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Adaptive Responses to Progressive Drought Stress in Two *Populus cathayana* Populations

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The young, vegetatively propagated cuttings of *Populus cathayana* Rehder were exposed to a progressive drought stress for 12 weeks in a greenhouse to characterize the physiological and biochemical basis of drought adaptation in woody plants. Two contrasting populations were employed in our study, which were from the wet and dry climate regions in western China, respectively. The results showed that the adaptive responses of *P. cathayana* to drought were affected by drought intensity and poplar genotype (population). The progressive drought stress significantly inhibited plant growth, increased carotenoid contents and, at the same time, accumulated soluble sugars and free proline in the plants of both populations tested. On the other hand, the gradually increasing drought also induced antioxidative systems including the increase of the activities of superoxide dismutase (SOD) and guaiacol peroxidase (POD). Moreover, there were different responses to progressive drought stress between the two contrasting populations. Compared with the wet climate population, the dry climate population had lower shoot height and growth rate, higher free proline content, and more efficient photoprotective system (such as higher carotenoid content and Car/Chl) and antioxidant system (such as higher POD activity), as a result of drought stress. These results suggest that the dry climate population possesses better drought tolerance than the wet climate population. The differences in drought tolerance may be closely related with efficient photoprotective system, accumulation of the osmoprotectant proline as well as the increased capacity of the antioxidative system to scavenge reactive oxygen species, and the consequent suppressed level of lipid peroxidation under drought conditions.

Keywords malondialdehyde, free proline, soluble sugars, antioxidant enzymes, drought tolerance, *Populus cathayana*
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1 Introduction

Drought is one of the most important abiotic stress factors that limit plant growth and ecosystem productivity worldwide (Passioura 1996). Because drought is a common occurrence in many environments, many perennial plant species have developed mechanisms to cope with an inadequate water supply (Arndt et al. 2001). Acclimation of plant to water deficit is the result of many different physiological and biochemical mechanisms, including a series of integrated events from stress signal perception, transduction to regulation of gene expression, which lead to the adaptive changes in plant growth and physio-biochemical processes, such as changes in plant structure, growth rate, stomatal conductance, tissue osmotic potential and antioxidant defenses (Kozlowski and Pallardy 2002, Zhang et al. 2005, Yin et al. 2005, Lei et al. 2006). Plants can avoid drought stress by maximizing water uptake (e.g., tapping ground water by deep roots) or minimizing water loss (e.g., stomatal closure and small leaves etc.) (Kozlowski and Pallardy 2002, Chaves et al. 2003). Apart from morphological structures contributing to drought stress tolerance, one of the potentially important mechanisms of drought tolerance is osmotic adjustment which can be achieved from the accumulation of compatible solutes (such as amino acids, glycine betaine, sugars, or sugar alcohols) in protoplasm (Bray 1997, Hare et al. 1998, Chaves et al. 2003, Bartels and Sunkar 2005). The osmotic adjustment allows cell enlargement and plant growth during severe drought stress, and allows stomata to remain partially open and CO_2 assimilation to continue during drought stress (Hare et al. 1998). Apart from playing a primary role of turgor maintenance, these compatible solutes may also be involved in stabilizing proteins and cell structures, as well as in scavenging reactive oxygen species (Bartels and Sunkar 2005). An increase in the proline levels in response to water deficit has been well-documented, and a number of studies have shown a positive correlation between proline accumulation and enhanced tolerance to abiotic stress (Bartels and Sunkar 2005, Lei et al. 2006, Ren et al. 2007). Increase in soluble sugar contents through inversion of some carbohydrates is another adjustment mechanism that improves tolerance toward osmotic stress as already reported (Bohnert and Jensen 1996). Therefore, the soluble sugar and proline accumulation seem to be a useful index of drought tolerance in many higher plants (Ain-Lhout et al. 2001, Ramanjulu and Bartels 2002). In addition, osmoadjustment mechanisms vary greatly among species and genotypes and may not be functional until severe dehydration occurs (Morgan 1984, Chaves et al. 2003).

Drought stress often leads to the accumulation of reactive oxygen species (ROS). ROS can act as second messengers involved in the stress signal transduction pathway, but excessive ROS production can cause oxidative stress to the photosynthetic apparatus and seriously impair the normal function of cells (Foyer et al. 1994, Smirnoff 1998, Niyogi 1999). In addition to proteolysis, ROS can damage lipids, terpenoids, carbohydrates and nucleic acids (Foyer and Noctor 2005, Moller et al. 2007). To keep the levels of active oxygen species under control, plants have evolved a series of antioxidative systems which are composed of metabolites such as ascorbate, glutathione, tocopherol, and enzymatic scavengers such as superoxide dismutase (SOD), peroxidase and catalase (Asada 1999). There are many cases that plants growing in hostile environments exhibit increased antioxidant enzyme activities to combat the deleterious effect of ROS (Duan et al. 2005, Jebara et al. 2005, Yin et al. 2005). The capability of scavenging ROS and reducing their damaging effects may correlate with the drought tolerance of plants (Tsugane et al. 1999).

