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Chemosphere 68 (2007) 686–694
CHEMOSPHERE
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Physiological and biochemical responses to high Mn concentrations in two contrasting *Populus cathayana* populations

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Received 18 October 2006; received in revised form 22 January 2007; accepted 25 January 2007
Available online 7 March 2007

Abstract

We exposed the cuttings of *Populus cathayana* to Hoagland's solution containing four different manganese (Mn) concentrations (0, 0.1, 0.5 and 1 mM) in a greenhouse to characterize the physiological and biochemical basis of Mn resistance in woody plants. Two contrasting populations of *P. cathayana* were used in our study, which were from the wet and dry climate regions in western China, respectively. The results showed that Mn treatments significantly decreased chlorophyll content and growth characteristics, including shoot height, basal diameter, biomass accumulation and total leaf area in the two populations. Mn treatments also significantly increased the levels of abscisic acid (ABA), polyamines and free amino acids especially proline (Pro), histidine (His) and phenylalanine (Phe) available for cellular signaling and heavy metal chelation. In addition, high Mn concentrations also caused oxidative stress indicated as the accumulation of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents. On the other hand, there were different responses to Mn stress between the two contrasting populations. Compared with the dry climate population, the wet climate population accumulated more Mn in plant tissues especially in leaves; it showed lower tolerance index and more pronounced decrease in growth and chlorophyll contents. The wet climate population not only accumulated less ABA, putrescine and free amino acids, but also exhibited lower activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX), thus suffering from more serious oxidative damage. Therefore, our results showed that the wet climate population was more susceptible to Mn stress than the dry climate population.

Keywords: Abscisic acid; Free amino acids; Manganese treatment; Oxidative stress; Polyamines; Poplar

1. Introduction

Heavy metal deposits in the soils, caused by bedrock weathering processes and human activities, such as mining, smelting and the use of pesticides and fertilizers, have resulted in serious contamination of terrestrial environments (Friedland, 1990). The heavy metal toxicity responses of plants may result from the binding of metals to the sulphydryl groups of proteins, leading to the inhibition of activity or disruption of structures, and to the displacement of an essential element, such as Zn, Mg, Ca and Fe, causing further deficiency effects. On the other hand, heavy metal excess may stimulate the formation of free radicals and reactive oxygen species, perhaps leading to oxidative stress (Hall, 2002; Metwally et al., 2005). Plants responses to heavy metals depend on an interrelated network of physiological and molecular mechanisms, such as: (i) a reduced uptake and accumulation of metals through binding to extracellular exudates and cell wall constituents; (ii) the compartmentation of metals in the vacuole by tonoplast-located transporters; (iii) the complexation of heavy metal ions inside the cell by various substances, e.g., organic acids, amino acids, phytochelatins and metallothioneins; (iv) general biochemical stress defense responses, such as the induction of antioxidative enzymes and the accumulation of free proline; and (v) the

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doi:10.1016/j.chemosphere.2007.01.066

activation or modification of plant metabolism to allow adequate functioning of metabolic pathways and rapid repair of damaged cell structures (Brune and Dietz, 1995; Prasad, 1999; Sanita di Toppi and Gabrielli, 1999; Hall, 2002; Cho et al., 2003).

Manganese (Mn), an essential trace element for plant systems, is involved in photosynthesis, respiration and in the activation of several enzymes. However, Mn is toxic when in excess, and, consequently, it represents an important factor in environmental contamination and causes various phytotoxic effects (Pittman, 2005). Remediation of sites contaminated with toxic metals is prohibitively challenging. Phytoremediation has been proposed as a cost-effective, environmental-friendly alternative technology (Lasat, 2002). Over the past 10 years, woody plants have been shown to be excellent candidates for phytoremediation purposes due to their rapid growth, high biomass, profuse root apparatus and low impact on the food chain and human health (Salt et al., 1998; Pilon-Smits, 2005). The majority of such work concerns the accumulation capacity and biomass production of woody plants as a response to high concentrations of pollutants (Pulford and Watson, 2003). However, only little is known about the absorption, accumulation and detoxification of heavy metals, especially about the reactions of Mn, in woody plants.

There is cross-tolerance in plants, e.g., the exposure of plants to one moderate stress can induce resistance to another stress (Kuznetsov et al., 1993; Collins et al., 1995; Capiati et al., 2006). In this study, we investigated two contrasting populations of *Populus cathayana* originating from wet and dry climate regions in western China, respectively, to examine whether there exists cross-tolerance between drought and Mn stress and to explore the physiological and biochemical responses to heavy metal stress. Furthermore, understanding the mechanisms mediating heavy metal accumulation and detoxification in woody plants will help us, eventually, to develop methods for phytoremediation.

2. Materials and methods

2.1. Plant materials and experimental design

Samples from two contrasting populations of *P. cathayana* were collected from their natural habitats in HanYuan and LeDu (Table 1). The mean annual rainfall in HanYuan and LeDu are 750 and 335 mm, respectively. Therefore, the populations from HanYuan and LeDu represent the wet and dry climate populations, respectively. The transpiration rates of the plants in the two populations are similar. Furthermore, in both regions the rainfall concentrates in the season from June to September, which contributes 70.1% and 84.8% of the yearly rainfall in HanYuan and LeDu, respectively. Cuttings from 20 trees from each population were collected in March 2006. After sprouting and growing for about one month, healthy cuttings of approximately equal height were selected and replanted into 5-l plastic pots filled with homogenized soil. The plants were then grown under semi-controlled environmental conditions in a naturally lit greenhouse with a temperature range of 18.0–32.0 °C and relative humidity range of 50–80%, and supplied with 800 ml Hoagland's solution every day. The selected properties of the soil used in this study were pH 7.2, 0.12% organic carbon, 0.02% organic nitrogen, 5.0 mg/kg manganese, 43 mg/kg potassium, 2 mg/kg phosphorus, 88.2% sand, 9.1% silt and 2.7% clay. Hoagland's nutrient solution (Hoagland and Arnon, 1950) consisted of 5 mM $Ca(NO_3)_2 \cdot 4H_2O$, 5 mM KNO_3, 2 mM $MgSO_4 \cdot 4H_2O$, 1 mM KH_2PO_4, 0.1 mM EDTA-Fe, 46 μM H_3BO_3, 9.1 μM $MnCl_2 \cdot 4H_2O$, 0.32 μM $CnSO_4 \cdot 5H_2O$, 0.76 μM $ZnSO_4 \cdot 7H_2O$ and 0.5 μM $H_2MoO_4 \cdot H_2O$.

