



Differences in growth and physiological traits of *Populus cathayana* populations as affected by enhanced UV-B radiation and exogenous ABA

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ABSTRACT

During one growing season, the effects of enhanced ultraviolet-B (UV-B) radiation, exogenous abscisic acid (ABA) and their combination on biomass accumulation, gas exchange, endogenous ABA, the concentration of UV-absorbing compounds, antioxidant system and on the carbon (C) and nitrogen (N) content and C/N ratio were investigated in two contrasting *Populus cathayana* Rehd. populations, originating from high and low altitudes in south-west China. Exogenous ABA was sprayed to the leaves, and enhanced UV-B treatments were applied using a square-wave system to expose the seedlings to ambient (1×) or twice ambient (2×) doses of biologically effective UV-B radiation (UV-B_{BE}). Enhanced UV-B radiation significantly decreased height, basal diameter, total leaf area, total biomass, net CO₂ assimilation rate (A), stomatal conductance (g_s), transpiration rate (E) and carbon (C) content in leaves, and significantly increased the activities of superoxide dismutase (SOD) and guaiacol peroxidase (GPx), and the contents of hydrogen peroxide (H₂O₂) and malonaldehyde (MDA), as well as the accumulation of UV-absorbing compounds and endogenous ABA concentrations among different organs in both populations. In contrast, exogenous ABA induced a significant decrease in A and significant increases in the activities of SOD and GPx, in the content of H₂O₂ and MDA, and in the endogenous ABA concentrations. Compared with the low altitude population, the high altitude population was more tolerant to enhanced UV-B and exogenous ABA. Significant interactions between UV-B and ABA were observed in A, E, and in the activities of SOD and GPx, as well as in endogenous ABA in the leaves and roots of both populations. Across all treatments, the C and N contents of leaves were strongly correlated with their contents in stems and roots. Additionally, the N content of leaves and stems were significantly correlated with the C content of stems.

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1. Introduction

A reduction in the thickness of the stratospheric ozone layer induced by anthropogenic emissions of pollutants, such as chlorofluorocarbons (CFCs), has been detected (Stolarski et al., 1992). According to the global climate model that incorporates simplified ozone depletion chemistry (Shindell et al., 1998), the direct result of the decrease in stratospheric ozone will be an increase in the biologically effective ultraviolet-B radiation (UV-B). This increase has been approximated to equal 1% per year since 1981 (Blumthaler and Ambach, 1990), with an expected severe increase occurring in

the northern hemisphere during the years 2010–2019. The recovery of stratospheric ozone to the early-1980s levels is not predicted until roughly 2050. Intensified current and projected UV-B radiation is known to affect plant growth, development and physiological processes (Nogués et al., 1998). UV-B effects on plants occur, e.g., through the dimerization of thymidin residues and oxidation of membrane lipids and proteins (Jansen et al., 1998). In addition, damage to photosystem II as well as reductions in photosynthetic rates has been observed (Sullivan et al., 2003). In rice (*Oryza sativa* L.) and cucumber (*Cucumis sativus* Linn.), an increase in the amount of antioxidant enzymes has also been reported in response to UV-B irradiation in laboratory conditions (Kim et al., 1996). Furthermore, studies conducted so far indicate that the effects of UV-B radiation together with other environmental variables, such as enhanced CO₂ (Björn et al., 1997), high temperature (Mark and Tevini, 1996) and water deficit (Alexieva et al., 2001) are both additive and interactive. However, little attention has been paid to possible simultaneous changes at the level of plant hormones, such as abscisic acid (ABA).

Abbreviations: A, net CO₂ assimilation rate; ABA, abscisic acid; C, carbon; CAT, catalase; E, transpiration rate; g_s, stomatal conductance; GPx, guaiacol peroxidase; H₂O₂, hydrogen peroxide; MDA, malonaldehyde; N, nitrogen; SOD, superoxide dismutase; UV-B, ultraviolet-B.

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ABA, a well-known stress-inducible plant hormone and growth inhibitor, has long been studied as a potential mediator for the induction of tolerance in plants (Zeevaart, 1999; Li et al., 2003a,b). It seems to play a predominant role in the conversion of environmental signals into changes in plant gene expression (Zhu, 2002). Different results have been reported regarding the effect of applied ABA, e.g., an inhibitory effect of ABA on the shoot and root growth on sunflowers (*Helianthus annuus*) seedlings (Lenzi et al., 1995), while ABA has been shown to promote the growth of excised soybean (*Glycine max* (Linn.) Merr.) roots (Yamaguchi and Street, 1977). The contradictory results suggest a complex interaction of hormone signaling in plants, because the application of a single hormone often affects many different plant processes and, conversely, different hormones can modulate the same developmental process (Gazzarrini and McCourt, 2001). At the same time, exogenous ABA has been shown to be involved in promoting drought tolerance, as detected in an experiment with exogenous ABA application to intact plants (Wang et al., 2003; Li et al., 2004) and also from the measurement of endogenous ABA levels (Li and Wang, 2003; Zhang et al., 2005). On the other hand, a considerable range of experimental evidence has shown that the physiological effects induced by salinity might be modulated by ABA. Guak and Fuchigami (2002) have shown in apple that the application of foliar ABA can enhance growth cessation and leaf senescence leading to an improved efficiency of nitrogen withdrawal from senescing leaves into woody tissues. However, the interaction of enhanced UV-B and exogenous ABA application has been only rarely studied, especially in tree species.

This study aims to analyze the interaction effects of elevated UV-B radiation and exogenous ABA on poplar, which is an economically and ecologically important tree species. Poplars have been studied in several field and greenhouse experiments to determine the effect of UV-B radiation on their growth and physiological responses (Bassman et al., 2003; Ren et al., 2006, 2007; Duan et al., 2008). In the present study, two contrasting *Populus cathayana* populations originating from low and high altitude in China were investigated. Earlier studies have demonstrated that species and populations originating from naturally high UV-B backgrounds (e.g., high altitude and low latitude) have more pronounced adaptive mechanisms than do those from low UV-B locations (e.g., low altitude and high latitude), and, thus, are less sensitive to enhanced levels of UV-B (Sullivan et al., 1992). Therefore, we hypothesized that the population originating from the high altitude site would be less affected by enhanced UV-B radiation than does the population originating from the low altitude location. On the other hand, based on previous results obtained about the interactions of exogenous ABA with other environmental factors, we supposed that exogenous ABA application may promote the growth and development of poplars when combined with enhanced UV-B radiation.

Specifically, we will answer the following questions: (1) how do enhanced UV-B radiation, exogenous ABA and their combination affect growth and physiological traits in two contrasting *P. cathayana* populations? (2) How do carbon (C) and nitrogen (N) content of leaves correlate with those in stems and roots, and how do C and N metabolism interact with each other? (3) Do the effects induced by the two stresses differ from each other in the two populations?