In addition, the responses to drought depend on the species and genotype, the length and severity of water deficit and the age and stage of development (Bray 1997). Therefore, for a long time there has been a considerable effort to elucidate the degree and nature of the genetic control over drought resistance or tolerance. However, most of these studies have focused on short-time responses to sharp water deficit rather than on long-term acclimation processes to moderate and gradually increasing water deficits. As far as drought stress is concerned, gradual soil water depletion is the most frequent situation in the field and in natural ecosystem. Furthermore, slowly increased water deficit usually has differ-

Table 1. The ecological and geographical parameters of the two *Populus cathayana* populations.

Population	Origin	Latitude (°N)	Longitude (°E)	Altitude (m)	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'	1500	750	800	17.7	33	–5	3600
LD	Ledu	36°31'	102°28'	3160	335	1500	6.9	38	–20	4500

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

ent physiological consequences compared with rapid tissue dehydration and due to this they may apply different gene network regulation mechanism (Chaves et al. 2003).

Poplar (*Populus* spp.) is fast-growing forest tree species, which has been widely used for timber, pulp and has potential as a source of biomass energy (Perry et al. 2001). It is a diverse and widely distributed genus and many species grow in arid and semiarid areas where they often subject to long periods of water deficits. Poplars are usually known as one of the most sensitive woody plants to drought (Tschaplinski et al. 1994), but their drought tolerance varies greatly among species, populations and clones (Tschaplinski et al. 1998, Zhang et al. 2004, Monclus et al. 2006). We hypothesized that there are a large set of parallel changes in the morphological, physiological, and biochemical responses when poplars are exposed to a gradually increasing drought stress; these responses could be dependant on time-course and intensity of drought. Therefore, in this study, we employed two contrasting populations of *Populus cathayana* Rehder, which were from the wet and dry climate regions in western China, respectively, as plant materials to investigate the morphological, physiological and biochemical responses to a progressive drought stress in woody plants. Our aim is not only to better understand the adaptive mechanisms that enable differently originated poplar population (different genotypes) to survive under prolonged drought stress, but also to provide some useful clues for forest tree breeding toward improved drought tolerance by selection and utilization of existing forest genetic resources.

2 Materials and Methods

2.1 Plant Materials and Experimental Design

Samples of two contrasting populations of *P. cathayana* were collected from their natural habitats in Hanyuan and Ledu, Southwest China for the study (Table 1). The mean annual rainfall in Hanyuan and Ledu are 750 and 335 mm, respectively. The transpiration rates of the plants in the two populations are similar. Furthermore, in both Hanyuan and Ledu the rainfall concentrates in the season from June to September, which contributes 70.1 % and 84.8 % of the yearly rainfall, respectively. Therefore, the populations from Hanyuan and Ledu represent the wet and dry climate populations, respectively. The experiment was carried out at Chengdu Institute of Biology, China. Cuttings from 20 trees from each population were collected. After sprouting and growing for about one month, 240 healthy cuttings of approximately equal height for each population were selected and replanted into 5-l plastic pots filled with homogenized soil. The selected properties of the soil used in this study were 88.2 % sand, 9.1 % silt and 2.7 % clay. During the experiment, a total of 12 g slow release fertilizer (13%N, 10%P, 14%K, Peters professional, the Scotts Company, USA) was added to each pot. The cuttings were grown in a naturally lit greenhouse under the semi-controlled environment with a temperature range of 18.0–33.0°C and relative humidity range of 50–80% during May to August 2006.

A completely randomized design with two factors (two populations and two watering regimes) was applied. The cuttings of each population were allocated randomly to two different watering regimes. In well-watered treatment (WW, as control), 120 pots of each population were always maintained at 100% of field capacity by supplying an amount of water equal to transpiration losses

every day. In progressive drought stress treatment (WS), six different watering stages which were kept at 100%, 85%, 70%, 55%, 40% and 25% of field capacity by watering every day were orderly used for other 120 pots of each population. The whole experiment lasted for 12 weeks, and each watering stage was maintained for 2 weeks. During the experiment, the pots were re-watered to designated soil water content by replacing the amount of water transpired every day. Evaporation from the soil surface was prevented by enclosing the pots in plastic bags that were tied to the stems of the plants. Transpiration water loss was measured gravimetrically by weighing all pots every day. Following periods of rapid growth, an empirical relationship between the plant fresh weight (Y, g) and height (X, cm), $Y = 0.975 + 0.112X$ ($R^2 = 0.968$, $P < 0.001$) (Li et al. 2004), was used to correct the amount of pot water for changes in plant biomass.

At the end of each stage, twenty cuttings including five replicates for each population and each treatment were harvested and every third to fifth fully expanded leaves from the top were used to determine various physiological and biochemical traits.

2.2 Measurement of Shoot Growth

Shoot height increments of all cuttings were recorded at the end of each watering stage, and shoot growth rate was then calculated as the shoot length increment divided by the days between two successive watering stages.

2.3 Extraction of Chlorophyll Pigments

About 0.1 g of leaves was pulverized in liquid nitrogen and added to 10 ml of 80% acetone solution. After 24 h incubation at 4°C in the dark, debris were separated by centrifugation at 10 000 × g for 10 min. The supernatants were collected, and absorption spectra at 663.8, 646.8 nm and 470 nm were recorded. The amounts of chlorophyll *a*, chlorophyll *b*, total chlorophyll (Chl) and the total carotenoids (Car) were calculated according to the experimental equations described by Lichtenthaler et al. (1987).