During the growth period, all cuttings maintained a unique stem. After culturing for one month, the seedlings were exposed to Mn stress. The Mn treatments were assigned as follows: basic Hoagland's solution containing 0 (control), 0.1, 0.5 or 1 mM Mn supplied as $MnSO_4 \cdot H_2O$. The pH of the solution was adjusted to 5.5 ± 0.2 with NaOH or HCl as required. The experimental layout was completely randomized with two factors (two populations and four Mn concentrations). Eighty cuttings of each population were allocated randomly to Mn stress for three months. Each treatment included five replications and four cuttings per replication.

2.2. Measurements for growth and tolerance index

All seedlings were harvested at the end of the experiment and divided into leaves, stem and roots. The longest root of each seedling was measured, and the tolerance index (TI) was calculated according to Wilkins (1978) using the following equation: TI % = 100 × (mean root length of the longest root developed in the Mn treatment)/(mean root of the longest root developed in the control). The total leaf area was determined using a Portable Laser Area Meter

Table 1
Origin of the two contrasting *P. cathayana* populations investigated in the study

Population	Origin	Latitude (°N)	Longitude (°E)	Annual rainfall (mm)	Annual transpiration (mm)	Mean annual temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Annual solar radiation (MJ m^{-2})
HY	HanYuan	29°25	102°40	750	800	17.7	33	−5	3600
LD	LeDu	36°31	102°28	335	1500	6.9	38	−20	4500

HY, the wet climate population; LD, the dry climate population.

(CI-203, CID Inc., Camas, WA). Biomass samples were dried (80 °C, 48 h) to constant dry weight (DW).

2.3. Extraction and estimation of chlorophyll content

At the moment of sampling, the leaves were separated into two groups by using the leaf plastochron index (LPI) as a classification system: 'young' (LPI = 1–7) leaves and 'mature' (LPI > 7) leaves. The LPI is based on the leaf rank starting from the first fully open, but not yet completely expanded, apical leaf (Dickmann, 1971; Di Baccio et al., 2005). About 0.5 g fresh young leaves (LPI = 4) were homogenized in 80% ice-cold acetone in dark and centrifuged at 10 000*g* for 10 min. The supernatants were collected and the absorption spectra at 663.8 nm and 646.8 nm were recorded for the estimation of total chlorophyll following the procedure of Inskeep and Bloom (1985).

2.4. Determination of Mn concentration

Some of the sampled plants were soaked in 0.2% EDTA for 2 h and rinsed thoroughly with deionized water to eliminate possible chemical contamination, separated into roots, stems and young leaves, then dried at 80 °C (48 h) and weighed. The samples were digested in a mixture of HNO_3:$HClO_4$ (4:1), and the Mn concentration was determined using an inductively coupled argon plasma emission spectrometry (ICP-ES; Model 1CAP 61E; Thermo-Jarrell Ash, Waltham, MA, USA).

2.5. Measurement for ABA and polyamines

ABA was analyzed using an ELISA (enzyme-linked immunosorbent assay) kit (purchased from Nanjing Agriculture University of China), as described by Xiong et al. (2006). Polyamines were extracted and measured according to Flores and Galston (1982). Equal-aged young leaves (LPI = 5) were collected, frozen in liquid nitrogen and stored at −80 °C. About 100 mg leaf without major veins from each sample was ground in liquid nitrogen and extracted with 1 ml of 0.2 M (v/v) $HClO_4$ (perchloric acid, PCA) for 1 h at 4 °C. To the homogenate, 2 μl of 38 mM 1,6-hexanediamine was added as an internal standard. The extract was centrifuged at 16 000*g* for 20 min at 4 °C. The pellet was washed twice with 1 ml of 0.2 M PCA. The supernatants were combined and the exact volume was measured with a syringe. The extracted polyamines were benzoylated, as suggested by Flores and Galston (1982). The standards, putrescine, spermidine and spermine were dissolved in 1 ml of PCA and benzoylated following the same procedure as with the samples.

2.6. Determination of free amino acids

For the determination of free amino acids contents, 0.5 g young leaves (LPI =5) were sampled, ground with 3 ml 3% sulfosalicylic acid, and extracted in boiling water for 10 min. After cooling to room temperature, the extract was centrifuged at 5000*g* at 4 °C for 10 min. Finally, 25 μl of the supernatants wasa analyzed with an amino acid analyzer (Hitachi Ltd., Tokyo, Japan).

2.7. Assay of ROS metabolism

The levels of H_2O_2 in young leaves (LPI = 6) were measured by monitoring the absorbance of the titanium–peroxide complex at 415 nm, following the method of Brennan and Frenkel (1977). Absorbance values were calibrated to a standard curve generated using known concentrations of H_2O_2. Leaf oxidative damage to lipids was expressed as equivalents of MDA contents. About 0.5 g leaf segments were homogenized in 10 ml of 10% trichloroacetic acid (TCA), and centrifuged at 12 000*g* for 10 min. After that, 2 ml 0.6% thiobarbituric acid (TBA) in 10% TCA was added to an aliquot of 2 ml from the supernatant. The mixture was heated in boiling water for 30 min, and then quickly cooled in an ice bath. After centrifugation at 10 000*g* for 10 min, the absorbance of the supernatant at 450, 532 and 600 nm was determined. The MDA content was calculated according to Hodges et al. (1999).

The SOD (EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) according to the method of Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm. The APX (EC 1.11.1.11) activity was analyzed by following the decrease in 290 nm (extinction coefficient 2.8 mM^{-1} cm^{-1}) for 1 min in 3 ml of a reaction mixture containing 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.1 mM sodium ascorbate, 2.5 mM H_2O_2 and 200 μl enzyme extract (Nakano and Asada, 1981). In addition, soluble protein contents were determined as described by Bradford (1976), using bovine serum albumin as a calibration standard.

