, including antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD), and catalase, as well as antioxidant metabolites like ascorbic acid (ASA) and glutathione (GSH). Studies indicated that antioxidant enzymes acted as key roles in plant antioxidant stress, which was proven by two major evidences: (1) stress induced the increase of antioxidant enzyme activities, which was correlated directly with stress tolerance; (2) plant pretreated with a kind of stress could produce cross-tolerance to other different stresses. For example, a breed tolerant to paraquat could induce increase of antioxidant enzymes to produce cross-tolerance to heat shock and cold stress [4]. Tsugane et al. [5] suggested that salt-tolerant *Arabidopsis* mutants might enhance oxidative stress tolerance.

Studies on salt tolerance of *Populus euphratica* focused mainly on plant growth, uptake, transport, distribution, and

compartmentation of saline ions; uptake, transport and osmotic adjustment of nutrients; and ABA signal conduction. However, less emphasis was put on salt-induced oxidative stress. Only effects of NaCl on the changes of SOD activities in leaves and roots of *P. euphratica* were reported in 1998 [6]. The objective of this study was to evaluate mechanisms of antioxidant stress of *P. euphratica* under salt stress compared with salinity-sensitive poplars.

2 Materials and methods

2.1 Plant materials

One-year-old seedlings of *P. euphratica* Oliv. and 1-year-old rooted cuttings of *P.* × *euramericana* cv. I-214 (hereafter abbreviated as *P.* cv. I-214) and *P. popularis* "35-44" (*P. popularis*) were used in this experiment. Hardwood cuttings of *P. popularis* and *P.* cv. Italica were obtained from the nursery of Beijing Forestry University and seedlings of *P. euphratica* from Xinjiang Uygur Autonomous Region. On April 6, 2003, the materials were planted in 10-L pots containing nursery soil and sands (soil/sand=2:1). Potted plants were well watered and fertilized by 1 L of full-strength Hoagland's nutrient solution every 2 weeks. Potted plants were placed in a greenhouse at the nursery of Beijing Forestry University prior to the salt treatment in July.

2.2 Salt treatments and sampling

Plants were subjected to 4 weeks of increasing NaCl stress. In the first 2 weeks, saline treatment was imposed by top watering of 1 L of 100 mmol/L NaCl and full-strength Hoagland's nutrient solution weekly. In the third and fourth weeks, plants were watered by 1 L of 150 and 200 mmol/L NaCl (in full-strength Hoagland's nutrient solution), respectively. Control plants were kept well watered and fertilized weekly by watering 1 L of full-strength Hoagland's nutrient solution without NaCl. Leaves were sampled after 1, 8, 21, and 28 days of exposure to the initial saline treatment, with three replications per treatment for every species. Immediately after the fully expanded leaves were sampled from upper part of shoots, leaf malondialdehyde (MDA) content, MP, and activities of SOD and POD were examined. Parts of sampled leaves were oven-dried at 65°C for 4 days, ground and passed through a 1.0-mm sieve, and stored for Na^+ and Cl^- measurements.

2.3 Leaf ion analysis

Extraction and determination of Na^+ and Cl^- were performed according to Chen et al. [7] with some modifications. Dried leaves (0.2–0.3 g) were incubated in 25 mL of 1 mol/L HNO_3 at 95–100°C for 12 h, then cooled, homogenized, and stored overnight. The extract was filtrated and the supernatant was diluted to 50 mL with deionized water for the measurement of Na^+ and Cl^- concentrations.

For Cl^-, abundant $AgNO_3$ (0.025 mol/L, 1–10 mL) was added to 20 mL of the extract to precipitate chloride in a 150-mL triangle bottle. The extract was heated at a moderate temperature after adding 10 mL of 1:1 HNO_3 (v/v); saturated $KMnO_4$ was added to clear the color of the extract and then cooled. Excessive Ag^+ was estimated by 0.01 mol/L NH_4SCN titration. $NH_4Fe(SO_4)_2$ was used as a color indicator for isoionic point determination.

For Na^+, Na^+ concentration was measured at 589.0 nm by atomic absorption spectrophotometer (Perkin-Elmer 2280).

2.4 Analysis of soil

According to Chen et al. [8], at each sampling time, soil was sampled at 20–30 cm depth in pots, and soil moisture content was determined. Extracts of soil samples (dried soil/deionized water=1:5, w/v) were used for Na^+ and Cl^- measurements. Na^+ and K^+ were measured by flame spectrophotometer (Perkin-Elmer 2280), Ca^{2+} and Mg^{2+} concentrations by atomic absorption spectrophotometer (Rili-180-80), and Cl^- by silver titration [7]. Ion concentration (mmol/L) was expressed as the ratio of ion content (mmol) per 1,000 g of dry soil to soil moisture content (kg) per 1,000 g of dry soil.