2.4 Estimation of Malondialdehyde Content

Oxidative damage to lipids was estimated by measuring the content of malondialdehyde (MDA) in leaf. Leaf segments (0.3 g) were homogenized in 10 ml of 10% trichloroacetic acid (TCA), and centrifuged at 12 000 × g for 10 min. Then 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 440, 532 and 600 nm was determined with a spectrometer (Unicam UV-330, Cambridge, UK). MDA content was calculated as described by Hodges et al. (1999).

2.5 Determination of Free Proline Content

Free proline content was measured according to the method of Bates et al. (1973). Briefly, 0.3 g leaves were homogenized in 8 ml 3% aqueous sulphosalicylic acid and then centrifuged at 8000 × g for 15 min. The supernatant was used for estimation of proline content. The reaction mixture consisted of 2 ml supernatant, 2 ml acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at 100°C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene, and the absorbance at 520 nm was determined. The free proline content was expressed as $\mu g\ g^{-1}$ FW.

2.6 Assessment of Total Soluble Sugar Content

Total soluble sugar was extracted and determined according to the method of Renaut et al. (2005). 0.3 g of frozen sample was ground in liquid nitrogen to a fine powder and total soluble sugars were extracted with 4 ml 80% ethanol. After centrifugation (10 000 × g, 10 min), the supernatant was collected and evaporated to dryness in vacuum. The dried residue was re-suspended in 5 ml of double-distilled water. Total soluble sugar content was estimated according to Yemm and Willis (1954).

2.7 Assays of Antioxidant Defense Systems

Frozen leaf segments (0.5 g) were homogenized into a fine powder with a mortar and pestle under liquid nitrogen. Soluble proteins were extracted by homogenizing the powder in 10 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12 000 × g for 20 min at 4°C and then the supernatant was used for the following enzyme assays. The amount of soluble proteins was quantified according to the method of Bradford (1976) with bovine serum albumin as standard.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured spectrophotometrically by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) (Beuchamp and Fridovich 1971), with the modification as follows: the reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 130 mM methionine, 750 μM NBT, 20 μM riboflavin and 0.1 ml enzyme extract. Riboflavin was added at last, and the reaction was initiated by placing the glass test tubes under fluorescent lamps. The reaction was terminated after 25 min by removal from the light source. An illuminated blank without protein gave the maximum reduction of NBT, thus, the maximum absorbance at 560 nm. In this assay, one unit of SOD was defined as the amount of enzyme inhibiting the photo-reduction of NBT by 50%. The specific activity of SOD was expressed as units g^{-1} FW.

Guaiacol peroxidase (POD, EC 1.11.1.7) activity was measured as described by Lin and Wang (2002). The reaction mixture contained 100 mM potassium phosphate buffer (pH 6.5), 16 mM guaiacol and 0.1 ml of 10% H_2O_2 in a 3 ml volume. The reaction was initiated by adding a 100 μl aliquot of the crude enzyme extract and was followed for 3 min. The activity of the mixture was determined spectrophotometrically at 470 nm.

2.8 Statistical Analysis

Statistical analyses were performed with the SPSS (Statistical Package for the Social Science, version 13.0) software (SPSS Inc., Chicago, IL, USA). Two-way analyses of variance (ANOVA) for the variables from the measurements were used to test the differences of drought treatment and populations at each watering stage, with watering regime and population as factors. Before ANOVA, data were checked for normality and the homogeneity of variances, and log-transformed to correct deviations from these assumptions when needed. The analyses were performed with the general linear ANOVA model (GLM) procedure of SPSS. Post-hoc comparisons were tested using the Tukey's test at a significance level of $P < 0.05$. Pearson's correlation coefficients were calculated to determine the relationships between variables for different treatments using individual plant data.

3 Results

3.1 Effects of Drought on Shoot Growth

Drought was a very important limiting factor at the initial phase of plant growth and establishment. The two populations had parallel changes in shoot height and growth rate with time course and progressively increasing water deficit (Fig. 1). Extended drought stress significantly slowed down shoot height growth in both populations. Although the wet climate population had greater height increment than the dry climate population, its growth was more sensitive to water deficit. Significant decline of shoot height increment of the wet climate population occurred at early drought stage (70% of FC), while for the dry climate population reduction of shoot growth was not significant until soil water content decreased to 40% of FC.