2.8. Statistical analyses

Analyses of variance (ANOVA) for all variables obtained from the measurements were used for testing the population and Mn treatment effects. Statistical analyses were conducted with the SPSS 11.0 for Windows statistical software package. The Duncan test was employed to detect possible differences between the treatments.

3. Results

3.1. Effect of Mn stress on tolerance index and growth

Treatments with increasing Mn concentrations significantly decreased the tolerance index (TI) in poplars originating from two contrasting conditions ($P < 0.001$). A significant difference in TI between the two populations

Table 2
Effect of manganese stress on tolerance index and growth characteristics in the two contrasting populations of *P. cathayana*

Population	Mn (mM)	Tolerance index (%)	Shoot height (cm)	Basal diameter (mm)	Total biomass (g)	Total leaf area (dm^2)
HY	0	100 a	117.4 ± 5.9 (100) a	8.69 ± 0.36 (100) a	34.22 ± 1.33 (100) a	2080.2 ± 31.7 (100) a
	0.1	61.56 ± 7.83 c	98.4 ± 5.8 (83.8) bc	7.12 ± 0.91 (81.9) bc	17.76 ± 1.39 (51.9) c	1726.4 ± 115.9 (83.0) b
	0.5	46.59 ± 3.91 d	85.6 ± 6.1 (72.9) d	6.25 ± 0.17 (71.9) d	15.03 ± 0.49 (43.9) d	1308.2 ± 192.9 (62.9) d
	1	36.05 ± 5.56 e	69.6 ± 9.3 (59.3) e	5.28 ± 0.36 (60.7) e	8.86 ± 1.08 (25.9) f	760.9 ± 142.9 (36.6) f
LD	0	100 a	105.8 ± 3.9 (100) b	8.61 ± 0.43 (100) a	25.91 ± 2.14 (100) b	2024.6 ± 160.6 (100) a
	0.1	71.36 ± 8.47 b	93.2 ± 6.0 (88.1) cd	7.38 ± 0.78 (85.7) b	16.70 ± 1.06 (64.4) cd	1523.9 ± 114.9 (71.4) c
	0.5	59.47 ± 8.53 c	85.0 ± 6.9 (80.3) d	6.42 ± 0.43 (74.6) cd	13.26 ± 1.78 (51.2) e	1097.2 ± 130.1 (59.5) e
	1	47.14 ± 9.63 d	66.2 ± 4.6 (62.6) e	5.52 ± 0.62 (64.2) e	8.45 ± 0.67 (32.6) f	437.6 ± 72.6 (47.1) g
Fp		0.000	0.000	0.000	0.000	0.000
Fm		0.000	0.013	0.404	0.000	0.000
Fp × m		0.163	0.267	0.889	0.165	0.000

The values (means of five replicates ± SE) in the same column followed by different letters are significantly different from each other at $P < 0.05$. Fp, population effect; Fm, manganese effect; Fp × m, population × manganese interaction effect.

was also observed: the wet climate population showed lower TI than did the dry climate population under the same Mn stress (Table 2). Shoot height, basal diameter, total biomass and total leaf area all gradually decreased with the increasing Mn concentration in both populations (Table 2). Compared with the dry climate population, the wet climate population showed a more pronounced decrease in shoot height, basal diameter and total biomass.

3.2. Effect of Mn stress on the chlorophyll and Mn content of plant tissues

The chlorophyll content significantly decreased with the increasing Mn concentration in both populations with the exception of the dry climate population at 0.1 mM Mn. ($P < 0.001$). This result was confirmed with the appearance of chlorosis especially under 1 mM Mn treatment (data not shown). On the other hand, the chlorophyll content of the dry climate population was higher than that of the wet climate population in all treatments except for the control (Fig. 1). The Mn content of plant tissues increased with increasing, exogenously applied Mn concentrations in both populations (Table 3). Under low Mn concentrations (0 and 0.1 mM), the dry climate population accumulated higher Mn contents, while under high Mn stress (0.5 and 1 mM), the Mn content of the wet climate population was much higher than that of the dry climate population. Under 0, 0.1 and 0.5 mM Mn treatments, Mn mostly accumulated in the root, less in the leaves and least in the stem, while under 1 mM, most Mn accumulated in the leaves in both populations.

3.3. Effect of Mn stress on the contents of ABA and polyamines

The ABA content increased significantly when the plants were exposed to Mn treatments ($P < 0.001$). However, compared with the wet climate population, the dry climate population exhibited a higher constitutive ABA content, which increased rapidly under all Mn stresses (Fig. 2). In

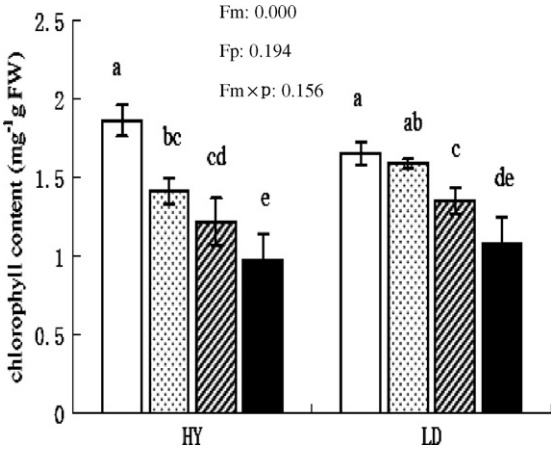

Fig. 1. Effect of manganese stress on chlorophyll contents in the young leaves of two contrasting populations of *P. cathayana*. HY, the wet climate population; LD, the dry climate population. □, 0 mM manganese; ▩, 0.1 mM manganese; ▨, 0.5 mM manganese; ■, 1 mM manganese. Fm, manganese effect; Fp, population effect; Fm × p, manganese × population interaction effect. The different letters above the bars (means of three replicates ± SE) are significantly different from each other at $P < 0.05$.

the wet climate population, putrescine increased significantly with the exception of 0.1 mM Mn, spermidine increased only under 0.1 and 1 mM Mn; and spermine changed very little under all Mn treatments. As for the dry climate population, significant increases in putrescine and spermidine were observed with the increasing Mn concentrations while spermine increased only under 1 mM Mn (Fig. 2).