2. Materials and methods

2.1. Plant materials and experimental design

Dormant *P. cathayana* ramets of two populations, 0.25–0.30 m long, were collected in their natural habitats in Sichuan (Jiuzhai, 32°33'N, 101°27'E) and Qinghai (Datong, 36°15'N, 101°40'E), south-

west China. The mean altitude of the collecting areas equals 1450 and 2840 m, and the parallel average daily biologically effective radiation (UV-B_{BE}) of those sites is 4.7 and 6.5 kJ m⁻², respectively, according to a mathematical model by Madronich et al. (1995). The mean annual rainfall at the origin of the low and high altitude population is 553 and 620 mm, respectively. In early spring in 2007, healthy cuttings of uniform height collected from each population were transplanted into 101 plastic pots filled with homogenized brown soil and grown in a greenhouse at the Maoxian Field Ecological Station of the Chinese Academy of Sciences under a semi-controlled environment with a day temperature range of 12–28 °C, a night temperature range of 9–15 °C and a relative humidity range of 35–85%. To avoid the effects of rainfall, a 0.08 mm thick polyethylene film (Chengguang Chem., Inc., Sichuan, China) was employed as a cover, which could transmit 80% of the ambient solar UV-B (280–320 nm) and 85% of the visible radiation (400–700 nm). The experiment was conducted during the growing season from 15 May to 30 September 2007.

During the greenhouse experiment, 80 cuttings of each population were subjected to four treatments as follows: (a) no exogenous ABA+no enhanced UV-B, (b) exogenous ABA+no enhanced UV-B, (c) no exogenous ABA+enhanced UV-B and (d) exogenous ABA+enhanced UV-B. In each treatment, there were 20 cuttings from each population, arranged into five blocks (4 cuttings per treatment and population in each block). Moreover, the locations of the five blocks in the greenhouse were randomized every 2 weeks to eliminate block effects. In the exogenous ABA treatment, all pots were given exogenous ABA (+ABA) [(±)-*cis*, *trans*-abscisic acid, Sigma, St. Louis, MO, USA] by spraying ABA on the leaves with 10 ml of 50 μM (±) ABA per day and seedling, as described by Li et al. (2003b). No exogenous ABA treatments were sprayed with 10 ml water per day and seedling as control (-ABA). In both with and without exogenous ABA treatments, half of the cuttings were exposed to enhanced UV-B radiation as described below, while another half of the cuttings were exposed to 80% ambient UV-B radiation as a control. All plants were watered to maintain soil water near 100% field capacity and fertilized with 4 g slow release fertilizer (13% N, 10% P and 14% K) to each pot during the experiment. Measurements of various morphological, physiological and biochemical parameters were conducted within a 2-week period at the end of the experiment.

2.2. Ultraviolet-B radiation treatments

The maximum value of UV-B radiation in our experimental location at the Maoxian Ecological Station (103°53'E, 31°41'N, altitude 1816 m) at summer solstice is 10 kJ m⁻² according to a mathematical model by Madronich et al. (1995). In this experiment, square-wave UV-B supplementation systems were used. Supplemental UV-B radiation was applied over an 8-h (from 9:00 to 17:00) period centered on solar noon using UV fluorescent lamps (ranging from 275 to 380 nm with a peak at 308 nm, Beijing Electronic Resource, Inc., Beijing, China) mounted in metal frames suspended above the pots. There were two UV-B radiation levels: with and without UV-B supplementation. The plants without UV-B supplementation treatment were kept under lamps covered with polyester films, which absorb radiation below 315 nm, to exclude both UV-B and UV-C radiation. Moreover, plants grown under no supplemental UV-B were also grown under 85% of the visible radiation (400–700 nm). For the UV-B supplementation treatments, the lamps were wrapped with cellulose diacetate film, which allowed the transmission of both UV-B and UV-A radiation. The cellulose diacetate films were changed weekly. The spectral irradiance from the lamps at the plant level was determined by USB2000 Fibre Optic spectrometer (wavelength steps of 0.36 nm in the UV and visible range, Ocean Optics, Inc., Dunedin, FL, USA) with CC-3-UV

Cosine Corrector. Before the measurements, the spectrometer was calibrated with DH2000-CAL Radiometric Calibrated Deuterium Tungsten Source (210–1050 nm National Institute of Standards and Technology-traceable Calibration, Ocean Optics, Inc.). The spectral irradiance was weighed according to the generalized plant action spectrum (Caldwell, 1971) and normalized at 300 nm to obtain UV-B_{BE}. For each population in each exogenous ABA regime, one half of the plants received 80% of ambient UV-B radiation, while another half of the plants received 80% of ambient UV-B radiation plus supplemental levels of UV-B radiation. The daily UV-B supplementation was 8.0 kJ m⁻² day⁻¹ (UV-B_{BE}). The high UV-B irradiance was used to examine the limits of UV-B tolerance for the two populations. For each population, four cuttings from each block received UV-B treatment from a single lamp. These cuttings were rotated weekly in order to minimize the effects of the microenvironment. UV-B radiation was maintained at the specified levels (measured at the top of the plants) throughout the experiment by adjusting the lamp-to-plant canopy distance every 4 days.

2.3. Growth and biomass measurements

For each population and treatment, 10 plants from five blocks (2 plants from each block) were randomly selected and harvested at the end of the experiment. Height, basal diameter and leaf number were measured. The biomass samples were dried (70 °C, 72 h) to constant weight and weighed. Leaf area was measured using a Portable Laser Area Meter (CI-203, CID, Inc., USA). Specific leaf mass (SLM, the leaf dry weight divided by the projected leaf area of the whole seedling) was then calculated.

2.4. Gas exchange

The net CO₂ assimilation rate (*A*), stomatal conductance (*g_s*) and transpiration rate (*E*) were measured on the second fully expanded leaf from five seedlings in each treatment using Portable Photosynthesis Systems (Model LI-6400, LI-COR, Inc., Lincoln, NE, USA) between 8:00 and 11:00 in August. The PAR, provided by a 6400-02 LED light source, was set to 1700 μmol m⁻² s⁻¹. The flow rate of air through the sample chamber was set at 500 μmol s⁻¹, and the leaf temperature was maintained at 25 ± 0.8 °C by thermoelec-

tric coolers. The CO₂ concentration of the chamber was adjusted to 400 μl l⁻¹ with the system's CO₂ injector (Model 6400-01, LI-COR).

2.5. Quantitative analysis of abscisic acid

For each population and treatment, fully expanded leaves, young stems and roots of five plants from five blocks (one plant from each block) were randomly selected for the endogenous ABA analysis. The ABA content was analyzed as described by Li et al. (2002). The samples were weighed, frozen in liquid nitrogen and freeze-dried. Of each sample, 0.3 g FW of plant material was homogenized in 5 ml of 50 mM sodium phosphate buffer, pH 7.0, with 0.02% sodium diethyldithiocarbamate as antioxidant and 30 ng ²H₄ ABA as internal standard. The endogenous ABA level was calculated in ng g⁻¹ FW.

2.6. Measurements of the concentration of UV-absorbing compounds

Samples of fully expanded leaves were collected for the determination of UV-absorbing compounds, which were immediately extracted from fresh leaf material with an acidified methanol (methanol:water:HCl = 79:20:1) solution (Dai et al., 2004). Absorbance at 300 nm of the solution was determined using spectrophotometry (Unicam UV-330, USA). The concentration of UV-absorbing compounds was calculated on the basis of leaf area.

