

BRIEF COMMUNICATION

Effect of increased alkalinity on Na⁺ and K⁺ contents, lipid peroxidation and antioxidative enzymes in two populations of *Populus cathayana*S. LU^{1,2}, S. ZHANG^{1,2}, X. XU³, H. KORPELAINEN⁴ and C. LI^{1*}*Chengdu Institute of Biology, Chinese Academy of Sciences, P.O.Box 416, Chengdu 610041, P.R. China¹**Graduate School of the Chinese Academy of Sciences, Beijing 100039, P.R. China²**College of Life Science, China West Normal University, Nanchong 637002, P.R. China³**Department of Applied Biology, University of Helsinki, FI-00014 Helsinki, Finland⁴***Abstract**

We compared two populations of *Populus cathayana* Rehder, originating from altitudes 2 840 m and 1 450 m, to determine whether trees from different altitudes exhibit different tolerance to alkalinity. The tree cuttings were exposed to nutrient solutions with pH 7.9, 8.8, 9.8 and 10.4 and the salt concentration 200 mM. Na⁺ and K⁺ contents, and Na⁺/K⁺ ratios in leaves and roots were greatly affected by pH values. At pH 10.4, the Na⁺/K⁺ ratios in both leaves and roots sharply dropped in the higher altitude population but were maintained at higher levels in the lower altitude population. The patterns of pH-induced changes in contents of malondialdehyde and free proline, and antioxidative enzyme activities indicated that the higher altitude population exhibits greater tolerance to alkalinity stress than does the lower altitude population.

Additional key words: free proline, malondialdehyde, poplar, salt-alkali mixed stress.

Soil alkalization frequently occurs simultaneously with salinization. High exogenous salt concentrations cause an imbalance of cellular ions resulting in osmotic stress, ion toxicity, and the production of ROS (Cheeseman 1988, Cramer *et al.* 1994). Alkalinity exerts similar reactions but with the added influence of stress due to high pH. The high pH environment surrounding the roots may directly inhibit ion uptake by causing Ca²⁺, Mg²⁺ and H₂PO₄⁻ to precipitate (Shi and Zhao 1997, Yang *et al.* 2007) and disrupt the ion homeostasis of plant cells. Plants develop defense strategies against salt and alkali stress. In osmotic adjustment, the accumulation of organic compounds, such as sugars and amino acids, in the cytoplasm plays an important role (Morgan 1992). However, because of the lower energy consumption needed when absorbing inorganic ions compared to synthesizing organic compounds, plants usually accumulate inorganic ions,

mainly Na⁺, in vacuoles to decrease water potential (Munns 2002). Plants accumulate cations, such as Na⁺ and K⁺, and simultaneously accumulate inorganic anions, such as Cl⁻ (Ghoulam *et al.* 2002, Melgar *et al.* 2008), or synthesize organic anions (Sagi *et al.* 1997) to keep ion balance. Previous studies have shown that to maintain pH stability, organic acid accumulation may be an important means for some plants, such as *Aneurolepidium chinense* (Shi and Wang 2005). Osmotic stress leads to the formation of ROS. To scavenge the ROS, the activities of antioxidative enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POD), glutathione reductase (GR) and glutathione peroxidase (GSH-Px), increase. The activities of these enzymes have been shown to correlate with salt tolerance (Benavides *et al.* 2000, Hernández *et al.* 2000, Sreenivasulu *et al.* 2000, He and Zhu 2008). To date, there

Received 15 December 2008, *accepted* 27 June 2009.

Abbreviations: ABA - abscisic acid; APX - ascorbate peroxidase; CAT - catalase; GR - glutathione reductase; GSH-Px - glutathione peroxidase; HAP - higher altitude population; LAP - lower altitude population; MDA - malondialdehyde; NBT - nitroblue tetrazolium; POD - guaiacol peroxidase; ROS - reactive oxygen species; SOD - superoxide dismutase.