3.4. Effect of Mn stress on the contents of free amino acids

The most abundant amino acids in both populations were Ser and Glu. The Mn treatment greatly altered the composition and concentration of free amino acids (Table 4). According to the pattern of concentration changes in response to Mn stress, free amino acids can be divided into three groups: The first group, including Glu, Ala and Asp, showed a decrease with the increasing Mn concentrations with the exception of Ala in the dry climate population.

Table 3
Effect of manganese stress on the manganese content of plant tissues in the two contrasting populations of *P. cathayana*

Population	Mn (mM)	Root (mg kg^{-1} DW)	Stem (mg kg^{-1} DW)	Leaf (mg kg^{-1} DW)
HY	0	34.5 ± 0.2 h	6.5 ± 0.1 g	28.6 ± 0.2 g
	0.1	116.9 ± 0.5 e	24.7 ± 0.2 e	74.7 ± 0.5 f
	0.5	227.4 ± 3.5 c	144.8 ± 2.1 c	218.0 ± 1.3 c
	1	481.0 ± 3.0 a	349.3 ± 1.5 a	713.1 ± 2.5 a
LD	0	54.4 ± 1.1 g	18.8 ± 0.1 f	28.3 ± 0.2 g
	0.1	98.8 ± 0.2 f	26.2 ± 0.1 e	91.9 ± 0.3 e
	0.5	157.9 ± 1.7 d	122.6 ± 1.5 d	144.2 ± 2.6 d
	1	352.9 ± 1.8 b	311.5 ± 6.2 b	681.2 ± 5.0 b
Fp		0.000	0.000	0.000
Fm		0.000	0.000	0.000
Fp × m		0.000	0.000	0.002

The values (means of five replicates ± SE) in the same column followed by different letters are significantly different from each other at $P < 0.05$. Fp, population effect; Fm, manganese effect; Fp × m, population × manganese interaction effect.

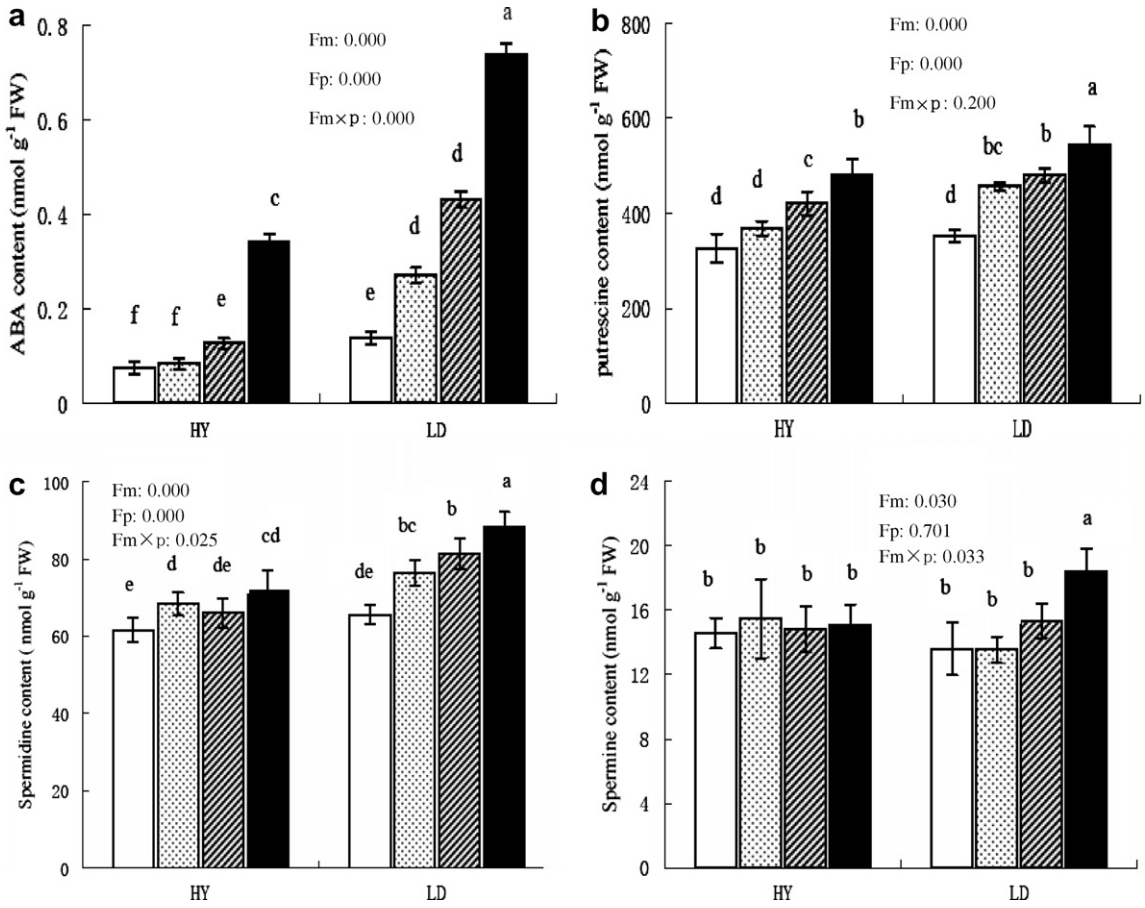

Fig. 2. Effect of manganese stress on ABA (a), putrescine (b), spermidine (c) and spermine (d) contents in the young leaves of the two contrasting populations of *P. cathayana*. HY, the wet climate population; LD, the dry climate population. □, 0 mM manganese; ▨, 0.1 mM manganese; ▨, 0.5 mM manganese; ■, 1 mM manganese. Fm, manganese effect; Fp, population effect; Fm × p, manganese × population interaction effect. The different letters above the bars (means of three replicates ± SE) are significantly different from each other at $P < 0.05$.

