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Adaptive Responses to Progressive Drought Stress in Two Poplar Species Originating from Different Altitudes

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Cuttings of *Populus kangdingensis* C. Wang et Tung and *Populus cathayana* Rehder, originating from high and low altitudes in the eastern Himalaya, respectively, were examined during one growing season in a greenhouse to determine the effects of progressive drought stress. The results manifested that the adaptive responses to progressive drought stress were different in these two species from different altitudes. Significant changes in stem height, leaf development, relative water content (RWC), malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) appeared earlier in *P. cathayana* than in *P. kangdingensis*, whereas changes in soluble protein, soluble sugar, free proline and antioxidant enzymes appeared earlier in *P. kangdingensis*. In addition, changes in these parameters became more and more significant when the drought stress progressed, especially under severe drought stress in *P. cathayana*. Plant growth showed significant positive correlations with soluble proteins and sugars, free proline and antioxidants and a significant negative correlation with RWC under water stressed treatment in two poplar species. Compared with *P. cathayana*, *P. kangdingensis* was able to maintain a superior height growth and leaf development under drought stress. Also, *P. kangdingensis* possessed greater increments in soluble protein, soluble sugar, free proline and antioxidant enzymes, but lower increments in MDA and H_2O_2 than did *P. cathayana* when the cuttings were exposed to progressive drought stress. Our results suggest that *P. kangdingensis* originating from the high altitude has a better drought tolerance than does *P. cathayana* originating from the low altitude. Furthermore, this study manifested that acclimation to drought stress are related the rapidity, severity, duration of the drought event and the altitude of two poplar species.

Keywords antioxidant enzymes, drought stress, free proline, hydrogen peroxide, malondialdehyde, *Populus*

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1 Introduction

Drought strongly influences the natural distribution of woody plants in arid and semi-arid regions. Many woody plants have developed mechanisms to cope with an inadequate water supply (Li et al. 2000, Capell et al. 2004, Arend and Fromm 2007). The adaptive responses to water deficit include morphological, physiological and biochemical changes, such as changes in growth rate, stomatal conductance, tissue osmotic potential and antioxidant defenses (Kozlowski and Pallardy 2002, Yin et al. 2005, Zhang et al. 2005, Duan et al. 2007). A number of studies have shown that soluble sugar, proline accumulation and efficient antioxidative characteristics could enhance the tolerance to drought stress (Lei et al. 2006, Ren et al. 2007, Xiao et al. 2008, Xu et al. 2008). Although many studies demonstrated the physiological and biochemical responses to drought stress on plants, sudden or punctual responses to drought stress were rarely studied.

Populus kangdingensis C. Wang et Tung and *Populus cathayana* Rehder, which belong to Sect. *Tacamahaca* Spach, occupy different natural habitats in the eastern Himalaya. *P. kangdingensis* occurs at high-altitude regions while *P. cathayana* occupies low-altitude regions. Both species play very important roles in preventing soil erosion and soil water loss, and in regulating climate, as well as in retaining ecological stability in the southwestern China. In our study, we employed these two poplar species as plant materials to investigate the physiological and biochemical responses of woody plants to progressive drought stress. We hypothesized that there are a set of parallel changes in *P. kangdingensis* and *P. cathayana* in the morphological, physiological and biochemical responses when exposed to progressive drought stress, and that the responses could be different, since the species originate from different altitudes. Therefore, the objectives of the study were (i) to assess whether there are a set of parallel changes in the morphological, physiological and biochemical responses when these two poplars are exposed to progressive drought stress; (ii) to determine different adaptive responses to progressive drought stress in the two species.

2 Materials and Methods

2.1 Plant Material and Experimental Design

One-year-old male and female cuttings of two poplar species of *Sect. Tacamahaca* Spach, *P. kangdingensis* and *P. cathayana*, were collected in their natural habitats in the eastern Himalaya (Table 1). The cuttings were planted in March 2007. After sprouting and growing for about 2 months, 288 healthy cuttings with approximately the same crown size and equal height were chosen and replanted into 10 L plastic pots filled with 8 kg homogenized soil (1 cutting per pot), which was sieved surface sandy soil (0–30 cm) taken from the field on the experimental site. The plants were moved to a naturally lit greenhouse at the Chengdu Institute of Biology, the Chinese Academy of Sciences, with a semi-controlled environment with a day temperature range of 22–31 °C and a night temperature range of 15–23 °C, and the relative humidity range of 55–85% between 16 May and 22 July 2007.

A completely randomized design with two factors (two species and two watering regimes) was employed. In the well-watered treatment (used as a control), the pots were weighed every day and re-watered to 100% of field capacity by replacing the amount of water transpired. In this case, the soil water content was always kept at 42.2% by daily watering. In the progressive drought stress treatment, six different watering levels, 100, 85, 70, 55, 40 and 25% of field capacity, were used. The set watering level was maintained by daily watering. The experiment lasted for two weeks for each watering stage, the whole experiment totaling 12 weeks. Six batches, six replications per each batch, four cuttings per replication within each species were used for six watering stages, respectively. Three replications of each batch were used for progressive drought stress treatment in each watering stage, and other three replications were used as control in each watering stage. At the end of each stage, twelve cuttings including three replicates for each species 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.

Table 1. The origins of the two poplar 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 days	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

2.2 Measurements of Growth and Relative Water Content

Stem height, leaf number and leaf area were recorded at the end of each watering stage, and their increments were calculated. Leaf area (LA) was determined by the Portable Laser Area Meter (CI-203, CIDInc., USA). The relative water content (RWC) of leaves was measured as described by Duan et al. (2005) and calculated as: RWC (%) = (FW–DW)/(TW–DW) × 100, where FW is the fresh weight, TW is the turgid weight after rehydrating samples for 24 h, and DW is the dry weight after oven-drying samples at 85 °C for 24 h.

2.3 Determinations of Free Proline, Soluble Sugar and Soluble Protein Concentrations

Free proline was measured as described by Bates et al. (1973) and Ren et al. (2007). The absorbance of the free proline was measured at 515 nm, and the free proline concentration was expressed as $\mu g\ g^{-1}$ DW. Soluble sugar was measured as described by Mohsenzadeh et al. (2006), and the concentration was expressed as $mg\ g^{-1}$ DW. Soluble protein contents were determined as described by Bradford (1976), using bovine serum albumin as a standard. Soluble protein concentration was expressed as $mg\ g^{-1}$ DW.

