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RESEARCH PAPER

Effect of cold acclimation on the photosynthetic performance of two ecotypes of *Colobanthus quitensis* (Kunth) Bartl.

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Abstract

The effects of cold acclimation of two ecotypes (Antarctic and Andes) of *Colobanthus quitensis* (Kunth) Bartl. Caryophyllaceae on their photosynthetic characteristics and performance under high light (HL) were compared. Non-acclimated plants of the Antarctic ecotype exhibited a higher (34%) maximal rate of photosynthesis than the Andes ecotype. In cold-acclimated plants the light compensation point was increased. Dark respiration was significantly increased during the exposure to 4 °C in both ecotypes. Cold-acclimated Antarctic plants showed higher Φ_{PSII} and *qP* compared with the Andes ecotype. In addition, the Antarctic ecotype exhibited higher heat dissipation (*NPQ*), especially in the cold-acclimated state, which was mainly associated with the fast relaxing component of non-photochemical quenching (NPQ_F). By contrast, the Andes ecotype exhibited a lower NPQ_F and a significant increase in the slowly relaxing component (NPQ_s) at low temperature and HL, indicating higher sensitivity to low temperature-induced photoinhibition. Although the xanthophyll cycle was fully operational in both ecotypes, cold-acclimated Antarctic plants exposed to HL exhibited higher epoxidation state of the xanthophyll cycle pigments (*EPS*) compared with the cold-acclimated Andes ecotype. Thus, the photosynthetic apparatus of the Antarctic ecotype operates more efficiently than that of the Andes one, under a combination of low temperature and HL. The ecotype differences are discussed in relation to the different climatic conditions of the two *Colobanthus*.

Key words: Antarctic plants, heat dissipation, low temperature, non-photochemical quenching, photoinhibition, photosynthesis.

Introduction

Excess irradiance may be harmful for plants that are unable to balance the absorbed/utilized energy ratio (Huner *et al*., 1998). This may be even worse when plants are exposed simultaneously to high light and low temperatures which decrease carbon and other enzymatic assimilation processes, creating a greater imbalance because light absorption is largely temperature insensitive (Huner *et al*., 1998). However, cold acclimation decreases susceptibility to photoinhibition (Krause, 1994) by causing several metabolic alterations and producing changes at the chloroplast level that may restore the energy balance. A widely accepted hypothesis is that cold acclimation may improve the ability of plants to maintain metabolism at low temperature by keeping Q_A, the

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Abbreviations: A, antheraxanthin; *EPS*, epoxidation state of the xanthophyll cycle pigments; F_o, instantaneous (dark) chlorophyll fluorescence at open PSII centres in dark-adapted samples; F_m, maximal fluorescence at closed PSII centres; F_v, variable fluorescence; HL, high light intensity; *LCP*, light compensation point; LL, low light intensity; *NPQ*, non-photochemical quenching; NPQ_F, NPQ_s, fast and slow relaxing component of the *NPQ*; respectively; Pn_{max}, maximum rate of net photosynthesis; Φ_{PSII}, quantum yield of PSII; Φ_{O_2}, quantum yield of oxygen evolution; *qE*, energy-dependent quenching of chlorophyll fluorescence; *qI*, photoinhibitory quenching; *qP*, photochemical quenching; *Rd*, dark respiration; VAZ, pool of the xanthophyll cycle pigments; V, violaxanthin; Z, zeaxanthin.

primary quinone electron acceptor, more oxidized (Huner *et al*., 1998; Melis, 1999). Other plants use different strategies. For instance, cold-acclimated *Pinus contorta* L. partially loses PSII reaction centres, reduces needle chlorophyll per unit area, and reduces its daily carbon gain (Savitch *et al*., 2002). All these changes are accompanied by an increased and sustained capacity for heat dissipation through non-photochemical quenching. It is also known that xanthophyll levels increase during cold acclimation and the half-time to develop *qE* decreases (Krause, 1994). All these cold acclimation-induced changes may help to restore the energy balance and hence reduce the incidence of low-temperature-induced photodamage.

Colobanthus quitensis (Kunth) Bartl. Caryophyllaceae extends from the Maritime Antarctic and along the Andes Mountains to Ecuador, with one site in Mexico (Lewis Smith, 2003). It usually grows above 2500 masl in the Andes Mountains. The Antarctic *C. quitensis* plants have been described as morphologically and physiologically adapted to succeed in these cold environments (Mantovani and Vieira, 2000; Perez-Torrez *et al*., 2004). For instance, its photosynthetic machinery is well adapted to low temperature (Xiong *et al*., 1999, 2000). The photosynthetic responses of a *C. quitensis* population from the Andes of Central Chile have been recently studied in the field (Casanova-Katny *et al*., 2006) and ecotypic differentiation of the Antarctic and Andes populations has been proposed (Gianoli *et al*., 2004; Sierra-Almeida *et al*., 2007).

Antarctic and Andean environments can differ significantly in solar radiation and temperature during the summer. *PFD* in the Antarctic summer can go up to 1500 μmol photons m^{-2} s^{-1} on sunny days; however, sunny days are infrequent, accounting for less than 20% in summer (Xiong *et al*., 1999; Xiong and Day, 2001). Average temperature in the Antarctic summer is about 3 °C (Alberdi *et al*., 2002). In the Andes mountains, *PFD* in summer is frequently as high as 2500 μmol photons m^{-2} s^{-1} and the average temperature at 2600 masl is about 13 °C (Cavieres and Arroyo, 1999). These differences in *PFD* and temperature regime during the growing season could have resulted in selection for altered plasticity of the photosynthetic apparatus to cope with high light and low temperature in Andes and Antarctic ecotypes, respectively. In order to test this hypothesis, the two ecotypes of *C. quitensis* described above (Gianoli *et al*., 2004) were grown, one from Antarctica (sea level) and the other from the Andes (2700 masl), under the same controlled laboratory conditions. The purpose of this study was to assess the effect of cold acclimation on the capacity of these ecotypes of *C. quitensis* to cope with excess irradiance and low temperature-induced photoinhibition.

Materials and methods

Plant material

Antarctic plants of *C. quitensis* were collected on King George Island, Maritime Antarctic (sea level; 62°10′ S; 58°29’ W) and transported to the laboratory. Plants of *C. quitensis*, ecotype Andes were collected on the slopes of Cerro La Parva (2650 masl; 33°19′ S; 70°17′ W). Both ecotypes were reproduced vegetatively in plastic pots, using a soil:peat mixture (3:1 v/v) and maintained at 13–15 °C in a growth chamber (Forma Scientific Inc.) with a photon flux density (*PFD*) of 120±20 μmol photons m^{-2} s^{-1} at the top of the canopy and a 16/8 h light/dark period. The light source consisted of cool-white fluorescent tubes F40CW (General Electric). Plants were fertilized with Phostrogen® (Solaris) using 0.2 g l^{-1} once every two weeks. One group of both Antarctic and Andes plants was cold-acclimated at 4 °C for 21 d. This treatment reduced the LT_{50} from –7 °C to –10 °C and from –7 °C to –14.5 °C in Andes and Antarctic ecotypes, respectively (Gianoli *et al*., 2004). Mature pre-existing leaves were sampled for each analysis.