Acknowledgements: The research was supported by the Program of "Knowledge Innovation Engineering" of the Chinese Academy of Sciences (No. KSCX2-YW-N-064) and the Outstanding Young Scientist Program of the National Natural Science Foundation of China (No. 30525036).

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are only a few reports about alkali stress, especially in trees. Based on previous studies, it is evident that the effects of alkali stress are more severe than those of salt stress (Hartung *et al.* 2002, Shi and Sheng 2005, Yang *et al.* 2007). As there are increasing areas of saline and alkaline soil in the world, it is necessary to do more research on salt-alkali stress and its effects on plants. In *Populus*, morphological, physiological and ecological differences between populations in responses to abiotic stresses, *e.g.*, drought and UV-B, have been found (Li *et al.* 2004, Zhang *et al.* 2005, Lei *et al.* 2006, Duan *et al.* 2008). However, little attention has been focused on what kind of differences there are between populations in response to salt-alkali mixed stress. The objective of this study was to investigate the physiological responses of *Populus cathayana* from different altitude regions in eastern Himalaya to mixed salt and alkali stress.

Cuttings of two populations of *Populus cathayana* Rehder were collected in their natural habitats in Jiuzhai and Datong with the altitudes 1450 and 2840 m, respectively. Therefore, the populations from Jiuzhai and Datong represent low and high altitude populations, respectively. Healthy cuttings of uniform height were chosen from each population and individually transferred to 10-dm³ plastic pots filled with homogenized soil, and grown in a greenhouse under natural irradiance, day/night temperature of 12 - 31/9 - 15 °C, and a relative humidity of 35 - 85 %. The 100 cuttings of each population were divided into five groups, and each group (four replications of five cuttings per replication for each population) was exposed to one of the five treatments. Two neutral salts (NaCl and Na₂SO₄) and two alkaline salts (NaHCO₃ and Na₂CO₃) were selected and mixed in various proportions according to Shi and Wang (2005). Plants in one treatment group were watered with Hoagland nutrient solution as control, while plants in the other four groups were watered with nutrient solution containing different salt mixtures which represent the pH 7.9, 8.8, 9.8 and 10.4 treatments. The total salt concentration was 200 mM. The cuttings each received 200 cm³ of respective solution twice a week. The pots were weighed twice a day to maintain the soil moisture at field capacity. After one-month treatment, all plants were harvested.

The Na⁺ and K⁺ contents were measured according to Peng *et al.* (2004) by the atomic absorption spectroscopy (AA-6300, Shimadzu, Japan) after the tissues were dried and extracted with HCl. The total superoxide dismutase (EC 1.15.1.1, SOD) activity was measured spectrophotometrically (UV-330, Unicam, UK) based on inhibition in the photochemical reduction of nitroblue tetrazolium, NBT (Beauchamp and Fridovich 1971). The catalase (EC 1.11.1.6, CAT) activity was determined by directly measuring the decomposition of H₂O₂ at 240 nm, as described by Aebi (1984). The glutathione peroxidase (EC 1.11.1.7, PODGSH-Px) activity of leaves was measured as described by Flohe and Gunzler (1984). Free proline was measured as described by Bates *et al.* (1973). The MDA content was measured as described by Kramer *et al.* (1991).

Statistical analyses were performed with the statistical software package for social science (SPSS), version 11.0. Among all treatments, the means were compared by Duncan's tests at $P < 0.05$.

When compared with cuttings grown in control conditions, the Na⁺ content of leaves and roots significantly increased and the K⁺ content of roots significantly decreased in both *P. cathayana* populations under salt-alkali mixed stress (Table 1). Increments of Na⁺ and decrements of K⁺ have been commonly observed in other plant species, not only with increasing salinity but also with increasing pH values (De Lacerda *et al.* 2003). Such responses induced by salinity or alkalinity have been attributed to the competition between Na⁺ and K⁺ (Munns 2002, Shi and Sheng 2005, Shi and Wang 2005). However, in contrast to some previous results, K⁺ contents in the leaves of *P. cathayana* did not decrease, and even slightly increased in the higher altitude population with increasing pH values in comparison with controls. Comparable results have been previously reported by De Costa *et al.* (2007) and Yang *et al.* (2008). The phenomenon might be due to the transfer of K⁺ from roots to shoots (Franklin and Zwiazek 2004). It is apparent that higher alkalinity affected K⁺ content and Na⁺/K⁺ ratio more in the lower altitude population than in the higher altitude population of *P. cathayana*.