2.4 Assessments of Malondialdehyde and Hydrogen Peroxide

The malondialdehyde (MDA) concentration was measured according to the method of Heath and Packer (1968) and modified as follows: fresh leaves (0.3 g) were homogenized in 5 ml 5% trichloroacetic acid (TCA) solution. The absorbance of MDA was measured at 532, 600 and 450 nm. The MDA concentration can be obtained through the following formula: $C\ (\mu mol\ l^{-1}) = 6.45(A_{532} - A_{600}) - 0.56A_{450}$. The MDA concentration was expressed as $\mu mol\ g^{-1}$ DW.

The concentration of hydrogen peroxide (H_2O_2) was determined as described by Mukherjee and Choudhuri (1983). First, fresh leaves (0.3 g) were homogenized in 3 ml of refrigerated 10% acetone, the mixture was centrifuged at 10 000 × g for 10 min, and 1 ml supernatant was mixed with 0.1 ml 5% $Ti(SO4)_2$ and 0.2 ml 35% thick ammonia. After the precipitate was formed, the reaction mixture was centrifuged at 10 000 × g for 10 min. The resulting pellet was washed with acetone 3 times and then dissolved in 2 M H_2SO_4, and the absorbance was recorded at 415 nm. The H_2O_2 level was calculated according to a H_2O_2 standard curve. The concentration of H_2O_2 was expressed as $nmol\ g^{-1}$ DW.

2.5 Assays of Antioxidant Defense Systems

Fresh leaves (0.4 g) were ground in liquid nitrogen using a mortar and pestle, and the ground samples were homogenized on an ice bath, one by one, in 4 ml of extraction solution containing 50 mM Tris-HCl (pH 7.0), 1 mM EDTA, 20% glycerin, 1 mM ascorbic acid (AsA), 1 mM dithiothreitol (DTT), 1 mM glutathione (GSH) and 5 mM $MgCl_2$. The homogenate was centrifuged at 8000 × g at 4 °C for 15 min. This method was modified from the method described by Knorzer et al. (1996). The supernatant was stored in volumes of 0.4 ml at –70 °C until the following determination of antioxidant enzymes. All experiments were performed at 25 °C and, each time, completed within 2 days (a total of six times).

Superoxide dismutase (EC 1.15.1.1, SOD): The SOD activity was measured spectrophotometrically based on inhibition in the photochemical reduction of nitroblue tetrazolium (NBT) (Giannopolitis and Ries 1977), modified as follows: The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.8), 0.1 mM EDTA and 13.37 mM methionine. The 5.7 ml reaction mixture was mixed with 200 μl 0.1 mM riboflavin (containing 50 mM Tris-HCl, 0.1 mM EDTA, pH 7.8) and 0.1 ml of the enzyme source. Riboflavin was added last, and the reaction was initiated by placing the glass test tubes under fluorescent lamps. The reaction was terminated after 30 min by removal from the light source. Non-illuminated identical tubes served as blanks. An illuminated blank without protein gave the maximum reduction of NBT, thus, the maximum absorbance at 560 nm. In this assay, 1 unit of SOD was defined as the amount of enzyme inhibiting the photo-reduction of NBT by 50%. The total activity of SOD was expressed as unit g^{-1} protein.

Catalase (EC 1.11.1.6, CAT): A modification of the procedure of Aebi (1984) was used to analyse the CAT activity. The CAT activity was determined by directly measuring the decomposition of H_2O_2 at 240 nm. The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.0) and 0.1 mM EDTA. The reaction mixture and 750 mM H_2O_2 were warmed up at 25 °C. Then, 50 μl enzyme solution was mixed with 2.9 ml of the reaction mixture, and 50 μl 750 mM H_2O_2 was added to start the reaction. The absorbance at 240 nm was read every 30 seconds. The CAT activity was followed by a decrease of absorbance between 0.5 and 3 min. The CAT activity was calculated and expressed as nmol H_2O_2 mg^{-1} protein min^{-1}.

Peroxidase (EC 1.11.1.7, POD): The guaiacol peroxidase (G-POD) activity was measured using a modification of the method of Chance and Maehly (1995). The assay mixture contained 50 mM Tris-HCl (pH 7.0), 0.1 mM EDTA, 10 mM guaiacol and 5 mM H_2O_2. First, 50 μl enzyme solution was added to the reaction mixture with a total volume of 3.0 ml. Changes in the absorbance of the brown guaiacol at 470 nm between 0.5 and 3.5 min were recorded to calculate the POD activity, which was expressed as μmol guaiacol mg^{-1} protein min^{-1}.

Ascorbate peroxidase (EC 1.11.1.11, APX): The APX activity was measured using a modification of the procedure of Nakano and Asada (1981). The reaction mixture contained 50 mM Tris-HCl buffer (pH 7.0), 0.1 mM EDTA and 0.1 mM H_2O_2. First 2.93 ml of the reaction mixture was homogenized with 50 μl enzyme solution, and the reaction was stimulated by 20 μl 30 mM ASA (a total volume of 3 ml). The H_2O_2-dependent oxidation of ascorbate peroxidase was followed as a decrease in the absorbance at 290 nm within 1 min. The enzyme activity was expressed as μmol AsA mg^{-1} protein min^{-1}.

Glutathione reductase (EC 1.6.4.2, GR): A modified method of Halliwell and Foyer (1978) was employed for the assay of GR activity. The reaction mixture contained 50 mM Tris-HCl (pH 7.5), 0.1 mM EDTA and 5 mM $MgCl_2$. The reaction mixture, 10 mM NADPH and 10 mM glutathione disulphide (GSSG) were warmed up at 25 °C. Then, 50 μl enzyme solution and 20 μl 10 mM NADPH were added to the 2.88 ml reaction mixture, and, finally, 50 μl 10 mM GSSG was added. The absorbance at 340 nm was recorded every 30 seconds. The GR activity was calculated and expressed as nmol NADH mg^{-1} protein min^{-1}.

