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Populus from high altitude has more efficient protective mechanisms under water stress than from low-altitude habitats: a study in greenhouse for cuttings

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Cuttings of *Populus przewalskii* and *P. cathayana*, which originated from high and low altitudes in southwest China, were used to examine the effect of water stress on the morphological, physiological and biochemical traits of plants in a greenhouse for one growing season. The dry mass accumulation and allocation, gas exchanges, extent of peroxidation damage, osmotic adjustment and antioxidative defenses, and amounts of pigments were measured to characterize the differences in peroxidation damage and protective mechanisms of two poplar species that contrast in drought tolerance. Under water stress, poplars showed a series of biochemical adjustments and morphological changes as follows: a decrease in leaf relative water content, gas exchanges, plant growth and dry mass accumulation; an increase in relative allocation to roots; an increase in the osmolyte contents (e.g. total amino acids). Additionally, water deficit induced an increase in peroxidation damage [as indicated by an increase in electrolyte leakage, malondialdehyde (MDA), carbonyl (C = O) and hydrogen peroxide (H_2O_2) content], enhanced activities or contents of antioxidants (e.g. ascorbate peroxidase, guaiacol peroxidase, glutathione redutase and ascorbic acid) and reduced amounts of leaf pigments (e.g. chlorophyll and carotenoid). Furthermore, there were significant differences in the extent of morphological and biochemical changes between the two poplar species. Compared with *P. cathayana*, *P. przewalskii* responded to water stress by allocating relatively more to root dry mass, possessing a higher net photosynthesis rate, and having more efficient protective mechanisms, such as more osmolyte accumulation, stronger antioxidant activities and lower chlorophyll/carotenoid ratio. Thus, *P. przewalskii* suffered less damage as deduced from lower levels of electrolyte leakage, MDA, C=O and H_2O_2 content. Therefore, *P. przewalskii* originating from high altitude could possess more efficient protective mechanisms than *P. cathayana*, which is from low-altitude habitats.

Abbreviations – APX, ascorbate peroxidase; AsA, ascorbic acid; CAT, catalase; C=O, carbonyl; DNPH, 2,4-dinitrophenylhydrazine; E, transpiration rate; EC, electrical conductivity; EDTA, ethylenediaminetetraacetic acid; FC, field capacity; GPX, guaiacol peroxidase; GR, glutathione reductase; g_s, stomatal conductance; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; O_2^-, superoxide; ·OH, hydroxyl radical; P_n, net photosynthesis rate; ROS, reactive oxygen species; RWC, relative water content; SOD, superoxide dismutase; TCA, trichloroacetic acid.

Introduction

Drought is a multifactorial syndrome in which water deficit, heat stress and oxidative stress all interact to reinforce one another (Sánchez et al. 2004). Therefore, drought tolerance is a complex phenomenon involving different traits. The physiological and developmental mechanisms that allow a species to tolerate prolonged periods of drought can involve numerous attributes. The most studied plant adaptations to dehydration include structural changes (Li 2000, Li et al. 2000, Yin et al. 2005a, b), stomatal closure (Cornic 2000, Yin et al. 2004, 2006), osmotic adjustment (Li 1998, Sánchez et al. 2004) and antioxidative protection (Guerrier et al. 2000).

It is well known that water stress leads to a reduction in water potential in the root zone, which makes water acquisition difficult (Bohnert et al. 1998). Lowering the osmotic potential as a result of solute accumulation, known as osmotic adjustment, allows cell enlargement and plant growth, stomata to remain partially open and CO_2 assimilation to continue at low water potentials during severe water stress (Pugnaire et al. 1994). Many solutes may be used in osmotic adjustment. These solutes are known as compatible (non-toxic), and include sugars, glycerol, amino acids, such as proline and glycinebetaine, sugar alcohols, such as mannitol, and other metabolites with low molecular weights (Morgan 1984). Plant species differ in their primary osmolytes. Variation in osmotic potential or in the extent of osmotic adjustment among and within tree species may be a useful basis for selection of dehydration tolerance (Gebre et al. 1998).

Drought stress may induce stomatal closure to reduce leaf transpiration and prevent development of an excessive water deficit in tissues, as suggested by many researchers (Jones and Sutherland 1991, Tyree and Sperry 1988), and so decreased in CO_2/H_2O gas exchanges (Yin et al. 2005b, 2006). When excess light energy cannot be converted to photosynthetic CO_2 fixation, and excess of excitation energy in chloroplasts cannot be dissipated, oxygen can serve as the final acceptor of electrons in a pseudocyclic phosphorylation process (Foyer et al. 1994), which leads to the production of reactive oxygen species [ROS, i.e. superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH)] (Asada 1999). ROS are necessary for inter and intracellular signaling (Van Breusegem et al. 2001), but at high concentrations they can cause damage at various levels of organization (Asada 1999). To counteract ROS toxicity, a highly efficient antioxidative defense system, including both enzymic and non-enzymic constituents, is present in plant cells. Antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and glutathione redutase (GR) (Reddy et al. 2004). Non-enzymatic antioxidants, such as carotenoids, α-tocopherol and ascorbate, cooperate to maintain the integrity of photosynthetic membranes under oxidative stress (Noctor and Foyer 1998, Smirnoff and Wheeler 2000). Carotenoids are a group of colored terpenoids with antioxidant properties (Phadwal 2005). They play diverse roles in photobiology, photochemistry and medicine (reviewed by Edge et al. 1997).

Populus cathayana Rehder and *P. przewalskii* Maximowicz, which belong to section Tacamahaca Spach., are from different natural habitats in the eastern Himalaya. *P. cathayana* occurs in low-altitude regions, whereas *P. przewalskii* occupies high-altitude regions. An earlier study (Yin et al. 2005a) indicated that the two sympatric species had different drought tolerances; hence, we hypothesize that the two poplar species investigated here probably differ in their ability to prevent peroxidation damages under water stress. *P. przewalskii*, which occurs at a high altitude, could possess better protective mechanisms than *P. cathayana*, which is from a low altitude. In our study, cuttings of *P. przewalskii* and *P. cathayana*, which originated from high and low altitudes in Aba autonomy state, Sichuan province, China, were used as a model system to characterize the differences in morphological traits, peroxidation damage and protective mechanisms under water stress in tree species.

Materials and methods

Plant material and experimental design

Cuttings of *P. cathayana* Rehder and *P. przewalskii* Maximowicz were collected from 30 different trees in their natural habitats (Table 1). The cuttings (about 15 cm in length) were pricked in 5-l plastic pots (one cutting per pot) and filled with homogenized soil of the same weight, at the beginning of March 2005. After the cuttings sprouted and grew for about 1 month, drought stress was initiated on May 1 and ended on August 1, 2005. During the experiment, the plants were grown in a greenhouse under semi-controlled conditions with a temperature range of 18–32°C, a relative humidity range of 50–80%, mean active photosynthetic irradiation range of 700 ± 50 μmol m^{-2} s^{-1}, and a total of 12 g slow release fertilizer (13% N, 10% P and 14% K) was added to each pot. The experimental layout was completely randomized with two factors (species and watering regime). Three watering treatments, i.e. 100, 50 and 25% of field capacity (FC, i.e. the soil water

Table 1. The origin of the two *Populus* species of section Tacamahaca Spach. investigated in the study.