Photoinhibitory treatment

Cold-acclimated and non-acclimated plants were subjected to high light treatment (HL) 1600±50 μmol photons m^{-2} s^{-1} and low light treatment (LL) 120±20 μmol m^{-2} s^{-1} both at low temperature (4 °C) for 2 h. Low and high *PFD* were provided by 1000 W halogen lamps. Pigment composition was monitored at the end of each treatment.

Net photosynthesis

Photosynthetic oxygen evolution was measured in detached leaves with a gas phase oxygen electrode unit, using an Oxylab and an oxygen electrode chamber (Model LD2/3 Hansatech Instruments Ltd., King’s Lynn, Norfolk, UK). Measurements were performed at either 4 °C and/or 15 °C under saturating CO_2 and irradiances over the range of 0–800 μmol m^{-2} s^{-1} given by an array of red light-emitting diodes, (Model LH36/2R, Hansatech Instruments Ltd., King’s Lynn, Norfolk, UK). Detached leaves were adapted for 10 min to each temperature. Quantum yield of oxygen evolution (Φ_{O_2}), maximum rate of net photosynthesis (Pn_{max}), and light compensation point (*LCP*) were determined on the bases of incident light measured with the quantum sensor (QSRED, Hansatech).

Fluorescence measurements

C. quitensis, possess narrow and short leaves, especially the Antarctic ecotype. This makes the fluorescence measurements in attached leaves difficult to perform. For this reason, in order to conduct measurements in the same way for both ecotypes, fully developed detached leaves from control and HL-treated cold-acclimated and non-acclimated plants were aligned parallel and immobilized using transparent tape and then dark-adapted for 30 min using the instrument leaf-clips to obtain open PSII centres to ensure maximum photochemical efficiency. Chlorophyll fluorescence recordings and calculations were performed by a pulse-amplitude modulated fluorimeter (FMS 2, Hansatech, Instruments Ltd., Norfolk, UK) according to Schreiber *et al*. (1986). The fibre-optic and its adapter were fixed to a ring located over the clip at about 10 mm from the sample and the different light pulses (see below) were applied following the standard routines programmed within the instrument. Minimal fluorescence (F_o) with all PSII reaction centres in the open state was determined by applying a weak modulated light (0.4 μmol m^{-2} s^{-1}). Maximal fluorescence (F_m) with all PSII reaction centres in the closed state was induced by a 0.8 s saturating pulse of white light (9000 μmol m^{-2} s^{-1}). After 10 s, the actinic light was turned on and the same saturating

pulse described previously was applied every 20 s, until steady-state photosynthesis was reached in order to obtain F_s and F'_m. Finally, F'_o was measured after turning the actinic light off and applying a 2 s far red light pulse. Definitions of fluorescence parameters (qP, F'_v/F'_m, and Φ_{PSII}) were used as described by van Kooten and Snel (1990), Non-photochemical quenching (NPQ) was calculated according to Walters and Horton (1991) Fluorescence measurements were performed at different actinic light intensities which was controlled by the light source of the FMS 2 apparatus and applied through an optic fibre. Light intensity at the leaf surface was calibrated using a LI-250 light meter (Li-Cor).

Determination of NPQ *components*

The components of non-photochemical quenching (NPQ) were determined at 4 °C and 15 °C in leaves of cold-acclimated and non-acclimated plants from both the Antarctic and Andes ecotypes of *C. quitensis*. NPQ was resolved into slow (NPQ_s) and fast (NPQ_F) components (equivalent to qI and qE, respectively) essentially as described by Walters and Horton (1991) by analysing the kinetics of F_m recovery after actinic light has been turned off. $NPQ_s=(F_m–F_{mr})/F_{mr}$ and $NPQ_F=(F_m–F'_m)–(F_m–F_{mr})$. F_{mr} (the value of F_m that would have been attained if only slowly relaxing quenching had been present) was obtained by extrapolation in a semi-logarithmic plot of maximum fluorescence yield versus time of data points recorded toward the end of the relaxation back to the time where the actinic light was removed.

Pigments

C. quitensis leaves were cut and placed immediately in a cold mortar. A tip of spatula (approximately 1 mg) of $CaCO_3$ was added before grinding in 100% (v/v) acetone at 4 °C under dim light. The supernatant was filtered through a 0.22 μm syringe filter and samples were stored at –80 °C until analysed. Pigments were separated and quantified by HPLC analysis as described previously (Ivanov *et al*., 1995) with some modifications. The HPLC system consisted of the Beckman System Gold programmable solvent module 126, a diode array detector module 168 (Beckman Instruments, San Ramon, California, USA), CSC-Spherisorb ODS-1 reverse-phase column (5 μm particle size, 25×0.46 cm ID) with an Upchurch Perisorb A guard column (both columns from Chromatographic Specialties Inc., Concord, Ontario, Canada). Pigments were eluted isocratically for 6 min with acetonitrile:methanol:0.1 M TRIS–HCl (pH 8.0), (72:8:3.5, by vol.), followed by a 2 min linear gradient to 100% methanol:hexane (4:1, v/v), which continued isocratically for 4 min with a flow rate of 2 ml min^{-1}. Absorbance was monitored at 440 nm. Retention times and response factors of Chl *a*, Chl *b*, lutein, and β-carotene were determined by injection of known amounts of pure standards purchased from Sigma (St Louis, MO, USA). The retention times of zeaxanthin, antheraxanthin, violaxanthin and neoxanthin were determined by using pigments purified by thin-layer chromatography as described by Diaz *et al*. (1990). Epoxidation state (EPS) of the pigments pool was estimated as: $EPS=(0.5A+V)/(V+A+Z)$, where A is antheraxanthin, V is violaxanthin, and Z is zeaxanthin.

Statistics

Differences in parameters extracted from light response curves of net photosynthesis were statistically evaluated using three-way ANOVA (level of significance was $P < 0.05$) using growth condition, ecotype, and measurement temperature as factors. Fluorescence parameters and pigment contents were statistically evaluated using three-way ANOVA (level of significance was $P < 0.05$) with growth condition, ecotype, and light intensity as factors. Tukey *post-hoc* tests were used to identify those means with significant differences. Statistical analyses were performed using SigmaStat 3.1 (Systat Software, Inc. Richmond CA, USA).