The second group, including Val, Leu and Thr, remained little changed or altered insignificantly under Mn stress. The third group of amino acids increased under Mn toxicity and this group can be further divided into two subgroups: under 1 mM Mn the contents of Ser, Tyr, Phe, His and Pro were more than three times when compared to their contents under control conditions, while the contents of Ile, Lys, Arg and Gly under 1 mM Mn were less than twice of their contents under control conditions. Meanwhile, the dry climate population accumulated more free amino acids than did the wet climate population under the same Mn concentration.

Table 4
Effect of manganese stress on the contents of free amino acids in the young leaves of the two contrasting populations of *P. cathayana*

Amino acid [mg (100 g)$^{-1}$ FW]	HY population				LD population			
	0 mM	0.1 mM	0.5 mM	1 mM	0 mM	0.1 mM	0.5 mM	1 mM
Glu	23.61 c	23.81 c	16.04 e	14.93 e	31.48 a	29.50 b	29.75 b	19.14 d
Ala	10.44 a	8.73 b	6.66 d	5.61 e	7.96 c	4.10 f	3.65 f	3.71 f
Asp	15.23 b	13.06 c	12.17 cd	11.96 d	16.63 a	14.74 c	11.46 d	11.07 d
Val	0.74 a	0.61 c	0.64 ab	0.75 a	0.62 ab	0.64 ab	0.72 ab	0.66 ab
Leu	0.49 c	0.60 ab	0.60 ab	0.59 ab	0.62 ab	0.55 bc	0.65 a	0.63 ab
Thr	5.08 a	5.15 a	4.46 bc	5.12 a	4.41 c	5.09 a	4.93 ab	5.17 a
Ser	11.78 f	12.62 ef	25.34 c	36.23 b	12.06 f	13.43 e	22.78 d	41.12 a
Tyr	0.48 d	0.47 d	0.81 b	2.05 a	0.56 bc	0.75 bc	0.79bc	2.03 a
Phe	0.73 f	1.86 d	2.76 b	2.81 b	0.75 f	1.23 e	2.44 c	4.62 a
His	0.55 f	1.38 d	1.35 d	2.35b	0.62 f	1.04 e	2.03 c	4.02 a
Pro	0.32 e	0.51 d	0.71 c	1.45 b	0.38 e	0.56 d	0.80 c	1.69 a
Ile	0.91 b	1.14 a	1.17 a	1.19 a	0.70 c	1.15 a	1.13 a	1.16 a
Lys	1.98 d	2.50 c	2.13 d	2.33 b	2.61 c	3.20 b	3.21 b	3.51 a
Arg	2.36 d	2.97 c	2.54 d	3.73 b	3.11 c	3.82 b	3.83 b	4.18 a
Gly	0.69 c	0.70 c	0.71 c	0.73 bc	0.67 c	0.84 ab	0.82 ab	0.93 a
Total	75.39 e	76.11 e	78.09 e	91.85 b	83.18 d	80.68 e	89.02 c	103.64 a

The values (means of three replicates) in the same line followed by different letters are significantly different from each other at the $P < 0.05$ levels.

3.5. Effect of Mn stress on ROS metabolism

The Mn treatments caused oxidative stress, as indicated by the significant increase of H_2O_2 and MDA contents in both populations ($P < 0.001$). The effects of population and population × Mn interaction also significantly affected the H_2O_2 and MDA contents ($P < 0.001$) (Fig. 3). The SOD activity increased under 0–0.5 mM Mn treatments and then decreased significantly under 1 mM Mn in the wet climate population. However, in the dry climate population, the SOD activity remained about the same and it was maintained at a higher level under all Mn concentrations. The APX activity increased in both populations with the exception of the wet climate population under 0.1 mM Mn, although the dry climate population exhibited a higher activity than did the wet climate population under the same Mn concentration (Fig. 3).

4. Discussion

The tolerance index of the dry climate population of *P. cathayana* was significantly higher than that of the wet climate population, which indicated higher Mn tolerance in the former and the existence of cross-tolerance between drought and Mn stress in poplars. A common response of plants to various stresses is growth adjustment, as also seen in the two poplar populations in the present study as the reduction of shoot height, basal diameter, total dry mass and total leaf areas with increasing Mn concentrations. Furthermore, the growth reduction detected in the wet climate population was more pronounced than that in the dry climate population. This also confirms the presence of higher Mn tolerance in the dry climate population. Chlorophyll contents were found to decrease significantly under Mn toxicity, which may be due to Fe deficiency induced by Mn. Excess Mn has been reported to inhibit the synthesis of chlorophyll through a Fe-concerning process (Fecht-Christoffers et al., 2003; Sarkar et al., 2004). It was also found that under 0 mM Mn the chlorophyll content of the dry climate population was lower than that of the wet climate population. It may be a 'cost' for resistant plants to grow in normal conditions, a phenomenon described in previous studies (Bradshaw, 1984; Liu et al., 2004). The Mn content of the plant tissues increased under all Mn treatments in both populations. Under high Mn concentrations, the dry climate population accumulated a lower level of Mn, which indicates reduced uptake and transporting or a strong ability of Mn sequestration to avoid excessive Mn toxicity (Table 3). Our result was consistent with previous studies on *Rumex dentatus*, *Holcus lanatus* (Coughttey and Martin, 1978) and *Salix* (Landberg and Greger, 1996). It was also discovered that under high Mn concentration, most Mn was accumulated in the leaves in both populations, which led to visible symptoms in leaves, as also reported by Demirevska-Kepova et al. (2004).

