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Influence of water stress and low irradiance on morphological and physiological characteristics of *Picea asperata* seedlings

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Abstract

The combined effects of water stress (WS) and low irradiance (LI) on growth, photosynthesis, osmotic adjustment, and lipid peroxidation were studied in dragon spruce (*Picea asperata* Mast.) seedlings grown under two water treatments (well watered, 100 % of field capacity, and water stressed, 30 % of field capacity) and two irradiances (HI, 100 % of full sunlight and LI, 15 % of full sunlight). WS reduced growth, chlorophyll (Chl) *a* and *b* contents, net photosynthetic rate, transpiration rate, stomatal conductance, and effective quantum yield of photosystem 2 (*Y*) but increased free proline and malondialdehyde contents. LI increased Chl contents and decreased *Y*, photochemical quenching (q_P), and non-photochemical quenching (q_N) under both water treatments. Hence the seedlings in the understory were more sensitive to drought than to LI.

Additional key words: carotenoids; chlorophyll fluorescence; growth; photosynthesis; photosystem 2; quantum yield; root/shoot ratio; specific leaf area; stomatal conductance; transpiration.

Introduction

The interactive effects of drought and shade on woody seedlings have often been reported (Aranda *et al.* 2005, Climent *et al.* 2006). However, there are many divergent questions asking whether a given drought has a stronger impact on seedling growth in deep shade or at higher irradiance. The most influential *Trade-off* hypothesis predicted that drought has a stronger impact on seedlings grown in deep shade than on those in higher irradiance (Sack and Grubb 2002). However, there was increasing evidence that drought has a weaker impact on plants in deep shade (Holmgren 2000). Indeed, under drought condition moderate shade (down to 20–40 %) often improved plant performance relative to plants in full sunlight (Rousset and Lepart 2000).

Water stress (WS) resulted in the reduction of shoot height, basal diameter, chlorophyll (Chl) content, and photosynthetic rate in Norway spruce (Wallin *et al.* 2002). Colom and Vazzana (2003) found that drought also affected photosystem 2 (PS2) photochemistry. In addition, higher content of free proline, an important osmolyte, was reported in drought-induced seedlings (Ashraf and Iram 2005), and drought brought damage of cell membrane through malondialdehyde (MDA) content (Tewari *et al.* 2005). Similarly, radiant energy may be an important limiting resource in community structure (Gefferson and Pennacchio 2005). Low irradiance has complex effects on plants through photosynthesis and morphological plasticity. For example, it can urge plant capturing more photon energy to improve photosynthetic rate by increasing Chl content. In turn, enhanced shoot/root ratio may be observed in low irradiance-adapted plants (Jefferson and Pennacchio 2005).

In this study, we made a greenhouse experiment to test the changes in morphological and physiological

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Abbreviations: AOS – active oxyen species; Car – carotenoid; Chl – chlorophyll; *E* – transpiration rate; F_v/F_m – maximal photochemical efficiency of photosystem 2; FC – field capacity; FM – fresh mass; g_s – stomatal conductance; MDA – malondialdehyde; P_N – net photosynthetic rate; PS – photosystem; q_N – non-photochemical quenching; q_P – photochemical quenching; RSR – root/shoot ratio; SLA – specific leaf area; *Y* – effective quantum yield of PS2.

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characteristics of *Picea asperata* seedlings subjected to WS under shade (LI) or sunny (HI) conditions. A question arose whether WS has a stronger impact on seedlings grown at LI (15 % of full sunlight) than on those grown at HI or not.

Dragon spruce (*Picea asperata* Mast.) is one of the dominant conifers of the sub-alpine forested areas in western Sichuan provinces of China (Liu 2002). It is widely used for artificial regeneration after clear-felling of natural sub-alpine coniferous forests because it is relatively easy to collect the seeds and raise the seedlings. We wanted to give a better theoretic understanding of regeneration progress affected by interaction of the *in situ* WS and LI in sub-alpine coniferous forest.