Results

Net photosynthesis

Light response curves of net photosynthesis (Pn) were performed in cold-acclimated and non-acclimated plants of both Antarctic and Andes ecotypes (Fig. 1). Pn was measured at 4 °C and 15 °C (Table 1). The Antarctic ecotype a exhibited higher maximum rate of net photosynthesis (Pn_{max}) value than the Andes ecotype, regardless of the measuring and/or growth temperature. The highest net photosynthesis was registered in non-acclimated Antarctic plants exposed to 15 °C, reaching 8.95 μmol O_2 m^{-2} s^{-1}. Cold acclimation did not significantly affect Pn_{max} of either ecotype ($P > 0.05$) (Table 1). However, measuring temperature had a significant and differential

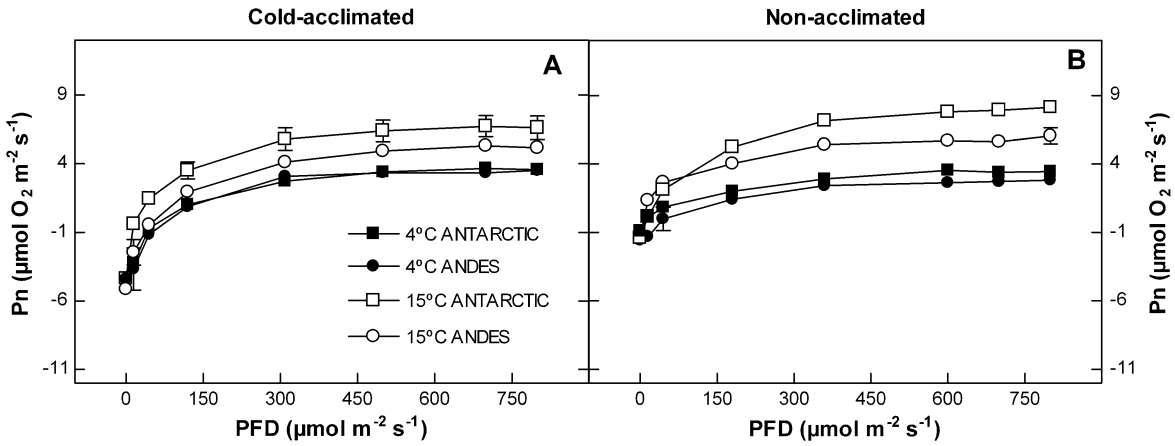

Fig. 1. Light response curve of photosynthetic oxygen evolution of *C. quitensis* in cold-acclimated (A) and non-acclimated (B) Antarctic (squares) and Andes (circles) ecotypes at either 4 °C and/or 15 °C under saturating CO_2. Results are means ±SE; $n=3$.

Table 1. Photosynthetic parameters in both Andes and Antarctic ecotypes of *C. quitensis*

These parameters were obtained from the analysis of light response curves (Fig. 1). Cold-acclimated (4 °C) and non-acclimated (15 °C) plants of both ecotypes were measured at 4 °C and 15 °C, respectively. (Φ_{O_2}, quantum yield of oxygen evolution; Pn_{max}, maximal rate of net photosynthesis; *LCP*, light compensation point; *Rd*, dark respiration rate. Different letters indicate statistically significant differences within each parameter. Results are means ±SE; n=3.

Parameters	Cold-acclimated				Non-acclimated			
	Andes		Antarctic		Andes		Antarctic	
	4 °C	15 °C	4 °C	15 °C	4 °C	15 °C	4 °C	15 °C
Φ_{O_2} (mol O_2 mol^{-1} photons)	0.04±0.01 a	0.07±0.01 ab	0.07±0.02 ab	0.12±0.01 bc	0.04±0.01 a	0.18±0.03 c	0.04±0.01 a	0.08±0.01 b
Pn_{max} (μmol O_2 m^{-2} s^{-1})	3.90±0.44 a	5.89±0.47 b	4.31±0.02 a	7.43±0.85 bc	3.11±0.18 a	6.67±0.62 b	4.14±0.20 a	8.95±0.41 c
LCP (μmol photons m^{-2} s^{-1})	55±17 a	29±6 c	35±4 c	19±3 b	32±6 c	7±1 d	19±3 b	13±3 b
Rd (μmol O_2 $m^{-2}s^{-1}$)	–4.0±0.7 a	–4.1±0.6 a	–4.0±0.2 a	–3.3±0.4 a	–1.8±0.2 b	–1.3±0.1 c	–0.9±0.3 d	–1.2±0.3 d

effect on *Pn* and higher Pn_{max} values were observed at 15 °C than at 4 °C. This effect depended on the ecotype and the increase of Pn_{max} in cold-acclimated Andes plants exposed to 15 °C was 51%, with respect to 4 °C, while in the Antarctic plants this increase was significantly higher (73%) (Table 1). This clearly implies an increased capacity for photosynthesis in the cold-acclimated Antarctic ecotype.

Cold acclimation significantly increased *LCP* in both ecotypes (Table 1), which is associated with an increase in dark respiration observed in cold-acclimated plants. At either temperature, *LCP* was higher in the Andes ecotype, except in non-acclimated plants exposed to 15 °C, where the Andes ecotype exhibited a lower *LCP* (7 μmol photons m^{-2} s^{-1}) than the Antarctic ecotype (13 μmol photons m^{-2} s^{-1}). Low measuring temperature increased *LCP* in both ecotypes independently of growth temperature, with the exception observed in the non-acclimated Antarctic ecotype which exhibited non-significant statistical differences between *LCP* at 4 °C and 15 °C. The effect of cold acclimation on Φ_{O_2} depends on the ecotype and measuring temperatures. The Φ_{O_2} was higher in non-acclimated plants of the Andes ecotype at 15 °C and it was reduced upon cold acclimation. On the other hand, the Antarctic ecotype exhibited a tendency to increase Φ_{O_2} upon cold acclimation at both measuring temperatures, although no statistically significant differences (P >0.05) were observed in this ecotype (Table 1).

Effect of light and growth temperature on steady state fluorescence yield

Light response curves of quantum yield of PSII (Φ_{PSII}), in the Andes and Antarctic ecotypes of *C. quitensis* under non-acclimated and cold-acclimated conditions, showed a decline of Φ_{PSII} with increasing *PFD* (Fig. 2A). Under cold-acclimated conditions, the Antarctic ecotype exhibited a significantly higher (P <0.05) quantum yield of PSII, compared with the Andes plants, except at the highest *PFD* (Fig. 2A). Under non-acclimated conditions, the Andes ecotype showed a slower decrease of Φ_{PSII} than the Antarctic ecotype from 200 to 700 μmol photons m^{-2} s^{-1} of *PFD* (Fig. 2B). The Antarctic ecotype also had a higher proportion of open reaction centres measured as F_v'/F_m' in the cold-acclimated state (Fig. 2C), while it showed no mayor differences with the Antarctic ecotype under non-acclimated conditions (Fig. 2D).

Cold-acclimated Antarctic plants demonstrated significantly higher (P <0.05) photochemical quenching (*qP*) compared with the Andes ecotype (Fig. 2E). The opposite effect was observed under non-acclimated conditions, where the Andes ecotype had higher values of *qP* than the Antarctic one in the range of 200–800 μmol photons m^{-2} s^{-1} (Fig. 2F).