ABA was found to accumulate significantly in the two contrasting populations under Mn treatments. Furthermore, the dry climate population possessed a higher constitutive ABA content and a more pronounced increase (Fig. 2). Hsu and Kao (2003) have also found that under treatments with $CdCl_2$, the ABA content rapidly increased in the leaves and roots of the Cd-tolerant cultivar (cv.Tainung 67, TNG67) but not in the Cd-sensitive cultivar (cv.Taichung Native 1, TN1). ABA has been thought to act as a messenger in stress – perception – response pathways in various environmental stresses (Zhu et al., 1997). Circumstantial evidence indicated that ABA may induce a number of genes and proteins involved in stress defenses, including SOD, GPX, APX, GR and pathogenesis-related

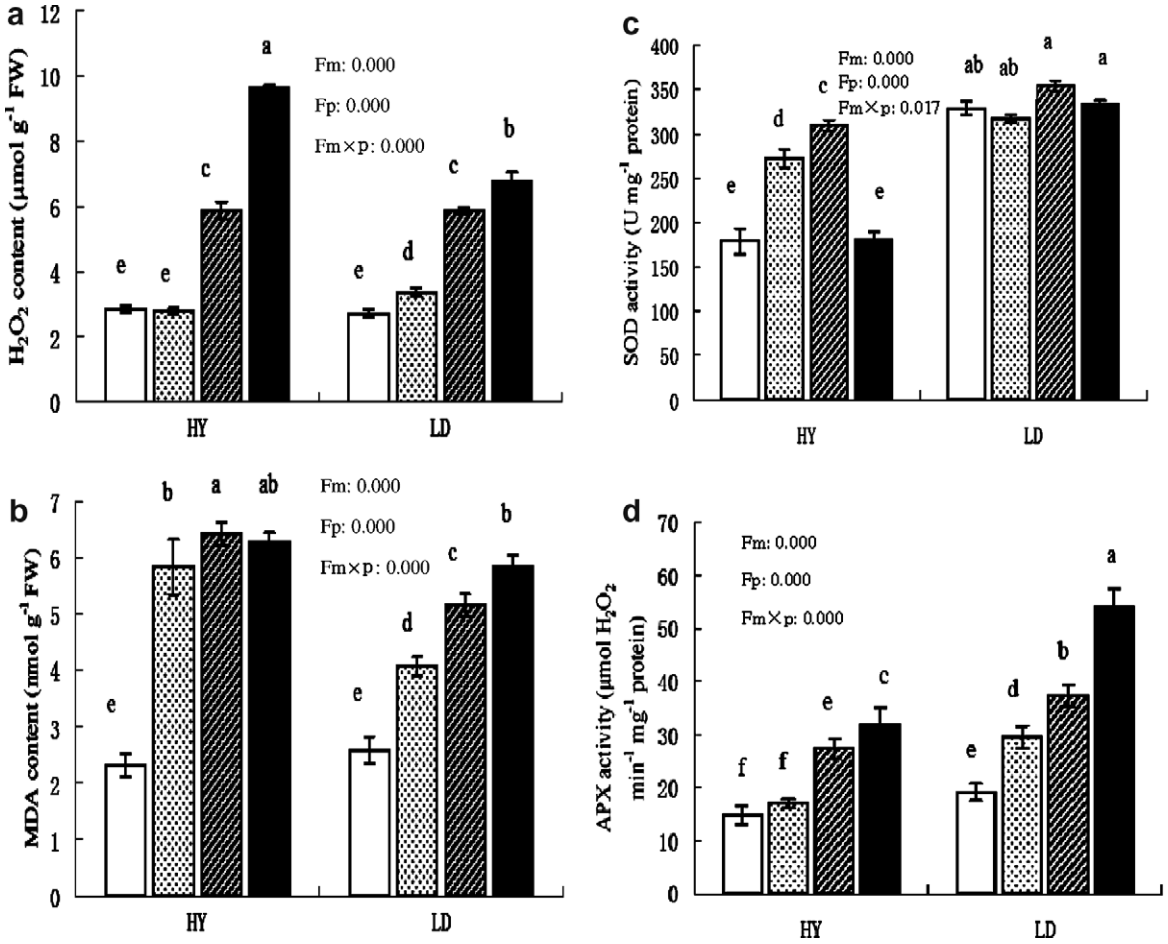

Fig. 3. Effect of manganese stress on H_2O_2 (a), MDA (b) contents and SOD (c), APX (d) activities in the young leaves of the two contrasting populations of *P. cathayana*. HY, the wet climate population; LD, the dry climate population. □, 0 mM manganese; ▩, 0.1 mM manganese; ▨, 0.5 mM manganese; ■, 1 mM manganese. Fm, manganese effect; Fp, population effect; Fm × p, manganese × population interaction effect. The different letters above the bars (means of three replicates ± SE) are significantly different from each other at $P < 0.05$.

(PR) proteins (Jiang and Zhang, 2001). Polyamine contents also alter in response to the exposure to heavy metals. Weinstein et al. (1986) have shown an up to 10-fold increment in the putrescine content in Cd-treated oat seedlings and detached leaves along with a marginal rise in the spermidine and spermine content. Our results were comparable, although the increase in the putrescine content was not as enormous. A pretreatment of sunflower leaf discs with exogenous spermine has been found to reverse almost completely the Cd- or Cu-induced lipid peroxidation (Groppa et al., 2001). Polyamines are cations due to protonation under cytoplasmic pH conditions, which accounts for their binding ability to negatively charged phospholipids and DNA, thereby stabilizing the function of the membranes and the nucleus (Groppa et al., 2001). Polyamines also have an antioxidative property by quenching the accumulation of O_2^- probably through the inhibition of NADPH oxidase (Papadakis and Roubelakis-Angelakis, 2005). Furthermore, polyamines block one of the major vacuolar channels, the fast vacuolar cation channel, and their accumulation could decrease ion conductance at the vacuolar membrane to facilitate metal ion compartmentation (Brüggemann et al., 1998).