3.2 Effects of Drought on Chlorophyll Pigments

Under severe drought stress (25% of FC), total chlorophyll content in the cuttings belonging to the wet climate population decreased significantly, while there was no statistically significant differences between drought and well-watered treatment in the dry climate population during

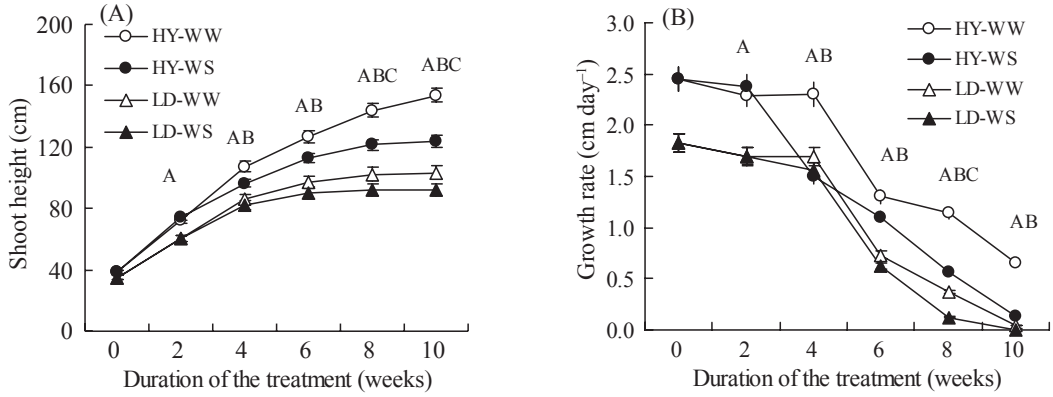


Fig. 1. The shoot height (A) and growth rate (B) in cuttings of the two contrasting poplar populations exposed to progressive drought stress. For progressive drought stress treatment (WS), six different watering stages which were kept at 100%, 85%, 70%, 55%, 40% and 25% of field capacity were orderly used, and each stage was maintained for 2 weeks. HY-WW, well-watered treatment of the wet climate population (HY); HY-WS, water-stressed treatment of the wet climate population (HY); LD-WW, well-watered treatment of the dry climate population (LD); LD-WS, water-stressed treatment of the dry climate population (LD). The values presented are means ± S.E. of five replicates. Letters A indicate significant difference between the populations tested at $P<0.05$; letters B indicate significant difference between the WW and WS treatment of HY population at $P<0.05$; letters C indicate significant difference between the WW and WS treatment of LD population at $P<0.05$.

the whole experiment (Fig. 2A). Under moderate drought (55% and 40% of FC), the carotenoid content increased significantly in the stressed leaves for both populations (Fig. 2B), but under severe drought (25% of FC), this value decreased in the wet climate population. The ratio of carotenoid/total chlorophyll (Car/Chl) in the dry climate population increased significantly under moderate and severe drought, while in the wet climate population Car/Chl kept relatively stable during the whole drought period (Fig. 2C). In addition, the wet climate population had higher contents of total chlorophyll than the dry climate population for the same treatment at each drought stage, but the dry population had higher carotenoid content from the third drought stage. During the whole experimental period, Car/Chl in the dry climate population was higher than that in the wet climate population.

3.3 Effects of Drought on Malondialdehyde Content

The effects of drought on the levels of lipid peroxides (i.e., MDA content) in leaves of poplar cuttings are shown in Fig. 3. In the wet climate population, drought resulted in significant MDA accumulation except at the last drought stage. As for the dry climate population, there was no statistically significant difference in MDA content between treatments during the whole experiment.

3.4 Effects of Drought on Free Proline and Total Soluble Sugar Accumulations

Mild drought did not lead to an increase in proline content. When soil water content decreased to 55% of FC, proline began to accumulate in droughted plants of the wet climate population, while for the dry population this occurred at 40% of FC and moreover, the accumulated proline content was much more than that in the wet cli-

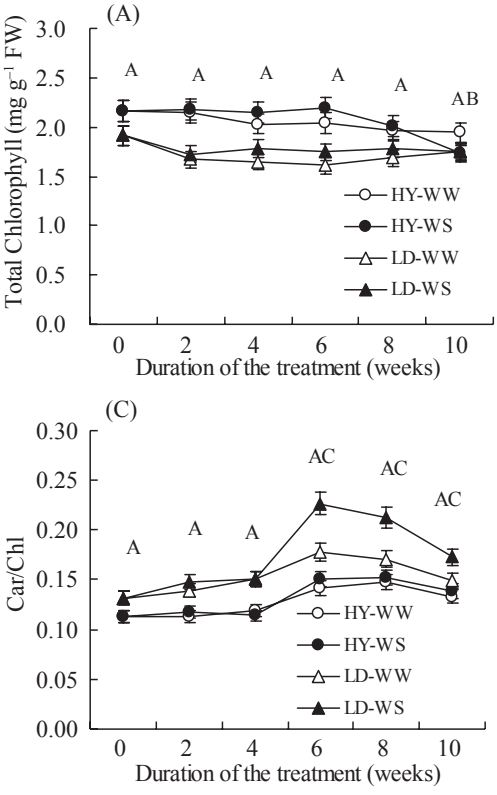


Fig. 3. The MDA content in cuttings of the two contrasting poplar populations exposed to progressive drought stress. For progressive drought stress treatment (WS), six different watering stages which were kept at 100%, 85%, 70%, 55%, 40% and 25% of field capacity were orderly used, and each stage was maintained for 2 weeks. HY-WW, well-watered treatment of the wet climate population (HY); HY-WS, water-stressed treatment of the wet climate population (HY); LD-WW, well-watered treatment of the dry climate population (LD); LD-WS, water-stressed treatment of the dry climate population (LD). The values presented are means ± S.E. of five replicates. Letters A indicate significant difference between the populations tested at $P < 0.05$; letters B indicate significant difference between the WW and WS treatment of HY population at $P < 0.05$; letters C indicate significant difference between the WW and WS treatment of LD population at $P < 0.05$.