Growth and measuring temperature and light effects on NPQ *components*

NPQ and its fast and slow relaxation components were studied in both ecotypes of *C. quitensis* under cold-acclimated and non-acclimated conditions at different *PFD* and at two different temperatures, 15 °C and 4 °C, which are the optimum temperature for photosynthesis and the temperature used for cold acclimation, respectively. In general, cold-acclimated plants exhibited higher *NPQ* values at lower *PFD* than non-acclimated ones when measured at 15 °C (Fig. 3B), while no major differences were observed at 4 °C (Fig. 3A). Cold-acclimated Antarctic plants exposed to the 15 °C measuring temperature exhibited the highest capacity for *NPQ*, reaching values over 5.0 at high *PFD* (Fig. 3A). Non-acclimated leaves of both *C. quitensis* ecotypes exposed to the low measuring temperature (4 °C) showed a greater capacity for *NPQ* at lower actinic light and a lower increase with increasing the light intensity than at 15 °C, reaching similar *NPQ* values at high *PFD* under both growth temperature regimes (Fig. 3B).

The fast relaxing component of the non-photochemical quenching (NPQ_F), which is associated with the energy-dependent *NPQ* (*qE*), reached the highest values at the 15 °C measuring temperature, being higher in the Antarctic ecotype at both measuring and growth temperatures

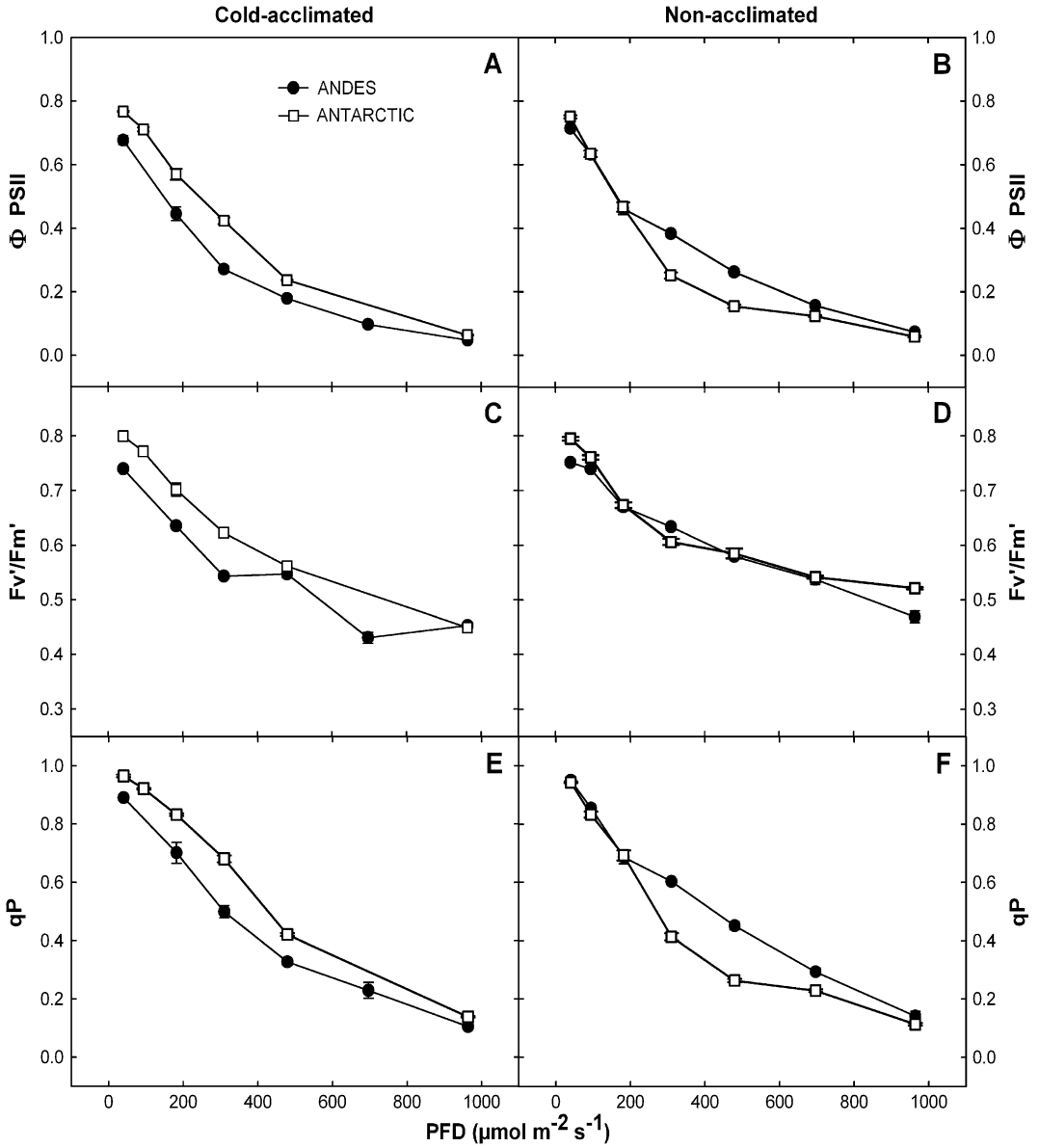

Fig. 2. Light response of fluorescence parameters in cold-acclimated (A, C, E) and non-acclimated (B, D, F) plants of both ecotypes of *C. quitensis*. Quantum yield of PSII (Φ_{PSII}) (A, B), open reaction centres (F_v'/F_m') (C, D), and photochemical quenching (*qP*) (E, F) were measured at 15 °C. Mean values ±SE were calculated from five independent experiments.

at *PFD*s higher than 600 μmol photons m^{-2} s^{-1} (Fig. 3C, D). The cold-acclimated Andes ecotype measured at 4 °C exhibited little NPQ_F, which was saturated at the lowest light intensity of about 100 μmol photons m^{-2} s^{-1}. In non-acclimated plants, NPQ_F was lower when measured at 4 °C than at 15 °C (Fig. 3D) and was similar to that of cold-acclimated plants of the Antarctic ecotype measured at 4 °C. Cold-acclimated plants exhibited minimal differences in NPQ_s regardless of the measuring temperature (Fig. 3E). No significant differences in NPQ_s were observed between the two non-acclimated ecotypes measured at 15 °C, compared with cold-acclimated plants (Fig. 3F). By contrast, there was a significantly higher NPQ_s in both ecotypes when the measuring temperature was 4 °C, with respect to 15 °C. The highest *NPQ* values were observed in non-acclimated Andes plants at high *PFD* (Fig. 3F).