Rising alkalinity caused membrane damage to the leaves. It has been shown that in sensitive genotypes the content of MDA increased at first and then declined when salinity was above a certain level (Tao *et al.* 2005). The gradually increasing MDA content observed in this study indicated that the cell membranes of both populations were damaged more at higher pH (Table 1). On the other hand, free proline content significantly increased at lower alkalinity in the lower altitude population and then sharply dropped when pH value reached 9.8 (Table 1). In contrast, the tendency was different in the higher altitude population: the proline concentration did not significantly increase until pH value was higher than 9.8 (Table 1). Different tendencies indicate that the higher altitude population could endure higher alkalinity better than does the lower altitude population, as proline is generally assumed to serve as a physiologically compatible solute that accumulates as needed to maintain not only osmotic potential but also ion balance (Kurkdjian and Guern 1989, Shi and Sheng 2005).

We suggest that certain factors are important when trying to explain the observed stress reactions. Firstly, alkalinity disturbs availability of mineral such as Mg, Ca, and P in soil (Shi and Zhao 1997). Secondly, the roles of antioxidative enzymes are different in scavenging ROS. In higher altitude population, GSH-Px activity significantly increased with increasing alkalinity until pH 9.8, but the maximum activity in lower altitude population was in pH 8.8. Besides, SOD activities in both populations changed slightly at lower alkalinity and reached maximum activity in pH 9.8. Moreover, the pH value when the CAT activities reached maximum were different in two populations, 9.8 and 10.4 for higher and lower altitude

Table 1. The leaf and root Na⁺ and K⁺ contents, Na⁺/K⁺ ratio, MDA content, free proline content and GSH-Px, SOD and CAT activities of *Populus cathayana* lower altitude (LAP) and higher altitude (HAP) populations when exposed to solutions with different pH (7.7, 8.8, 9.8, 10.4). Means \pm SE, $n = 5$. Means within a row followed by the same letter indicate non-significant differences at $P < 0.05$.