2.7. Enzyme assays

For each population and treatment, fully expanded leaves of five plants from five blocks (one plant from each block) were randomly selected for enzyme assays. The samples were transported to the laboratory in darkness on a moist cloth on ice at a temperature near 0 °C. The enzymes were extracted using an ice-cold mortar and pestle, with 60 mg polyvinylpyrrolidone and 1 ml of the following optimized extraction media: superoxide dismutase (SOD) guaiacol peroxidase (GPx) (100 mM K-phosphate buffer, pH 7.8, 0.1 mM EDTA and 0.1% Triton X-100). The resulting slurry was centrifuged at 12,000 × *g* for 20 min at 4 °C. The supernatants were collected and used for the enzyme activity assays.

Table 1

Growth and biomass accumulation in two contrasting *P. cathayana* populations exposed to different UV-B levels with or without exogenous BA application.

Population	Treatment	Height (cm)	Basal diameter (mm)	Total leaf area (dm ²)	Leaf number	Specific leaf mass (g dm ⁻²)	Total biomass (g)
JZ	-A-U	184.0 ± 4.3b	12.18 ± 0.48c	37.14 ± 1.41c	40.2 ± 1.6a	0.58 ± 0.02a	66.13 ± 1.87c
	+A-U	173.2 ± 1.2a	10.55 ± 0.34b	27.59 ± 1.75b	46.8 ± 1.8b	0.67 ± 0.02ab	56.33 ± 1.83b
	-A+U	164.2 ± 2.2a	9.42 ± 0.10a	20.98 ± 1.22a	47.4 ± 1.6b	0.65 ± 0.04ab	40.64 ± 0.97a
	+A+U	169.0 ± 3.6a	10.12 ± 0.34ab	21.56 ± 0.78a	48.8 ± 1.8b	0.70 ± 0.02b	43.97 ± 1.55a
<i>P</i> _(A)		0.021	0.004	0.002	0.031	0.563	0.001
<i>P</i> _(U)		0.001	0.000	0.000	0.015	0.122	0.000
<i>P</i> _(A×U)		0.340	0.143	0.004	0.143	0.033	0.060
DT	-A-U	199.6 ± 2.8b	11.69 ± 0.50b	29.50 ± 0.85c	49.0 ± 2.3a	0.69 ± 0.02a	73.79 ± 1.86d
	+A-U	200.8 ± 2.3b	11.56 ± 0.55b	28.77 ± 0.79b	49.0 ± 1.9a	0.64 ± 0.03a	65.33 ± 1.48c
	-A+U	174.6 ± 1.7a	9.67 ± 0.17a	24.87 ± 0.89a	49.2 ± 0.4a	0.72 ± 0.02b	58.89 ± 0.86b
	+A+U	176.2 ± 1.2a	9.92 ± 0.13a	22.74 ± 0.32a	53.2 ± 1.6b	0.73 ± 0.03b	54.69 ± 0.85a
<i>P</i> _(A)		0.510	0.887	0.074	0.255	0.519	0.000
<i>P</i> _(U)		0.000	0.000	0.000	0.212	0.021	0.000
<i>P</i> _(A×U)		0.925	0.628	0.366	0.255	0.206	0.129
<i>P</i> _(P)		0.000	0.591	0.650	0.001	0.028	0.000
<i>P</i> _(P×A)		0.093	0.040	0.000	0.409	0.979	0.000
<i>P</i> _(P×U)		0.002	0.650	0.001	0.323	0.693	0.006
<i>P</i> _(P×A×U)		0.393	0.214	0.019	0.063	0.360	0.015

JZ, the population from the low altitude; DT, the population from the high altitude. ANOVA: *P*_(A): ABA effect; *P*_(U): UV-B effect; *P*_(A×U): ABA × UV-B effect; *P*_(P): population effect; *P*_(P×A): population × ABA effect; *P*_(P×U): population × UV-B effect; *P*_(P×A×U): population × ABA × UV-B effect; -A-U, control; +A-U, exogenous ABA application; -A+U, enhanced UV-B treatment; +A+U, exogenous ABA and enhanced UV-B radiation condition. Values followed by the same letter in the same column are not significantly different at *P* < 0.05 level according to Duncan multiple range test. Values are means ± S.E., *n* = 10.

Table 2

Photosynthesis measurements and concentrations of UV-absorbing compounds in two contrasting *P. cathayana* populations exposed to different UV-B levels with or without exogenous ABA application.

Population	Treatment	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g _s ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	UV-absorbing compounds ($A_{300} \text{ cm}^{-2}$)
JZ	–A–U	21.15 ± 0.42c	0.89 ± 0.03d	5.80 ± 0.02c	4.56 ± 0.04a
	+A–U	18.73 ± 0.05b	0.76 ± 0.00c	5.25 ± 0.06b	4.61 ± 0.06a
	–A+U	15.64 ± 0.02a	0.34 ± 0.00a	3.98 ± 0.15a	5.32 ± 0.16b
	+A+U	18.13 ± 0.20b	0.43 ± 0.02ab	3.94 ± 0.12a	5.32 ± 0.11b
$P_{(A)}$		0.000	0.564	0.025	0.829
$P_{(U)}$		0.000	0.000	0.000	0.000
$P_{(A \times U)}$		0.004	0.000	0.048	0.806
DT	–A–U	21.82 ± 0.22c	0.76 ± 0.06c	5.38 ± 0.15c	5.04 ± 0.04a
	+A–U	18.86 ± 0.18a	0.72 ± 0.03bc	5.44 ± 0.11c	5.03 ± 0.04a
	–A+U	19.69 ± 0.39b	0.64 ± 0.04b	4.99 ± 0.12b	5.90 ± 0.08c
	+A+U	18.69 ± 0.08a	0.48 ± 0.01a	4.59 ± 0.04 ^a	5.71 ± 0.01b
$P_{(A)}$		0.000	0.011	0.120	0.821
$P_{(U)}$		0.000	0.000	0.000	0.000
$P_{(A \times U)}$		0.001	0.123	0.040	0.039
$P_{(P)}$		0.000	0.047	0.000	0.000
$P_{(P \times A)}$		0.000	0.078	0.460	0.281
$P_{(P \times U)}$		0.000	0.000	0.000	0.744
$P_{(P \times A \times U)}$		0.816	0.001	0.004	0.595

JZ, the population from the low altitude; DT, the population from the high altitude. ANOVA: $P_{(A)}$: ABA effect; $P_{(U)}$: UV-B effect; $P_{(A \times U)}$: ABA × UV-B effect; $P_{(P)}$: population effect; $P_{(P \times A)}$: population × ABA effect; $P_{(P \times U)}$: population × UV-B effect; $P_{(P \times A \times U)}$: population × ABA × UV-B effect; –A–U, control; +A–U, exogenous ABA application; –A+U, enhanced UV-B treatment; +A+U, exogenous ABA and enhanced UV-B radiation condition. Values followed by the same letter in the same column are not significantly different at the $P < 0.05$ level according to Duncan multiple range test. Values are means ± S.E., $n = 5$.

The total SOD activity was determined by measuring its ability to inhibit photochemical reduction of nitrobluetetrazolium (NBT) as described by Becana et al. (1986). The reaction mixture with a total volume of 3 ml contained 0.3 ml each of 20 μM riboflavin, 150 mM L-methionine and 600 μM NBT, and 0.1 ml of the extract. The reaction was started with the addition of riboflavin and carried out for 20 min under irradiance of 170 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ provided by a white fluorescent lamp. The absorbance at 560 nm was determined, and the extract volume causing 50% inhibition of NBT reduction was taken as one unit of activity.