Pigments and xanthophyll cycle under photoinhibitory conditions

Chlorophylls and carotenoids were measured in non-inhibited control (LL) and photoinhibited (HL) at 4 °C leaves of both Antarctic and Andes ecotypes of *C. quitensis* under non-acclimated and cold-acclimated growth conditions. In general, plants of the Andes ecotype showed lower total chlorophyll (Chl *a*+*b*) and carotenoid

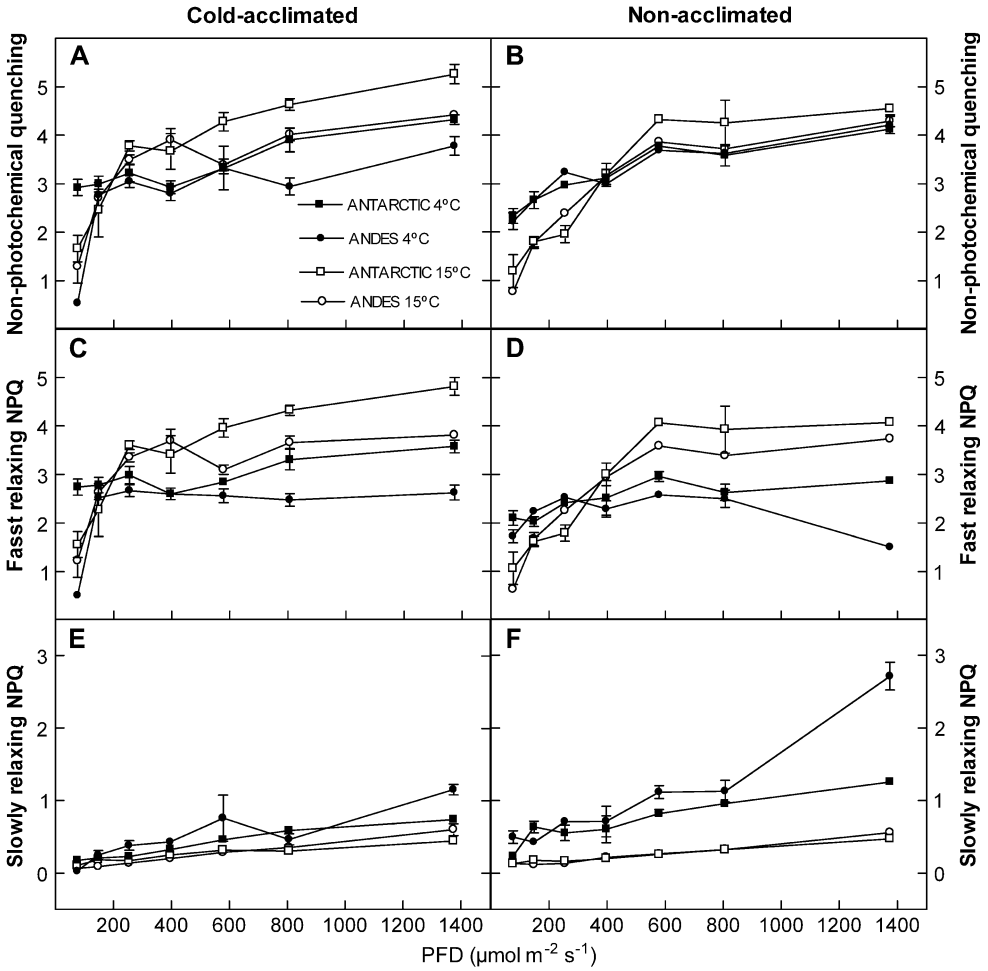

Fig. 3. Analyses of fast (NPQ_F) and slow (NPQ_s) relaxing components of *NPQ* in *C. quitensis*. *NPQ* components were determined at 4 °C (empty symbols) and 15 °C (solid symbols) using non-acclimated and cold-acclimated leaves of both ecotypes of *C. quitensis*. Results are means ±SE; n=5.

contents than the Antarctic ecotype independently of light and temperature treatments (Table 2). Cold-acclimated plants of both ecotypes also exhibited lower chlorophyll and carotenoid contents, the lowest content of pigments being observed in HL-treated cold-acclimated Andean plants, which also showed the lowest *Chl*/*Car* ratio (Table 2). Chl *a*/Chl *b* ratios were similar for both ecotypes in all treatments. Cold-acclimated and non-acclimated plants of both ecotypes showed an increase of chlorophylls and carotenoids under HL compared with LL treatment, and this increase was smaller in cold-acclimated plants (Table 2). This effect was not observed for the total pool of xanthophyll cycle pigments (VAZ). On the contrary, significantly higher levels of the VAZ pool (P <0.05) were observed in either cold-acclimated or non-acclimated *C. quitensis* plants of both ecotypes under HL conditions. The highest VAZ content was observed in the non-acclimated HL-treated Antarctic ecotype (Fig. 4). These results clearly indicate that some *de novo* synthesis of xanthophyll cycle pigments occurs during the HL treatments in both ecotypes, especially in non-acclimated plants. The epoxidation state (*EPS*) of the xanthophyll pool, which represents the inverse of the efficiency of violaxanthin conversion to zeaxanthin via antheraxanthin (Demmig-Adams and Adams III, 1996) was about 0.9 for both ecotypes at LL with no significant effect of cold-acclimation (Fig. 4). As expected, HL treatment caused a significant decrease in *EPS* reaching, in both non-acclimated ecotypes and in cold-acclimated Andes plants, values around 0.4 (Fig. 4B). Interestingly, cold-acclimated Antarctic plants showed a significantly (P <0.05) higher *EPS* (0.62) corresponding to less efficient conversion of violaxanthin to zeaxanthin under HL exposure than non-acclimated ones (Fig. 4).

Discussion

Concomitant with earlier studies, net O_2 evolution measured in laboratory-grown *C. quitensis* plants was higher in the Antarctic ecotype compared with the Andes one (Table 1) and the values for Pn_{max} were similar to

Table 2. *Effects of low temperature-induced photoinhibition on pigments composition of* C. quitensis *ecotypes*

Cold-acclimated and non-acclimated plants of Andes and Antarctic ecotypes were exposed to high light (HL, 1600 μmol photons m^{-2} s^{-1}) and low light intensity (LL, 100 μmol photons m^{-2} s^{-1}).at 4 °C for 2 h. Different letters indicate statistically significant differences within each parameter. Results were means ±SE; n=5.

Pigments (μg g^{-1} FW)	Cold-acclimated				Non-acclimated			
	Andes		Antarctic		Andes		Antarctic	
	LL	HL	LL	HL	LL	HL	LL	HL
Chl *a*	478±30 a	416±29 a	538±23 ab	601±41 bc	643±33 c	719±25 c	731±6 d	831±25 e
Chl *b*	176±2 a	153±1 b	177±5 a	204±9 c	229±4 d	259±4 e	250±4 f	287±6 g
Chl *a*/Chl *b*	2.7±0.1 a	2.7±0.2 a	3.0±0.1 a	2.9±0.1 a	2.8±0.2 a	2.8±0.1 a	2.9±0.1 a	2.9±0.1 a
β-Carotene	43±3 a	41±2 a	53±2 b	56±4 b	58±3 bc	61±1 c	66±2 d	70±2 d
Lutein	66±3 a	71.2±0.8 ab	70±1 a	80±4 b	83±1 b	100.1±0.5 c	86±4 b	98±1 c
Neoxanthin	10.4±0.6 a	10.2±0.4 a	14.5±0.7 b	14.4±0.5 b	14±1 b	17.8±0.8 c	16.8±0.8 c	18.2±0.5 c
Chl/Car	4.6±0.1 a	3.7±0.1 b	4.3±0.1 a	4.4±0.1 a	4.8±0.2 a	4.5±0.1 a	4.8±0.1 a	4.8±0.1 a