Species	Locality	Latitude (°N)	Longitude (°E)	Altitude (m)	Mean annual rainfall (mm)	Mean annual temperature (°C)	Soil type
P. przewalskii	Aba	32°33′	101°27′	3100	712	4.8	Meadow soils
P. cathayana	Jiuzhai	34°32′	105°04′	1100	550	12.7	Cinnamon soils

contents were 52.8, 26.4 and 13.2%, respectively) were used. Twenty seedlings from each species were exposed to each watering treatment. The pots were weighed every day and re-watered to 100, 50 and 25% FC by replacing the amount of water that had transpired. In each species and treatment, five replications, each including four cuttings, were used. To avoid systematic error because of the possible differences in fluctuating environmental condition, the cuttings were rotated every 10 days during the experiment. All cuttings from each species and treatment were measured for the analysis of morphological, physiological and biochemical traits.

The procedure to maintain soil water content was as follows: before the onset of the experiment, we used the 5-l pots filled with the same soil without cuttings to measure the soil water content at FC. At least five pots were thoroughly watered and kept in a basin with water overnight to let them reach FC, the pots were assumed to be at FC and weighed after they were removed from the water basin and allowed to drain. The measured soil water content was 52.8% when at FC. After the beginning of experiment, a 2-day cyclic watering schedule was applied throughout the experiment. Water loss was estimated every second day by weighing five randomly selected pots of each species and treatment. The used expression was as follows: water loss = the weight of the soil with the expected soil water content + plastic pot weight + cutting weight − the actual weight. Following changes in plant biomass, an empirical relationship between seedling fresh weight (Y, g) and seedling height (X, cm), $Y = 0.975 + 0.112\,X (R^2 = 0.968, P < 0.001)$ (Li et al. 2004), was used to correct the amount of water in each pot.

Growth measurements

At the onset and end of the experiment, plant height, basal diameter and leaf number were measured. At the end of the experiment, whole plants of both species from each treatment were harvested and divided into leaves, stems and roots. Biomass samples were dried (70°C, 48 h) to a constant weight and weighed. The root/weight ratio was then calculated by dividing root mass by total biomass.

Measurement of gas exchanges of leaves

Measurements of gas exchanges were made on July 20, 2005 on a sunny and windless day with the LI-6400 Portable Photosynthesis System (Li-cor, Lincoln, NE) and a 6-cm^2 leaf chamber for the third to the fifth non-detached fully expanded leaf from the top of the plant. Levels of net photosynthesis rate (P_n), stomatal conductance (g_s) and transpiration rate (E) were measured at reference chamber CO_2 concentration 400 ± 1 μmol mol^{-1} by using a CO_2 injector mixer, air temperature 30–31°C, leaf–air vapor pressure deficit 1.8 ± 0.2 kPa, relative air humidity $57 \pm 1.5\%$ and photosynthetic active radiation 1400 μmol m^{-2} s^{-1} in the leaf chamber using a 6400–02B LED Light Source. Five seedlings from each treatment were selected for the measurements, and a measurement was made for three leaves of each seedling.

Determination of relative water content of leaves

Five fresh leaves from each treatment were excised from five randomly selected plants, weighed (Fresh wt.), then kept in water for 24 h, recorded for turgid weight (Turgid wt.), and then dried in hot air oven (70°C) till constant weight (Dry wt.) was achieved. Relative water content (RWC) was estimated as follows (Whetherley 1950): RWC (%) = (Fresh wt. − Dry wt.) ×100/ (Turgid wt. − Dry wt.)

Analyses of soluble sugar and total amino acid contents

The upper fully expanded and exposed leaves were randomly selected from each species and treatment and used for the analyses. Soluble sugar was estimated by the anthrone method (Yemm and Willis 1954). Leaves (about 0.2 g) were homogenized in a mortar, added to 50 ml distilled water and extracted in boiling water for 20 min. Thereafter, the extract was cooled to room temperature and centrifuged. For the reaction, 1 ml of the supernatant was added to 5 ml anthrone reagent, mixed and heated in boiling water for 10 min, cooled using ice water, and the absorbance was determined using glucose as the standard at 620 nm.

The quantitative measurement of total amino acids was conducted using the ninhydrin reaction (Correia et al. 2005). Approximately 2 ml of buffered ninhydrin solution (0.8 g ninhydrin and 0.12 g hydrindantin dissolved in 30 ml 2-methoxyethanol plus 10 ml acetate buffer 4 *M*, pH 5.5) was added to 1 ml supernatant and heated in boiling water for 15 min. The mixture was cooled to room temperature, 3 ml 50% ethanol was added, and the absorbance was read at 570 nm after 10 min. The amount of amino acids was determined by reference to a standard curve that was previously prepared with arginine.

Measurement of electrolyte leakage, malondialdehyde, carbonyl and H_2O_2 contents

The relative intactness of the plasma membrane was measured as the leakage percentage of electrolytes, as described by Gong et al. (1998). Five leaf discs (1 cm^2) were placed in test tubes containing 30 ml distilled deionized water. The tubes were incubated in a water bath at 30°C for 4 h, and the initial electrical conductivity of the medium (EC_1) was measured. Then the samples were boiled at 100°C for 15 min to release all electrolytes and cooled before the final electrical conductivity (EC_2) was measured. The leakage percentage of electrolytes was calculated using the formula: $EC_1/EC_2 \times 100$.

The level of oxidative damage to lipids was assessed by determining the concentration of malondialdehyde (MDA), which is a product of the oxidation of polyunsaturated fatty acids. MDA concentration was determined as follows (Hodges et al. 1999): leaves (about 1 g) were homogenized in 10 ml 10% trichloroacetic acid (TCA), centrifuged at 12 000 *g* for 10 min, and then 2 ml 0.6% thiobarbituric acid in 10% TCA was added to 2 ml the supernatant. The mixture was heated in boiling water for 15 min, and then quickly cooled using ice. After centrifugation at 12 000 *g* for 10 min, the absorbance of the supernatant was determined with a spectrometer (Unicam, UV330) at 450, 532 and 600 nm. The concentration of MDA was calculated by the following formula: $C(\mu mol\, l^{-1}) = 6.45(A_{532} - A_{600}) - 0.56A_{450}$.