2.6 Statistical Analyses

For all variables recorded in the measurements, analyses of variance (ANOVA) were conducted to test the differences. Statistical analyses using the Duncan's method were performed with the SPSS12.0 for Windows statistical software package.

3 Results

3.1 Effects of Progressive Drought Stress on Plant Size Traits

In the well watered treatment, the better plant growth was found in *P. cathayana* than in *P. kangdingensis*. When the cuttings were exposed to progressive drought stress, the two species had parallel changes in the stem height, leaf number increment and leaf area increment. However, the two species significantly differed in their response to progressive drought stress, especially under severe drought stress. In *P. cathayana,* these parameters were significantly inhibited when the soil water content was depleted to 55% field capacity, whereas in *P.*

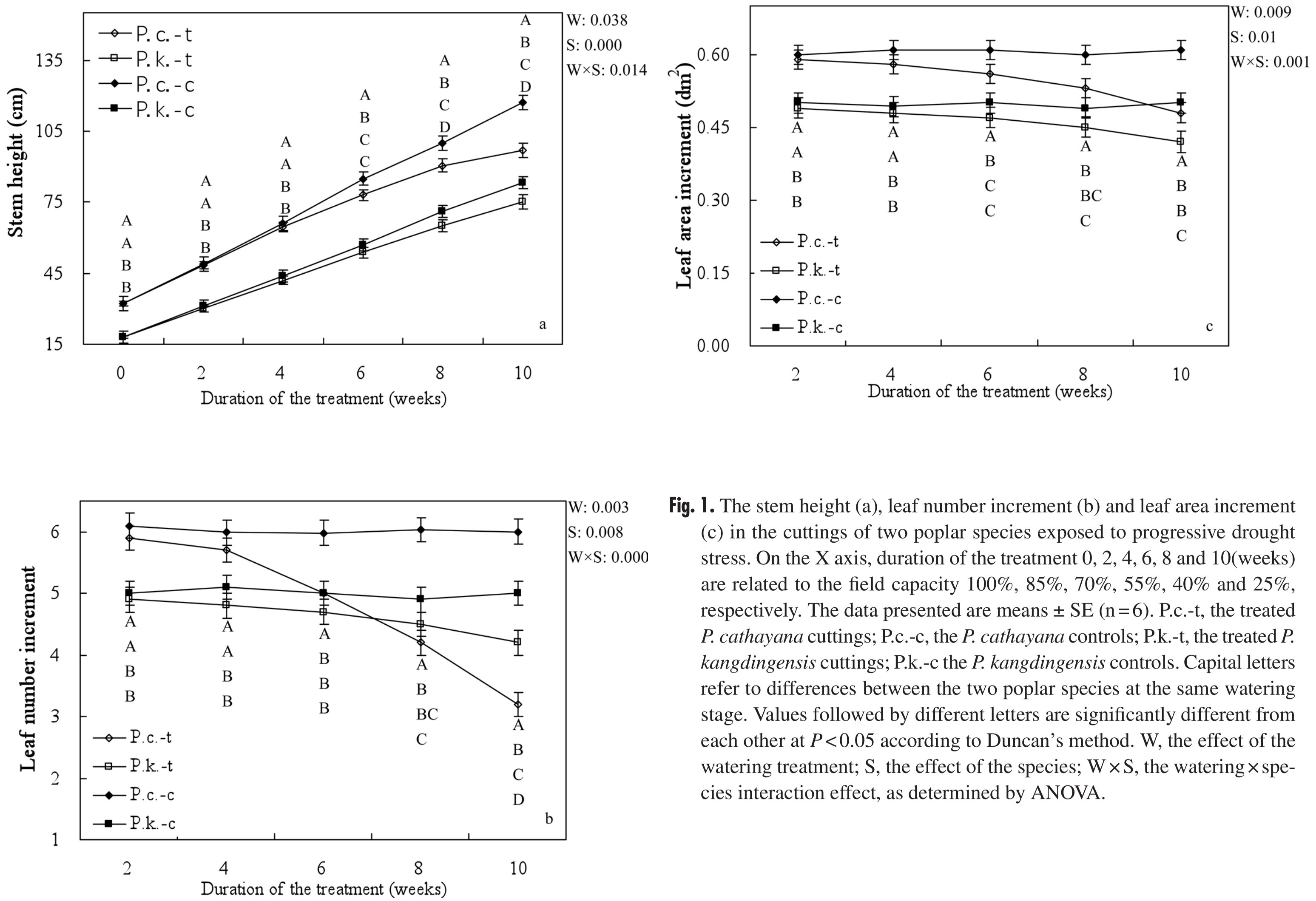


Fig. 1. The stem height (a), leaf number increment (b) and leaf area increment (c) in the cuttings of two poplar species exposed to progressive drought stress. On the X axis, duration of the treatment 0, 2, 4, 6, 8 and 10(weeks) are related to the field capacity 100%, 85%, 70%, 55%, 40% and 25%, respectively. The data presented are means ± SE (n = 6). P.c.-t, the treated *P. cathayana* cuttings; P.c.-c, the *P. cathayana* controls; P.k.-t, the treated *P. kangdingensis* cuttings; P.k.-c the *P. kangdingensis* controls. Capital letters refer to differences between the two poplar species at the same watering stage. Values followed by different letters are significantly different from each other at $P < 0.05$ according to Duncan's method. W, the effect of the watering treatment; S, the effect of the species; W × S, the watering × species interaction effect, as determined by ANOVA.