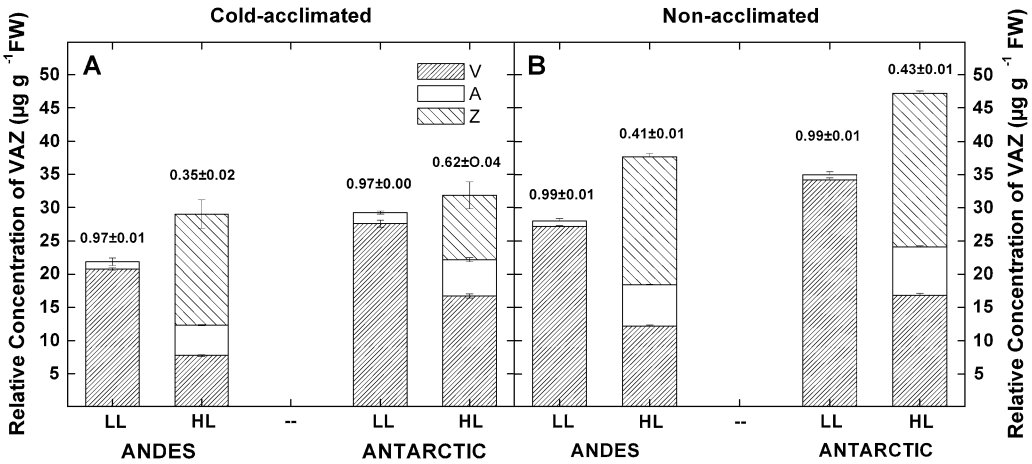

Fig. 4. Xanthophyll cycle pigment contents and epoxidation state (numbers on top of the bars) in cold-acclimated (A) and non-acclimated (B) plants of both ecotypes of *C. quitensis* exposed to high light, (HL) 1600 μmol photons m^{-2} s^{-1} and low light, (LL) 100 μmol photons m^{-2} s^{-1} at 4 °C for 2 h. Results are means ±SE; n=5.

those observed in field experiments. For instance, A_{max} values of 8 μmol CO_2 m^{-2} s^{-1} have been reported in the Antarctic plants (Xiong *et al.*, 1999), while lower net photosynthesis (5.0 μmol CO_2 m^{-2} s^{-1}) was measured in plants from the Andes under field conditions (Casanova-Katny *et al.*, 2006). It has been suggested that the moderately higher rate of net photosynthesis in the Antarctic ecotype may be associated with the thicker leaves of this ecotype (Gianoli *et al.*, 2004). This is consistent with the higher chlorophyll content in the Antarctic ecotype on fresh weight bases (Table 2) and the lack of significant differences between the Antarctic and Andes ecotypes when net photosynthesis was expressed on chlorophyll bases (data not shown). The higher net photosynthesis and lower *LCP* at low temperature demonstrates the ability of the Antarctic ecotype to maximize its photosynthetic performance, which optimizes the energy allocated for growth and reproduction in a short period with favourable temperature and very unstable light supply for photosynthesis (Xiong and Day, 2001). It is interesting to note that in cold-acclimated plants of *C. quitensis* dark respiration was enhanced (Table 1). This confirms a previous observation of CO_2 uptake in the cold-acclimated Antarctic ecotype of *C. quitensis* measured at low temperature (Pérez-Torres *et al.*, 2006). The increase in dark respiration caused by cold-acclimation may be associated with the ability of this species to survive several months covered by snow. In fact, it has been shown that, in over-wintering winter wheat, the activity of respiratory enzymes is increased during the autumn to maintain the adenylate energy charge (Sagisaka *et al.*, 1991). Lower growth temperature exacerbates the alternative oxidase pathway (Vanlerberghe and McIntosh, 1992). Overexpression of alternative oxidase has been shown to alleviate oxidative stress in transgenic *A. thaliana* under low temperature (Sugie *et al.*, 2006). Furthermore, it has recently been reported that up-regulation of mitochondrial alternative oxidase occurs

concomitantly with chloroplast over-reduction by excess light in *A. thaliana* (Yoshida *et al*., 2007). These authors suggest that the alternative pathway can dissipate the excess reducing equivalents, which are transported from the chloroplasts, and serve in efficient photosynthesis. It is not yet clear whether this increased dark respiration in cold-acclimated *C. quitensis* is due to alternative oxidase up-regulation.

Steady-state fluorescence yield and fluorescence quenching analyses demonstrated that the photosynthetic apparatus of both non-acclimated *C. quitensis* ecotypes responded similarly to light intensity. However, cold acclimation induced differential responses of PSII photochemical performance measured as Φ_{PSII}, F_v'/F_m', and *qP* in these two ecotypes. Overall, growth at low temperature (cold acclimation) increased photochemical performance of the Antarctic ecotype showing higher values of Φ_{PSII}, F_v'/F_m', and *qP*, while PSII photochemistry in cold-acclimated Andes plants was slightly suppressed (Fig. 2A–F).

In both ecotypes, heat dissipation of absorbed excess light energy (*NPQ*) was mostly associated with the fast relaxing component, or NPQ_F (Fig. 3) related to the ΔpH- and zeaxanthin-dependent energy quenching (*qE*) within the LHCII antenna (Walters and Horton, 1991; Xu *et al*., 2000). Cold acclimation significantly modified the xanthophyll cycle activity in the Antarctic ecotype where a higher *EPS* was observed during exposure to HL (Fig. 4) as compared to non-acclimated plants and the Andes ecotype at both growth conditions. This unexpected result is quite intriguing, because the cold-acclimated Antarctic ecotype exhibited the highest *NPQ* and almost 90% of it was associated with NPQ_F (Fig. 3C, E). One possible explanation would be a greater contribution of an additional quenching process independent of zeaxanthin-mediated *NPQ*. The existence of such an additional quenching mechanism is consistent with earlier observations that significant levels of *NPQ* can occur independent of zeaxanthin (Hurry *et al*., 1997; Demmig-Adams *et al*., 1999) and cannot be accounted for by antenna quenching (Kramer, 2004). It has been proposed recently that dissipation of excess light energy via PSII reaction centre quenching might serve as such an additional quenching mechanism in cold-acclimated plants when the enzymatic conversion of violaxanthin to zeaxanthin within the xanthophyll cycle is thermodynamically restricted by low temperatures (Ivanov *et al*., 2003). Indeed, reaction centre quenching of excess light was suggested to play a substantial role in supplementing the antenna-based *NPQ* in cold-acclimated Scots pine (Ivanov *et al*., 2002) and cold-hardened *Arabidopsis* (Sane *et al*., 2003) and barley plants (Ivanov *et al*., 2006). Alternatively, the apparent uncoupling of *NPQ* from the *EPS* levels observed in the cold-acclimated Antarctic ecotype could also be due to the fact that only a few molecules of zeaxanthin are required for a fully active heat dissipation mechanism (Bukhov *et al*., 2001). Another interesting observation is that both ecotypes exposed to low temperature (4 °C), regardless of their acclimation state, exhibited higher values of NPQ_F even at very low *PFD* compared with plants exposed or acclimated to 15 °C (Fig. 3C, D). At very low *PFD*, electron transport rates would be too low to create and sustain a stable ΔpH within the magnitude required for the conversion of violaxanthin to zeaxanthin. In addition, no zeaxanthin and/or antheraxantin are present in neither ecotype at low *PFD* (Fig. 4). Considering these two facts, it appears plausible to suggest that under very low light intensities non-radiative energy dissipation within PSII reaction centres may be induced prior to the detection of antenna quenching as proposed earlier (Finazzi *et al*., 2004).