Oxidative damage to proteins was quantified as total carbonyl (C=O) content (Levine et al. 1994). Leaves (about 1 g) were homogenized in 6 ml 50 m*M* phosphate buffer (pH 7.0) containing 1 m*M* phenylmethylsulfonyl fluoride and soybean trypsin inhibitor, the homogenate was centrifuged at 10 000 *g* for 5 min and the supernatant was added to 1% (w/v) streptomycin sulfate to remove possible contaminating nucleic acids. Afterward, the solution was centrifuged again at 11 000 *g* for 10 min. The supernatant was divided into two shares as a 'test' and 'control', 4 ml 10 m*M* 2,4-dinitrophenylhydrazine (DNPH) prepared in 2.5 *M* HCl and 4 ml 2.5 *M* HCl was added to the test sample and the control sample, respectively. The contents were mixed thoroughly and incubated in the dark at room temperature for 1 h, and tubes were shaken intermittently every 15 min. Then 5 ml of 20% TCA (w/v) were added to both tubes, left in ice for 10 min and centrifuged at 3500 *g* for 20 min to obtain the protein pellet. After aspirating and discarding the supernatant carefully, a second wash was made with 10% TCA as described above. Finally, the precipitates were washed thrice with 4 ml ethanol:ethyl acetate (1:1, v/v) to remove unreacted DNPH and lipid remnants. The final protein pellet was dissolved in 2 ml 6 *M* guanidine hydrochloride, incubated at 37°C for 10 min and the insoluble materials were removed by centrifugation. C=O content was determined by taking the spectra of the representative samples at 355–390 nm. Each sample was read against the control sample (treated with 2.5 *M* HCl). The C=O content was calculated from peak absorption (370 nm) using an absorption coefficient (ε) of 22 mM^{-1} cm^{-1} and expressed as nmol g^{-1} DW.

The level of H_2O_2 was measured by monitoring the absorbance of the titanium–peroxide complex at 415 nm, following the method of Brennan and Frenkel (1977). Chilled leaves (about 0.3 g) were homogenated in 6 ml cold acetone and centrifuged at 3000 *g* for 10 min. The supernatant (1 ml) was treated with 0.1 ml titanium reagent (20% titanic tetrachloride in concentrated HCl, v/v) and 0.2 ml concentrated ammonia solution to precipitate the titanium–hydroperoxide complex. After centrifugation (at 10 000 *g* for 10 min) precipitate was dissolved in 5 ml 2 *M* H_2SO_4 and the final volume was made with the addition of 6 ml cold double-distilled water. Absorbance values were calibrated to a standard curve, which was generated using known concentrations of H_2O_2, and H_2O_2 content expressed as µmol g^{-1} DW, at 415 nm.

Assays of APX, CAT, GPX and GR activity, soluble protein and ascorbic acid content

Frozen leaf segments of about 0.5 g were crushed into a fine powder using a mortar and pestle with liquid nitrogen. Crude enzyme extract was prepared by homogenizing the powder in 10 ml 50 m*M* potassium phosphate buffer (pH 7.0) containing 1 m*M* ethylenediaminetetraacetic acid (EDTA) and 1% polyvinylpyrrolidone, with the addition of 1 m*M* ascorbic acid (AsA) in the case of an APX assay. The homogenate was centrifuged at 12 000 *g* for 20 min at 4°C, and the supernatant was used for the following soluble protein and enzyme assays.

Soluble protein contents were determined as described by Bradford (1976), using bovine serum albumin as a calibration standard.

APX (EC 1.11.1.11) activity was analyzed by following the decrease in A_{290}($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$) for 1 min in 3 ml of a reaction mixture containing 50 m*M* sodium phosphate buffer (pH 7.0), 0.1 m*M* EDTA, 0.1 m*M* sodium ascorbate, 2.5 m*M* H_2O_2 and 200 µl enzyme extract (Nakano and Asada 1981). CAT (EC 1.11.1.6) activity was determined using the method described by Córdoba-Pedregosa et al. (2003) in 50 m*M* phosphate buffer (pH 7.0) with 20 m*M* of H_2O_2. The reaction was induced by the addition of the sample, and the decomposition of H_2O_2 at 240 nm ($\varepsilon = 43.6\ mM^{-1}\ cm^{-1}$) was monitored. GPX (EC 1.11.1.7) activity was based on the determination of guaiacol oxidation ($\varepsilon = 26.6\ mM^{-1}\ cm^{-1}$) at 470 nm by H_2O_2. The reaction mixture contained 100 m*M* potassium phosphate buffer (pH 6.5), 16 m*M* guaiacol and 0.1 ml 10% H_2O_2 in a 3 ml volume. The reaction was initiated by adding 50 µl of enzyme extract and then followed for 3 min (Lin and Wang 2002). GR (EC 1.6.4.2) activity was determined by following the oxidation of NADPH at 340 nm ($\varepsilon = 6.2\ mM^{-1}\ cm^{-1}$) for 3 min in 3 ml of assay mixture containing 50 m*M* potassium phosphate buffer (pH 7.8), 2 m*M* Na_2EDTA, 0.15 m*M* NADPH, 0.5 m*M* GSSG and 200 µl enzyme extract (Schaedle and Bassham 1977).

In addition, reduced AsA was determined according to the method of Hodges et al. (1996). About 0.5 g leaf segments were homogenized in 5 ml cold 5% *m*-phosphoric acid and centrifuged at 10 000 *g* for 15 min. For 5 min, 300 µl supernatant was incubated in a 700 µl total volume of 100 m*M* KH_2PO_4 and 3.6 m*M* EDTA. Color was developed with 400 µl 10% TCA, 400 µl 44% *o*-phosphoric acid, 400 µl 65 m*M* α,α'-dipyridyl in 70% ethanol and 200 µl 110 m*M* $FeCl_3$. The reaction mixtures were then incubated at 40°C for 1 h and quantified at 525 nm.

Estimation of chlorophyll and carotenoid contents

Leaf tissues were homogenized in chilled *N*, *N*-dimethylformamide using a mortar and pestle in the dark at 4°C, and the homogenates were centrifuged at 8800 *g* for 10 min. The supernatants were collected and the absorption spectra were recorded at 663.8 and 646.8 nm for the estimation of chlorophyll *a*, chlorophyll *b* and total chlorophyll following the procedure described by Inskeep and Bloom (1985). For the estimation of total carotenoid, leaf tissues (0.5 g) were homogenized in chilled 80% (v/v) acetone, and then centrifuged at 8800 *g* for 10 min in the dark at 4°C. The absorbance of the acetone extracts was measured at 663, 645 and 470 nm. Total carotenoid content was calculated as described by Lichtenthaler and Wellburn (1983).

Statistical analyses

All variables obtained from the measurements were analyzed by two-way ANOVA to test the effect of watering, species and watering × species interaction. The Duncan test was used to detect possible differences between the treatments. Statistical analyses were conducted with the SPSS 11.0 statistical software package for Windows.

Results

Interspecific differences in RWC of leaves

All of the effects of watering, species and the watering × species interaction on the RWC were significant. Water stress distinctly decreased the RWC of leaves in both species (Fig. 1). However, compared with *P. cathayana*, *P. przewalskii* showed higher RWC under water stress (i.e. 50 and 25% FC).