Materials and methods

Plants and experiment design: Two-years-old and healthy *Picea asperata* seedlings of uniform size from 301 forest farm (102º50′E, 31º50′N, 2 800 m a.s.l.) in Miyaluo area, Aba Tibetan and Qiang People Autonomous Prefecture, China were selected based on plant height and fresh mass (FM). The average plant height, stem basal diameter, length of root, and FM were 7.35±0.16 cm, 1.91±0.17 mm, 7.88±0.16 cm, and 1.76±0.11 g. Each seedling was transplanted to 1 500 cm^3 plastic pots filled with homogenized soil. They (one per pot) were grown in a naturally lit greenhouse under the semi-controlled environment (only shelter from rainfall) with a day temperature of 18–32 °C and a night temperature of 10–16 °C at local forest farm from 10 May to 10 September 2006.

The experimental layout was completely randomized with two fixed factors: two water treatments (C = well watered, 100 % of field capacity; WS, 30 % of field capacity) and two irradiances (HI, 100 % of full sunlight; LI, 15 % of full sunlight). Adjustment of the intended water contents was accomplished on a mass basis every two days. The experiment consisted of four treatments. Each treatment had 4 blocks and each block had 10 pots. Seedlings at LI were grown in shade house covered with neutral shade clothes, which had a neutral effect on radiation quality. All current-year needles from the four treatments were used for the measurements of morphological and physiological parameters.

Growth and relative water content (RWC): 8 seedlings from each treatment were sampled randomly. Needles, shoots, and roots were separated. Shoot height and stem diameter were measured. For dry mass (DM) determination samples were oven dried (65 °C for 48 h) to constant mass and then weighed. Root/shoot ratio was then calculated. Thirty current-year needles per seedling were scanned by a scanner (*Microtek Phantom 3500*, Shanghai, China) and then analyzed with an *UTHSCSA* Image Tool analysis system (University of Texas Health Science Center, San Antonio, Texas, USA) to determine leaf area. We estimated the specific leaf area (SLA) as the ratio between leaf area and leaf biomass.

Needle relative water content (RWC) was investigated as follows: 30 needles from the first whirl of branches were chosen, cleaned, and then their FM was recorded. After these weighed needles were immersed in distilled water for 24 h in dark, the turgid mass (TM) was also recorded. At last, these dipped needles were oven-dried at 65 °C for 24 h to a constant DM. RWC was calculated as RWC [%] = (FM – DM)/(TM – DM)×100.

Photosynthetic pigments, gas exchange, and Chl fluorescence: 0.2 g of current-year needles were ground in 80 % acetone and the homogenate was centrifuged at 1 000×*g* for 5 min. Absorbance of the supernatant was determined at 663, 645, and 470 nm using a spectrophotometer *UNICAM UV330* (*Thermo Spectronic*, USA). Chl and total carotenoid (Car) contents were calculated according to Lichtenthaler (1987) on DM basis.

With a portable photosynthesis system (*LI-6400,* Lincoln, NE, USA) in open-circuit mode, net photosynthesis rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were determined at 10:00–12:00 on a cloudless day. Photosynthetic photon flux density (PPFD) and needle temperature were maintained at 1 000±50 µmol m^{-2} s^{-1} and 25±1.5 °C, respectively. Gas exchange parameters were measured by enclosing 14–20 current-year needles in the chamber of the photosynthesis system. Results were expressed on a projected leaf area basis. The needles used for gas exchange measurements were collected and their projected area was determined using a scanner and an *UTHSCSA* Image Tool analysis system (University of Texas Health Science Center, San Antonio, Texas, USA).