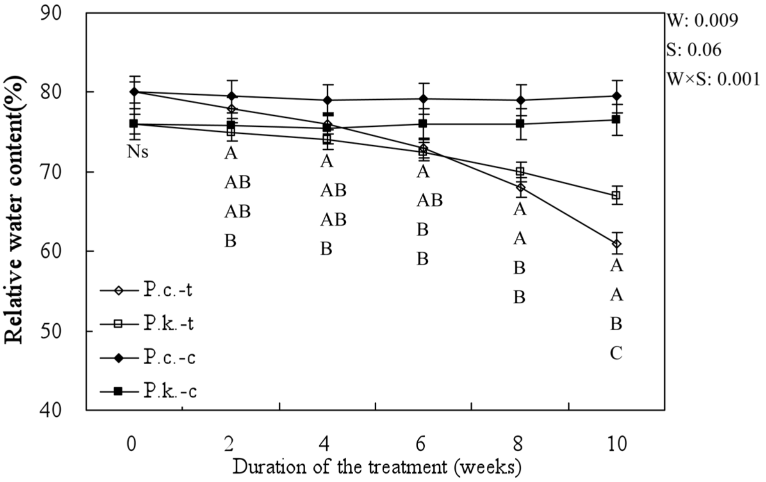


Fig. 2. Relative water content in the cuttings of two poplar species exposed to progressive drought stress. On the X axis, duration of the treatment 0, 2, 4, 6, 8 and 10(weeks) are related to the field capacity 100%, 85%, 70%, 55%, 40% and 25%, respectively. The data presented are means ± SE (n=6). P.c.-t, the treated *P. cathayana* cuttings; P.c.-c, the *P. cathayana* controls; P.k.-t, the treated *P. kangdingensis* cuttings; P.k.-c the *P. kangdingensis* controls. Capital letters refer to differences between the two poplar species at the same watering stage. Values followed by different letters are significantly different from each other at $P<0.05$ according to Duncan's method. W, the effect of the watering treatment; S, the effect of the species; W × S, the watering × species interaction effect, as determined by ANOVA.

kangdingensis, the significant inhibition happened until the soil water content was reduced to 40% field capacity (Fig. 1). At the end of the experiment, compared with the well-watered cuttings, the rate of the inhibition in the height increment, leaf number increment and leaf area increment of the water-stressed cuttings were 58.8%, 46.7% and 21.3% in *P. cathayana*, respectively, whereas they were only 24.2%, 36.1% and 16.2% in *P. kangdingensis.* In addition, size traits were significantly affected by the interaction of severity, duration of the drought event and species.

3.2 Effects of Progressive Drought Stress on RWC

During the whole experiment, there were no significant differences between the species in RWC of the well-watered cuttings (Fig. 2). However, when the cuttings were exposed to progressive drought stress, RWC decreased gradually. The significant decrease occurred at earlier drought stage in *P. cathayana* (55% field capacity) than in *P. kangdingensis* (40% field capacity). At the end of the experiment, compared with the well-watered cuttings, the decrease of RWC in the water-stressed cuttings was 23.3% in *P. cathayana*, respectively, whereas it was 16% in *P. kangdingensis.* RWC was affected by the interaction of severity, duration of the drought event and species.

3.3 Effects of Progressive Drought Stress on Free Proline, Soluble Sugar and Soluble Protein Concentrations

The concentrations of free proline, soluble sugar and soluble protein in the well-watered cuttings did not significantly differ between the two species during the whole experiment. However, the concentration gradually increased when the cuttings were exposed to progressive drought stress (Fig. 3), and then the significant differ-

