Responses to drought stress in two poplar species originating from different altitudes

F. YANG[1,2], X. XU[1,2], X. XIAO[1,2] and C. LI[1]*

Chengdu Institute of Biology, Chinese Academy of Sciences, P.O.Box 416, Chengdu 610041, P.R. China[1]
Graduate School of the Chinese Academy of Sciences, Beijing 100039, P.R. China[2]

Abstract

Cuttings of *Populus kangdingensis* and *Populus cathayana*, originating from high and low altitudes in the eastern Himalaya, respectively, were examined during one growing season in a greenhouse to determine their responses to drought stress (soil moisture decreased from 100 to 55 or 25 % field capacity). Compared to control plants grown under 100 % field capacity, those poplars grown under 55 and 25 % field capacity possessed lower increases in height and stem diameter, and higher contents of soluble sugars, free proline, malondialdehyde (MDA) and hydrogen peroxide, and higher activities of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR). Compared with *P. cathayana* with greater leaf area, *P. kangdingensis* with greater root/shoot ratio exhibited lower MDA and H_2O_2 contents, higher soluble sugar and free proline contents, and higher activities of CAT, SOD, POD, APX and GR. These results suggested that *P. kangdingensis* was more drought tolerant than *P. cathayana*.

Additional key words: antioxidant enzymes, free proline, hydrogen peroxide, malondialdehyde, soluble sugars.

Introduction

Plants can avoid drought stress by maximizing water uptake (*e.g.*, deep roots) or minimizing water loss (*e.g.*, small leaves) (Kozlowski and Pallardy 2002, Ruiz-Sánchez *et al.* 2007). Apart from morphological structures contributing to drought stress tolerance, plants have evolved a variety of physiological and biochemical processes, which act as components of drought tolerance (Ren *et al.* 2007, Wang *et al.* 2007). Plants have also developed enzymatic antioxidant systems to cope with drought stress and to avoid oxidative damage (Shvaleva *et al.* 2006, Horváth *et al.* 2007).

Poplars (*Populus* spp.) which are fast-growing forest tree species, are widely used for timber, pulp and paper, and have potential as a source of biomass energy (Yin *et al.* 2004). They play a very important role in preventing soil erosion and soil-water loss and in regulating climate as well as in retaining ecological stability (Yin *et al.* 2005). We hypothesized that the response to drought could be different in species from different altitudes and different during vegetative season. Therefore, in this study we used two contrasting species originating from different natural habitats in the eastern Himalaya, *P. kangdingensis* occurs at the high-altitude zone (altitude of about 3 500 m), whereas *P. cathayana* inhabits the low-altitude zone (altitude of about 1 500 m), to investigate the possible different responses to drought.

Materials and methods

Plants and experimental design: Healthy cuttings of a uniform height of two poplar species *Populus kangdingensis* C. Wang *et* Tung and *Populus cathayana* Rehder, were collected in their natural habitats in the eastern Himalaya (Table 1). Then the cuttings were grown in pots with soil in a naturally lit greenhouse under a

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Abbreviations: APX - ascorbate peroxidase; AsA - ascorbic acid; CAT - catalase; d.m. - dry mass; GR - glutathione reductase; LA - leaf area; MDA - malondialdehyde; NBT - nitroblue tetrazolium; POD - peroxidase; ROS - reactive oxygen species; RS - root/shoot ratio; RWC - relative water content; SOD - superoxide dismutase.
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* Corresponding author; fax: (+86) 28 85222753, e-mail: licy@cib.ac.cn

semi-controlled environment with a day/night temperature of 12 - 38/9 - 30 °C, and relative humidity of 35 - 85 % from 16 March to 30 August 2006 at the Chengdu Institute of Biology.

A completely randomized design with two factors (species and watering regime) was employed. The cuttings of each species were allocated randomly to three different watering regimes as follows: a well-watered treatment (100 % of field capacity) and two water-stressed treatments (50 and 25 % of field capacity). Five replications, each with 18 cuttings, were used in each species and each watering treatment. In the well-watered treatment, the pots were weighed every day and re-watered to field capacity by replacing the amount of water transpired; in this case, the soil water content was always kept at 40.2 % by daily watering. In the water-stressed treatment, the pots were watered to 50 and 25 % of field capacity by watering every day, in this case, the soil water contents were always kept at 20.1 and 10.1 % by daily watering, respectively. Evaporation from the soil surface was prevented by enclosing all pots in plastic bags sealed at the base of the stem of each cutting. During the experimental period, the fully expanded leaves were harvested to measure the biochemical parameters every two weeks, total six times.