Catalase (CAT) activity was measured spectrophotometrically, as previously described by Beers and Sizer (1952). CAT activity was detected in 3 ml 50 mM potassium phosphate buffer (pH 7.8) containing 3 mM H_2O_2 . One unit was defined as the decomposition of 1 mmol H_2O_2 per min per g FW.

Guaiacol peroxidase (GPx): GPx activity of the fully expanded and exposed leaves was measured as described by Chance and Maehly (1955). The reaction mixture (3.0 ml final volume) consisted of 50 μl of 10 mM guaiacol, 2.9 ml of 50 mM phosphate buffer (pH 6.0) and 50 μl 10% H_2O_2 . A 100 μl aliquot of the crude enzyme extract was then added to start the reaction. The activity of the mixture was determined spectrophotometrically at 470 nm.

2.8. Hydrogen peroxide (H_2O_2) and lipid peroxidation

The levels of H_2O_2 were measured by monitoring the absorbance of the titanium-peroxide complex at 415 nm, following the method of Brennan and Frenkel (1977). Absorbance values were calibrated to a standard curve generated using known concentrations of H_2O_2 . Leaf oxidative damage to lipids was expressed as equivalents of

Table 3

The effects of exogenous ABA, UV-B and their combination on the activities of SOD, CAT and GPx, as well as on H_2O_2 and MDA in two contrasting *P. cathayana* populations.

Population	Treatment	SOD (unit g FW ⁻¹)	CAT (mmol H_2O_2 g FW ⁻¹)	GPx (mmol H_2O_2 g FW ⁻¹)	H_2O_2 ($\mu\text{mol g FW}^{-1}$)	MDA (nmol g FW ⁻¹)
JZ	–A–U	178.27 ± 4.26a	0.68 ± 0.01a	0.10 ± 0.03a	5.39 ± 0.21a	0.57 ± 0.02a
	+A–U	193.87 ± 0.71b	0.74 ± 0.02ab	0.12 ± 0.02a	7.05 ± 0.19c	0.95 ± 0.06b
	–A+U	201.43 ± 1.09b	0.80 ± 0.02b	0.17 ± 0.02a	9.19 ± 0.25d	1.16 ± 0.08c
	+A+U	199.60 ± 0.40b	0.81 ± 0.04b	0.36 ± 0.03b	6.19 ± 0.21b	0.94 ± 0.03b
$P_{(A)}$		0.015	0.180	0.007	0.000	0.000
$P_{(U)}$		0.000	0.007	0.000	0.000	0.000
$P_{(A \times U)}$		0.005	0.309	0.002	0.015	0.154
DT	–A–U	194.67 ± 2.39a	0.85 ± 0.03a	0.17 ± 0.01a	4.95 ± 0.04a	0.65 ± 0.01a
	+A–U	211.63 ± 1.07b	1.06 ± 0.03b	0.12 ± 0.01a	5.26 ± 0.11b	0.75 ± 0.03b
	–A+U	224.33 ± 1.90c	1.29 ± 0.03c	0.19 ± 0.02a	6.15 ± 0.10c	0.87 ± 0.02c
	+A+U	224.80 ± 0.59c	1.07 ± 0.03b	0.49 ± 0.04b	5.46 ± 0.10b	0.79 ± 0.02 b
$P_{(A)}$		0.001	0.015	0.001	0.001	0.005
$P_{(U)}$		0.000	0.000	0.000	0.000	0.000
$P_{(A \times U)}$		0.001	0.000	0.000	0.069	0.676
$P_{(P)}$		0.000	0.000	0.062	0.000	0.000
$P_{(P \times A)}$		0.518	0.311	0.062	0.000	0.002
$P_{(P \times U)}$		0.023	0.006	0.063	0.005	0.013
$P_{(P \times A \times U)}$		0.868	0.000	0.063	0.060	0.227

JZ, the population from the low altitude; DT, the population from the high altitude. ANOVA: $P_{(A)}$: ABA effect; $P_{(U)}$: UV-B effect; $P_{(A \times U)}$: ABA × UV-B effect; $P_{(P)}$: population effect; $P_{(P \times A)}$: population × ABA effect; $P_{(P \times U)}$: population × UV-B effect; $P_{(P \times A \times U)}$: population × ABA × UV-B effect; –A–U, control; +A–U, exogenous ABA application; –A+U, enhanced UV-B treatment; +A+U, exogenous ABA and enhanced UV-B radiation condition. FW, fresh weight. Values followed by the same letter in the same column are not significantly different at the $P < 0.05$ level according to Duncan multiple range test. Values are means ± S.E., $n = 5$.

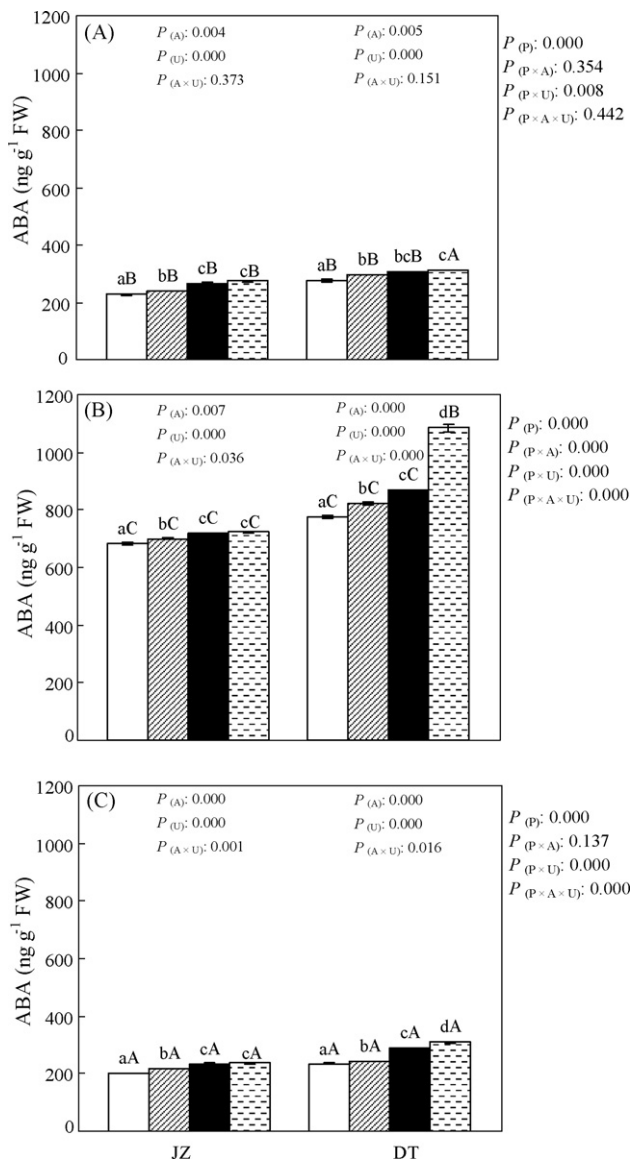


Fig. 1. The effects of exogenous ABA, enhanced UV-B and their combination on the concentrations of endogenous ABA in stems (A), leaves (B) and roots (C) in two contrasting *P. cathayana* populations. Values are means \pm S.E. Values followed by the same small letters above the bars are not significantly different at the $P < 0.05$ level according to the Duncan multiple range test. Different capital letters above the bars refer to significant differences between the organs in the same population and treatment at the $P < 0.05$ level according to the Duncan multiple range test. JZ, the population from the low altitude; DT, the population from the high altitude. FW: fresh weight. Treatments: no exogenous ABA + no enhanced UV-B application (white area); exogenous ABA + no enhanced UV-B application (lined area); no exogenous ABA + enhanced UV-B application (black area); exogenous ABA + enhanced UV-B application (stippled area). *P*-Values and significance levels (ANOVA): $P_{(A)}$, ABA effect; $P_{(U)}$, UV-B effect; $P_{(A \times U)}$, ABA \times UV-B interaction effect; $P_{(P)}$, population effect; $P_{(P \times A)}$, population \times ABA effect; $P_{(P \times U)}$, population \times UV-B effect; $P_{(P \times A \times U)}$, population \times ABA \times UV-B effect.