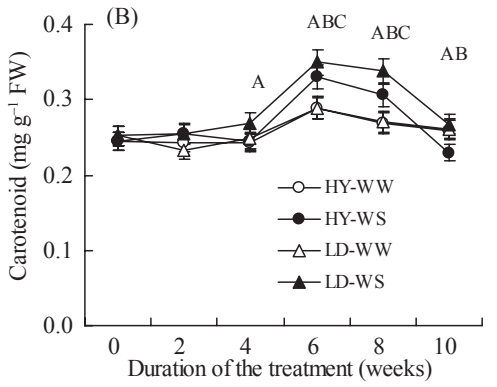
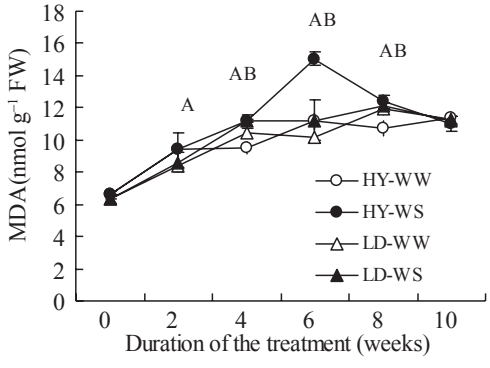


Fig. 2. The total chlorophyll content (A), carotenoid content (B) and Car/Chl (C) in cuttings of the two contrasting poplar populations exposed to progressive drought stress. For progressive drought stress treatment (WS), six different watering stages which were kept at 100%, 85%, 70%, 55%, 40% and 25% of field capacity were orderly used, and each stage was maintained for 2 weeks. HY-WW, well-watered treatment of the wet climate population (HY); HY-WS, water-stressed treatment of the wet climate population (HY); LD-WW, well-watered treatment of the dry climate population (LD); LD-WS, water-stressed treatment of the dry climate population (LD). The values presented are means ± S.E. of five replicates. Letters A indicate significant difference between the populations tested at $P < 0.05$; letters B indicate significant difference between the WW and WS treatment of HY population at $P < 0.05$; letters C indicate significant difference between the WW and WS treatment of LD population at $P < 0.05$.



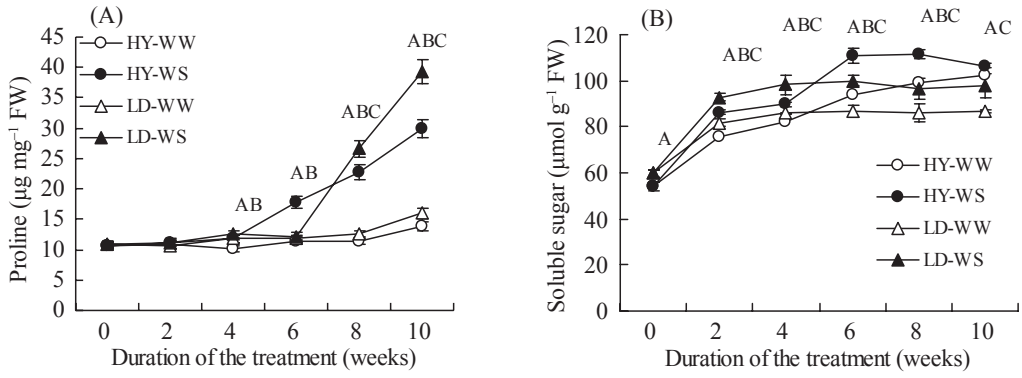


Fig. 4. The proline content (A) and soluble sugar content (B) in cuttings of the two contrasting poplar populations exposed to progressive drought stress. For progressive drought stress treatment (WS), six different watering stages which were kept at 100%, 85%, 70%, 55%, 40% and 25% of field capacity were orderly used, and each stage was maintained for 2 weeks. HY-WW, well-watered treatment of the wet climate population (HY); HY-WS, water-stressed treatment of the wet climate population (HY); LD-WW, well-watered treatment of the dry climate population (LD); LD-WS, water-stressed treatment of the dry climate population (LD). Letters A indicate significant difference between the populations tested at $P<0.05$; letters B indicate significant difference between the WW and WS treatment of HY population at $P<0.05$; letters C indicate significant difference between the WW and WS treatment of LD population at $P<0.05$.

mate population (Fig. 4A). Under well-watered conditions, the amounts of free proline in both populations remained stable during the whole experimental period and there was no difference between the two populations. As for soluble sugar content, the cuttings in both populations experienced parallel changes when the drought prolonged. Soluble sugar content in the drought stressed leaves significantly increased at the early drought stage and then remained at a relatively higher level then the control in both populations (Fig. 4B).