Chl fluorescence was measured on intact needles with a portable *PAM-2100* fluorometer (*Walz*, Effeltrich, Germany) following the procedures of Streb *et al.* (1998). Needles were kept in clip curettes for dark adaptation for 30 min before the measurements. Fluorescence was excited with a saturating beam of "white light" (PPFD = 8 000 µmol m^{-2} s^{-1}, 1.0 s) to determine the maximum (F_m) and the minimum (F_0) fluorescence of the dark-adapted sample. Maximal photochemical efficiency of PS2 was estimated from the variable to maximum fluorescence ratio F_v/F_m defined as $(F_m - F_0)/F_m$. Effective quantum yield of PS2 (Y) was defined as $F_v'/F_m' = (F_m' - F_0')/F_m'$. The photochemical ($q_P$) and non-photochemical (q_N) quenchings were determined following Schreiber *et al.* (1986).

Free proline and lipid peroxidation: Free proline was extracted from 0.5 g of current-year needles in 3 % (m/v) aqueous sulphosalicylic acid and its content was estimated by using ninhydrin reagent and calculated from (determination was done by using calibration curve of Bates *et al.* 1973).

Lipid peroxidation was determined by estimating the MDA content with slight modification (Heath and Packer 1968) using the thiobarbituric acid (TBA) reaction. 1 g of current-year needles were homogenized with 5 cm^3 of 20 % trichloroacetic acid (TCA) and homogenate was centrifuged at 3 500×*g* for 20 min. 2 cm^3 of 20 % TCA containing 0.5 % TBA and 100 mm^3 of 4 % butylated hydroxytoluene in ethanol were added to 2 cm^3 of the supernatant. The mixture was heated at 95 °C for 30 min and then quickly cooled on ice. The resulting mixture the absorbance of fraction with toluene aspired at 520 nm was centrifuged at 10 000×*g* for 15 min. The content of MDA was calculated from the absorbance at 532 nm (correction was done by using extinction coefficient of 155 $\mathrm{mM}^{-1}\,\mathrm{cm}^{-1}$).

Statistical analyses were conducted with a *SPSS13.0 for Windows* statistical software package. All data were subjected to an analysis of variance that tested water, irradiance, and water×irradiance interactions. Comparisons with $p<0.05$ were considered significantly different. We used Duncan comparisons at a threshold $p=0.05$ to test the differences between the treatments.

Results

Effect of WS and LI on growth parameters and RWC: WS significantly decreased shoot height, basal diameter, and total biomass under both irradiances ($p<0.001$). However, WS significantly reduced root/shoot ratio (RSR) and SLA in HI seedlings, and increase in RSR and SLA was found under WS and LI treatments. Moreover, LI decreased shoot height, basal diameter, and RSR. The effect of irradiance×water was mainly found in the basal diameter, total biomass, RSR, and SLA ($p<0.05$, Table 1). WS significantly decreased RWC in HI seedlings, whereas there was not a prominent drought-induced decrease in RWC in LI seedlings (Table 1).

Table 1. Growth parameters and needle relative water content of *Picea asperata* seedlings grown under high (HI) and low (LI) irradiances submitted to two watering regimes. Means±S.E. *Different letters in the same row* indicate significant differences between treatments ($p<0.05$, $n = 8$). C – control, F_{I} – irradiance effect, F_{W} – water effect, $F_{\mathrm{I\times W}}$ – interactive effect of water and irradiance.

Parameter	HI		LI		F_{I}	F_{W}	$F_{\mathrm{I\times W}}$
	C	WS	C	WS			
Height [cm]	12.44±0.78a	10.80±0.77b	11.95±0.49a	9.90±0.94c	0.017	0.000	0.478
Basal diameter [mm]	2.54±0.24a	1.98±0.22bc	2.03±0.16b	1.80±0.18c	0.000	0.000	0.027
Biomass [g]	1.81±0.23a	0.52±0.21d	0.70±0.09b	0.55±0.10c	0.000	0.000	0.000
RSR	0.92±0.17a	0.28±0.11b	0.26±0.04b	0.27±0.08b	0.000	0.000	0.000
SLA [$\mathrm{m}^2\,\mathrm{kg}^{-1}$]	10.61±0.54a	8.03±0.17c	7.90±0.53c	9.32±0.46b	0.026	0.056	0.000
RWC [%]	81.63±3.45a	67.94±1.62b	85.27±3.37a	77.31±3.42ab	0.087	0.012	0.425