Height and stem diameter were measured every two weeks. Stem diameter was determined at the site of the first leaf by a vernier caliper. At the end of the whole experiment, leaf area (LA) was determined by the portable laser area meter (*CI-203*, *CID*, Vancouver, USA), and the relative water content of leaves (RWC) was measured as described by Duan *et al.* (2005).

Determinations of contents of soluble sugars, free proline, MDA and H_2O_2: Soluble sugar was measured as described by Mohsenzadeh *et al.* (2006). Free proline was measured as described by Lei *et al.* (2007) and Ren *et al.* (2007). The absorbance of the free proline concentration was measured at 515 nm. The content of malondialdehyde (MDA) was measured as described by Heath and Packer (1968) and Fazeli *et al.* (2007). The absorbance of MDA was measured at 532, 600 and 450 nm. The MDA content was calculated according to the formula: MDA [µM] = $6.45(A_{532} - A_{600}) - 0.56A_{450}$. The H_2O_2 content was determined as described by Mukherjee and Choudhuri (1983).

Assays of antioxidant enzymes: The modified method of Knörzer *et al.* (1996) and Fazeli *et al.* (2007) was used. Fresh leaf samples (0.4 g) were ground in liquid nitrogen and homogenized on ice bath in 4 cm^3 of single extraction solution containing 50 mM Tris-HCl (pH 7.0), 1 mM EDTA, 20 % glycerin, 1 mM ascorbic acid (AsA), 1 mM dithiothreitol, 1 mM glutathione, 5 mM $MgCl_2$. The homogenate was centrifuged at 8 000 *g* at 4 °C for 15 min. The supernatant was stored in at -70 °C. Soluble protein contents were determined as described by Bradford (1976) using bovine serum albumin as a standard.

Superoxide dismutase (EC 1.15.1.1, SOD): The SOD activity was measured spectrophotometrically based on the inhibition in the photochemical reduction of nitroblue tetrazolium (NBT) (Giannopolitis and Ries 1977, Fazeli *et al.* 2007). 1 unit of SOD was defined as the amount of enzyme inhibiting the photo-reduction of NBT by 50 %.

The procedure of Aebi (1984) was used to analyse the catalase (EC 1.11.1.6, CAT) activity. The CAT activity was determined by directly measuring the decomposition of H_2O_2 at 240 nm. The CAT activity was followed by a decrease of absorbance between 0.5 and 3 min.

The guaiacol peroxidase (EC 1.11.1.7, POD) activity was measured using the method of Chance and Maehly (1955) and Horváth *et al*. (2007). Changes in the absorbance of the brown guaiacol at 470 nm between 0.5 and 3.5 min were recorded. The ascorbate peroxidase (EC 1.11.1.11, APX) activity was measured using the procedure of Nakano and Asada (1981). The H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm within 1 min.

The method of Halliwell and Foyer (1978) was employed for the assay of glutathione reductase (EC 1.6.4.2, GR) activity. The GR activity was followed by a decrease of absorbance at 340 nm between 0.5 and 3.5 min.

Statistical analyses: Statistical analyses were performed using the statistical software package for social science (*SPSS*) version *12.0*. Two-way analyses of variance (*ANOVA*) were used to test the main effects of the drought treatment, species and their interaction. Within each species, pairwise comparisons were made using the Student-Newman-Keuls multiple range test. Differences were considered significant at the $P < 0.05$ level.

Results

Drought significantly inhibited the stem height and diameter increment, especially under severe drought stress (25 % field capacity) (Fig. 1). Drought also significantly decreased LA and RWC, whereas it significantly increased the RS (Table 2). The cuttings of *P. cathayana* had a greater height (Fig. 1*A*) and stem diameter (Fig. 1*B*) than *P. kangdingensis* under the well-watered treatment. RS increased much more in *P. kangdingensis* than in *P. cathayana* when cuttings were exposed to drought stress, whereas *P. cathayana* had a greater LA than *P. kangdingensis*. However, there were no significant differences in RWC between species.

In both species, the contents of soluble sugar (Fig. 1*C*) and free proline (Fig. 1*D*) significantly increased when cuttings were exposed to drought stress for 4 weeks. *P. kangdingensis* exhibited always higher contents of soluble sugar and free proline than did *P. cathayana*. At the end of experiment, the soluble sugar and free proline contents at 25 % field capacity were higher by 87 and 80 %, respectively, than those at 100 % field capacity in *P. cathayana,* whereas they were by 144 and 138 % higher in *P. kangdingensis*.