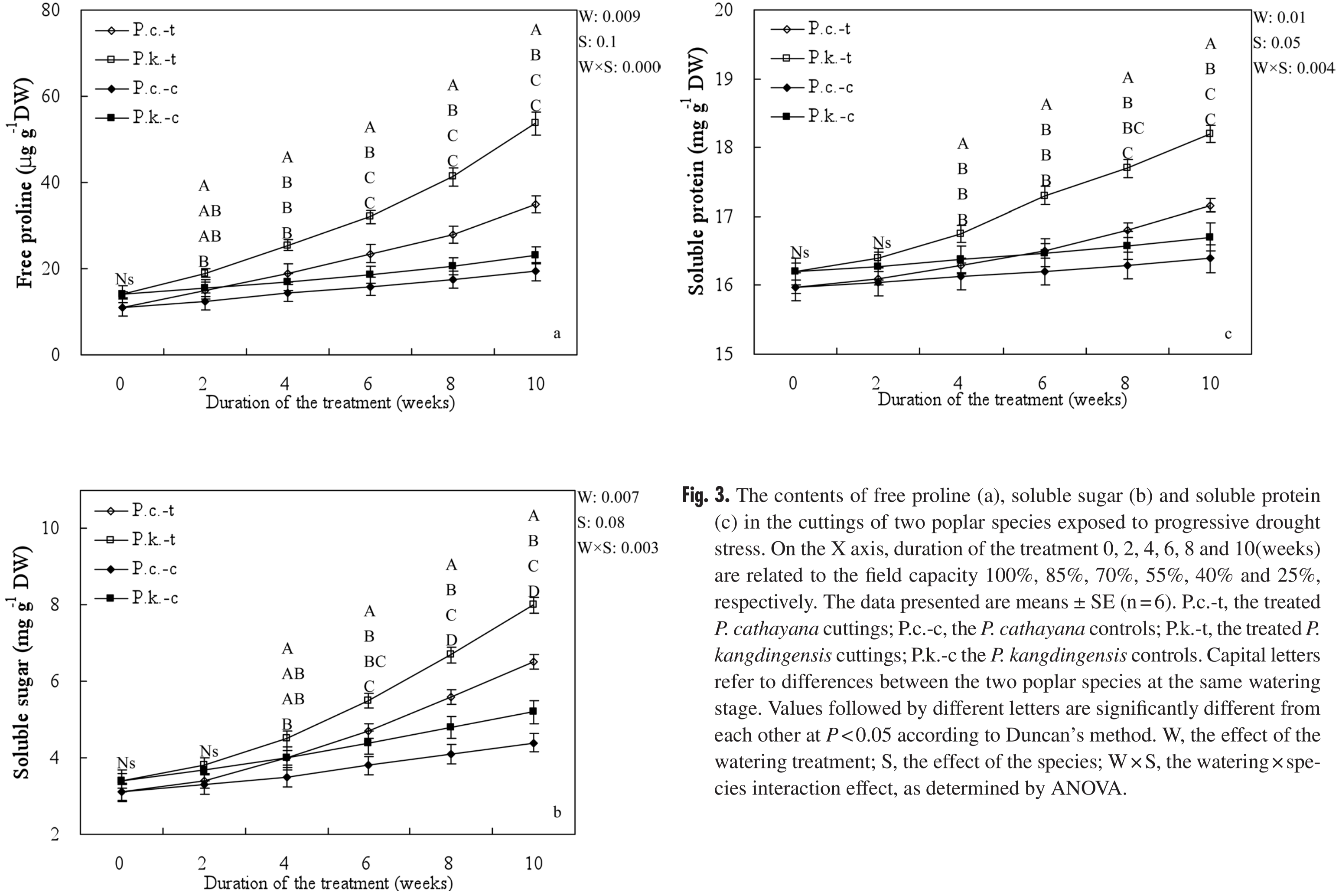


Fig. 3. The contents of free proline (a), soluble sugar (b) and soluble protein (c) in the cuttings of two poplar species exposed to progressive drought stress. On the X axis, duration of the treatment 0, 2, 4, 6, 8 and 10(weeks) are related to the field capacity 100%, 85%, 70%, 55%, 40% and 25%, respectively. The data presented are means ± SE (n = 6). P.c.-t, the treated *P. cathayana* cuttings; P.c.-c, the *P. cathayana* controls; P.k.-t, the treated *P. kangdingensis* cuttings; P.k.-c the *P. kangdingensis* controls. Capital letters refer to differences between the two poplar species at the same watering stage. Values followed by different letters are significantly different from each other at $P < 0.05$ according to Duncan's method. W, the effect of the watering treatment; S, the effect of the species; W × S, the watering × species interaction effect, as determined by ANOVA.

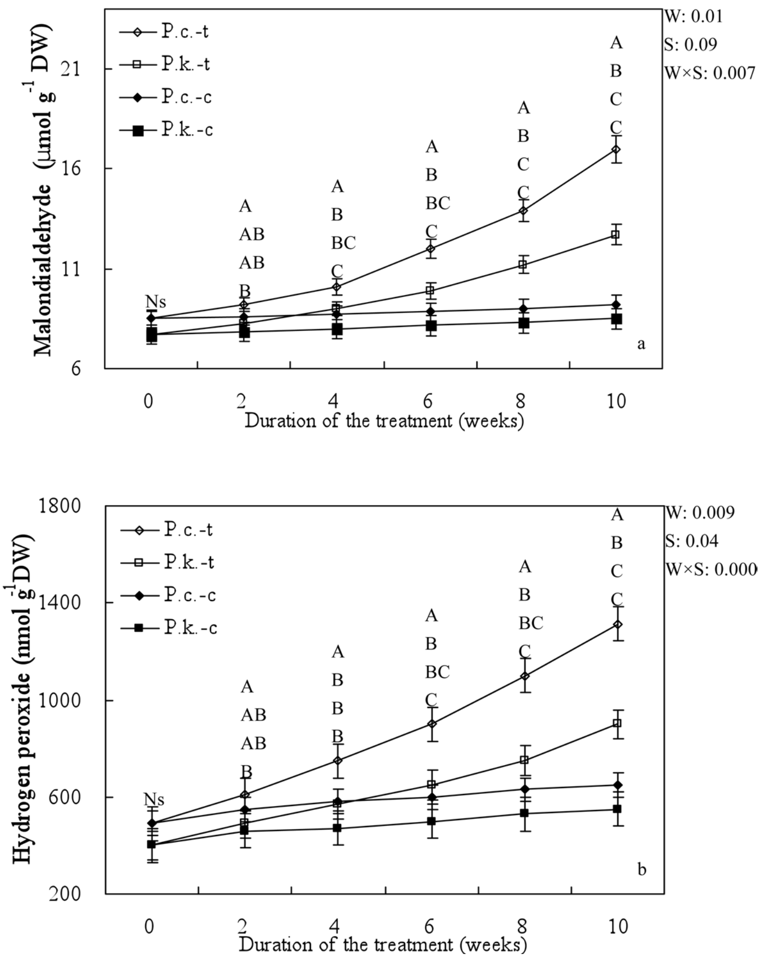


Fig. 4. The contents of malondialdehyde (a) and hydrogen peroxide (b) in the cuttings of two poplar species exposed to progressive drought stress. On the X axis, duration of the treatment 0, 2, 4, 6, 8 and 10(weeks) are related to the field capacity 100%, 85%, 70%, 55%, 40% and 25%, respectively. The data presented are means ± SE (n=6). P.c.-t, the treated *P. cathayana* cuttings; P.c.-c, the *P. cathayana* controls; P.k.-t, the treated *P. kangdingensis* cuttings; P.k.-c the *P. kangdingensis* controls. Capital letters refer to differences between the two poplar species at the same watering stage. Values followed by different letters are significantly different from each other at $P<0.05$ according to Duncan's method. W, the effect of the watering treatment; S, the effect of the species; W×S, the watering×species interaction effect, as determined by ANOVA.

ences between the two species were detected. Compared with the well-watered cuttings, significant increments in the concentrations of free proline, soluble sugar and soluble protein occurred at 75%, 55%, 75% field capacity in *P. kangdingensis*, respectively, whereas at 55%, 55%, 40% field capacity in *P. cathayana.* In the other words, significant changes in free proline and soluble protein appeared at earlier drought stage in *P. kangdingensis* than in *P. cathayana*.

P. kangdingensis exhibited higher increases of free proline, soluble sugar and soluble protein than did *P. cathayana* under the same drought stages. At the end of the experiment, compared with the well-watered cuttings, the increments of free proline, soluble sugar and soluble protein in the water-stressed cuttings were 81.3%, 50.4% and 4.7% in *P. cathayana,* respectively, whereas they were 133.6%, 53.8% and 8.9% in *P. kangdingensis.* In all, the free proline, soluble sugar and soluble protein were significantly affected by the interaction of severity, duration of the drought event and species.