Table 2. Contents of photosynthetic pigments in needles of *Picea asperata* seedlings grown under high (HI) and low (LI) irradiances subjected to two water treatments. Means±S.E. *Different letters in the same row* indicate significant differences between treatments ($p<0.05$, $n = 3$). C – control, Chl – chlorophyll, Car – carotenoids, F_{I} – irradiance effect, F_{W} – water effect, $F_{\mathrm{I\times W}}$ – interactive effect of water and irradiance.

Parameter	HI		LI		F_{I}	F_{W}	$F_{\mathrm{I\times W}}$
	C	WS	C	WS			
Chl *a* [g kg^{-1}(DM)]	0.61±0.01b	0.46±0.10c	0.87±0.08a	0.71±0.09b	0.000	0.008	0.848
Chl *b* [g kg^{-1}(DM)]	0.08±0.01b	0.06±0.02b	0.16±0.02a	0.13±0.02a	0.000	0.071	0.716
Chl *a/b*	7.64±0.37a	8.22±2.61a	5.56±0.29b	5.57±1.38b	0.026	0.744	0.748
Chl *a*+*b* [g kg^{-1}(DM)]	0.69±0.03b	0.52±0.06c	1.03±0.02a	0.84±0.04b	0.000	0.007	0.801
Car [g kg^{-1}(DM)]	2.44±0.11a	1.55±0.27b	2.59±0.09a	1.85±0.29b	0.103	0.000	0.573

Effect of WS and LI on pigment contents: In both irradiance treatments, contents of Chl *a* and *b* and Car decreased markedly in response to WS ($p<0.05$), but an increase in these characteristics was found in seedlings at LI under both water treatments (Table 2). No significant change in Chl *a/b* ($p=0.744$) was found between the two watering treatments due to a parallel decreasing trend in Chl *a* and Chl *b*.

Effect of WS and LI on gas exchange and Chl fluorescence: Significant reductions in P_{N}, g_{s}, and C_{i} were found in seedlings submitted to WS under both HI and LI.

However, the magnitude of the decrease was slightly smaller in LI seedlings (decrease by 56.02, 80.39, and 13.40 %, respectively) than in HI seedlings, where the decrease was 71.83, 63.16, and 85.71 %, respectively. In addition, a distinct interaction of irradiance×water was observed on P_N, g_s, and C_i ($p<0.001$, Fig. 1)

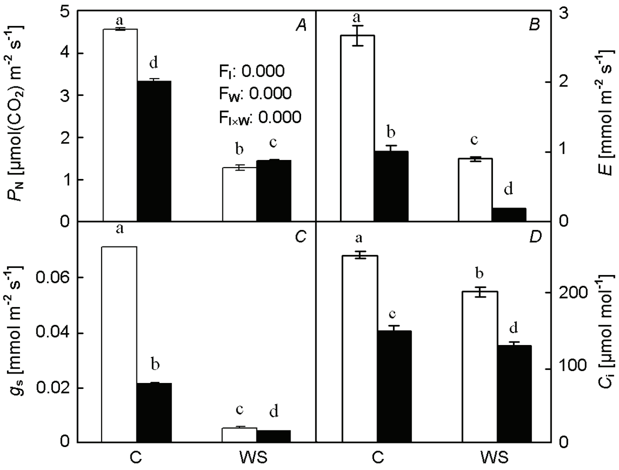

Fig. 1. Net photosynthetic rate, P_N (*A*), transpiration rate, *E* (*B*), stomatal conductance, g_s (*C*), and intercellular CO_2 concentration, C_i (*D*) of *Picea asperata* seedlings affected by water stress (WS) and low irradiance. C – control; □, HI – high irradiance; ■, LI – low irradiance. *Different letters above the bars* indicate significant differences between treatments ($p<0.05$, $n = 8$). F_I – irradiance effect, F_W – water effect, $F_{I×W}$ – interactive effect of water and irradiance.