Interspecific differences in plant growth, dry mass accumulation and allocation

After 3 months growth, the plant height, basal diameter and leaf number were affected to a different extent

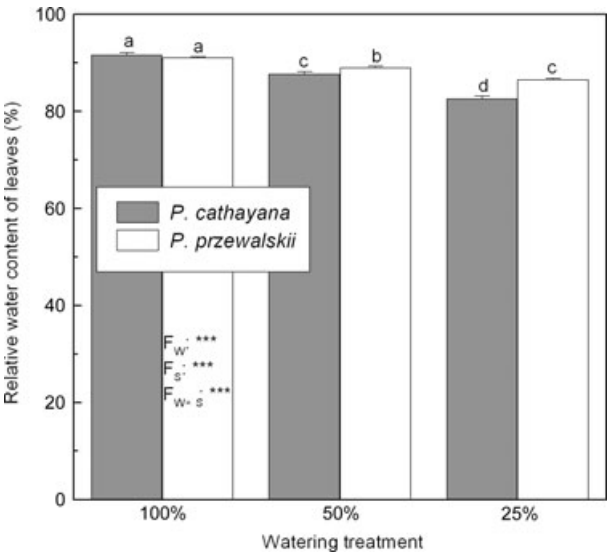

Fig. 1. The effect of watering on relative water content of leaves (%) in *P. cathayana* and *P. przewalskii* (means and standard deviation bars shown). Different letters indicate significant differences between treatments ($P < 0.05$, a Duncan test). F_W, watering effect; F_S, species effect; $F_{W\times S}$, watering × species interaction effect. ***$P < 0.001$.

by watering, species and the interaction (Fig. 2). Moreover, water stress significantly decreased biomass production and relatively increased root mass in both species (Fig. 3). However, compared with *P. cathayana*, *P. przewalskii* showed higher biomass production under all watering treatments. The root/weight ratio of *P. przewalskii* was lower under the well-watered treatment and higher under the water stress treatment (particularly at 25% FC) than that of *P. cathayana*. In addition, the effects of the watering × species interaction on dry matter accumulation and allocation were also significant.

Interspecific differences in gas exchanges

Gas exchanges (i.e. P_n, E and g_S) was significantly decreased by water stress in both species. Interspecific differences were observed in gas exchanges, and P_n

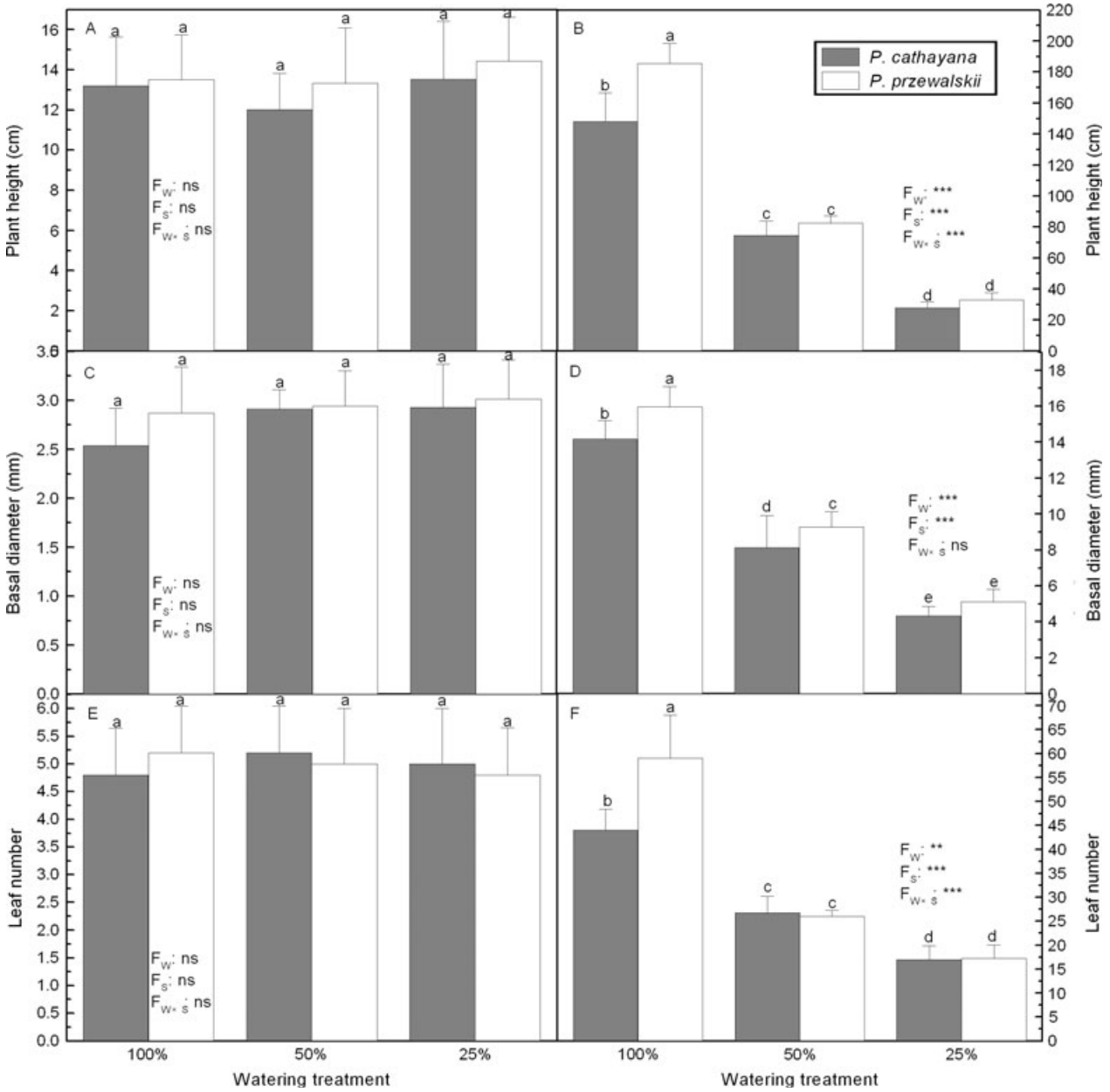

Fig. 2. The plant height, basal diameter and leaf number (means and standard deviation bars shown) of *P. cathayana* and *P. przewalskii* at the onset (A, C and E) and at the end (B, D and F) of the experiment. Different letters indicate significant differences between treatments ($P < 0.05$, a Duncan test). F_W, watering effect; F_S, species effect; $F_{W\times S}$, watering × species interaction effect; ns, not significant. $***P < 0.001$; $**P < 0.01$.