malonaldehyde (MDA) contents. About 0.5 g leaf segments were homogenized in 10 ml of 10% trichloroacetic acid (TCA), and centrifuged at $12,000 \times g$ for 10 min. After that, 2 ml 0.6% thiobarbituric acid (TBA) in 10% TCA was added to an aliquot of 2 ml from the supernatant. The mixture was heated in boiling water for 30 min, and then quickly cooled in an ice bath. After centrifugation at $10,000 \times g$ for 10 min, the absorbance of the supernatant at 450, 532 and 600 nm was determined. The MDA content was calculated according to Hodges et al. (1999).

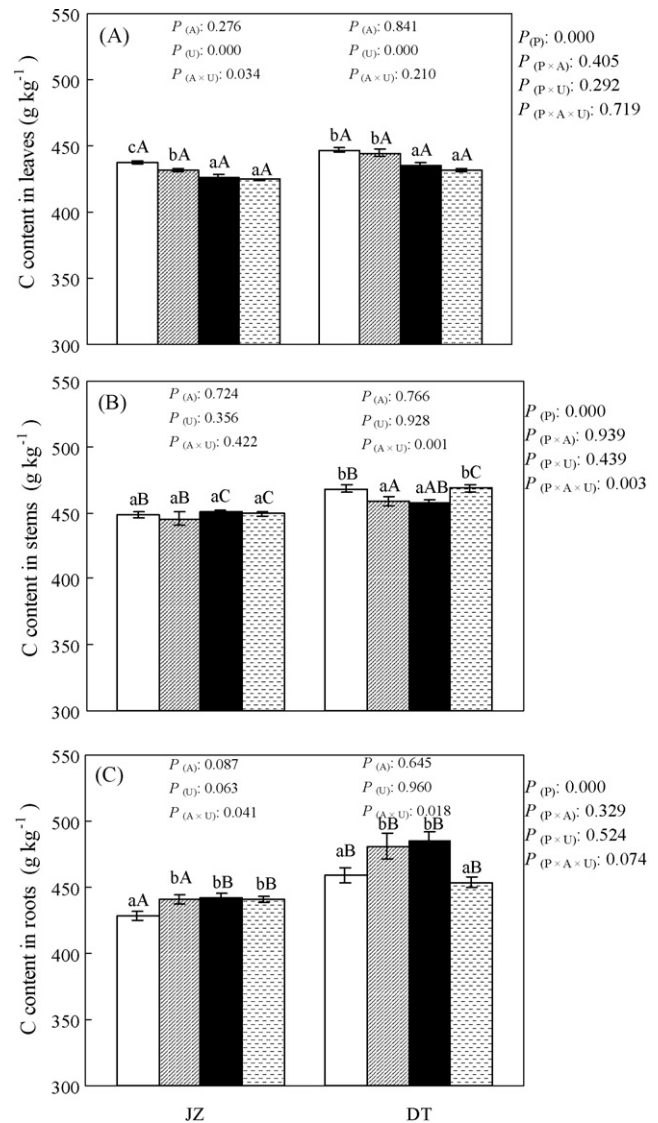


Fig. 2. The effects of exogenous ABA, enhanced UV-B and their combination on carbon contents in leaves (A), stems (B) and roots (C) in two contrasting *P. cathayana* populations. Values are means \pm S.E. Values followed by the same small letters above the bars are not significantly different at the $P < 0.05$ level according to the Duncan multiple range test. Different capital letters above the bars refer to significant differences between the organs in the same population and treatment at the $P < 0.05$ level according to the Duncan multiple range test. JZ, the population from the low altitude; DT, the population from the high altitude. Treatments: no exogenous ABA + no enhanced UV-B application (white area); exogenous ABA + no enhanced UV-B application (lined area); no exogenous ABA + enhanced UV-B application (black area); exogenous ABA + enhanced UV-B application (stippled area). *P*-Values and significance levels (ANOVA): $P_{(A)}$, ABA effect; $P_{(U)}$, UV-B effect; $P_{(A \times U)}$, ABA \times UV-B interaction effect; $P_{(P)}$, population effect; $P_{(P \times A)}$, population \times ABA effect; $P_{(P \times U)}$, population \times UV-B effect; $P_{(P \times A \times U)}$, population \times ABA \times UV-B effect.

2.9. Determination of carbon and nitrogen contents

Samples of leaves, stems and roots were ground and passed through a 20 mesh screen after being dried at 80°C for 36 h. The total contents of nitrogen (N) and organic carbon (C) were determined by the semi-micro Kjeldahl method and the rapid dichromate oxidation technique (Nelson and Sommers, 1982), respectively. The total C–N ratio (C/N) was calculated as an estimate for long-term nitrogen use efficiency (Livingston et al., 1999).

2.10. Statistical analyses

Statistical analyses were performed with the statistical software package SPSS, version 11.0. Three-way analyses of variance (ANOVA) were used to determine the significance of the effects of population, the interaction of population \times ABA, the interaction of population \times UV-B, as well as the interaction of population \times ABA \times UV-B. Two-way ANOVAs were conducted to evaluate the significance of the effects of ABA, UV-B and their interaction in each population. One-way ANOVAs were used to determine differences between the populations under each treatment. The means were compared by the Duncan's test at a significance level of $P < 0.05$. Bivariate correlation coefficients were applied to determine the relationships between the C content, N content and C/N ratio in leaves, stems and roots.

3. Results

3.1. The effects of enhanced UV-B and exogenous ABA on growth and biomass accumulation

In both populations, height, basal diameter, total leaf area and total biomass all significantly decreased when the cuttings were exposed to enhanced UV-B radiation alone (Table 1). In contrast, the exogenous ABA application induced a significant decrease in height, basal diameter and total leaf area, and a significant increase in leaf number only in the low altitude population, but had no effect on these parameters in the high altitude population. SLM was not affected by exogenous ABA alone in either population, while it was significantly increased by enhanced UV-B radiation in the high altitude population (Table 1). Significant population differences were found in height, leaf number, total biomass and SLM. In addition, a significant interaction between UV-B and ABA was detected in total leaf area and SLM in the low altitude population.