WS decreased slightly F_v/F_m under both irradiances as though there was not statistically significant difference. Significant reduction induced by WS in *Y* was found in HI. F_v/F_m and *Y* in drought-induced decreases was more noticeable at HI than LI. LI decreased markedly q_P in both water treatments, whereas no prominent difference induced by WS was found. On the other hand, WS increased significantly q_N under HI and decreased it under LI whatever significant or not. In addition, compared with HI, LI increased F_v/F_m but decreased *Y*, q_P, and q_N. The interaction effects of water and irradiance were only found in *Y* and q_N ($p<0.05$, Fig. 2).

Effects of WS and LI on free proline accumulation and lipid peroxidation: A prominent WS-induced increase in free proline content of *P. asperata* seedlings was found under both irradiances ($p<0.05$). LI significantly increased the content of free proline under C treatment which was accompanied by an opposite reduction in the WS treatment. On the other hand, an interaction of irradiance×water effect was found for free proline content ($p<0.05$, Fig. 3).

WS resulted in evidently higher MDA content under both irradiances ($p<0.05$). In addition, LI also reduced MDA content under each watering treatment whether significantly or not. The interactive effect between irradiance and water was also significant for MDA content ($p=0.008$, Fig. 2).

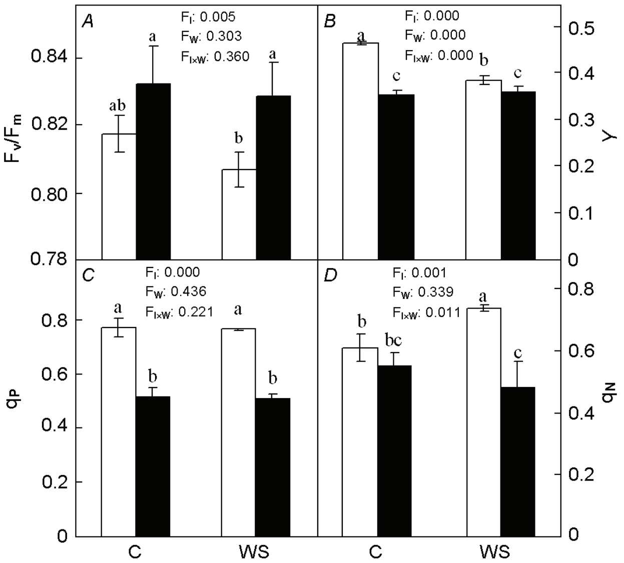

Fig. 2. Maximal photochemical efficiency of photosystem 2 (PS2), F_v/F_m (*A*), effective quantum yield of PS2, *Y* (*B*), and photochemical, q_P (*C*) and non-photochemical, q_N (*D*) quenchings of *Picea asperata* seedlings affected by water stress (WS) and low irradiance. C – control; □, HI – high irradiance; ■, LI – low irradiance. *Different letters above the bars* indicate significant differences between treatments ($p<0.05$, $n = 8$). F_I – irradiance effect, F_W – water effect, $F_{I×W}$ – interactive effect of water and irradiance.