Table 1. The origin of the two *Populus* species examined, and climatic data from the collection areas, expressed as mean annual values.

Species	Latitude [N]	Longitude [E]	Altitude [m]	Evaporation [mm]	Rainfall [mm]	Frostless [d]	Temperature [°C]
P. kangdingensis	30°12′	102°35′	3500	1301.7	924	188	7.1
P. cathayana	32°25′	104°31′	1500	1056.8	553	255	12.6

Table 2. Root/shoot ratio (RS), leaf area (LA) and the relative water content of leaves (RWC) in two poplar species as affected by drought stress. Each value represents the mean ± SE of four replicates. Values followed by the same letter within a column indicate no significant difference at $P < 0.05$.

Species	Soil moisture [%]	RS	LA [cm^2]	RWC [%]
P. cathayana	100	0.19 ± 0.05d	104.89 ± 16.29a	80.44 ± 1.53a
	55	0.31 ± 0.03c	66.56 ± 6.12b	68.22 ± 0.97b
	25	0.38 ± 0.02b	61.07 ± 5.08b	64.55 ± 1.09b
P. kangdingensis	100	0.20 ± 0.05d	69.13 ± 3.15b	76.92 ± 0.96a
	55	0.33 ± 0.04c	51.94 ± 5.41c	67.93 ± 1.21b
	25	0.48 ± 0.04a	42.35 ± 4.36d	65.60 ± 1.70b

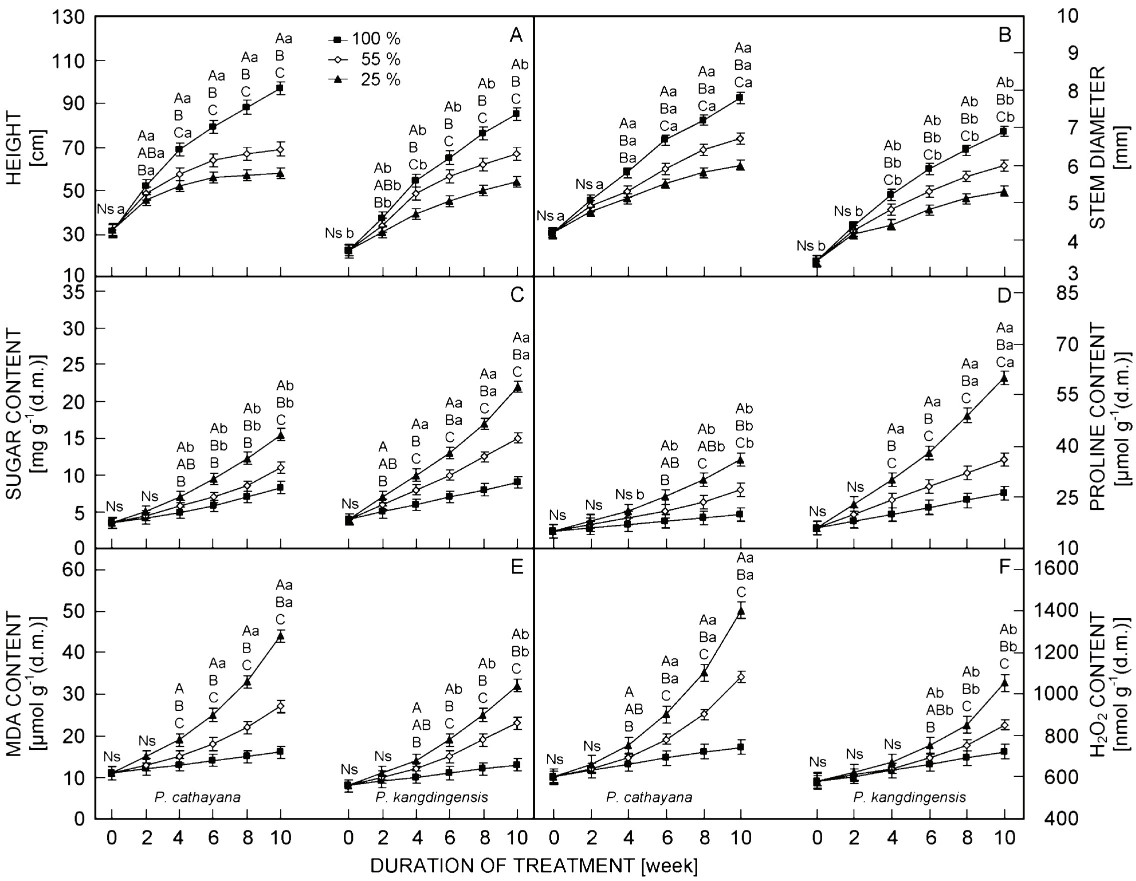

Fig. 1. Height (*A*), stem diameter (*B*) and contents of soluble sugar (*C*), free proline (*D*), MDA (*E*) and H_2O_2 (*F*) in cuttings of two poplar species exposed to drought stress. The data presented are means ± SE of four replicates. Capital letters refer to differences in the same species under different treatments. Small letters refer to differences between species under the same treatment. Values followed by different letters are significantly different from each other at $P < 0.05$.