3.2. The effects of enhanced UV-B and exogenous ABA on gas exchange and on the concentration of UV-absorbing compounds

Significant decreases in A , g_s and E , and a significant increase in UV-absorbing compounds were observed in both populations under enhanced UV-B radiation. Exogenous ABA induced a significant reduction in A in both populations, in g_s in the high altitude population and in E in the low altitude population. Significant population differences were found in all four parameters. The UV-B \times ABA interaction effect was found in A and E in both populations, in UV-absorbing compounds in the high altitude population and in g_s in the low altitude population (Table 2).

3.3. The effects of enhanced UV-B and exogenous ABA on enzyme activities, H_2O_2 and lipid peroxidation (MDA content)

In both populations, the activities of SOD, and the GPx, H_2O_2 and MDA contents all showed a significant increase in response to UV-B and exogenous ABA alone, while an even more pronounced increase occurred in GPx when the two stresses were applied together (Table 3). The activity of CAT was significantly affected by enhanced UV-B alone in both populations, while it was significantly increased by ABA only in the high altitude population. Population differences were significant for the activities of SOD and CAT, MDA and H_2O_2 but not on the activities of GPx. A significant interaction between UV-B and ABA was observed in the activities of SOD and GPx in both populations, in the activity of CAT in the high altitude population and in H_2O_2 in the low altitude population.

3.4. The effects of enhanced UV-B and exogenous ABA on endogenous ABA concentrations in different organs

Endogenous ABA concentrations in stems, leaves and roots were all significantly accumulated by exogenous ABA, UV-B and their combination (Fig. 1). Compared with leaves, the endogenous ABA concentrations were lower in stems and roots in both populations under all treatments. The supply of exogenous ABA and enhanced UV-B together caused a greater increase in endogenous ABA in leaves and roots, especially in the high altitude population. Compared with the low altitude population, the high altitude population showed a higher level of endogenous ABA concentration in all three organs. In addition, the ABA \times UV-B interaction significantly affected the endogenous ABA concentrations of leaves and roots in both populations (Fig. 1).

3.5. The effects of enhanced UV-B and exogenous ABA on C content, N content and C/N ratio among different organs

In both populations, the leaf C content significantly decreased when the cuttings were exposed to enhanced UV-B radiation under two exogenous ABA levels (Fig. 2). Each stress supplied alone decreased the stem C content in the high altitude population, while this decrease was balanced by increased amounts of C partitioned to roots. When the two stresses were combined, both the stem and root C content were recovered to the control level. There were significant population differences in the C contents of all organs. Significant ABA \times UV-B interaction effects were detected in root C in both populations, in stem C in the high altitude population and in leaf C in the low altitude population. Across all treatments, leaf C content strongly correlated with C content in stems and roots, and stem C content significantly correlated with root C content (Table 4).

Table 4

Correlation coefficients between C content, N content and C/N ratio in leaves, stems and roots.

	C_L	C_S	C_R	$(C/N)_L$	$(C/N)_S$	$(C/N)_R$	N_L	N_S	N_R
C_L	1.000								
C_S	0.384*	1.000							
C_R	0.502**	0.372*	1.000						
$(C/N)_L$	0.278	0.469***	0.054	1.000					
$(C/N)_S$	0.291	0.606**	-0.082	0.581**	1.000				
$(C/N)_R$	0.193	0.492**	0.481***	0.338*	0.472***	1.000			
N_L	-0.032	-0.398*	0.072	-0.962**	-0.542**	-0.297	1.000		
N_S	-0.207	-0.433***	0.203	-0.602**	-0.968**	-0.384*	0.583**	1.000	
N_R	0.143	-0.277	0.103	-0.329*	-0.558**	-0.818**	0.371*	0.543**	1.000

C_L , carbon content in leaves; C_S , carbon content in stems; C_R , carbon content in roots; $(C/N)_L$, C/N ratio in leaves; $(C/N)_S$, C/N ratio in stems; $(C/N)_R$, C/N ratio in roots; N_L , nitrogen content in leaves; N_S , nitrogen content in stems; N_R , nitrogen content in roots.

* A significant correlation between variables at $P < 0.05$.

** A significant correlation between variables at $P < 0.001$.

*** A significant correlation between variables at $P < 0.01$.

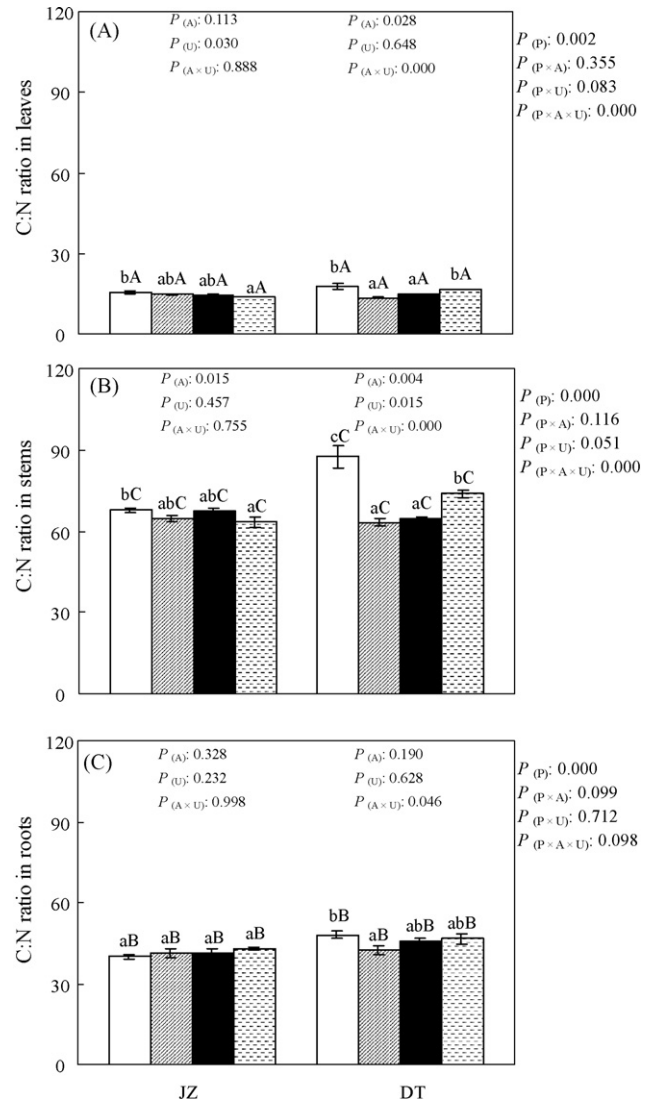
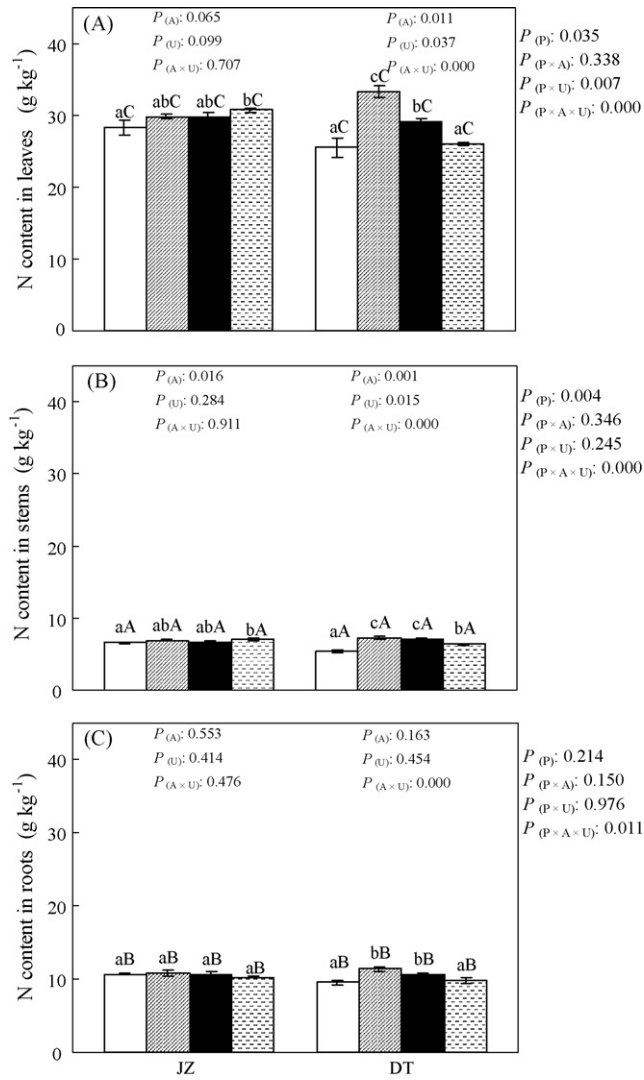