The Mn treatment greatly altered the composition and concentration of free amino acids and the greatest increase was found in the His, Pro, Phe, Tyr and Ser contents. Kramer et al. (1996) have previously detected a 36-fold increase in the His content of the xylem sap on exposure to Ni in the Ni-hyperaccumulating plant *Alyssum lesbiacum*. On the other hand, supplying His to a non-accumulating species greatly increased both its Ni tolerance and the capacity for Ni transport to the shoot. As for Pro, an increase of up to 20-fold content in the leaves of metal non-tolerant *Silene vulgaris* has been observed (Schat et al., 1997), and a large body of data suggests its beneficial functions under a metal stress. In general, Pro is supposed to have three major functions, namely osmoregulation, metal chelation and antioxidant defense (Hall, 2002; Wu et al., 2004). As an osmolyte, Pro might offset the water deficit developed due to an exposure to heavy metals, and it has also been reported to bring about stomatal closure to restrict metal uptake and translocation (Rajagopal, 1981). The Phe con-

tent also increased significantly under Mn toxicity in both populations. It is the substrate of many phenolics biosynthesis catalyzed by phenylalanine ammonia-lyase, which is believed to be involved in the resistance to a variety of biotic and abiotic stresses, including heavy metal stress (Ye et al., 2006).

Oxidative stress is a phenomenon which has been implicated as one of the main agents causing cellular damage in plants exposed to a wide variety of stress conditions, including heavy metal toxicity. In the two poplar populations investigated in the present study, the Mn treatments significantly enhanced the generation of H_2O_2 and then caused lipid peroxidation as indicated by an increase in the MDA content. To cope with oxidative damage, antioxidant enzymes, such as SOD and APX are activated, as shown in many earlier studies (Yin et al., 2005; Lei et al., 2006). Compared with the dry climate population, the wet climate population exhibited lower SOD and APX activities, thus decreasing its ability of scavenging free radicals resulting from Mn stress and leading into a more serious membrane damage.

Our results clearly showed that the population originating from dry climate conditions was more tolerant to Mn stress than the wet climate population. It is apparent that there exists cross-tolerance between Mn and drought resistance in poplars. A proposed explanation is that Mn tolerance may result from a less specific mechanism that confers a broad tolerance to several different stresses (co-tolerance) or it may involve a series of independent metal-specific mechanisms (multiple tolerance) (Hall, 2002). In our study, poplars were found to extract a considerable amount of Mn into their leaves. This feature can be taken into account when planning actions for the remediation of contaminated soils. However, their growth rate and biomass accumulation were also greatly retarded under Mn stress. The future research should be focused on the improvement of their growth under heavy metal toxicity, maybe through genetic manipulation. In fact, *Populus* has been chosen as a model system for tree plant research targeted at genetic manipulation, because of its rapid growth, high biomass, ease of cloning, relatively small genome, facile transformation and propagation techniques (Pulford and Watson, 2003).

In conclusion, poplars employ a broad spectrum of mechanisms to cope with Mn stress, including the enhanced synthesis of ABA and polyamines, the accumulation of free amino acids, especially His and Pro, and the activation of the enzymes SOD and APX. The dry climate population exhibited a lower Mn uptake and transportation into leaves, higher contents of ABA, polyamines and free amino acids, and higher activities of antioxidant enzymes. All these features make it superior in Mn tolerance in comparison with the wet climate population.

Acknowledgements

The research was supported by the Outstanding Young Scientist Program of the National Natural Science Foundation of China (No. 30525036) and the China National Key Program of the International Cooperation for Science and Technology (No. 2005DFA30620).

References

Bradford, M.M., 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Bradshaw, A.D., 1984. Adaptation of plants to soils containing toxic metals – a test for conceit. In: Evered, D., Collins, G.M. (Eds.). In: Proceedings of the CIBA Foundation Symposium on Origin and Development of Adaptation, vol. 102. Pitman, London, pp. 4–19.

Brennan, T., Frenkel, C., 1977. Involvement of hydrogen peroxide in the regulation of senescence in pear. Plant Physiol. 59, 411–416.

Brüggemann, L.I., Pottosin, I.I., Schonknecht, G., 1998. Cytoplasmic polyamines block the fast-activating vacuolar cation channel. Plant J. 16, 101–105.

Brune, A., Dietz, K.J., 1995. A comparative analysis of element composition of barley roots and leaves under cadmium-, molybdenum-, nickel-, and zinc-stress. J. Plant Nutr. 18, 853–868.

Capiati, D.A., País, S.M., Téllez-Iñón, M.T., 2006. Wounding increases salt tolerance in tomato plants: evidence on the participation of calmodulin-like activities in cross-tolerance signaling. J. Exp. Bot. 57, 2391–2400.

Cho, M., Chardonnens, A.N., Dietz, K.J., 2003. Differential heavy metal tolerance of *Arabidopsis halleri* and *Arabidopsis thaliana*: a leaf slice test. New Phytol. 158, 287–293.

Collins, G., Nie, X.L., Saltveit, M., 1995. Heat shock proteins and chilling sensitivity of mung bean hypocotyls. J. Exp. Bot. 46, 795–802.

Coughttey, P.J., Martin, M.H., 1978. Cadmium uptake and distribution on tolerant and non-tolerant populations of *Holcus lanarus* grown in solution culture. Oikos 30, 555–560.

Demirevska-Kepova, K., Simova-Stoilova, L., Stoyanova, Z., Hölzer, R., Feller, U., 2004. Biochemical changes in barley plants after excessive supply of copper and manganese. Environ. Exp. Bot. 52, 253–266.

Di Baccio, D., Kopriva, S., Sebastiani, L., Renenberg, H., 2005. Does glutathione metabolism have a role in the defence of poplar against zinc excess? New Phytol. 167, 73–80.

Dickmann, D.I., 1971. Photosynthesis and respiration by developing leaves of cottonwood (*Populus deltoides* Bartr.). Bot. Gaz. 132, 253–259.

Fecht-Christoffers, M.M., Maier, P., Horst, W.J., 2003. Apoplastic peroxidases and ascorbate are involved in manganese toxicity and tolerance of *Vigna unguiculata*. Physiol. Plant. 117, 237–244.

Flores, H.E., Galston, A.W., 1982. Analysis of polyamines in higher plants by high performance liquid chromatography. Plant Physiol. 69, 701–706.

Friedland, A.J., 1990. The movement of metals through soils and ecosystems. In: Shaw, A.J. (Ed.), Heavy Metal Tolerance in Plants: Evolutionary Aspects. CRC Press, Boca Raton, pp. 7–19.