After 1, 8, 21, and 28 days of NaCl treatment, NaCl concentrations in the soil reached 58.5, 81.3, 137.2, and 213.8 mmol/L, respectively, whereas soil NaCl in the control treatment was maintained at approximately 10 mmol/L throughout the experiment. There is a notable difference in K^+, Ca^{2+}, and Mg^{2+} concentration between NaCl-treated and control soil.

2.5 Superoxide dismutase activity measurements

Total SOD activity was assayed by monitoring the superoxide radical-induced nitroblue tetrazolium (NBT) reduction at 560 nm [9]. One unit of SOD was defined as the amount of enzyme that causes a 50% inhibition of the aforementioned reaction in comparison with a blank sample. Briefly, approximately 0.500,0 g (fresh weight) of leaf tissue was homogenized on ice in 2 mL of precooled 0.05 mol/L sodium phosphate buffer (pH 7.8) containing 1% PVPP-40. The homogenate was centrifuged at 1,500×g for 10 min at 4°C, and the supernatant was diluted to 25 mL. The diluted enzyme extract (20 μL) was then added into a 3-mL reaction system containing 1.5 mL of 0.05 mol/L sodium phosphate buffer (pH 7.8), 0.3 mL of 30 mmol/L methionine, 0.3 mL of 750 μmol/L NBT, 0.3 mL of 100 μmol/L EDTA–Na_2, 0.3 mL of 120 μmol/L riboflalivin, and 100 μL of distilled water. In blank controls, 200 μL of 0.05 mol/L sodium phosphate buffer (pH 7.8) was added into the reaction system instead of the enzyme extract. Afterward, the mixtures were illuminated by cool white light of 4,000 lx [30 μmol/(m^2·s)] for 8 min; then, colorimetry at 560 nm and absorbencies (A) were recorded.

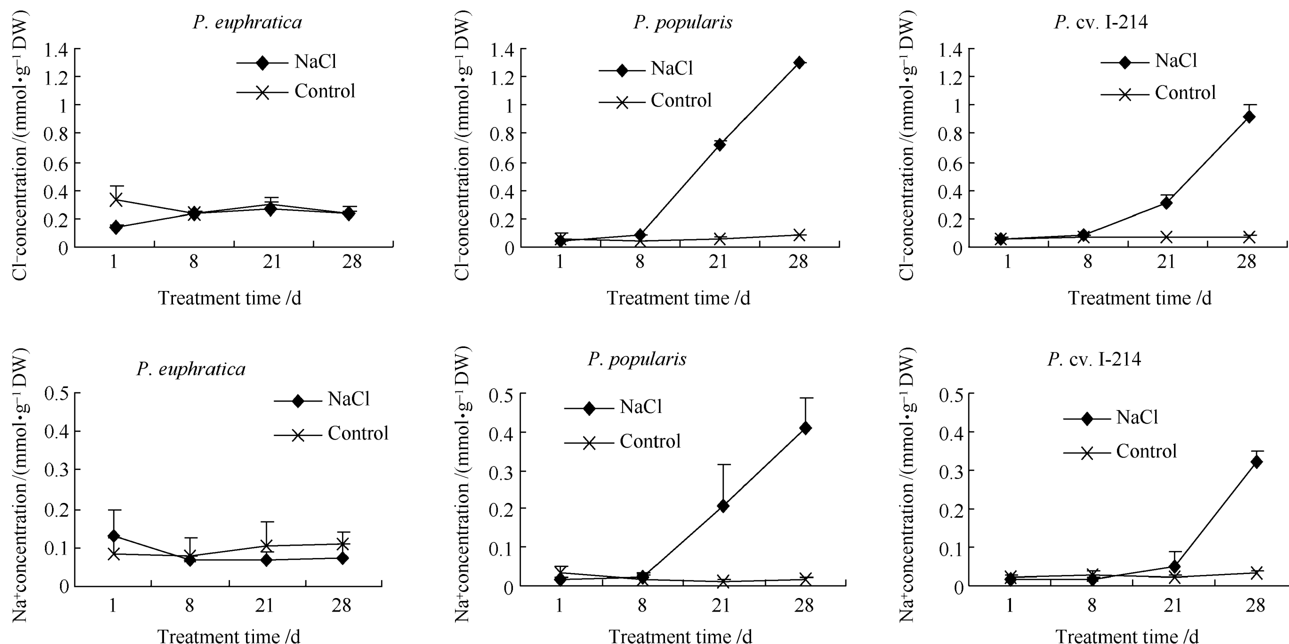

Fig. 1 Effects of NaCl on Na^+ and Cl^- concentration in leaves of *P. euphratica*, *P. popularis*, and *P.* cv. I-214. Each *point* is the mean of three plants, and *bars* represent the standard error of the mean (also applicable in the succeeding figures)

The absorbency used for SOD activity calculation was $A_{\text{enzyme}}-A_{\text{control}}$.