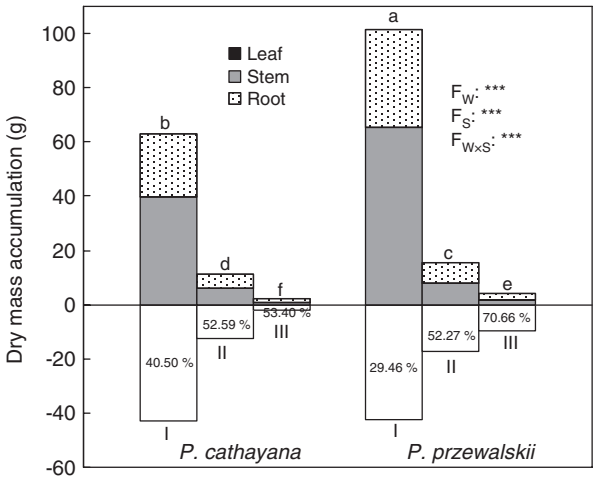

Fig. 3. The effect of watering (I, 100% FC; II, 50% FC and III, 25% FC) on the dry mass accumulation and allocation in *P. cathayana* and *P. przewalskii*. Different letters above the columns indicate significant differences between the total biomass accumulated in different treatments ($P < 0.05$, a Duncan test). The absolute values of the negative values in the figure refer to the root mass underground. The values shown within columns are the root/weight ratios. F_W, watering effect; F_S, species effect; $F_{W\times S}$, watering × species interaction effect. ***$P < 0.001$.

and E were also affected by the interaction of watering × species (Fig. 4). Under 100% FC, there were no differences between *P. przewalskii* and *P. cathayana* in P_n or E and g_s. Under 50% FC and 25% FC, *P. przewalskii* showed higher P_n than *P. cathayana,* but an interspecific difference was not found in g_s.

Interspecific differences in contents of soluble sugar and total amino acid

As one of the familiar osmolytes, soluble sugar and total amino acid contents were significantly affected by water stress (Fig. 5), but soluble protein content did not change significantly (data not shown). Soluble sugar content of *P. przewalskii* gradually increased with increasing water stress, whereas that of *P. cathayana* increased by 50% FC and then decreased by 25% FC when compared with 100% FC (Fig. 5A). In addition, *P. przewalskii* showed higher total amino acid content than *P. cathayana* under 100 and 25% FC (Fig. 5B).

Interspecific differences in electrolyte leakage, MDA, C=O and H_2O_2 contents

The electrolyte leakage, MDA, C=O and H_2O_2 contents, which were used to express the extent of damage, were significantly affected by the effects of watering, species and the watering × species interaction (Fig. 6). The

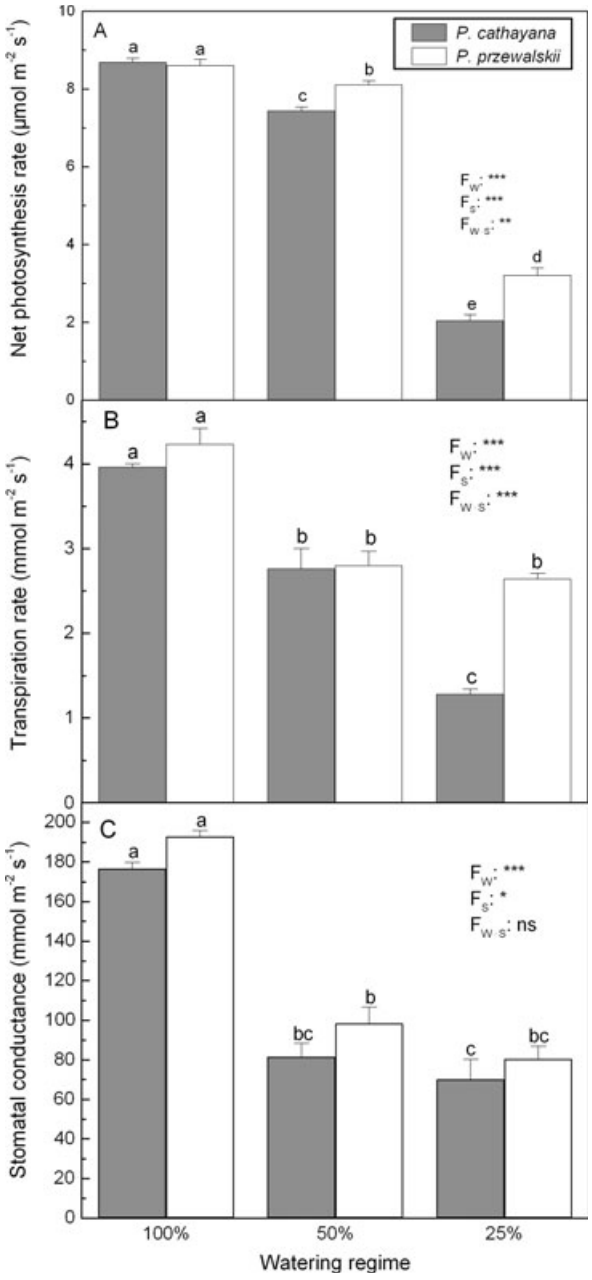

Fig. 4. The effect of watering on (A) net photosynthesis rate, (B) transpiration rate and (C) stomatal conductance in *P. cathayana* and *P. przewalskii* (means and standard deviation bars shown). Different letters indicate significant differences between treatments ($P < 0.05$, a Duncan test). F_W, watering effect; F_S, species effect; $F_{W\times S}$, watering × species interaction effect; ns, not significant. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

damage became more serious with increasing water stress in both species. Generally, *P. przewalskii* showed lower electrolyte leakage, and MDA, C=O and H_2O_2 contents than *P. cathayana* under all watering regimes (Fig. 6).

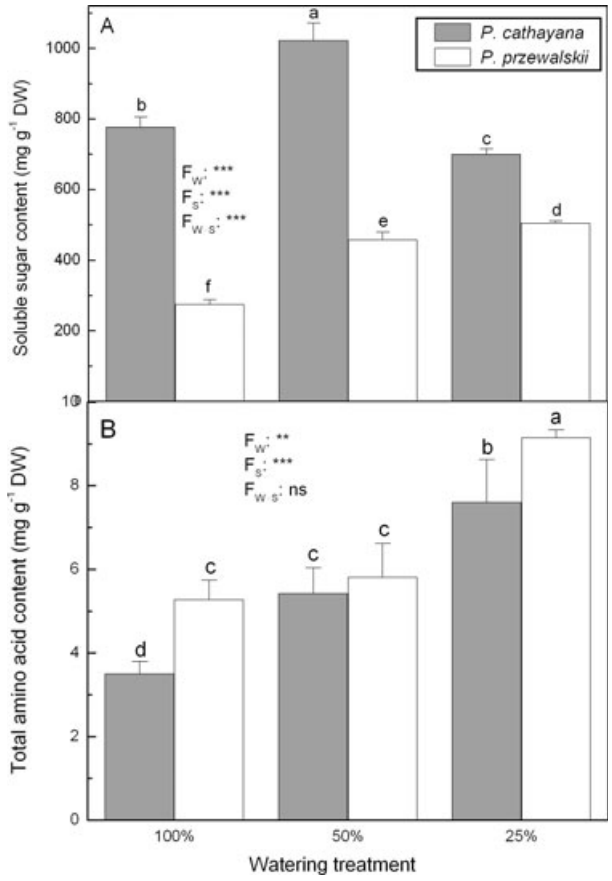

Fig. 5. The effect of watering on (A) soluble sugar content and (B) total amino acid content in *P. cathayana* and *P. przewalskii* (means and standard deviation bars shown). Different letters indicate significant differences between treatments ($P < 0.05$, a Duncan test). F_W, watering effect; F_S, species effect; $F_{W\times S}$, watering × species interaction effect; ns, not significant. $^{***}P < 0.001$; $^{**}P < 0.01$.