3.5 Effects of Drought on Antioxidant Enzyme Systems

Drought stress significantly activated antioxidant system of leaves in both poplar populations. When soil water content dropped to 55% of FC, the activity of SOD remarkably increased in both populations and no statistically significant difference was found between the two populations (Fig. 5A). Mild water deficit resulted in a remarkable increase of POD activity in the two populations tested (Fig. 5B). Furthermore, as water deficit was prolonged and intensified, the activities of POD in both populations increased continually. Compared with the wet population under the same treatment, the dry climate population had higher activity of POD under two watering regimes at each drought stage.

3.6 Correlation Coefficients among Some Physiological Properties

Plant growth rate showed a significant positive correlation with Chl content and a significant negative correlation with Car/Chl under two watering regimes (Table 2). In addition, under drought stress, Plant growth rate showed a significant positive correlation with contents of MDA, Proline, soluble sugars and activities of antioxidant enzyme systems.

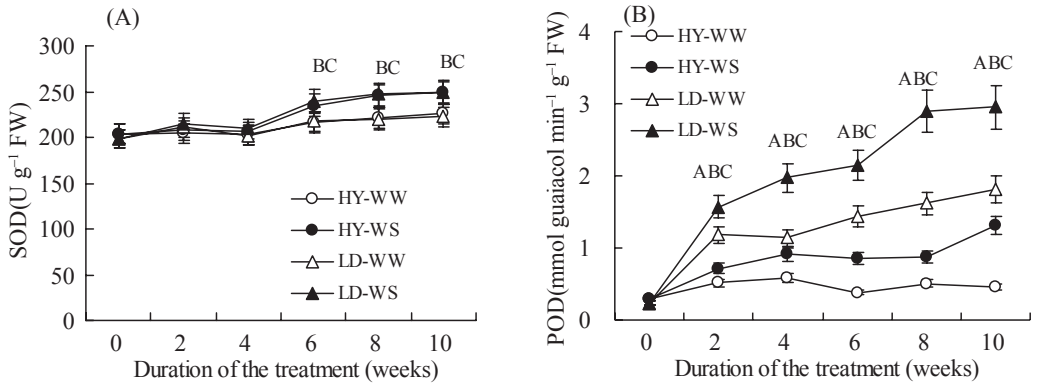


Fig. 5. The SOD activities (A) and POD activities (B) in cuttings of the two contrasting poplar populations exposed to progressive drought stress. For progressive drought stress treatment (WS), six different watering stages which were kept at 100%, 85%, 70%, 55%, 40% and 25% of field capacity were orderly used, and each stage was maintained for 2 weeks. HY-WW, well-watered treatment of the wet climate population (HY); HY-WS, water-stressed treatment of the wet climate population (HY); LD-WW, well-watered treatment of the dry climate population (LD); LD-WS, water-stressed treatment of the dry climate population (LD). The values presented are means ± S.E. of five replicates. Letters A indicate significant difference between the populations tested at $P<0.05$; letters B indicate significant difference between the WW and WS treatment of HY population at $P<0.05$; letters C indicate significant difference between the WW and WS treatment of LD population at $P<0.05$.

Table 2. Correlation coefficients among some growth and physiological properties in *P. cathayana* populations under well-watered treatment (upper triangle, bold numbers) and progressive drought stress treatment (lower triangle, numbers in italic).

	Ht	Gr	Chl	Car	Car/Chl	MDA	Proline	Sugars	SOD	POD
Ht	**1.000**	**0.744****	**0.662***	**–0.522**	**–0.702***	**–0.301**	**0.543**	**0.085**	**–0.063**	**0.185**
Gr	*–0.338***	**1.000**	**0.968****	**–0.466**	**–0.941****	**0.108**	**0.053**	**0.664***	**0.573**	**0.716****
Chl	*0.167*	*0.297**	**1.000**	**–0.451**	**–0.965****	**0.057**	**0.057**	**0.663***	**0.669***	**0.742****
Car	*0.236*	*–0.072*	*0.422***	**1.000**	**0.669***	**–0.394**	**–0.709****	**–0.392**	**0.065**	**–0.469**
Car/Chl	*0.031*	*–0.438***	*–0.663***	*–0.334***	**1.000**	**–0.172**	**–0.250**	**–0.676***	**–0.547**	**–0.745****
MDA	*0.462***	*–0.503***	*0.050*	*0.552***	*0.040*	**1.000**	**–0.263**	**0.752****	**0.337**	**0.488**
Proline	*0.154*	*–0.651***	*–0.136*	*0.014*	*0.152*	*0.363***	**1.000**	**–0.355**	**–0.605***	**–0.118**
Sugars	*0.391***	*–0.583***	*0.032*	*0.392***	*0.184*	*0.565***	*0.240*	**1.000**	**0.801****	**0.811****
SOD	*0.473***	*–0.730***	*0.151*	*0.246*	*0.102*	*0.429***	*0.439***	*0.623***	**1.000**	**0.664***
POD	*–0.390***	*–0.508***	*–0.516***	*–0.271**	*0.503***	*0.016*	*0.510***	*0.074*	*0.180*	**1.000**

Ht – shoot height; Gr – growth rate; Chl – total chlorophyll content; Car – carotenoid content; Car/Chl – the carotenoid content/Chlorophyll content ratio; MDA – malondialdehyde content
*** = P < 0.001; ** = P < 0.01; * = P < 0.05.