2.6 Peroxidase activity measurements

The measurement of total POD activity was carried out based on the determination of guaiacol oxidation [extinction coefficient 26.6 mmol/(L·cm)] at 470 nm by H_2O_2 [10]. In brief, approximately 0.50 g (fresh weight) of leaf tissue was homogenized on ice in 2 mL of 0.05 mmol/L sodium phosphate buffer (pH 5.5) containing 1% PVPP-40. The homogenate was centrifuged at 1,500×*g* for 10 min at 4°C, and then, the supernatant was diluted to 25 mL for assays. We added 300 μL of the diluted enzyme extract into a 5-mL reaction system that contained 2.7 mL of 0.05 mmol/L sodium phosphate buffer (pH 5.5), 1.0 mL of 2% H_2O_2, and 1.0 mL of 0.05 mol/L guaiacol that had been incubated at 34°C (the optimum temperature for enzyme reaction). Immediately after the addition of the enzyme extracts, the absorbency of the mixture was measured at 470 nm and recorded every 30 s during a 150-s observation period. One unit of POD was defined as the amount of enzyme that causes a 0.01 absorbency increase at 470 nm in comparison with a blank control. In blank controls, the same amount of 0.05 mol/L sodium phosphate buffer (pH 5.5; 300 μL) was added into the reaction system instead of the enzyme extract.

2.7 Malondialdehyde content measurements

Standard procedures required for MDA measurement were followed as Heath and Pacher [11]. Approximately 0.50 g (fresh weight) of leaf tissue was homogenized in 1.5 mL of 5% trichloroacetic acid (TCA; w/v). The homogenate was centrifuged at 1,500×*g* for 10 min, and then, the supernatant was diluted to 10 mL. We mixed 2 mL of the diluted extract with 2 mL of 0.67% 2-thiobarbtiuric acid (TBA; w/v). The mixture was incubated in boiled water (95–100°C) for 30 min, then centrifuged at 1,500×*g* for 10 min. Absorbencies of the aqueous phase at 450, 532, and 600 nm were measured, respectively. MDA content in the

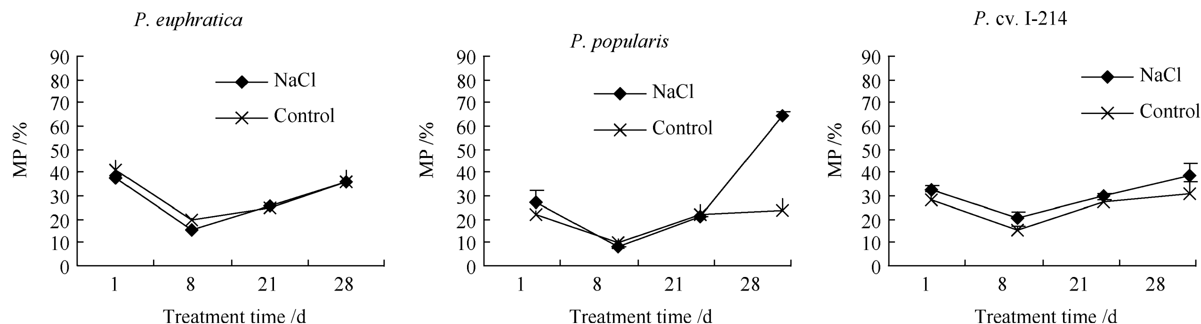

Fig. 2 Effects of NaCl on MP (%) in leaf of *P. euphratica*, *P. popularis*, and *P.* cv. I-214

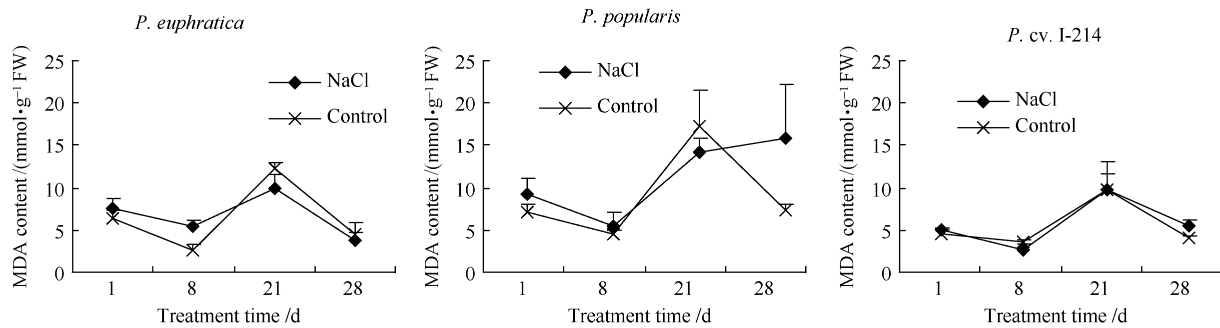

Fig. 3 Effects of NaCl on MDA contents in leaves of *P. euphratica*, *P. popularis*, and *P.* cv. I-214

aqueous phase was calculated according to the following format: C (μmol/L)=6.45×(A_{532}–A_{600})–0.56×A_{450}.