Interspecific differences in APX, GPX and GR activities, and AsA content

For the antioxidants contents or activities, APX, GPX and GR activities, and AsA content were significantly increased, whereas CAT activity (data not shown) was not significantly affected by water stress. Compared with *P. cathayana*, *P. przewalskii* showed higher APX, GPX and GR activities, and a lower AsA content under all watering regimes (Fig. 7). The effects of the watering × species interaction on these traits were also significant.

Interspecific differences in contents of pigments, and chlorophyll/carotenoid ratio

The contents of pigments were significantly decreased, and the chlorophyll/carotenoid ratio of *P. cathayana* was significantly increased by water stress, but had little effect on *P. przewalskii* (Fig. 8). *P. przewalskii* had higher contents of chlorophyll and carotenoid under all watering regimes and a lower chlorophyll/carotenoid ratio at 25% FC than *P. cathayana* (Fig. 8).

Discussion

In this study, water stress decreased the RWC of leaves in both poplar species (Fig. 1). However, compared with *P. cathayana*, *P. przewalskii* showed higher RWC under water stress at 50 and 25% FC. The results indicated that *P. przewalskii* had better water use than *P. cathayana* under the same soil water content. In addition, water stress was found to have a significant effect on plant growth, dry matter accumulation and allocation in the two *Populus* species investigated in this study (Figs 2 and 3). The main results, which showed that water stress inhibited growth and relatively increased root mass in *Populus* trees, were similar to reports in many previous studies (Li 2000, Li et al. 2004, Yin et al. 2005a, Zhang et al. 2004). Compared with *P. cathayana*, *P. przewalskii* responded to water stress by allocating relatively more dry mass to root, and it maintained higher biomass production under water stress. The higher biomass of *P. przewalskii* under water stress was attributed to higher gas exchanges, especially higher P_n (Fig. 4).

The soluble sugar and total amino acid contents showed different changes under water stress (Fig. 5). The soluble sugar content of *P. przewalskii* increased gradually when the soil water content changed from 100 to 25% FC, whereas that of *P. cathayana* first increased and then decreased. However, the total amino acid contents rapidly increased with water stress in both species. The total amino acid content of *P. przewalskii* was much higher than that of *P. cathayana*. Our results were supported by earlier studies that show sugars and amino acids are major constituents of osmoregulation in expanded leaves of many species (reviewed by Morgan 1984). Similarly, Sánchez et al. (2004) reported that water stress induced the accumulation of soluble sugars and free proline in pea, and that the consequent contribution to osmotic adjustment was very important but varied depending on the cultivar in question. In addition to osmotic adjustment, proline, which is one of the amino acids, can function as a ·OH scavenger to prevent membrane damage and protein denaturation (Ain-Lhout et al. 2001); hence, the accumulation of total amino acids was closely related to oxidative response.

Stomata closure (i.e. decrease in g_s) and decrease in gas exchanges under drought stress (Fig. 4) induced an excess of energy in chloroplast that cannot be dissipated, which lead to the production of a high ROS concentration. With respect to the damage of ROS to plants, the electrolyte leakage, MDA, C=O and H_2O_2

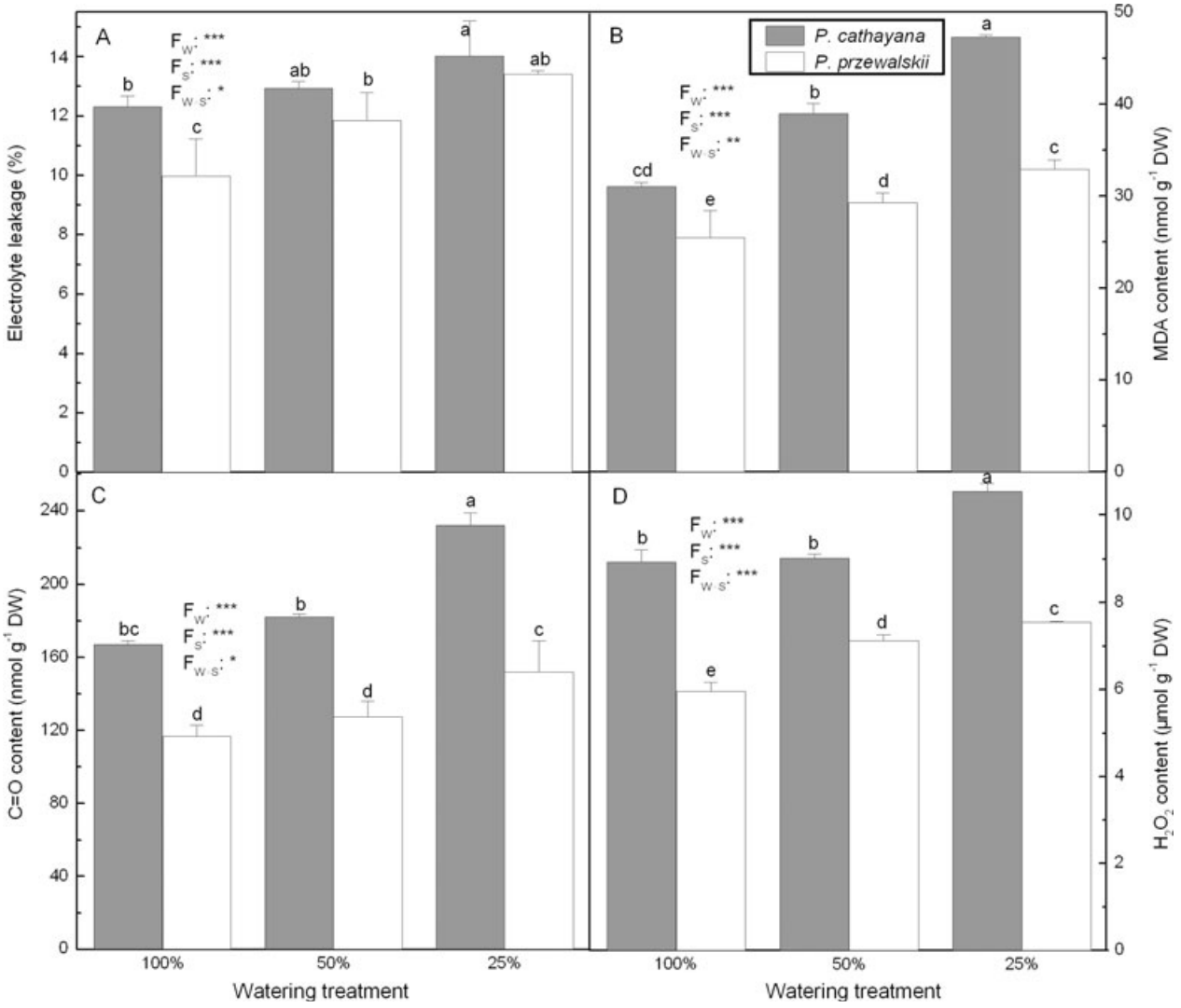

Fig. 6. The effect of watering on (A) electrolyte leakage; (B) malondialdehyde (MDA) content; (C) C=O content and (D) H_2O_2 content in *P. cathayana* and *P. przewalskii* (means and standard deviation bars shown). Different letters indicate significant differences between treatments ($P < 0.05$, a Duncan test). F_W, watering effect; F_S, species effect; $F_{W\times S}$, watering × species interaction effect. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$.