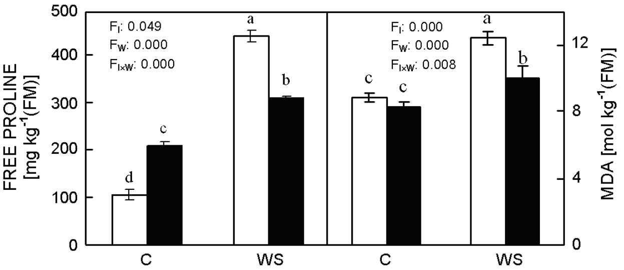

Fig. 3. Free proline and malondialdehyde (MDA) contents of *Picea asperata* seedlings affected by water stress (WS) and low irradiance. C – control; □, HI – high irradiance; ■, LI – low irradiance. Means±S.E. *Different letters above the bars* indicate significant differences between treatments ($p<0.05$, $n = 8$). F_I – irradiance effect, F_W – water effect, $F_{I×W}$ – interactive effect of water and irradiance.

Discussion

An early morphological response to drought was the avoidance mechanism through adjustment of plant growth such as reduction in seedling height, basal diameter, and total biomass (Lei *et al.* 2006). In our study, drought significantly reduced shoot height, stem diameter, and total biomass under both irradiances. This may also be attributed to the reduction in Chl content that limited photosynthesis (Table 2, Fig. 1). In addition, reduction in RSR under HI found in our study may be due to a parallel decreasing trend in total biomass (Table 1). On the other hand, we found LI-reduced shoot height, basal diameter, and RSR in both water treatments. Similar results were found by Groninger *et al.* (1996): an increase in irradiance resulted in increased seedling growth. In nature, it was not certain if the increase in growth was due solely to increased irradiance since this increase was associated with a decrease in overstory basal area and the resultant increase in soil resources (Jose *et al.* 2003). We found that availability of radiant energy was a major factor that determined seedling growth. The decrease in growth of LI seedlings might be due to the fact that seedlings allocating more biomass to lateral growth had greater capacity to capture more photons in irradiance-limited environments (Wang *et al.* 2006).

The reduction in contents of photosynthetic pigments played a key role in reduced P_N under WS. We found that WS induced significant reduction in Chl *a* and Car contents under both irradiances, which might be due to Chl *a/b*-protein synthesis restrained in chloroplast segments resulting from WS (Alberte *et al.* 1977). Car is the quenching agent of short-wave radiation with high energy and can protect Chl from photo-oxidative destruction (Singh 1996). However, under stress Car might be rapidly destroyed and therefore were no longer available to protect against oxidative damage (Munné-Bosch and Alegre 2000). The reduction in Car indicated that WS would induce substantive oxidative stress by accumulation of AOS (active oxygen species). In addition, Chl content in LI seedlings was larger than in the HI ones, which would improve quantum use efficiency and allow needles to capture more photons to increase the photosynthetic capacity (Niinemets *et al.* 1998). Observed lower Chl *a/b* (Table 2) in LI seedlings was similar to that reported in *Ginkgo* and *Fagus* (Sarijeva *et al.* 2006), indicating a higher proportion of LHC proteins which are associated with higher and broader grana thylakoid stacks and a higher stacking degree, also a higher portion of appressed thylakoid membranes.

The reductions of growth and Chl content were closely related to a strong reduction in photosynthesis under WS (Llorens *et al.* 2003). In addition, the parallel decrease in RWC and P_N under WS (Table 1, Fig. 1) indicated that needle RWC was correlated positively with P_N. As demonstrated here, drought induced significant decrease in P_N, g_s, and C_i under both irradiances (Fig. 1). C_i is a reliable indicator discriminating the changes of P_N between stomatal and non-stomatal limitations (Farquhar and Sharkey 1982). Thus, low P_N in seedlings suffering from WS has often been reported, usually as a consequence of stomatal closure (Dixon *et al.* 1998). Our results support the idea that the reduction in photosynthesis of WS seedlings was more closely related to stomatal limitations.