2.8 Membrane permeability measurements

Thirty leaf discs, 0.2 cm in diameter, were immersed in 10 mL of distilled water, and a subsequent 30 min vacuum was applied. Then, electrical conductivity (E1) was measured with DDS-307 conductivity meter (Shanghai Precision & Scientific Instrument) at room temperature. Afterward, leaf discs were incubated in boiled water (95–100°C) for 30 min, and electrical conductivity (E2) was measured at room temperature. MP of leaf was calculated as E1/E2×100%.

2.9 Data analysis

All data were subjected to ANOVA, and significant differences between means were determined by Duncan's multiple-range test. Unless otherwise stated, differences were considered statistically significant when $P<0.05$.

3 Results

3.1 Na^+ and Cl^- concentrations

Leaf Na^+ and Cl^- levels in control *P. euphratica* plants were typically higher than those in control plants of *P. popularis* and *P.* cv. I-214 during the observation period (Fig. 1). Leaf Na^+ and Cl^- concentrations in NaCl-treated plants of the three tested genotypes did not significantly increase in the first 8 days of salt stress; however, a genotypic difference in buildup of Na^+ and Cl^- was observed in following days of salinity (Fig. 1). Leaf Na^+ and Cl^- levels in *P. popularis* and *P.* cv. I-214 increased sharply corresponding to the NaCl increment in the soil (Fig. 1). Salinized *P. popularis* accumulated Na^+ 17.4-fold higher and Cl^- 12.2-fold higher than control plants on the 21st day (Fig. 1); for NaCl-treated *P.* cv. I-214, it was Na^+ 2.5-fold higher and Cl^- 4.2-fold higher, respectively. Compared with control plants, Na^+ and Cl^- concentrations of NaCl-treated *P. popularis* and *P.* cv. I-214 reached the highest level on the 28th day, Na^+ concentrations in leaves of *P. popularis* and *P.* cv. I-214 increased 22.8- and 9.3-fold, and Cl^- concentrations increased 15.8- and 10.5-fold. In contrast to *P. popularis* and *P.* cv. I-214, there was no significant increase in Na^+ and Cl^- in *P. euphratica* leaves during 4 weeks of increasing salt stress (Fig. 1).

3.2 Membrane permeability

Compared with nonstressed plants, MP in *P. popularis* leaves remained unchanged in the first 3 weeks of salt stress, but a notable rise (124%) was observed on the 28th day (Fig. 2). The trend of MP in salinized *P.* cv. I-214 plants was similar to *P. popularis* except for a lower MP increase (26%) on the 28th day (Fig. 2). In contrast, leaf MP in *P. euphratica* did not notably increase compared with control plants during the period of experiment (Fig. 2).

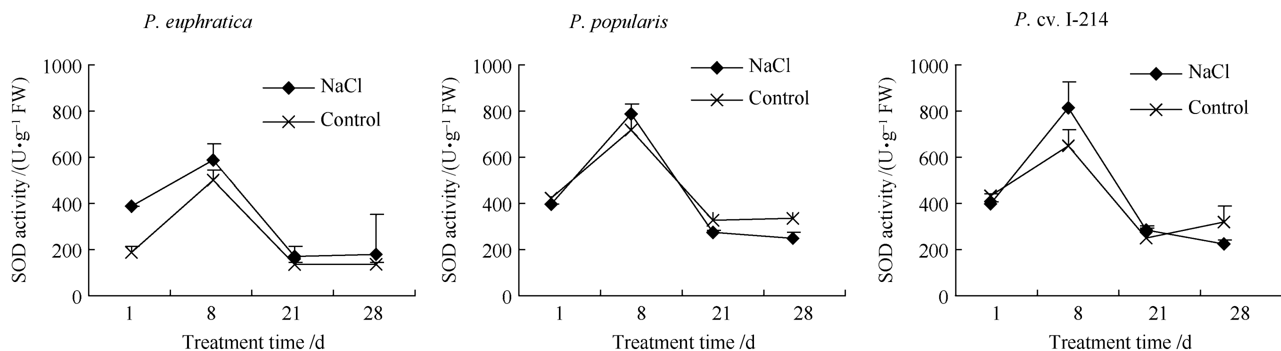

Fig. 4 Effects of NaCl on SOD activity in leaves of *P. euphratica*, *P. popularis*, and *P.* cv. I-214

3.3 Malondialdehyde content

Leaf MDA contents in NaCl-treated plants of *P. popularis* and *P.* cv. I-214 remained at a level similar to those in controls during the first 21 days of salt stress, even though MDA levels varied greatly over the observation period (Fig. 3). On the 28th day, an evident increase in MDA was observed in salinized plants of both genotypes, and MDA increased by 114% in *P. popularis* and 26% in *P.* cv. I-214 (Fig. 3). In contrast to *P. popularis* and *P.* cv. I-214, MDA contents in leaves of salinized *P. euphratica* plants did not significantly differ from those in control plants during the period of salt stress (Fig. 3).