3.4 Effects of Progressive Drought Stress on MDA and H_2O_2

Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels had parallel changes (gradually increments) in the two poplar species when the cuttings were exposed to progressive drought stress (Fig. 4). Compared with the well-watered cuttings, significant increments in H_2O_2 levels occurred at 75% field capacity in *P. cathayana*, whereas at 55% field capacity in *P. kangdingensis.* Although there were no significant differences between the species in MDA and H_2O_2 in the well-watered cuttings during the whole experiment, MDA and H_2O_2 accumulations were more significant in *P. cathayana* than in *P. kangdingensis* when the cuttings were exposed to progressive drought stress. *P. kangdingensis* showed a slower increase in the concentrations of MDA and H_2O_2 than did *P. cathayana* during the whole water stress treatment. At the end of the experiment, compared with the well-watered cuttings, the increments of the MDA and H_2O_2 concentrations in the water-stressed cuttings were 88.9% and 99.7% in *P. cathayana*, respectively, whereas they were only 44% and 63.6% in *P. kangdingensis*. The contents of MDA and H_2O_2 were significantly affected by the interaction of severity, duration of the drought event and species.

3.5 Effects of Progressive Drought Stress on Antioxidant Defense Systems

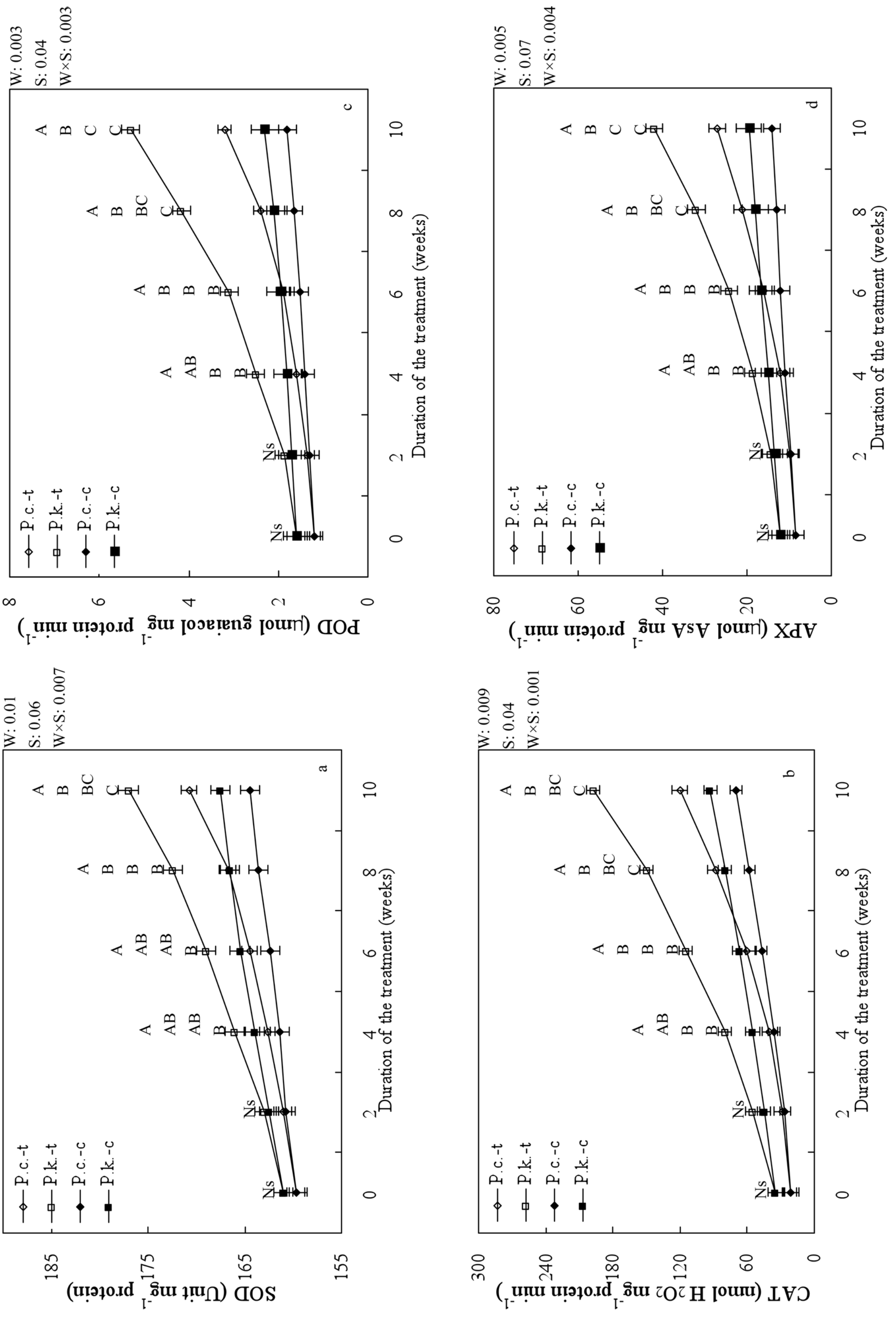
When the cuttings were exposed to progressive drought stress, the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) gradually increased in the two species (Fig. 5). Compared with the well-watered cuttings, significant increments appeared much earlier in *P. kangdingensis* than in *P. cathayana.* Significant changes in SOD, CAT, POD, APX and GR occurred at 40%, 75%, 75%, 75% and 55% field capacity in *P. kangdingensis*, respectively, whereas at 25%, 40%, 40%, 40% and 25% field capacity in *P. cathayana.* Although there were no significant differences between the species in the activities of these antioxidant enzymes in the well-watered cuttings during the whole experiment, *P. kangdingensis* always possessed significantly higher increments in the activities of antioxidant enzymes than did *P. cathayana* during the whole water stress treatment, especially under severe drought stress. At the end of the experiment, compared with the well-watered cuttings, the increments in the activities of SOD, CAT, POD, APX and GR of the water-stressed cuttings were 5.6%, 112.3%, 130.4%, 115.4% and 55% in *P. kangdingensis*, respectively, whereas they were only 3.7%, 71.4%, 77.8%, 92.8% and 29% in *P. cathayana*. Activities of these antioxidant enzymes were significantly affected by the interaction of severity, duration of the drought event and species.

3.6 Correlations for Stem Height with Selected Variables

Although significant correlations for stem height with RWC were not found under well-water treatment in two poplar species, significant negative correlations were found under water stressed treatment in two poplar species (Table 2). Plant growth showed significant positive correlations with MDA, H_2O_2, soluble proteins and sugars, free proline and antioxidants under two watering regimes in two poplar species.