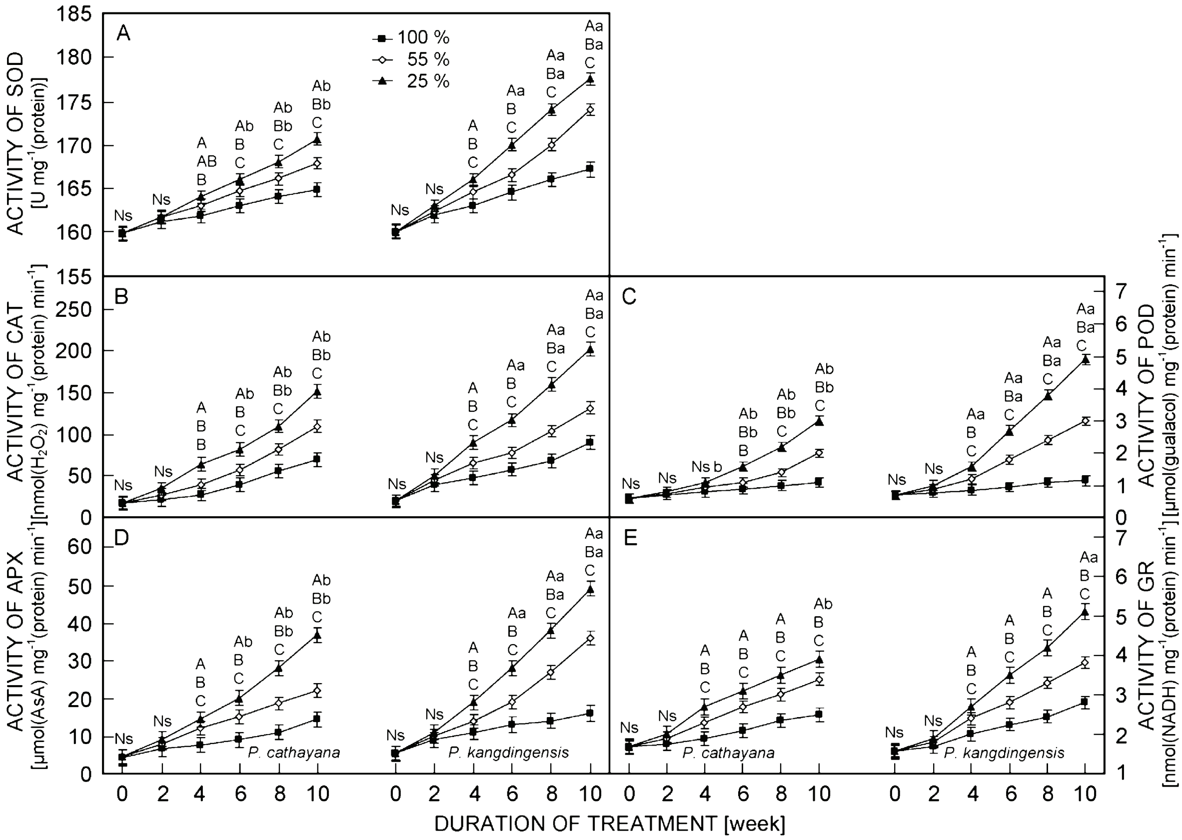

Fig. 2. The activities of SOD (*A*), CAT (*B*), POD (*C*), APX (*D*) and GR (*E*) in cuttings of two contrasting poplar species exposed to drought stress. The data presented are means ± SE of four replicates. Capital letters refer to differences in the same species under different treatments. Small letters refer to differences between species under the same treatment. Values followed by different letters are significantly different from each other at $P < 0.05$.