Giannopolitis, C.N., Ries, S.K., 1977. Superoxide dismutase in higher plants. Plant Physiol. 59, 309–314.

Groppa, M.D., Tomaro, M.L., Benavides, M.P., 2001. Polyamines as protectors against cadmium or copper-induced oxidative damage in sunflower leaf discs. Plant Sci. 161, 481–488.

Hall, J.L., 2002. Cellular mechanisms for heavy metal detoxification and tolerance. J. Exp. Bot. 53, 1–11.

Hoagland, D.R., Arnon, D.I., 1950. California agricultural experimental station. Circular 347.

Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K., 1999. Improving the thiobarbituric acid-reactive substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207, 604–611.

Hsu, Y.T., Kao, C.H., 2003. Role of abscisic acid in cadmium tolerance of rice (*Oryza sativa* L.) seedlings. Plant Cell Environ. 26, 867–874.

Inskeep, W.P., Bloom, P.R., 1985. Extinction coefficients of chlorophyll a and b in *N*,*N*-dimethylformamide and 80% acetone. Plant Physiol. 77, 483–485.

Jiang, M., Zhang, J., 2001. Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. Plant Cell Physiol. 42, 1265–1273.

Kramer, U., Cotter-Howells, J.D., Charnock, J.M., Baker, A.J.M., Smith, J.A.C., 1996. Free histidine as a metal chelator in plants that accumulate nickel. Nature 379, 635–638.

Kuznetsov, V., Rakutin, V., Boisova, N., Rotschupkin, B., 1993. Why does heat shock increase salt resistance in cotton plants? Plant Physiol. Biochem. 31, 181–188.

Landberg, T., Greger, M., 1996. Differences in uptake and tolerance to heavy metals in *Salix* from unpolluted and polluted areas. Appl. Geochem. 11, 175–180.

Lasat, M.M., 2002. Phytoextraction of toxic metals: a review of biological mechanisms. J. Environ. Qual. 31, 109–120.

Lei, Y., Yin, C., Li, C., 2006. Differences in some morphological, physiological, and biochemical responses to drought stress in two contrasting populations of *Populus przewalskii*. Physiol. Plant. 127, 182–191.

Liu, J., Xiong, Z., Li, T., Huang, H., 2004. Bioaccumulation and ecophysiological responses to copper stress in two populations of *Rumex dentatus* L. from Cu contaminated and non-contaminated sites. Environ. Exp. Bot. 52, 43–51.

Metwally, A., Safronova, V.I., Belimov, A.A., Dietz1, K.J., 2005. Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. J. Exp. Bot. 56, 167–178.

Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.

Papadakis, A.K., Roubelakis-Angelakis, K.A., 2005. Polyamines inhibit NADPH oxidase-mediated superoxide generation and putrescine prevents programmed cell death induced by polyamine oxidase-generated hydrogen peroxide. Planta 220, 826–837.

Pilon-Smits, E., 2005. Phytoremediation. Annu. Rev. Plant Biol. 56, 15–39.

Pittman, J.K., 2005. Managing the manganese: molecular mechanisms of manganese transport and homeostasis. New Phytol. 167, 733–742.

Prasad, M.N.V., 1999. Metallothioneins and metal binding complexes in plants. In: Prasad, M.N.V., Hagemeyer, J. (Eds.), Heavy metal stress in plants: from molecules to ecosystems. Springer Verlag, Berlin, Heidelberg, pp. 51–72.

Pulford, I.D., Watson, C., 2003. Phytoremediation of heavy metal-contaminated land by trees – a review. Environ. Int. 29, 529–540.

Rajagopal, V., 1981. The influence of exogenous proline on the stomatal resistance in *Vicia faba*. Physiol. Plant. 52, 292–296.

Salt, D.E., Smith, R.D., Raskin, I., 1998. Phytoremediation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 643–668.

Sanita di Toppi, L., Gabrielli, R., 1999. Response to cadmium in higher plants. Environ. Exp. Bot. 41, 105–130.

Sarkar, D., Pandey, S.K., Sud, K.C., Chanemougasoundharam, A., 2004. In vitro characterization of manganese toxicity in relation to phosphorus nutrition in potato (*Solanum tuberosum* L.). Plant Sci. 167, 977–986.

Schat, H., Sharma, S.S., Vooijs, R., 1997. Heavy metal-induced accumulation of free proline in a metal-tolerant and a non-tolerant ecotype of *Silene vulgaris*. Physiol. Plant. 101, 477–482.

Weinstein, L., Kaur-Sawhney, R., Venkat Rajam, M., Wettlaufer, S.H., Galston, A.W., 1986. Cadmium-induced accumulation of putrescine in oat and bean leaves. Plant Physiol. 82, 641–645.

Wilkins, D.A., 1978. The measurement of tolerance to edaphic factors by means of roots growth. New Phytol. 80, 623–633.

Wu, F., Chen, F., Wei, K., Zhang, G., 2004. Effect of cadmium on free amino acid, glutathione and ascorbic acid concentrations in two barley genotypes (*Hordeum vulgare* L.) differing in cadmium tolerance. Chemosphere, 447–454.

Xiong, Y., Xing, G., Li, F., Wang, S., Fan, X., Li, Z., Wang, Y., 2006. Abscisic acid promotes accumulation of toxin ODAP in relation to free spermine level in grass pea seedlings (*Lathyrus sativus* L.). Plant Physiol. Biochem. 44, 161–169.

Ye, S., Zhou, Y., Sun, Y., Zou, L., Yu, J., 2006. Cinnamic acid causes oxidative stress in cucumber roots, and promotes incidence of *Fusarium* wilt. Environ. Exp. Bot. 56, 255–262.

Yin, C., Peng, Y., Zang, R., Zhu, Y., Li, C., 2005. Adaptive responses of *Populus kangdingensis* to drought stress. Physiol. Plant. 123, 445–451.

Zhu, J., Hasegawa, P.M., Bressan, R.A., 1997. Molecular aspects of osmotic stress in plants. Crit. Rev. Plant Sci. 16, 253–277.