3.4 Superoxide dismutase activity

Leaf SOD activity in control plants of the three genotypes varied markedly over the observation period (Fig. 4). SOD activity in salinized plants of *P. popularis* and *P.* cv. I-214 was similar to controls by the 21st day, but a 30% and 29% decline of leaf SOD was found on the 28th day, respectively (Fig. 4). In contrast, there was no significant decrease in SOD activity in *P. euphratica* during the period of salt stress (Fig. 4). Notably, leaf SOD activity in *P. euphratica* increased by 100% after salt stress was initiated, whereas there was no corresponding change in *P. popularis* and *P.* cv. I-214.

3.5 Peroxidase activity

There were gentle genotypic differences in the pattern of POD response to salinity. Leaf POD activity in *P. euphratica* increased by 100% after 1 day of salt treatment, and then kept at a similar level to controls in following days of salt stress (Fig. 5). There was a slight increase in POD activity in *P. popularis* and *P.* cv. I-214 at the beginning of salt treatment (Fig. 5). POD activity in salinized *P. popularis* plants increased remarkably on the 21st day, and then decreased gently (Fig. 5). *P.* cv. I-214 had a marked increase in POD activity on the 28th day, which was 1 week later than *P. popularis* (Fig. 5; data not shown).

4 Discussion

4.1 Leaf salt concentration, membrane permeability, and malondialdehyde content

Leaf Na^+ and Cl^- concentrations in *P. euphratica* leaves had no marked increase during the period of salt stress; however, those in leaves of *P. popularis* and *P.* cv. I-214 greatly increased along with increasing soil NaCl although a slower increase of salt was observed in *P.* cv. I-214 (Fig. 1). Results indicate that *P. euphratica* leaves had a greater ability to exclude salt, which is consistent with our previous study. The greatest capacity for salt exclusion in *P. euphratica* is likely the result of root-to-shoot salt transport restriction [8] because Na^+ and Cl^- concentrations in roots of three poplars increased significantly during the salt treatment (data not shown).

Results showed that the increased MP in the two salt-sensitive genotypes was associated with Na^+ and Cl^- accumulation in leaves. Following the great buildup of Na^+ and Cl^- on the 28th day, leaf MP in *P. popularis* and *P.* cv. I-214 increased by 124% and 26%, respectively (Fig. 2). However, there was no similar change of MP in *P. euphratica* due to lower levels of Na^+ and Cl^- (Fig. 2). Increased MP indicates the destruction of cellular membrane integrity. In accordance, salinized plants of *P. popularis* and *P.* cv. I-214 exhibited a clear symptom of leaf injury, whereas there was no leaf necrosis in *P. euphratica*.

Leaf MDA content in *P. popularis* and *P.* cv. I-214 increased considerably after 28 days of salt treatment, suggesting that the elevated MP resulted from lipid peroxidation because MDA content is used as an indicator of lipid peroxidation under oxidative stress [12–14] (Fig. 3). Recent research found that H_2O_2 content in leaves increased under salt stress, followed by the rise of MDA content and MP [15]. Compared with control plants, MDA content in leaves of *P. euphratica* did not increase notably during the whole salt treatment, whereas MDA content in the most salt-sensitive *P. popularis* increased by 114% on the 28th day when salt ion in leaves built up greatly, which indicated a serious lipid peroxidation took place. The trend of MDA content in salinized *P.* cv. I-214 plants was similar

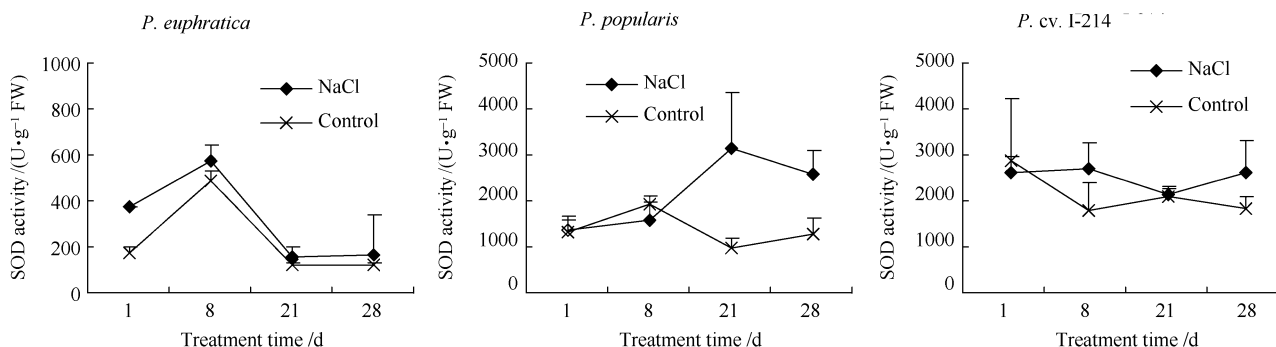

Fig. 5 Effects of NaCl on POD activity in leaves of *P. euphratica*, *P. popularis* and *P.* cv. I-214