Moreover, decreased photosynthetic capacity and increased dark respiration was observed in cold-acclimated ecotypes (Table 1). The enhanced rate of dark respiration has been reported earlier in cold-hardened plants such as cereals (Hurry *et al*., 1995) and conifers (Krivosheeva *et al*., 1996). It has been suggested that enhanced dark respiration, coupled to an enhanced activity of NADP-malate dehydrogenase, may contribute electrons for non-photochemical reduction of PQ (Savitch *et al*., 2000). This implies that low temperature may favour a light-independent process that can maintain a *trans*-thylakoid proton gradient at low light, possibly chlororespiration (Field *et al*., 1998). It has been suggested that the contribution of the chlororespiratory electron flux involving the NDH-complex and PTOX to total electron flow in the chloroplast and its photoprotective role as an alternative electron sink is rather limited under optimal growth conditions (Ort and Baker, 2002; Rosso *et al*., 2006). However, chlororespiration has been demonstrated to play an important photoprotective role in the high alpine plant species *Ranunculus glacialis* acclimated to low temperature (Streb *et al*., 2005). Furthermore, up-regulation of PTOX and the chloroplast NDH-complex have been reported in oat plants subjected to heat and high light stresses (Quiles, 2006). These data support the role of chlororespiration as an alternative electron sink in alleviating over-reduction of the PQ pool under unfavourable environmental conditions, which might be important for *C. quitensis* plants acclimated to the harsh environment of the Andes and Antarctica.

The increased contribution of the slow relaxing component (NPQ_S) of *NPQ*, which corresponds to photoinhibitory damage of PSII (Walters and Horton, 1991) in non-acclimated plants of both ecotypes exposed to 4 °C and high light compared to cold-acclimated plants (Fig. 3E, F), indicates that growth at low temperatures stabilizes the photosynthetic apparatus. The highest NPQ_S values were observed in non-acclimated Andes plants exposed to 4 °C and high light. This indicates that this

ecotype is more susceptible to low temperature induced photoinhibition of PSII. In the field, under clear sky days the Andes ecotype is often under high irradiances (2500 μmol photons m^{-2} s^{-1}), where temperature close to the soil can approach to 25 °C. Conversely, the Antarctic ecotype rarely experiences 1500 μmol photons m^{-2} s^{-1} and air temperature close to the soil is near 10 °C in snow free areas of the Maritime Antarctic. Therefore, the Andes ecotype is prepared to cope with high irradiances at relatively high temperatures, whereas the Antarctic ecotype can withstand low temperature and high irradiances better than the Andes one. Furthermore, freezing resistance of these ecotypes is consistent with the behaviour of their photosynthetic machineries. While the Antarctic ecotype is more freezing tolerant, reaching an LT_{50} of about –15 °C after 21 d of cold acclimation, the Andes ecotype only reaches an LT_{50} of –10 °C after the same acclimation time (Gianoli *et al*., 2004). It is suggested here that different selective pressure imposed by constant low temperature, shorter growing season, and a variable and sometimes limited light resource in the Antarctic relative to the Andes (Xiong *et al*., 1999; Xiong and Day, 2001) have genetically conditioned the Antarctic ecotype to an improved disposition for cold acclimation and, in this state, to maximize its photosynthetic performance.

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References

Alberdi M, Bravo LA, Gutiérrez A, Gidekel M, Corcuera LJ. 2002. Ecophysiology of Antarctic vascular plants. *Physiologia Plantarum* **115,** 479–486.

Bukhov NG, Kopecky J, Pfündel EE, Klughammer Heber U. 2001. A few molecules of zeaxanthin per reaction centre of photosystem II permit effective thermal dissipation of light energy in photosystem II of a poikilohydric moss. *Planta* **212,** 739–748.

Casanova-Katny M, Bravo LA, Molina Montenegro M, Corcuera LJ, Cavieres LA. 2006. Photosynthetic performance of *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) in a high-elevation site of the Andes of central Chile. *Revista Chilena de Historia Natural* **79,** 41–53.

Cavieres LA, Arroyo MTK. 1999. Tasa de enfriamiento adiabático del aire en el valle del río Molina, provincia de Santiago, Chile central (33° S). *Revista Geográfica de Chile Terra Australis* **44,** 79–86.

Demmig-Adams B, Adams III WW. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends in Plant Science* **1,** 21–26.

Demmig-Adams B, Adams WW, Ebber V, Logan BA. 1999. Ecophysiology of the xanthophyll cycle. In: Frank HA, Young AJ, Britton G, Cogdell RJ, eds. *The photochemistry of carotenoids*, Vol. 8. Dordrecht: Kluwer Academic Publishers, 245–269.

Diaz M, Ball E, Luttge U. 1990. Stress-induced accumulation of the xanthophyll rhodoxanthin in leaves of *Aloe vera*. *Plant Physiology and Biochemistry* **28,** 679–682.

Field TS, Nedbal L, Ort DR. 1998. Nonphotochemical reduction of the plastoquinone pool in sunflower leaves originates from chlororespiration. *Plant Physiology* **116,** 1209–1218.

Finazzi G, Johnson GN, Dallosto L, Joliot P, Wollman F-A, Bassi R. 2004. A zeaxanthin-independent nonphotochemical quenching mechanism localized in the photosystem II core complex. *Proceedings of the National Academy of Sciences, USA* **101,** 12375–12380.

Gianoli E, Inostroza P, Zuñiga-Feest A, Reyes-Diaz M, Cavieres LA, Bravo LA, Corcuera LJ. 2004. Ecotypic differentiation in morphology and cold resistance in population of *Colobanthus quitensis* (Cariophyllaceae) from the Andes of central Chile and Maritime Antarctica. *Arctic, Antarctic and Alpine Research* **36,** 484–489.

Huner NPA, Öquist G, Sarhan F. 1998. Energy balance and acclimation to light and cold. *Trends in Plant Science* **3,** 224–230.

Hurry V, Anderson JM, Chow WS, Osmond CB. 1997. Accumulation of zeaxanthin in abscisic acid-deficient mutants of *Arabidopsis* does not affect chlorophyll fluorescence quenching or sensitivity to photoinhibition *in vivo*. *Plant Physiology* **113,** 639–648.

Hurry V, Tobieson M, Krömer S, Gardeström P, Öquist G. 1995. Mitochondria contribute to increased photosynthetic capacity of leaves of winter rye (*Secale cereale* L.) following cold-hardening. *Plant, Cell and Environment* **18,** 69–76.