content were significantly increased (Fig. 6). The extent of damage in *P. przewalskii* was much lower than that of *P. cathayana*. The increases in membrane, lipid and protein peroxidation observed here are in agreement with the results obtained in other studies (Leitao et al. 2003, Lima et al. 2002), and the enhanced levels of oxidized proteins also correlated with the decline of total protein as previously observed (Hernández-Jiménez et al. 2002). Although ROS, especially H_2O_2 at a proper concentration, are involved in a cellular signaling process that induces a number of genes and proteins included in stress defenses (Jiang and Zhang 2001), they are highly reactive and can seriously damage plants by lipid peroxidation, protein degradation, DNA breakage and cell death in the absence of an effective protective mechanism (Beligni and Lamattina 1999) followed by an increase in electrolyte leakage, MDA and C=O contents. Increases in cellular damage seemed to reflect that the repairing mechanisms do not keep pace with damage, and that water deficit can influence the composition and turnover of membrane lipids and proteins (Smirnoff 1993).

To prevent ROS accumulation, many plants have evolved several mechanisms of photo- and antioxidative protection to withstand drought-induced oxidative stress. Apart from the xanthophylls cycle, photorespiration and other changes in metabolic activity (Kosaki and Takeba 1996), a number of enzymatic and non-enzymatic

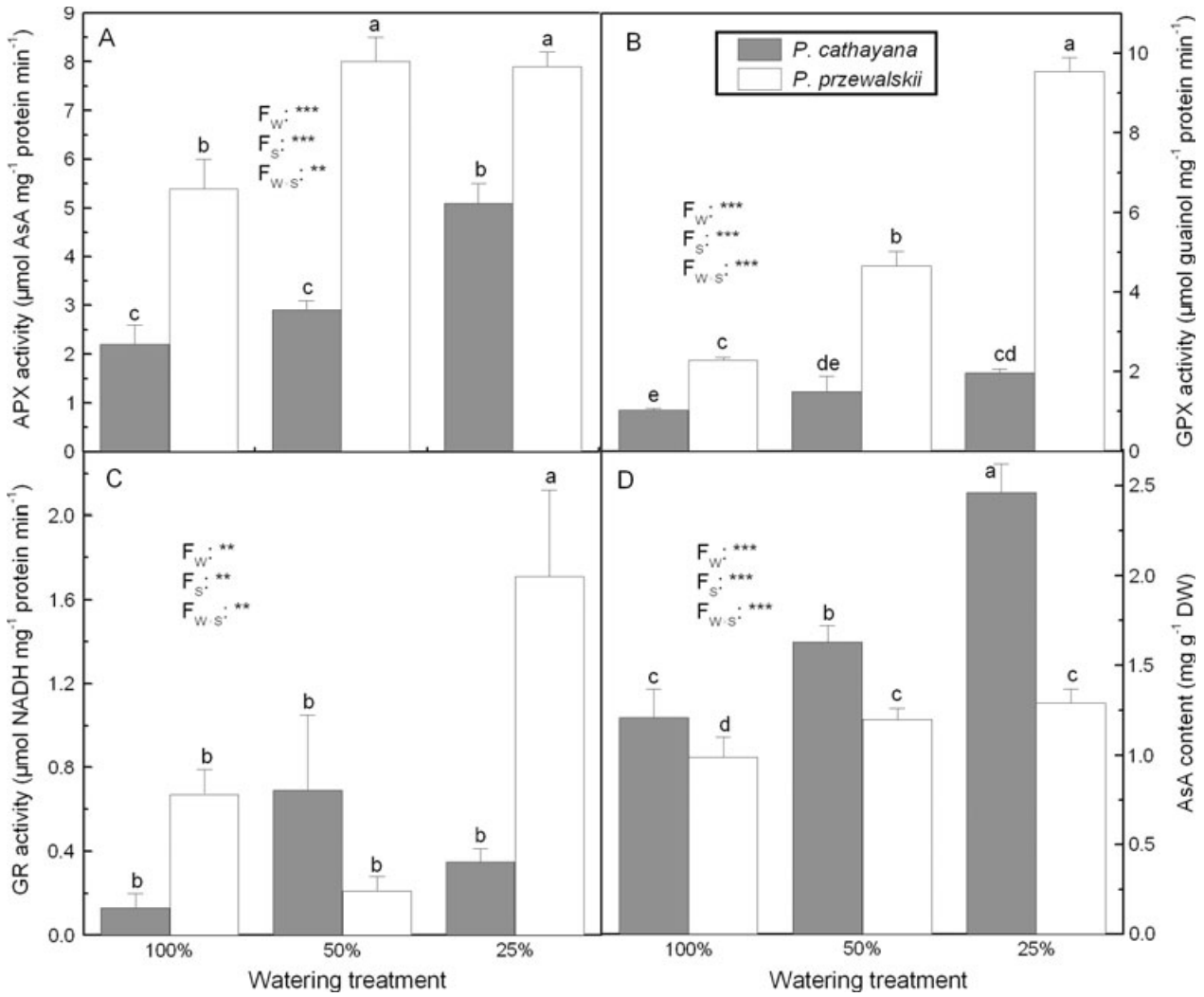

Fig. 7. The effect of watering on (A) APX activity; (B) GPX activity; (C) GR activity and (D) AsA content in *P. cathayana* and *P. przewalskii* (means and standard deviation bars shown). Different letters indicate significant differences between treatments ($P < 0.05$, a Duncan test). F_W, watering effect; F_S, species effect; $F_{W\times S}$, watering × species interaction effect. $***P < 0.001$; $**P < 0.01$.

antioxidants are also present in cells (Dietz 2003, Loggini et al. 1999). Under water stress, the APX, GPX and GR activities and the AsA content of poplars were increased. However, compared with *P. cathayana*, the antioxidant (except for AsA) activity of *P. przewalskii* was maintained at relatively higher level under water stress (Fig. 7). Therefore, our data showed that *P. przewalskii* possesses more efficient antioxidative characteristics, which could provide better protection against oxidative stress in leaves under water stress compared with *P. cathayana*. In agreement with our results, the activities of antioxidant enzymes, including SOD, CAT, APX, GPX and GR, were significantly increased, and the contents of AsA showed variable levels in mulberry under severe water stress (Reddy et al. 2004). Edjolo et al. (2001) have investigated the ascorbate–glutathione cycle in the cytosolic and chloroplastic fractions of drought-tolerant and drought-sensitive poplars and have found that a drought-tolerant clone exhibits a more efficient ROS-scavenging system than drought-sensitive clones. In our study, the activities of enzymatic antioxidants (i.e. APX, GPX and GR) were higher in *P. przewalskii* than in *P. cathayana*, and, therefore, the peroxidation damage to *P. cathayana* was more serious than that to *P. przewalskii*.