to *P. popularis* except for a lower MP increase on the 28th day (Fig. 2). Therefore, it can be concluded that salt-induced MP in salt-sensitive poplars was the result of salt accumulation in leaves, which accelerated membrane lipid peroxidation and, thus, caused ion leakage and leaf damage.

4.2 Salt-induced oxidative stress and activities of antioxidant enzymes

Lipid peroxidation is correlated with ROS levels in cells. When the accumulation of ROS exceeds the scavenging capacity of antioxidant systems, ROS began to accumulate, reduced activities of antioxidant enzymes, and increased membrane peroxide and permeability [12,13,15–18]. SOD and POD have been considered two key enzymes in ROS elimination [19–22], and variations of their activities reflect the ability to scavenge oxygen free radicals under environmental stresses [16,21,23]. An increasing number of studies proved that, under salt stress, antioxidant enzyme activities in plants declined and MDA accumulated rapidly, which resulted in an increase in permeability of plasma membranes [13,24,25]. In this study, the decreased leaf SOD activity in *P. popularis* on the 21st day suggested the reduced ability in O_2^- elimination (Fig. 4). At this time, POD activity in *P. popularis* leaves increased significantly and reached the highest level (Fig. 5), but the levels of MDA and MP did not increase compared with control plants (Figs. 2 and 3), indicating that salinized *P. popularis* plants were able to maintain the lower level of H_2O_2, which reduced cell damage by 7OH produced by Haber–Weiss reaction of H_2O_2 and O_2^-. On the 28th day, leaf POD activity in salt-stressed *P. popularis* was significantly reduced (Fig. 5), but both MDA content and MP increased markedly (Figs. 2 and 3), indicating that H_2O_2 production had exceeded the scavenging capacity of POD. Although leaf POD activity in *P. popularis* remained at a relatively high level (Fig. 5), H_2O_2 accumulation induced Haber–Weiss reaction and produced a large amount of 7OH, resulting in increased lipid peroxidation and MP. Changes of SOD and POD activities in *P.* cv. I-214 leaves followed a trend similar to *P. popularis* except for the fact that the marked SOD reduction and POD increase occurred 1 week later (Figs. 4 and 5). In contrast to *P. popularis* and *P.* cv. I-214, leaf SOD and POD activities in *P. euphratica* did not decline over the duration of salt stress (Figs. 4 and 5), and there were no marked elevation of MDA content and MP (Figs. 2 and 3), indicating that antioxidant enzymes in *P. euphratica* leaves were able to scavenge stress-induced ROS, and thus maintained a balance of ROS generation and elimination in the long period of salt stress.

It is noteworthy that SOD and POD activities in *P. euphratica* leaves increased by 100% after the onset of salt stress, whereas there was no corresponding change in SOD and POD activities in the other two salt-sensitive genotypes, *P. popularis* and *P.* cv. I-214 (Figs. 4 and 5). This suggested that a lower soil NaCl (NaCl concentration was 58.5 mmol/L) increased the activities of antioxidant enzymes, which was favorable for *P. euphratica* to adapt to salt stress through reducing ROS and uncontrolled oxidation at the beginning of salt stress. A rapid rise of antioxidant enzymes in leaves of *P. euphratica* is probably associated with salt-induced ABA. In previous studies, we found that salt-tolerant *P. euphratica* could sense soil salt stress and the root system had a higher ability to synthesize ABA, which triggers adjustments that confer salt tolerance [7,26]. Similar results were observed in the present study. ABA has been suggested to induce activities of antioxidant enzymes, including total SOD, catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) [27–34], and related gene expression [27–31]. Endogenesis ABA is correlated with the upgrade of antioxidative systems induced by stresses, and the upgrade of antioxidative systems is closely associated with stress tolerance. Pretreatment with ABA can reduce heat-stress-induced lipid peroxide and increase the survival rate of *Arabidopsis* [35]. ABA inhibitor fluridone can inhibit improvement of antioxidative stress ability under water stress [36–38]. Recent researches found that ABA can increase the ability of antioxidative stress under salt stress [34]. It was also found that ABA could increase SOD and POD activities of *Medlar* callus, decrease MDA content, and increase stability of membrane under saline conditions [39]. Our experiment indicated that the upgrade of SOD and POD activities in leaves of *P. euphratica* at the beginning of light salt stress might be induced by considerable ABA synthesis in roots; in contrast, SOD and POD activities in leaves of *P. popularis* and *P.* cv. I-214 did not change under the same condition, which might result from little ABA synthesis in roots.