4 Discussion

Investigations on progressive drought stress are a very useful way to gain insight into the sudden or


a
SOD (Unit mg^{-1} protein)
155
165
175
185
W: 0.01
S: 0.06
W×S: 0.007
b
CAT (nmol H$_2$O$_2$ mg^{-1} protein min^{-1})
0
60
120
180
240
300
W: 0.009
S: 0.04
W×S: 0.001
c
POD (μmol guaiacol mg^{-1} protein min^{-1})
0
2
4
6
8
W: 0.003
S: 0.04
W×S: 0.003
d
APX (μmol AsA mg^{-1} protein min^{-1})
0
20
40
60
80
W: 0.005
S: 0.07
W×S: 0.004
P.c.-t
P.k.-t
P.c.-c
P.k.-c
Ns
A
AB
B
BC
C
0
2
4
6
8
10
Duration of the treatment (weeks)

punctual responses to drought stress. In particular, the impact of progressive drought stress on plants should be assessed by examining drought effects during the time course using a wider range of water availability, since the physiological and biochemical processes of plants depend on the rapidity, severity and duration of the drought event (Kozlowski and Pallardy 2002). The existence of a large number of species and varieties of poplars growing in very diverse habitats enables the selection of a poplar species and seed source for almost any environmental condition, including very high altitude regions. In our study, the different physiological and biochemical responses to progressive drought stress attributed to explain the difference in drought adaptation between the two poplar species of *Sect. Tacamahaca* Spach originating from different altitudes in the eastern Himalaya.

Plant growth is responsive to progressive drought stress, and the reactions depend on the adaptation to the rapidity, severity, duration of the drought event. In this study, we discovered that *P. cathayana* possessed better height growth and leaf development in well water condition than did *P. kangdingensis*, whereas *P. kangdingensis* maintained a better height growth and leaf development than did *P. cathayana* under severe drought stress, which suggested that *P. cathayana* was more sensitive to drought stress than did *P. kangdingensis* and *P. kangdingensis* had stronger drought tolerance. Our results showed that drought adaptation are closely related to the environmental factors of their natural habitats in the two poplar species. Analyses of correlations for stem height with selected variables (Table 2) manifested that plant growth was closely related the RWC, MDA, H_2O_2, soluble proteins and sugars, proline and antioxidants. The leaf relative water content directly reflects the water status of plants. In our study, the results showed that drought stress significantly affected leaf relative water content, and stem height showed significant negative correlations with RWC under water stressed treatment. Similar results have been reported in previous studies on poplars (Marron et al. 2002, Liang et al. 2006). Earlier significant decline and greater extent decrease in RWC were found in *P. cathayana* than in *P. kangdingensis*, which suggested *P. kangdingensis* possessed better drought

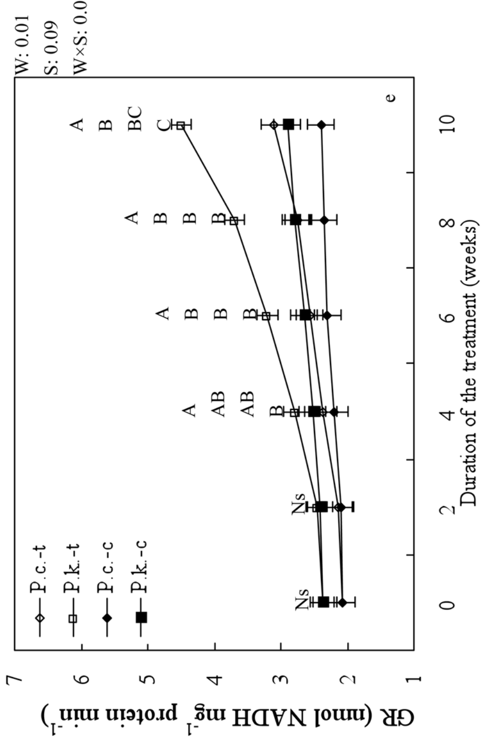


Fig. 5. The activities of superoxide dismutase (SOD) (a), catalase (CAT) (b), peroxidase (POD) (c), ascorbate peroxidase(APX) (d) and glutathione reductase (GR) (e) in the cuttings of two poplar species exposed to progressive drought stress. On the X axis, duration of the treatment 0, 2, 4, 6, 8 and 10 (weeks) are related to the field capacity 100%, 85%, 70%, 55%, 40% and 25%, respectively. The data presented are means ± SE (n=6). P.c.-t, the treated *P. cathayana* cuttings; P.c.-c, the *P. cathayana* controls; P.k.-t, the treated *P. kangdingensis* cuttings; P.k.-c the *P. kangdingensis* controls. Capital letters refer to differences between the two poplar species at the same watering stage. Values followed by different letters are significantly different from each other at $P<0.05$ according to Duncan's method. W, the effect of the watering treatment; S, the effect of the species; W×S, the watering×species interaction effect, as determined by ANOVA.

Table 2. Correlation coefficients of stem height with RWC, MDA, H_2O_2, soluble proteins and sugars, proline and antioxidants in two poplar species properties under different soil water contents.

		RWC	Free proline	Soluble sugar	Soluble protein	MDA	H_2O_2	SOD	CAT	POD	APX	GR
Stem height	P.c.-c	0.053	0.916**	0.937**	0.847**	0.224	0.911**	0.047	0.994**	0.868**	0.954**	0.842**
	P.c.-t	–0.884**	0.941**	0.943**	0.906**	0.915**	0.968**	0.918**	0.928**	0.902**	0.910**	0.768**
	P.k.-c	0.065	0.955**	0.977**	0.932**	0.434*	0.930**	0.938**	0.991**	0.962**	0.955**	0.955**
	P.k.-t	–0.819**	0.975**	0.973**	0.975**	0.942**	0.960**	0.973**	0.984**	0.965**	0.962**	0.963**

P.c.-t, the water-stressed P. cathayana cuttings; P.c.-c, the P. cathayana controls; P.k.-t, the water-stressed P. kangdingensis cuttings; P.k.-c the P. kangdingensis controls. *P< 0.05; **P< 0.01.

adaptation than did *P. cathayana.* Our results also showed that drought adaptations are closely related to the environmental factors of their natural habitats in the two poplar species. The different significant changes in different watering stage in two poplars also manifested that drought adaptations are closely related to the rapidity, severity, duration of the drought event and their individual altitude.