The chlorophyll and carotenoid contents of leaves were decreased, and the chlorophyll/carotenoid ratio was increased by water stress in *P. cathayana*, but had little effect on *P. przewalskii* (Fig. 8). Furthermore, *P. przewalskii* had a higher content of pigments under

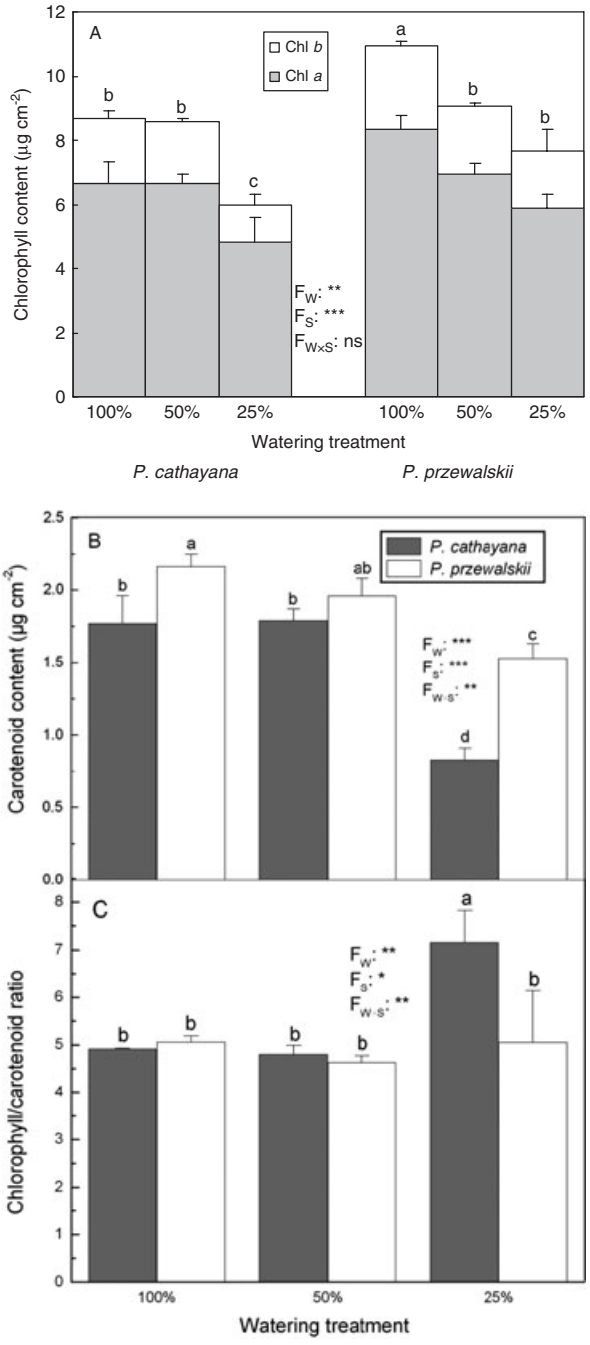

Fig. 8. The effect of watering on (A) chlorophyll content; (B) carotenoid content and (C) chlorophyll/carotenoid ratio in *P. cathayana* and *P. przewalskii* (means and standard deviation bars shown). Different letters indicate significant differences between treatments ($P < 0.05$, a Duncan test). F_W, watering effect; F_S, species effect; $F_{W\times S}$, watering × species interaction effect; ns, not significant. $***P < 0.001$; $**P < 0.01$; $*P < 0.05$.

all watering regimes and a lower chlorophyll/carotenoid ratio than *P. cathayana* at 25% FC. A drought-induced reduction in pigments was previously reported in strawberry(Munné-Bosch and Peñuelas 2004). However, a salt-induced increase in the carotenoid content in the leaves of *Plantago* has also been found (Koyro 2006). Under water stress, chlorophyll contents usually decrease, which may be as a result of either slow synthesis or fast breakdown (Ashraf 2003). In addition, carotenoid could prevent chlorophyll-photosensitized formation of $^1O_2$ by intercepting the chlorophyll triplet states (Demmig-Adams and Adams 1996). The decrease in carotenoid content suggested that drought stress caused marked oxidative stress by accumulation of ROS (Lei et al. 2006). Moreover, the decrease in chlorophyll content contributes not only to the increase in the ratio of xanthophyll to chlorophyll but also to the increase in the ratio of carotenoids, α-tocopherol and lutein to chlorophyll. It is now well documented that carotenoid was involved in dissipation of excess energy in photosystems I and II as heat, in non-damaging chemical reactions (Lu et al. 2003.), stabilization of membranes in chloroplasts (Havaux 1998), and as chain-breaking antioxidants by scavenging and deactivating free radicals (Bast et al. 1998). Therefore, the antioxidant capacity of leaves per amount of photons absorbed is enhanced by water stress conditions (Munné-Bosch and Alegre 2000).

In conclusion, under water stress poplars showed the following changes: a decrease in leaf RWC, gas exchanges, plant growth and dry mass accumulation, an increase in relative dry mass allocation to roots, the contents of osmolytes (such as sugar and total amino acid), worsened peroxidation damage (evaluated by an increase in electrolyte leakage and in the MDA, C=O, and H_2O_2 content), enhanced activities or contents of antioxidants (i.e. APX, GPX, GR and AsA) and reduced amounts of leaf pigments (chlorophyll and carotenoid). There were significant differences in these changes between the two *Populus* species. Compared with *P. cathayana*, which originated from low altitudes, *P. przewalskii* from high altitudes responded to water stress by allocating relatively more dry mass to roots, possession of a higher P_n and more efficient protective mechanisms, such as more osmolyte (i.e. total amino acids) accumulation and stronger antioxidants (APX, GPX and GR) activities, and a lower chlorophyll/carotenoid ratio. It suffered from less damage, as deduced from lower levels of electrolyte leakage, MDA, C=O and H_2O_2 contents. Considering the ecology of the species studied (Table 1; *P. przewalskii* originates from a region with higher rainfall but lower temperature than *P. cathayana*), it is possible that colder soils reduce water uptake of the root system and consequently induce water stress (James et al. 1994, Magnani and Borghetti 1995); hence, as the

result of chronical adaptation to lower water availability caused by low temperature, *P. przewalskii* was more drought-tolerant than *P. cathayana*.

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