Fig. 3. The effects of exogenous ABA, enhanced UV-B and their combination on nitrogen contents in leaves (A), stems (B) and roots (C) in two contrasting *P. cathayana* populations. Values are means \pm S.E. Values followed by the same small letters above the bars are not significantly different at the $P < 0.05$ level according to the Duncan multiple range test. Different capital letters above the bars refer to significant differences between the organs in the same population and treatment at the $P < 0.05$ level according to the Duncan multiple range test. JZ, the population from the low altitude; DT, the population from the high altitude. Treatments: no exogenous ABA + no enhanced UV-B application (white area); exogenous ABA + no enhanced UV-B application (lined area); no exogenous ABA + enhanced UV-B application (black area); exogenous ABA + enhanced UV-B application (stippled area). P -Values and significance levels (ANOVA): $P_{(A)}$, ABA effect; $P_{(U)}$, UV-B effect; $P_{(A \times U)}$, ABA \times UV-B interaction effect; $P_{(P)}$, population effect; $P_{(P \times A)}$, population \times ABA effect; $P_{(P \times U)}$, population \times UV-B effect; $P_{(P \times A \times U)}$, population \times ABA \times UV-B effect.

Fig. 4. The effects of exogenous ABA, enhanced UV-B and their combination on the C/N ratio in leaves (A), stems (B) and roots (C) in two contrasting *P. cathayana* populations. Values are means \pm S.E. Values followed by the same small letters above the bars are not significantly different at the $P < 0.05$ level according to the Duncan multiple range test. Different capital letters above the bars refer to significant differences between the organs under the same population and treatment at the $P < 0.05$ level according to the Duncan multiple range test. JZ, the population from the low altitude; DT, the population from the high altitude. Treatments: no exogenous ABA + no enhanced UV-B application (white area); exogenous ABA + no enhanced UV-B application (lined area); no exogenous ABA + enhanced UV-B application (black area); exogenous ABA + enhanced UV-B application (stippled area). P -Values and significance levels (ANOVA): $P_{(A)}$, ABA effect; $P_{(U)}$, UV-B effect; $P_{(A \times U)}$, ABA \times UV-B interaction effect; $P_{(P)}$, population effect; $P_{(P \times A)}$, population \times ABA effect; $P_{(P \times U)}$, population \times UV-B effect; $P_{(P \times A \times U)}$, population \times ABA \times UV-B effect.

In relation to the N content, significant increases were detected in all organs in the high altitude population, as induced by exogenous ABA and enhanced UV-B, separately (Fig. 3). In comparison, there was hardly any effect on leaf and stem N in response to ABA and UV-B alone in the low altitude population. When exposed to the combination of two stresses, the leaf and stem N in the low altitude population recovered to the control level. Compared with leaves, the N contents were lower in stems and roots in both populations under all treatments. Significant population differences were detected in the leaf and stem N contents, but not in root N content. There was a significant ABA \times UV-B interaction in the N contents of all organs in the high altitude population. A significant correlation was found in N content between the three organs among all treat-

ments. Besides, leaf and stem N contents significantly correlated with stem C content (Table 4).

The ratio C/N in roots was unaffected by exogenous ABA and enhanced UV-B alone, and by the combination of both factors in the low altitude population. However, under the combination of the two stresses, the C/N ratio of leaves and stems significantly decreased (Fig. 4). In the high altitude population, exogenous ABA and enhanced UV-B alone induced a significant decrease in the C/N ratio of leaves and stems. In comparison, a significant reduction in the C/N ratio of roots was observed only under exogenous ABA. The origin of population affected the C/N ratio of all organs. In addition, a significant ABA \times UV-B interaction was observed in the C/N ratio of all organs in the high altitude population.

4. Discussion

4.1. The effects on biomass accumulation, gas exchange and UV-absorbing compounds concentration

The enhanced UV-B treatment caused a stronger effect than did the exogenous ABA application on the main growth parameters of *P. cathayana* seedlings. Height, basal diameter, total leaf area and total biomass all significantly decreased when the cuttings were exposed to enhanced UV-B radiation alone. Comparable results have been obtained in previous studies (Ren et al., 2006, 2007; Duan et al., 2008). By contrast, the exogenous ABA application hardly affected those parameters in the high altitude population, while a significant decrease in height, basal diameter and total leaf area, and a significant increase in leaf number were observed in the low altitude population. These differences indicate that the two contrasting populations possess different responses and that the high altitude population is more responsive to the exogenous ABA stress. On the other hand, an increase in SLM induced by enhanced UV-B radiation was observed only in the high altitude population. This result corroborates with previous studies where the leaves of oak saplings (*Quercus robur*) from an outdoor experiment, and leaves of *Populus trichocarpa* and *Q. rubra* growing in a greenhouse, exposed to a UV-B treatment, had thicker and smaller leaves relative to the leaves of plants exposed to ambient levels of radiation (Nagel et al., 1998). Thicker leaves enable plants to attenuate more UV-B radiation and, hence, to protect palisade layers from the deleterious effects of irradiation (Newsham et al., 1998). This protective response occurring under enhanced UV-B could be related to the amount of UV-absorbing compounds. Increased production of UV-absorbing compounds is the most widely reported plant response to enhanced UV-B radiation, and it has been observed in several tree species (Yang et al., 2005; Ren et al., 2006; Lu et al., 2007; Duan et al., 2008). In the present study, the high altitude population had thicker leaves and a higher amount of UV-absorbing compounds in comparison with the low altitude population, which suggests that it is more tolerant to enhanced UV-B.