On the other side, in a similar study Valladares and Pearcy (2002) found that shade plants exhibited a greater decrease in P_N and g_s in response to drought than sun plants. They concluded this might be responsible for plant ability to maintain higher relative gas exchange rates than shade plants have under drought. On the contrary, we found that seedlings grown at HI showed a greater decrease in P_N and $g_{s,}$ suggesting that a greater increase of stomatal limitation caused by severe WS appeared under HI than under LI. Enhanced limitation resulted in stomatal closure. For similar results see Holmgren (2000) or Quero *et al.* (2006). Our results suggested that HI would not induce greater P_N and improve seedling growth unless soil water was limited (Jose *et al.* 2003).

F_v/F_m experienced slight decrease in needles of WS seedlings under both irradiances (Fig. 2), which may be attributed to the resistance of the photosynthetic machinery to WS (Grieu *et al.* 1995, Lawlor and Cornic 2002). The decreases in F_v/F_m observed in stressed plants at predawn were mostly due to sustained increases in the de-epoxidation status of the xanthophyll cycle and not to photoinhibitory damage (Adams *et al.* 1994). A prominent drought-induced decrease in *Y* was found in the HI seedlings, which was related to the decrease in P_N and total biomass. The changes might be due to a more decreased ability to recycle CO_2 (Scheuermann *et al.* 1991). HI seedlings under both water treatments maintained higher q_P than the LI ones (Fig. 2*C*). These results indicate that *P. asperata* seedlings differed in their responses to different irradiances, suggesting that a protective mechanism was present in HI seedlings. This might be due to electron transport rate partially blocked after the PS2 (Romanowska *et al.* 2006). The q_N was a reflection of plant photo-protective capacity. We found that higher q_N was typical for HI seedlings (Fig. 2*D*), indicating some protection conferred in *P. asperata* photochemical apparatus (Yamane *et al.* 1997) as a result of dissipation of excessive energy. On the other hand, an increase in q_N under HI and WS was shown in our study, suggesting lesser capacity for non-radiative dissipation of excessive heat energy and a limitation of the photoprotective capacity (Lu and Zhang 2000).

Free proline content may serve as a means of osmotic adjustment when stress condition leads to less growth. In addition, it could function as a hydroxyl radical scavenger to prevent membrane damage and protein de-naturation

(Ain-Lhout *et al.* 2001). We found a greater proline accumulation in seedlings grown at WS under both irradiances. We suppose that seedlings at WS made an ecological adaptation by osmotic adjustment. But accumulation of proline under WS did not improve needle RWC, seedling growth, and Chl content suggesting that proline accumulation in seedling needles was possibly related to increased arginine availability and ammonia detoxification (Lazcano-Ferrat and Lovatt 1999).

The MDA content is an indicator of membrane lipid peroxidation which could reflect the degree of damage at adverse condition. We showed that MDA content in needles grown at both irradiances was augmented by WS, suggesting an enhancement in lipid peroxidation of cell membrane. The increase might be due to stomatal closure resulting in a decrease in the needle C_i (Türkan *et al.* 2005). LI decreased MDA content under both water treatments, and the prominent difference under WS indicated that LI could alleviate the damage caused by drought on cell membrane.

In conclusion, we show that WS had weaker impact on seedlings grown at LI (15 % of full sunlight) than on those grown at HI. Although soil water content and precipitation was usually abundant at high altitude, WS induced by frozen, unavailable soil water or damaged foliage was severe for seedling growth. WS led to marked decline in growth parameters, P_N, RWC, content of photosynthetic pigments, and F_v/F_m under both irradiances, but the extent of decrease in LI seedlings was obviously lower than that in the HI ones. This might be due to the fact that LI environment exerted less water damages and less impact on morphological and physiological characteristics. On the other hand, WS increased the content of free proline under whatever irradiance. But the accumulation in free proline can not protect seedling cells against peroxidative processes adequately, owing to the increase in MDA content under both irradiances. In addition, lower free proline and MDA contents were found in LI seedlings than in the HI ones. These results reinforces the idea that WS has a weaker impact on LI seedlings and provides a theoretic evidence for seedling regeneration process in sub-alpine area.

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