The plant's defense against drought stress requires osmotic adjustment, which, to a certain degree, can be achieved through a synthesis of intracellular solutes (Serrano et al. 1999). Proline may protect protein structures by maintaining their structural stability (Bates et al. 1973), and, accordingly, drought stress significantly increases proline accumulation (Sofo et al. 2004, Ren et al. 2006). Soluble sugars acting as osmoprotectors stabilize proteins and membranes, most likely substituting the water in the formation of hydrogen bonds with polypeptide polar residues (Crowe et al. 1992) and phospholipid phosphate groups (Strauss and Hauser 1986). An increase in the soluble protein concentration under drought stress could be related to an increase in the protein synthesis related to acclimation and reprogramming to new conditions as well as to cell protection against these stresses (Chen and Plant 1999). Free proline, soluble sugar and soluble protein act as osmotic agents or osmoprotectors that play a major role in the osmotic adjustment of water deficit. In the two poplar species, the amounts of free proline, soluble sugar and soluble protein gradually increased with drought stress progressed, which suggested they possessed drought tolerance at some extent. Significant changes in free proline and soluble protein appeared at earlier drought stage in *P. kangdingensis* than in *P. cathayana*, *P. kangdingensis* possessed higher increases in these parameters than did *P. cathayana*, which suggested that *P. kangdingensis* possessed a better osmotic adjustment and higher drought tolerance than did *P. cathayana*. Our results suggested that the contents of intracellular solutes are affected by the interaction of the rapidity, severity, duration of the drought event and their individual altitude of two poplars.

As one of the end products of lipid peroxidation, the MDA content reflects the degree of the peroxidation of membrane lipids (Taulavuori et al. 2001). H_2O_2 as a reactive oxygen species (ROS) damages the membrane lipids, and induces protein denaturation and DNA mutation (Bowler et al. 1992, Foyer and Halliwell 1997). The MDA and H_2O_2 contents significantly increased with drought stress progressed in the two poplar species, but MDA and H_2O_2 possessed negative effects on drought tolerance. Therefore, plant growth actually showed significant negative correlations with MDA, H_2O_2 in spite of significant positive relationships between stem height with MDA, H_2O_2 were shown in Table 2. The significant increase of MDA and H_2O_2 contents with drought stress progressed in the two poplar species suggested drought stress caused oxidative damages in both two species, similarly as detected in olive trees (Sofo et al. 2004), sunflower (Bailly et al. 1996) and *Coffea arabica* (Queiroz et al. 1998). Significant differences in the levels of MDA and H_2O_2 were found between the two poplar species when the cuttings were exposed to progressive drought stress. Compared with *P. kangdingensis*, *P. cathayana* possessed higher increased rate in contents of MDA and H_2O_2

and earlier significant changes were found in *P. cathayana*, which suggested that *P. kangdingensis* possessed better drought tolerance and stronger drought adaptation. Our results also manifested that oxidative stress caused by drought stress are related to the interaction of the rapidity, severity, duration of the drought event and their individual altitude of two poplars.

Drought can cause an oxidative stress in higher plants through breaking the balance between the production of reactive oxygen species (ROS) and the antioxidant defense (Elstner 1982, Jung 2004). The accumulation of ROS induces oxidative stress to proteins, membrane lipids and other cellular components (Herbinger et al. 2002). As a consequence, higher plants possess efficient antioxidant systems, e.g., in chloroplasts to protect them against oxidative injury. It has been proved that efficient antioxidative characteristics can provide better protection against oxidative stress in leaves under drought stress (Reddy et al. 2004). We observed that *P. kangdingensis* always showed higher increased rate in the activities of antioxidant enzymes than did *P. cathayana* with drought stress progressed, significant increments in the activities of antioxidant enzymes were found at earlier watering stage in *P. kangdingensis* than in *P. cathayana*, which suggested that *P. kangdingensis* could remove superoxide anion free radicals more easily, as there were lower levels of lipid peroxidation and H_2O_2 in *P. kangdingensis* than in *P. cathayana*. Therefore, *P. kangdingensis* possesses a higher antioxidant capacity to defend stress. The ability to increase the activities of antioxidant enzymes in order to limit cellular damages may be an important attribute of *P. kangdingensis*. However, the increases in the levels of MDA and H_2O_2 in the two poplar species showed that the increased activities of antioxidant enzymes may not be enough to prevent the peroxidation of lipid membranes and to scavenge ROS under drought stress. Our results suggested antioxidant defense systems caused by drought were affected by the interaction of the rapidity, severity, duration of the drought event and their individual altitude of two poplars.

In conclusion, when the cuttings were exposed to progressive drought stress, punctual changes appeared earlier in height growth inhibition, leaf development, relative water content, MDA and H_2O_2 in *P. cathayana* than in *P. kangdingensis*, whereas later effects in the levels of free proline, soluble sugar, soluble protein and antioxidant enzymes occurred in *P. cathayana*. It appears that *P. kangdingensis* originating from the high altitude possesses a better drought tolerance and stronger drought adaptation than does *P. cathayana* originating from the low altitude, which can be explained that in spite of soil-water contents are high and precipitation is abundant due to much rainfall at high altitude, the trees at high altitudes may be water stressed due to wind and ice blasting in the winter time and colder soils reduce the water uptake of the root system, and then they possessed better acclimation to drought stress than ones at low altitude (Landhäusser et al. 2001, Li et al. 2004).Compared earlier study (Ren et al. 2006, Yang et al. 2009), our results manifested that acclimation to drought stress are not only with the environmental factors of plant’s natural habitats but also related with the rapidity, severity, duration of the drought event and their interaction. Different responses to different field capacity in two poplars improved our understanding of the mechanisms that enable plants to survive under different drought stress.

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