Ivanov AG, Krol M, Maxwell D, Huner NPA. 1995. Abscisic acid induced protection against photoinhibition of PSII correlates with enhanced activity of the xanthophyll cycle. *FEBS Letters* **371,** 61–64.

Ivanov AG, Sane PV, Hurry V, Krol M, Sveshnikov D, Huner NPA, Öquist G. 2003. Low temperature modulation of redox properties of the acceptor side of photosystem II: photoprotection through reaction centre quenching of excess energy. *Physiologia Plantarum* **119,** 376–383.

Ivanov AG, Sane PV, Krol M, Gray GR, Balseris A, Savitch LV, Öquist G, Hüner NPA. 2006. Acclimation to temperature and irradiance modulates PSII charge recombination. *FEBS Letters* **580,** 2797–2802.

Ivanov AG, Sane PV, Zeinalov Y, Simidjiev I, Huner NPA, Öquist G. 2002. Seasonal responses of photosynthetic electron transport in Scots pine (*Pinus sylvestris* L.) studied by thermoluminescence. *Planta* **215,** 457–465.

Kramer DM, Johnson G, Kiirats O, Edwards GE. 2004. New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. *Photosynthesis Research* **79,** 209–218.

Krause GH. 1994. Photoinhibition induced by low temperatures. In: Baker NR, Bowyer JR, eds. *Photoinhibition of photosynthesis from molecular mechanism to the field*. Oxford, UK: BiosScientific Publishers Ltd, 301–348.

Krivosheeva A, Tao D-L, Ottander C, Wingsle G, Dube SL, Öquist G. 1996. Cold acclimation and photoinhibition of photosynthesis in Scots pine. *Planta* **200,** 296–305.

Lewis Smith RI. 2003. The enigma of *Colobanthus quitensis* and *Deschampsia antartica* in Antarctica. In: Huiskes AHL, Gieskes WWC, Rozema J, Schorno RML, van der Vies SM, Wolff WJ, eds. *Antarctic biology in a global context*. Leiden, The Netherlands: Backhuys Publishers, 234–239.

Mantovani A, Vieira RC. 2000. Leaf micromorphology of Antarctic pearlwort *Colobanthus quitensis* (Kunth) Bartl. *Polar Biology* **23,** 531–538.

Melis A. 1999. Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage *in vivo*? *Trends in Plant Science* **4,** 130–135.

Ort DR, Baker NR. 2002. A photoprotective role for O_2 as an alternative electron sink in photosynthesis? *Current Opinion in Plant Biology* **5,** 193–198.

Pérez-Torres E, Bascuñan L, Sierra A, Bravo LA, Corcuera LJ. 2006. Robustness of activity of Calvin cycle enzymes after high light and low temperature conditions in Antarctic vascular plants. *Polar Biology* **29,** 909–916.

Pérez-Torres E, Dinamarca J, Bravo LA, Corcuera LJ. 2004. Responses of *Colobanthus quitensis* (Kunth) Bartl. to high light and low temperature. *Polar Biology* **27,** 183–189.

Quiles MJ. 2006. Stimulation of chlororespiration by heat and high light intensity in oat plants. *Plant. Cell and Environment* **29,** 1463–1470.

Rosso D, Ivanov AG, Fu A, *et al*. 2006. IMMUTANS does not act as a stress-induced safety valve in the protection of the photosynthetic apparatus of *Arabidopsis thaliana* during steady-state photosynthesis. *Plant Physiology* **142,** 574–585.

Sagisaka S, Matauda Y, Okuda T, Ozeki S. 1991. Comparative studies of changes in enzymatic activities in hardy and less hardy cultivars in winter wheat in late fall and in winter under snow. *Soil Science and Plant Nutrition* **37,** 545–550.

Sane PV, Ivanov AG, Hurry V, Huner NPA, Öquist G. 2003. Changes in the redox potential of primary and secondary electron-accepting quinones in photosystem II confer increased resistance to photoinhibition in low-temperature-acclimated *Arabidopsis*. *Plant Physiology* **132,** 2144–2151.

Savitch LV, Leonardos ED, Krol M, Jansson S, Grodzinski B, Huner NPA, Öquist G. 2002. Two different strategies for light utilization in photosynthesis in relation to growth and cold acclimation. *Plant, Cell and Environment* **25,** 761–771.

Savitch LV, Massacci A, Gray GR, Huner NPA. 2000. Acclimation to low temperature or high light mitigates sensitivity to photoinhibition: roles of the Calvin cycle and Mehler reaction. *Australian Journal of Plant Physiology* **27,** 253–264.

Schreiber U, Schliwa W, Bilger U. 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorimeter. *Photosynthesis Research* **10,** 51–62.

Sierra-Almeida A, Casanova-Katny MA, Bravo LA, Corcuera LJ, Cavieres LA. 2007. Comparing the photosynthetic responses to temperature and light between Antarctic and Andean populations of *Colobanthus quitensis* (Caryophylaceae). *Revista Chilena de Historia Natural* (in press).

Streb P, Josse E-M, Gallouet E, Baptist F, Kuntz M, Cornic G. 2005. Evidence for alternative electron sinks to photosynthetic carbon assimilation in the high mountain plant species *Ranunculus glacialis*. *Plant, Cell and Environment* **28,** 1123–1135.

Sugie A, Naydenov N, Mizuno N, Nakamura C, Takumi S. 2006. Overexpression of wheat alternative oxidase gene *Waox1a* alters respiration capacity and response to reactive oxygen species under low temperature in transgenic *Arabidopsis*. *Genes, Genetic and Systematics* **81,** 349–354.

Vanlerberghe GC, McIntosh L. 1992. Lower growth temperature increases alternative pathway capacity and alternative oxidase protein in tobacco. *Plant Physiology* **100,** 115–119.

van Kooten OV, Snel JFH. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research* **25,** 147–150.

Walters RG, Horton P. 1991. Resolution of components of non-photochemical chlorophyll fluorescence quenching in barley leaves. *Photosynthesis Research* **27,** 121–133.

Yoshida K, Terashima I, Noguchi K. 2007. Up-regulation of mitochondrial alternative oxidase concomitant with chloroplast over-reduction by excess light. *Plant Cell Physiology* **48,** 606–614.

Xiong FS, Day TA. 2001. Effect of solar ultraviolet-B radiation during springtime ozone depletion on photosynthesis and biomass production of Antarctic vascular plants. *Plant Physiology* **125,** 738–751.

Xiong FS, Mueller EC, Day TA. 2000. Photosynthetic and respiratory acclimation and growth response of Antarctic vascular plants to contrasting temperature regimes. *American Journal of Botany* **87,** 700–710.

Xiong FS, Ruhland CT, Day TA. 1999. Photosynthetic temperature response of the Antartic vascular plants *Colobanthus quitensis* and *Deschampsia antartica*. *Physiologia Plantarum* **106,** 276–286.

Xu C-C, Li L, Kuang T. 2000. Photoprotection in chilling-sensitive and resistant plants illuminated at a chilling temperature: role of the xanthophylls cycle in the protection against lumen acidification. *Australian Journal of Plant Physiology* **27,** 669–675.