The exposure of *P. cathayana* cuttings to increased UV-B radiation impairs the main processes of photosynthesis, including the stomatal diffusion of CO₂ into the leaf and CO₂ assimilation, as proposed by Teramura and Sullivan (1994) for other terrestrial plants. Meanwhile, as reported by Borzenkova et al. (2001), there is a higher assimilate export from *P. cathayana* leaves at higher ABA concentrations. This phenomenon suggests that ABA participates in activating the processes of assimilate transport, as proposed by Zeevaert (1999). In addition, numerous studies in several plant species have reported that ABA plays a critical role in the negative regulation of stomata (Dodd, 2003) and has a predictable negative relationship on g_s. However, modes of stomatal behaviour are too complex to be explained by ABA alone (Liang and Zhang, 1999), and other phytohormones have also been found to participate in the responses. CKs appear to have an antagonistic relationship with ABA in mediating physiological processes (Cowan et al., 1999), and evidence is increasing that they play a role in the regulation of stomata and gas exchange (Dodd, 2003). These results may well explain why the enhanced UV-B induced more significant effects on the gas exchange of the two poplar populations than did the exogenous ABA application.

4.2. The effects on antioxidant systems and endogenous ABA concentrations

Simultaneous enhanced UV-B radiation and exogenous ABA stresses induced oxidative stress in *P. cathayana* seedlings, as shown by the accumulation of H₂O₂ and lipid peroxide and by the increase in the MDA content, similarly as reported for other species (Correia

et al., 2006). Removing H₂O₂ requires the effects of several antioxidant enzymes cooperating with each other. Since the capacity and responsiveness of antioxidant systems contribute to the ability of plants to withstand oxidative stresses, inquisition of these protective measures has been considered being especially important for the acclimation of trees to fluctuating environmental conditions (Polle, 1996). In our plant system, an increase in the activities of SOD and CAT was evident after UV-B irradiation alone as well as in combination with ABA. Especially GPx activities increased significantly under the combination of enhanced UV-B and exogenous ABA in comparison with no change observed under a single stress. Compared with the low altitude population, the high altitude population exhibited higher activities of SOD and CAT, and lower MDA and H₂O₂ contents, which increased its ability to scavenge free radicals induced by two stresses and resulted in a less serious membrane damage.

An important function of endogenous ABA that has been revealed in recent studies in maize (*Zea mays* L.), tomato (*Lycopersicon esculentum* Mill.), and *Arabidopsis*, is to limit ethylene production. Meanwhile, the restriction of ethylene production may be a widespread function of ABA, which may often function to maintain rather than to inhibit plant growth (Sharp, 2002). Nevertheless, the regulation of endogenous ABA content is complex, involving ABA synthesis as well as ABA catabolism and conjugation (Zeevaert, 1999). A homeostasis mechanism involved in ABA metabolism may prevent the excess ABA accumulation and match the ABA content to the type and severity of the stress to which the plant is exposed. In our study, the endogenous ABA concentration of stems, leaves and roots all accumulated significantly as a result of exogenous ABA application, enhanced UV-B radiation and the two stresses together. In addition to the homeostasis mechanism, the most straightforward explanation accounting for increased endogenous ABA content in response to exogenous ABA is that the genes in question are involved in a feedback mechanism that regulates the turnover of ABA (Verslues and Bray, 2006). The high altitude population accumulated much more endogenous ABA in the three organs in all treatments in comparison with the low altitude population, which indicates that the high altitude population possesses a better ability to respond to these two stresses. In addition, compared with leaves, the endogenous ABA concentration was lower in stems and roots in both populations, similarly as previously observed in *Picea asperata* (Duan et al., 2007). As is well known, endogenous ABA produced in roots has been implicated in a series of studies as the likely chemical substance for root-to-shoot signal. Identical with the opinion that roots can be the primary location of ABA production, while leaves accumulate and metabolize ABA originating from other organs. On the other hand, the coordination of ABA with the xylem sap pH and ions has been proposed to regulate ABA distribution among different compartments of plant tissues (Davies et al., 2002), which also supplies evidence to this phenomenon. Moreover, our results are supported by earlier studies suggesting that genes that are responsible for stress-induced ABA production also seem to express differently in leaves and roots (Jia et al., 2002).

4.3. The effects on C content, N content and C/N ratio

Carbohydrate accumulation in leaves during photosynthesis is a common phenomenon that can be enhanced by a low sink demand (Neales and Incoll, 1968). As UV-B treated plants are known to have a tendency to have a lower sink capacity (Correia et al., 2000), the observed decrease in the C content of leaves induced by UV-B indicates that the main response is mediated by a lower net photosynthetic rate. On the other hand, two stresses supplied separately were found to decrease stem C content, while the decrease was balanced by increased C partitioned to roots in the high alti-

tude population. It can be explained by the “functional equilibrium” model of [Poorter and Nagel \(2000\)](#) that under stress conditions the plant may “rank” the different organs and C is partitioned more to roots to aid in the osmotic function and to increase capability to overcome stresses. Thus, significant correlations observed in our study between C content, N content and also between C and N in the three organs are logical. Nutrients can be freely distributed among different organs according to sink demand, and our results may be consistent also with the “functional equilibrium” model of [Poorter and Nagel \(2000\)](#). Compared with leaves, the N content of stems and roots was significantly lower in all treatments in both populations. We believe that this might be an allometric effect, since larger stems usually have a lower N content. In addition, the C/N ratio has been used to estimate the long-term nitrogen use efficiency (NUE) ([Livingston et al., 1999](#)), and [Martin et al. \(2002\)](#) have suggested that the C/N ratio may be an important signal for the control of gene expression in plants. In our study, the observed decrease in the C/N ratio in leaves and stems under enhanced UV-B and exogenous ABA in the high altitude population indicates that the two stresses decrease the long-term NUE of poplar cuttings due to the reduction of the C content and the increase of the N content, and that they may also induce parallel changes in the metabolite levels following the C/N ratio as an important signal of gene expression.

Interestingly, leaf and stem N contents correlated significantly with stem C content in all treatments in our study. This may be because C and N metabolisms are linked by shared intermediates and products, and also by a complex network of cross-talking signal pathways, which are better documented for shoots than for roots ([Coruzzi and Bush, 2001](#)). It is known that the C and N status of the plant is used to regulate gene expression and enzyme activity, and a further layer of complexity is introduced by the fact that carbohydrate metabolism is also implicated in the control of N metabolism, and *vice versa* ([Miller and Cramer, 2004](#)). For example, the domination of the enzymes NR and PEPc (in the main points of regulation in inorganic N reduction and assimilation) greatly contributes to the integration of C and N metabolism: some additional C may be taken up by the roots in the form of organic N or from other sources, and some C is assimilated through the activity of PEPc and other carboxylating enzymes, as also proposed by [Miller and Cramer \(2004\)](#). In addition to the C–N cross-talk discussed above, a genetic analysis by [Coruzzi and Bush \(2001\)](#) in Glc-insensitive *Arabidopsis* mutants has shown that C-metabolite signaling is also linked to abscisic acid response pathways ([Laby et al., 2000](#)), but the mechanism is uncovered to date.

In conclusion, enhanced UV-B radiation significantly affected the growth and physiological traits of *P. cathayana*. However, the overall effect of an exogenous ABA treatment was not as pronounced as expected, and the interactions between enhanced UV-B and exogenous ABA were not always significant. The plants from the low altitude population appeared to represent stress-sensitive ecotypes, while the plants from the high altitude population were more stress-tolerant. Therefore, it is suggested that *P. cathayana* seedlings from the high altitude population with qualities of stress-tolerant ecotype are preferable material for reforestation. These plants are better adjusted to the predicted climate changes as well as to simultaneous changes in the level of plant hormones.

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