In conclusion, the increased leaf MP in *P. popularis* and *P.* cv. I-214 was associated with accumulation of salts in a longer period of salt stress. The buildup of salts may initiate membrane lipid peroxidation that was characterized by increased MDA levels. *P. popularis* and *P.* cv. I-214 were not able to control a balance of ROS generation and elimination due to salt-induced reduction of SOD activity and the limited POD activity; consequently, ROS accelerated lipid peroxidation and resulted in an ion leakage. Compared with the two salt-sensitive genotypes, *P. euphratica* exhibited a greater ability in salt-induced antioxidative stress. *P. euphratica* rapidly activated antioxidant enzymes after the onset of salt stress, which might reduce ROS accumulation and the subsequent acceleration of lipid peroxidation. *P. euphratica* leaves exhibited a higher capacity to exclude salt in a longer period of increasing salinity, thus limiting salt-induced lipid peroxidation and MP, which contributed to membrane integrity maintenance and salt tolerance of *P. euphratica*.

Acknowledgements This research was financially supported by the Key Project of the National Natural Science Foundation of China (grant no. 30430430), the Foundation for the Author of National Excellent Doctoral Dissertation of China (grant no. 200152), and the Teaching and Research Award Program for Outstanding Young Teachers in Higher Education Institution of MOE, China (grant no. 2002-323). Mr. Lai Kunxiang is greatly acknowledged for his kindly providing plant materials.