4 Discussion

Plant adaptations to drought stress are complex and affected by internal constitutive drought tolerance mechanisms and external environmental factors such as water availability, or their interaction. An early morphological response of plants to drought stress is the avoidance mechanism through adjustment of plant growth rate such as a reduction in shoot height. Moreover, plants can exploit the limiting water resource in a more efficient way by increasing the proportion of water-absorbing root biomass relatively to the water-loosing leaf biomass (Li 1999, Li et al. 2000, Zhang et al. 2004, Duan et al. 2005). In our experiment, plant growth was significantly inhibited in the two contrasting populations of *P. cathayana* when subjected to the progressive drought stress. Moreover, significant differences between the two populations were observed in shoot height and growth rate under two watering regimes. The dry climate population had smaller shoot height and relative growth rate under both two watering regimes, but its growth was less affected by drought stress. Our results are consistent with many previous studies (Arndt et al. 2001, Li and Wang 2003, Zhang et al. 2004, 2005). These differences may be explained by the different strategies that the two populations have evolved in adapting to their native habitats (Gibson 1995, Arndt et. al. 2001). In general, *P. cathayana* populations have evolved two contrasting water-use strategies for survival and growth under limited water availability. The dry climate population, which grows under a natural habitat of prolonged annual drought, has developed a conservative water use strategy and intrinsic character of slow growth rates during long-term adaptation to its habitat. The wet climate population, with a prodigal water-use strategy, is adapted to mild drought of short duration where plants consume available water rapidly until almost all water from the soil is exhausted. This strategy contributes to fast growth rates. This study also provided evidence that plant growth adjustments are important adaptative mechanisms of *P. cathayana* to water limitation.

The change in chlorophyll contents has been used to evaluate the influence of environmental stress on plant growth and yield, and earlier study proved that chlorophyll contents usually decreased under drought stress due to their slow synthesis or fast breakdown (Majumdar et al. 1991). In our study, mild and moderate drought stress resulted in small increase in total chlorophyll content per unit leaf fresh weight, which in a certain degree might happen due to the decline in relative water content (RWC) of leaves under drought. The significant decline of chlorophyll content in the wet climate population under severe drought stress suggested that chlorophyll might to a certain degree be subjected to breakdown. This also indicates that the wet climate population is more sensitive to water deficit than the dry climate population. On the other hand, the carotenoid contents significantly increased in both populations under moderate drought stress, but there was significant decrease of carotenoids in the wet climate population under severe drought. In addition, the ratio of Car/Chl significantly increased under drought in the dry climate population, while no significant change was observed in the wet climate population during the whole experiment. Carotenoid content and Car/Chl ratio are correlated with the capacity of light protecting mechanisms (Boardman 1977). Carotenoids have essential functions in photosynthesis and photoprotection. Besides their structural roles, they are well known for their antioxidant activity by quenching ^{3}Chl and $^1O_2$, inhibiting lipid peroxidation, and stabilizing membranes (Demmig-Adams and Adams 1992, Frank and Cogdell 1996, Niyogi 1999). They also play a critical role in the assembly of the light-harvesting complex and in the radiationless dissipation of excess energy (Streb et al. 1998, Munné-Bosch and Alegre 2000). In our study, the Car content and Car/Chl ratio in the wet climate population was obviously less than that in the dry climate population under two watering regimes at each drought stage. The significant decrease in content of carotenoids in the wet climate population under severe drought suggested that drought caused considerable oxidative stress by accumulation of ROS. The differences in chlorophyll and carotenoid contents between the two populations indicated that the dry climate population provided stronger photoprotective system against drought stress compared with the wet climate population.

In our study, when the intensity of drought stress increased to a certain degree (varying between two different populations), the levels of free proline significantly increased in both populations, which could be interpreted as a mechanism of osmotic adjustment to lower the osmotic potential and protect plants from damages of dehydration. Osmotic adjustment, utilizing synthesis and accumulation of some small compatible solutes known as osmoprotectants, has been considered as one of the crucial mechanisms that plants have evolved for adaptation to a series of various stresses, such as cold, drought, salt (Morgan 1984, Chaves et. al. 2003, Mittler 2006). Proline accumulation may serve as a means of osmotic adjustment and play a highly protective role in plants that are subjected to abiotic stresses. In addition to osmotic adjustment, its function is involved in prevention of protein denaturation, preservation of enzyme structure and activity (Rajendrakumar et al. 1994, Samul et al. 2000), and protection of membrane from damage of reactive oxygen species (ROS) produced under drought and other stressful conditions (Kishor et al. 1995, Ain-Lhout et al. 2001). These functions of proline may be even more important than its role in osmotic adjustment (Hare et al. 1998).

We also found that the cuttings of the dry climate population did not significantly accumulate proline under mild drought stress, but as the drought was prolonged and intensified, the amount of free proline in the dry climate population sharply increased to a level much higher than that in the wet climate population. This may be due to the difference in drought tolerance between two genotypes. Under mild drought, the dry population did not need to accumulate proline to tolerate such mild drought as the damage caused by stress was limited. Limited increase of MDA in the dry population also suggested that severe membrane injury did not occur under such conditions. This result indicated that the dry climate population is not sensitive to mild drought but tolerates drought stress better than the wet climate population.