Parameter	Population	Control	pH 7.9	pH 8.8	pH 9.8	pH 10.4
Leaf Na ⁺	LAP	14.0 \pm 1.6e	194.7 \pm 9.2a	206.3 \pm 5.8a	205.6 \pm 3.3a	111.2 \pm 0.5c
[$\mu\text{mol g}^{-1}$ (d.m.)]	HAP	15.7 \pm 0.7e	187.2 \pm 3.6a	143.8 \pm 17.5b	163.3 \pm 0.1b	76.3 \pm 4.7d
Leaf K ⁺	LAP	164.3 \pm 11.6c	196.6 \pm 9.2ab	201.6 \pm 5.3ab	214.3 \pm 3.7a	126.3 \pm 11.9d
[$\mu\text{mol g}^{-1}$ (d.m.)]	HAP	162.9 \pm 5.8c	179.5 \pm 9.7bc	181.6 \pm 10.9bc	183.1 \pm 2.8bc	185.8 \pm 8.3bc
Leaf Na ⁺ /K ⁺	LAP	0.09 \pm 0.01e	0.99 \pm 0.00abc	1.02 \pm 0.00ab	0.96 \pm 0.00abc	0.90 \pm 0.09bc
	HAP	0.10 \pm 0.007e	1.05 \pm 0.05a	0.79 \pm 0.05c	0.89 \pm 0.02c	0.41 \pm 0.01d
Root Na ⁺	LAP	27.4 \pm 0.3d	110.2 \pm 1.7c	139.0 \pm 3.3a	118.0 \pm 3.5bc	106.9 \pm 17.9c
[$\mu\text{mol g}^{-1}$ (d.m.)]	HAP	24.0 \pm 0.2d	134.6 \pm 2.2ab	144.7 \pm 2.3a	111.2 \pm 3.0c	125.6 \pm 3.9abc
Root K ⁺	LAP	69.6 \pm 9.5a	44.4 \pm 0.6b	43.4 \pm 1.1b	35.7 \pm 1.0bc	22.8 \pm 3.3d
[$\mu\text{mol g}^{-1}$ (d.m.)]	HAP	65.1 \pm 0.4a	43.0 \pm 0.5b	31.9 \pm 0.3cd	22.8 \pm 0.9d	37.8 \pm 1.6bc
Root Na ⁺ /K ⁺	LAP	0.34 \pm 0.01f	2.48 \pm 0.01e	3.20 \pm 0.02cd	3.30 \pm 0.01c	4.66 \pm 0.13b
	HAP	0.37 \pm 0.01f	3.13 \pm 0.02d	4.53 \pm 0.09b	4.88 \pm 0.09a	3.33 \pm 0.04c
MDA	LAP	0.31 \pm 0.02e	0.32 \pm 0.01de	0.37 \pm 0.01bc	0.35 \pm 0.00cd	0.41 \pm 0.02ab
[$\mu\text{mol g}^{-1}$ (f.m.)]	HAP	0.41 \pm 0.01ab	0.37 \pm 0.00bc	0.38 \pm 0.02bc	0.46 \pm 0.01a	0.49 \pm 0.02a
Proline	LAP	0.27 \pm 0.03cd	0.45 \pm 0.04a	0.47 \pm 0.06a	0.25 \pm 0.01cd	0.17 \pm 0.01d
[$\mu\text{g g}^{-1}$ (f.m.)]	HAP	0.30 \pm 0.02bc	0.30 \pm 0.00bc	0.25 \pm 0.01cd	0.44 \pm 0.05a	0.39 \pm 0.01ab
GSH-Px [$\mu\text{mol(quiacol)}$	LAP	8.29 \pm 0.32g	14.83 \pm 0.60cde	19.17 \pm 0.62ab	17.53 \pm 0.59bcd	6.82 \pm 0.23g
mg^{-1} (prot.) min^{-1}]	HAP	12.92 \pm 1.19ef	13.65 \pm 1.57ef	18.33 \pm 2.67abc	22.00 \pm 1.73a	10.00 \pm 1.16fg
SOD	LAP	187.04 \pm 7.03bc	190.75 \pm 9.41b	203.01 \pm 16.33b	244.34 \pm 5.71a	187.01 \pm 7.03bc
[U mg^{-1} (prot.)]	HAP	151.03 \pm 15.01cde	124.34 \pm 9.02e	174.74 \pm 7.32bc	188.73 \pm 16.34b	147.02 \pm 14.24de
CAT [$\mu\text{mol}(\text{H}_2\text{O}_2)$	LAP	197.33 \pm 14.54c	219.02 \pm 7.81bc	225.74 \pm 12.73b	221.71 \pm 10.13bc	306.32 \pm 11.94a
mg^{-1} (prot.) min^{-1}]	HAP	214.04 \pm 3.83bc	147.04 \pm 5.03d	165.04 \pm 7.62d	306.31 \pm 5.62a	211.34 \pm 9.83bc

populations, respectively (Table 1). The different responses may be related to both temporal regulation of the specific isoenzymes and to the induction of new isoforms (Sreenivasulu *et al.* 2000, Sekmen *et al.* 2007). The isoforms of these antioxidative enzymes may be different in the two *P. cathayana* populations.

In conclusion, the physiological properties of both high and low altitude populations of *P. cathayana* were greatly

affected by increasing alkalinity. Although the Na⁺, K⁺ content, Na⁺/K⁺ ratio, MDA content, free proline content and antioxidative enzyme activities were significantly affected by increasing pH in both populations, the results indicated that higher altitude population exhibited greater tolerance to alkalinity stress than does the lower altitude population.

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