References

1. Chen S.-Y., Injury of membrane lipid peroxidation to plant cell, Plant Physiol. Commun., 1991, 27(2): 84 –90
2. Imlay J.-A. and Linn S., DNA damage and oxygen radical toxicity, Science, 1998, 240: 1,302–1,309
3. Byrd S., Reins D. and Doetsch P.-W., Effects of oxidative DNA damage on transcription by RNA polymerases, Free Radic. Biol. Med., 1990, 9(1): 47–57
4. Foyer C.-H., Descourvieres P. and Kunert K.-J., Protection against oxygen radicals: an important defense mechanism studied in transgenic plants, Plant Cell Environ., 1994, 17: 507–523
5. Tsugane K., Koboyashi K. and Niwa Y., A recessive *Arabidopsis* mutant that grows photoautotrophically under salt stress shows enhanced active oxygen detoxification, Plant Cell, 1999, 11: 1,195–1,206
6. Ma H.-C. and Wang S.-S., The stability of the membrane and metabolic response of *P. euphratica* under salt stress, J. Southwest For. Coll., 1998, 18(1): 15–22
7. Chen S., Li J., Wang S. and Hüttermann A., Salt, nutrient uptake and transport and ABA of Populus euphratica; a hybrid in response to increasing soil NaCl, Trees, 2001, 15(3): 186–194
8. Chen S., Li J. and Fritz E., Sodium and chloride distribution in roots and transport in three poplar genotypes under increasing NaCl stress, For. Ecol. Manag., 2002, 168(1–3): 217–230
9. Giannopolits C.-N. and Ries S.-K., Superoxide dismutases: occurrence in higher plants, Plant Physiol., 1977, 59: 309–314
10. Kochba J., Lavce S. and Spiegel R.-P., Difference in peroxidase activity and isoenzymes in embryogenic orange ovular callus lines, Plant Cell Physiol., 1977, 18: 463– 467
11. Heath R.-L. and Pacher L., Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation, Arch. Biochem. Biophys., 1968, 125: 189–198
12. Fadzilla N.-M., Finch R.-P. and Burdon R.-H., Salinity, oxidative stress and antioxidant responses in shoot culture of rice, J. Exp. Bot., 1997, 48: 325–331
13. Hernández J.-A., Corpass F.-J. and Gome M., Salt-induced oxidative stress mediated by active oxygen species in pea leaf mitochondria, Physiol. Plant., 1993, 89: 103–110
14. Smirnoff N., Antioxidant Systems and Plant Response to the Environment. In: Smirnoff N. (ed.) Environment and Plant Metabolism: Flexibility and Acclimation, Oxford: Bios Scientific Press, 1995: 217–243
15. Bandeoglue E., Eyidogan F. and Yücel M, Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress, Plant Growth Regul., 2004, 42: 69–77
16. Abed S. and Peter M.-N., Exogenous ascorbic (vitamin C) increases resistance to salt stress and reduces lipid peroxidation, J. Exp. Bot., 2001, 52(364): 2,207–2,211
17. Halliwell B. and Gutteridge J.-M.-C., Free Radicals in Biology and Medicine, 3rd edn., Oxford: Oxford University Press, 1999, 936–975
18. Liu Y.-L., Mao C.-L. and Wang L.-J., Advances in salt tolerance in plants, Commun. Plant Physiol., 1987, 23: 1–7
19. Ruth G.-A., Neval E. and Lenwood S.-H., Role of superoxide dismutases in controlling oxidative stress in plants, J. Exp. Bot., 2002, 53(372): 1n331–1n341
20. Asada K. and Takahashi M., Production and scavenging of active oxygen in photosynthesis. In: Kyle D.-J., Osmond C.-B., Arntzen C.-J. (eds.) Photoinhibition, Amsterdam: Elsevier Science Publishers, 1987: 227–287
21. Hernández J.-A., Olmo E. and Corpass F.-J., Salt-induced oxidative stress in chloroplast of pea plants, Plant Sci., 1995, 105: 151–167
22. Shigeru S., Takahiro I. and Masahiro T., Regulation and function of ascorbate peroxidase isoenzymes, J. Exp. Bot., 2002, 53(372): 1,305–1,319
23. Li M., Wang G.-X., Lin J.-S., Application of external calcium in improving the PEG-induced water stress tolerance in liquorice cells, Bot. Bull. Acad. Sin., 2003, 44: 275–284
24. Liang Y.-C., Chen Q. and Liu Q., Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.), J. Plant Physiol., 2003, 160: 1,157–1,164
25. Lutts S., Kinet J.-M. and Bouharmont J., NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance, Ann. Bot., 1996, 78: 389–398
26. Chen S., Li J. and Fritz E., Osmotic stress and ion-specific effects on xylem abscisic acid the relevance to salinity tolerance in poplar, J. Plant Growth Regul., 2002, 21(3): 224 –233
27. Anderson M.-D., Prasad T.-K. and Martin B.-A., Differential gene expression in chilling-acclimated maize seedlings and evidence for the involvement of abscisic acid in chilling tolerance, Plant Physiol., 1994, 105: 331–339
28. Bueno P., Piqueras A. and Kurepa J., Expression of antioxidant enzymes in response to abscisic acid and high osmoticum in tobacco BY-2 cell cultures, Plant Sci., 1998, 138: 27–34
29. Guan L. and Scandalios J.-G., Effects of the plant growth regulator abscisic acid and high osmoticum on the developmental expression of the maize catalase genes, Physiol. Plant., 1998, 104: 413– 422
30. Guan L. and Scandalios J.-G., Two structurally similar maize cytosolic superoxide dismutase genes, *Sod4* and *Sod4A*, respond differentially to abscisic acid and high osmoticum, Plant Physiol., 1998, 117: 217–224
31. Guan L.-M., Zhao J. and Scandalios J.-G., *Cis*-element and transfactors that regulate expression of maize *Cat1* antioxidant gene in response to ABA and osmotic stress: H_2O_2 is the likely intermediary signaling molecule for the response, Plant J., 2000, 22: 87–95
32. Prasad T.-K., Anderson M.-D. and Stewart C.-R., Acclimation, hydrogen peroxide, and abscisic acid protect mitochondria against irreversible chilling injury in maize seedlings, Plant Physiol., 1994, 105: 619–627
33. Gong M., Li Y. and Chen S.-Z., Abscisic acid-induced thermotolerance in maize seedlings is mediated by calcium and associated with antioxidant systems, J. Plant Physiol., 1998, 153: 488– 496
34. Bellaire B.-A., Carmody J. and Gossett D.-R., Involvement of abscisic acid-dependent and -independent pathways in the upregulation of antioxidant enzyme activity during NaCl stress in cotton callus tissue, Free Radic. Res., 2000, 33: 531–545
35. Larkindale J. and Knight M.-R., Protection against heat stress-induced oxidative damage in Arabidopsis involves calcium, abscisic acid, ethylene, and salicylic acid, Plant Physiol., 2002, 128: 682–695
36. Jiang M. and Zhang J., Role of abscisic acid in water stress induced antioxidant defense in leaves of maize seedlings, Free Radic. Res., 2002, 36: 1,001–1,015
37. Jiang M. and Zhang J., Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves, J. Exp. Bot., 2002, 53: 2,401–2,410
38. Jiang M. and Zhang J., Involvement of plasma membrane NADPH oxidase in abscisic acid and water stress-induced antioxidant defense in leaves of maize seedlings, Planta, 2002, 215: 1,022–1,030
39. Xu X., Mao G.-L. and Li S.-H., Effect of salt stress and abscisic acid on membrane-lipid peroxidation and resistant-oxidation enzyme activities of *Lycium barbarum* callus, Acta Bot. Boreal-occident. Sin., 2003, 23(5): 745–774