MDA (Fig. 1*E*) and H_2O_2 (Fig. 1*F*) contents significantly increased in the two contrasting poplar species exposed to drought stress for 4 to 10 weeks. MDA and H_2O_2 accumulation was much higher in the 25 % field capacity than in the 55 % field capacity. *P. kangdingensis* had slower MDA and H_2O_2 accumulation (146 and 45 %, respectively, under 25 % field capacity compared with 100 % field capacity) than *P. cathayana* (175 and 89 %, respectively). In addition, the species × watering interaction was significant for MDA and H_2O_2.

The activities of CAT, SOD, POD, APX and GR significantly increased after 4 to 6 weeks of the treatment (Fig. 2). Among three watering treatments, the cuttings possessed the highest activities of antioxidant enzymes at the 25 % field capacity. *P. kangdingensis* exhibited always higher activities of antioxidant enzymes under the same water-stress treatments. At the end of experiment, compared with 100 % field capacity, at 25 % field capacity the increased activities of CAT, SOD, POD, APX and GR were 4.3, 103, 172, 208 and 56 % in *P. cathayana*, respectively, whereas they were 8.1, 125, 326, 276 and 78 % in *P. kangdingensis.* Drought stress significantly affected the activities of antioxidant enzymes and the watering × species interaction effects were also significant.

Discussion

Acclimation to water deficit results from a series of integrated events occurring at morphological, physiological and biochemical levels. Such adjustments help in the retention and/or acquisition of water, and in the protection of the chloroplast functions and the antioxidant system (Hernández *et al.* 2004). In our study, *P. kangdingensis* could still keep rapid height increment during the whole duration of the treatment at 25 % field capacity, whereas *P. cathayana* grew very slowly after 6-week treatment, which suggests that *P. kangdingensis* possess better drought adaptation. In contrast, *P. cathayana* grew more rapidly at 100 % field capacity. Compared with *P. cathayana* with a greater LA, *P. kangdingensis* with greater RS could avoid drought stress by maximizing water uptake and minimizing water loss.

The plant's defense against drought stress requires osmotic adjustment, which can be achieved through a synthesis of intracellular solutes (Serrano *et al.* 1999). In our study, the accumulation of soluble sugar and free proline was related to the degree of drought stress. Similar results have been detected also in *Aeluropus lagopoides* (Mohsenzadeh *et al.* 2006) and in *Radix astragali* (Tan *et al.* 2006). In *P. kangdingensis*, soluble sugar and free proline contents increased more rapidly than in *P. cathayana* under the same watering treatments. Changes in these two parameters manifested that *P. kangdingensis* possesses better osmotic adjustment than *P. cathayana*.

MDA is one of the end products of lipid peroxidation, and the MDA content reflects the degree of the peroxidation of membrane lipids. H_2O_2 as a reactive oxygen species (ROS) can damage the membrane lipids, proteins and DNA (Bowler *et al.* 1992, Foyer and Halliwell 1997). In our study, the MDA and H_2O_2 contents increased significantly in the two contrasting poplars under drought stress. Similar results have also been detected in olive trees (Sofo *et al.* 2004). The increase in the MDA content detected after 4 weeks of exposure to drought showed that the increased activities of APX and GR may have not been enough to prevent the peroxidation of lipid membranes under drought stress. On the other hand, the increase in the MDA level detected after 4 weeks of drought treatment may also be correlated with inadequate activities of SOD and CAT to scavenge ROS in poplar leaves. Compared with *P. kangdingensis*, *P. cathayana* possessed higher contents of MDA and H_2O_2, which demonstrates that oxidative stress is more serious in *P. cathayana*. On the other hand, *P. kangdingensis* always had higher antioxidant enzyme activities than did *P. cathayana* and can remove ROS more easily. Therefore, *P. kangdingensis* possesses a higher antioxidative capacity. Thus, the ability to increase the antioxidant system activity in order to limit cellular damages may be an important attribute of *P. kangdingensis*.

Tolerance to drought may be seen from two points of view: survival (better detoxification, reduction of water use through reduction of shoot growth) or maintenance of productivity. Compared with *P. cathayana*, *P. kangdingensis* grew slower at 100 % field capacity, but it could keep growth under serious drought stress and possessed higher contents of soluble sugar, free proline and activities of antioxidant enzymes, and lower contents of MDA and H_2O_2. All these pieces of evidence manifest our hypotheses that the biochemical responses to drought stress could be different in the species from different altitude. *P. kangdingensis* from the high altitude has better drought tolerance than *P. cathayana* originating from low altitude. In this study, we also proved that plant acclimation processes depend on the rapidity, severity and duration of the drought event. This study provides a comprehensive analysis of drought acclimation in poplar. We hope that this study could help to select species with an improved ability to cope with drought in the future.

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