From the changes of total soluble sugar content in poplar leaves, it was observed that soluble sugar content increased at the early drought stage and then remained relatively stable in drought stressed leaves in both populations. This was in agreement with the results observed in other studies (Bogeat-Triboulot et. al. 2007). A strong correlation between sugar accumulation and osmotic stress tolerance has been widely reported, including transgenic experiments (Chaves et al. 2003, Bartels and Sunkar 2005). Sugars have different functions in plants (from energy storage to signaling), and plants utilize several sugar-based strategies to adapt to environmental stresses (Anderson and Kohorn 2001, chaves et al. 2003). The current hypothesis is that sugars act as osmotica and/or protect specific macromolecules and contribute to the stabilization of membrane structures (Bartels and Sunkar 2005). In general, soluble sugar content tends to be maintained in the leaves of drought-stressed plants, although rates of carbon assimilation were partially reduced. The maintenance of soluble sugar content may be achieved at the expense of starch which drastically declines (Chaves 1991). Increase in soluble sugar contents through inversion of some carbonhydrates may contribute to enhanced desiccation tolerance and allows metabolic activity to be maintained.

ROS (Reactive Oxygen Species), including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH), which are inevitably generated during the course of normal cellular metabolism such as photosynthesis and photorespiration (Foyer 1994, Polle 1997), can cause oxidative stress to photosynthetic apparatus and seriously impair the normal function of plants by lipid peroxidation, protein degradation and DNA nicking if they can not be efficiently metabolized (Foyer 1994, Smirnoff 1998, Niyogi 1999, Foyer and Noctor 2005). ROS, which occur more under stress (Mittler 2002), can lead to the oxidation of unsaturated fatty acids in membranes yielding lipid hydroperoxides. Malonedialdehyde (MDA), as a breakdown product resulting from lipid peroxidation, has been used as an index for the occurrence of oxidative stress. In our study, drought resulted in significant MDA accumulation in the wet climate population except for the last drought stage, indicating that drought brought considerable damage on the cellular membranes. This result indicated that the wet climate population was more sensitive to drought stress and more prone to oxidative stress. On the other hand, no direct correlation was found between water deficit level and MDA content because MDA concentra-

tions did not increased continually as drought was prolonged and intensified, which is consistent with the results observed in some other studies (Bogeat-Triboulot et. al. 2007). Increases in cellular damage appeared to reflect impairments in the equilibrium between the ROS production and antioxidant defence systems, which also indicated that photoprotection and antioxidant substances were not sufficient enough to protect against cell membrane damage caused by ROS. In addition, there was no statistically significant difference in MDA content between treatments in the dry climate population during the whole drought period. These results showed that the dry climate population controlled the overproduction of ROS more efficiently than did the wet climate population.

It is known that the balance between ROS and antioxidant ability is important to plants and the ability of plants to overcome the effects of various stresses and to sustain their productivity may be related to the scavenging of ROS by antioxidant systems. Mechanisms of ROS detoxification exist in all plants and can be categorized as enzymatic (such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD)) and non-enzymatic (such as flavanones, anthocyanins, carotenoids and ascorbic acid) (Reddy et al. 2004). In our study, during the whole drought period, POD activities continually increased with the prolonged and intensified drought stress and SOD activities were also activated under moderate drought in both populations. There were no differences in the activities of SOD between the two populations, but the dry climate population showed much higher POD activity than the wet climate population, indicating that it may have stronger ability of scavenging ROS and avoiding damage of oxidative stress, and thus, stronger tolerance to drought stress.

Moreover, the observed correlations among different growth and physiological parameters showed that plant growth rate is closely correlated with Chl content and Car/Chl ratio. High growth rate is usually corresponding to high Chl content, while high Car/Chl ratio is associated with low growth rate and stronger drought tolerance. In general, high Car/Chl ratio implies that there is a greater investment in Car to provide photoprotection rather than in Chl to enhance C assimilation. In addition, under drought stress, the negative correlation between plant growth rate and other drought tolerance-related indices, such as contents of proline and sugars and activities of antioxidant enzymes, also showed that plants will invest more in stress protection mechanisms rather than in growth when subjected to drought stress.

In conclusion, the growth and the physiological properties of the two *P. cathayana* populations were affected by water availability and these adaptative responses to drought stress depended on the time course and intensity of water deficit; the progressive drought stress significantly inhibited plant growth, increased carotenoid contents and accumulated free proline and soluble sugar with the prolonged drought. In addition, antioxidant systems including SOD and POD were also activated by drought stress. On the other hand, there were different responses to progressive drought stress between the two contrasting populations of *P. cathayana*. Compared with the wet climate population, the dry climate population had lower shoot height and growth rate, higher free proline content, as well as more efficient photoprotective system (such as higher carotenoid content and Car/Chl) and antioxidant system (such as higher POD activity), as a result of drought stress. All these results indicate that the dry climate population possesses better drought tolerance than the wet climate population. These differences in drought responses also provide some useful clues for forest tree breeding toward improved drought tolerance by selection and utilization of existing forest genetic resources.

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