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ORIGINAL PAPER

# Leaf functional and micro-morphological photoprotective attributes in two ecotypes of *Colobanthus quitensis* from the Andes and Maritime Antarctic

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**Abstract** *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) is distributed from Mexico to the Maritime Antarctic. It grows forming inconspicuous populations in humid and cold sites along high elevations in the Andes Mountains. Mediterranean Andes is characterized by a wider oscillation of diurnal and seasonal temperature, while the Maritime Antarctic is characterized by permanent low temperatures. Both places may experience high irradiance during sunny days (reaching up to 2,000 μmol photons $m^{-2}$ $s^{-1}$); however, the frequency of sunny days in the Maritime Antarctica is significantly lower (less than 20% of the whole growing season). We study whether acclimation to each environment relies on different photoprotective mechanisms. The Andean ecotype that has a longer growing season and a higher light integral reduces light absorption by the development of smaller chloroplasts with lower stacking granum area and down-regulation of Lhcb2. It also enhances the dissipation of the excess of absorbed energy by higher level of de-epoxidation of xanthophylls pool. On the other hand, the Antarctic ecotype which has developed under a shorter growing season, with lower total irradiance and continuous low temperatures, maximizes photochemical process even at low temperatures and it has a lower light-harvesting/core complex ratio and higher level of photoprotection supplied by an unusually high $\beta$-carotene and xanthophylls cycle pool. It resembles a well full light acclimated plant, probably due to higher excitation pressure imposed by lower temperature even at moderate irradiance. It is suggested that the biochemical plasticity of this species, highlighted by the development of these different strategies, is essential to cope successfully with these particular environments.

**Keywords** Antarctic plants · Andean plants · Ecotypes · Low temperatures stress · High light stress · Photoprotection strategies

## Introduction

Higher plants growing at low temperature or/and high light intensity often experience an imbalance between energy absorption and photosynthetic light utilization (Logan et al. 1998). This effect is based on the fact that under high light intensity, plants are not able to utilize all the absorbed light for photosynthesis and this imbalance is increased at low temperature when the Calvin cycle reactions are considerably slower (Huner et al. 1993). Under these conditions, light absorbed in excess may potentially cause photo-oxidative damage of photosystem II, PSII (Aro et al. 1993). To prevent photo-damage, plants have developed different strategies to cope with spatial and temporal variations in environmental conditions. These strategies demand adjustment of primary (light reactions) and secondary (Calvin cycle) photosynthetic processes in order to avoid the harmful

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effects of the overexcitation of the photosynthetic apparatus (Huner et al. 1993).

Two main mechanisms protect plant tissues from the excess absorbed energy: the modulation of light capture and the enhancement of energy dissipation. Thus, plants growing at high light typically decrease the probability of light absorption by the development of a thicker parenchyma and the presence of smaller chloroplasts, with less grana per chloroplast and less thylakoids per granum (Anderson 1986; Melis 1991). In addition, these plants develop a smaller antenna complex; therefore, the light harvesting to reaction center ratio is lower. This is reflected in a higher Chl *a*/Chl *b* ratio (considering that core complex of PSII mainly has Chl *a* and the antenna complex has both Chl *a* and *b*) (Ensminger et al. 2006) and *β*-carotene/neoxanthin ratio (the first is not present in light-harvesting complexes while the latter is not present in reaction centers; Croce et al. 1999).

Once light has been absorbed by the antenna, photoprotection may be achieved by dissipation through the photochemical use of the energy (photochemical quenching, qL) or by thermal dissipation of excess absorbed light in the pigment bed (Demmig-Adams et al. 1996a). Most, but not all, photochemical use of energy is dependent on $CO_2$ fixation and carbon assimilation. When the energy utilization capacity through the Calvin cycle is exceeded, as is the case of low temperature stress combined with high irradiance (Ensminger et al. 2006), other photochemical processes become relevant. These processes include cyclic electron transport, photorespiration and reactive oxygen species (ROS) production through Mehler reaction (Bendall and Manasse 1995; Osmond 1981; Kozaki and Takeba 1996; Asada 1999). On the other hand, the thermal dissipation can be monitored by the non-photochemical quenching (NPQ) of chlorophyll fluorescence. NPQ has a fast relaxing component ($NPQ_f$) and a slow relaxing component ($NPQ_s$) (Maxwell and Johnson 2000). $NPQ_f$ is related to thermal energy dissipation, also called qE and involves intrathylakoidal acidification, activation of the xanthophylls cycle with the conversion of violaxanthin (V) to anteraxanthin (A) and zeaxanthin (Z) (Demmig-Adams et al. 1996b; Niyogi et al. 1998) and a conformational change of the antenna complex protein, PsbS (Li et al. 2000). $NPQ_s$ reflects sustained thermal dissipation most likely caused by retention of Z or by photo-damage of PSII induced by excess absorbed energy (Müller et al. 2001). The excess energy results in the unavoidable generation of ROS when absorbed energy exceeds the capacity for photochemical and non-photochemical dissipation. To prevent ROS accumulation, plants contain several protective enzymes and antioxidant molecules. Antioxidants are present at high concentrations in the chloroplasts and can be classified as hydrophilic and lipophilic; the latter include tocopherols and *β*-carotene (Polle and Rennenberg 1994). Tocopherols are present in plastoglobules, thylakoid membranes and in the chloroplast envelope. They act as efficient quenchers of $^1O_2$, but are also able to stop lipid peroxidation chain reactions (Sattler et al. 2004). *β*-Carotene is present in the core complex of PSII, protecting it from the generation of triplet excited chlorophyll and $^1O_2$ (Young et al. 1997). Whenever the antioxidant mechanisms are exceeded, D1 suffers photo-oxidative damage (De Las Rivas et al. 1992). To maintain PSII activity, repair mechanisms are involved in the turnover and replacement of this protein (Kyle et al. 1984; Ohad et al. 1984; Andersson and Aro 2006). Only when the rate of damage is greater than the rate of repair, there is a net loss of PSII activity (Aro et al. 1993).

*Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) is one of the few vascular plants that have been able to colonize the Maritime Antarctic. This species is also found along the Andes Mountains in South and Central America (Lewis Smith 2003). Based on freezing resistance, morphology and ITS sequence studies, ecotype differentiation between central Chilean Andes and Antarctic populations has been established (Gianoli et al. 2004). The Antarctic ecotype grows under sustained but stable low temperature (−2 to 6°C air temperature) and usually low PPFD (300–600 μmol photons $m^{-2}$ $s^{-1}$), with a few days with high PPFD (2,000 μmol photons $m^{-2}$ $s^{-1}$) (Alberdi et al. 2002; Xiong et al. 1999; Xiong and Day 2001). The Andean ecotype grows under a wider diurnal temperature oscillation (usually between 0 and 22°C) and frequently exposed to high PPFD at noon (>2,000 μmol photons $m^{-2}$ $s^{-1}$) (Casanova-Katny et al. 2006; Cavieres and Arroyo 1999). Their life cycles occur once snow melts (from December to March in the Antarctic, and from October to April in the Andean ecotypes). In spite of this very harsh environmental conditions, neither the Antarctic nor the Andean ecotypes present photo-damage symptoms in the field (Xiong and Day 2001; Casanova-Katny et al. 2006). When both ecotypes grow under comparable conditions in growth chambers, the Antarctic ecotype has a higher qL and $NPQ_f$ than the Andean ecotype (Bravo et al. 2007). However, their photoprotective strategies in the field have not been studied. These contrasting environments in which this species grows suggest that photoprotective strategies could be different in Andean and Antarctic ecotypes. We propose that in the Andean ecotype, which experiences a higher light integral during the growing season, the main regulatory mechanism is thermal dissipation or the decrease in the capability to absorb light. In the Antarctic ecotype, instead, the maintenance of photochemical processes at low temperature could be crucial to grow under a lower seasonal light availability and permanent cold conditions. The aim of this paper was to verify whether different photoprotective

mechanisms are employed by both ecotypes growing under their natural conditions.

## Materials and methods

### Site description and plant material

Two ecotypes *C. quitensis* (Kunth) Bartl., Andean and Antarctic (Gianoli et al. 2004), were studied in their natural environments at the Andes Mountains (La Parva, 2,650 m a.s.l. 33°19′S; 70°17′W) and King George Island (Near Arctowski base, see level; 62°10′S; 58°29′W). Measurements of chlorophyll fluorescence and collection of leaf samples were performed during three sunny days by the end of October 2007 for the Andean ecotype and beginning of February 2008 for the Antarctic one. These months were selected because we were able to find *C. quitensis* plants in vegetative stage in both study sites. Xanthophylls cycle pigments and PSII proteins were analyzed in the samples obtained from both sites. The daily course of instantaneous temperature and PPFD were recorded as a single measure with an interval of 1 h using dataloggers LiCor LI1400 with sensors of temperature and PPDF (LiCor Inc., Lincoln, NE, USA) located at 1.5 m from the soil in the case of the Antarctic, and using a HOBO Micro-Station, H-21-002 located at 1.5 m from the soil (Onset Computer Corporation, Bourne, MA) in the Andes Mountains.

Due to the natural differences in light intensity and temperature during in situ measurements (Fig. 1), it was not possible to compare fluorescence parameters under natural conditions between ecotypes. Comparisons were restricted to daily patterns within each ecotype. Comparative measurements of fluorescence parameters under controlled conditions were done in plants collected from each study site.

### Light and electron microscopy analysis

Fully developed leaves of *C. quitensis* from the two ecotypes were collected at midday of one sunny day. The samples were immediately fixed in 4% glutaraldehyde. In the laboratory, leaves were sectioned in 2–3 mm in length fragments and fixed again in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4°C room temperature. After a short rinse with 0.1 M phosphate buffer (three exchanges), the plant material was post-fixed 2 h in 2.5% osmium tetroxide. The fixed tissue was then washed in buffer, dehydrated in a graded ethanol series, transferred to mixtures with increasing ratios of Araldite Resin, and finally embedded in pure Araldite Resin. Both semi-thin (150 nm) and ultra-thin (50 nm) sections were prepared on an ultramicrotome Reichert Sorval MT-2. Semi-thin sections were stained with toluidine blue and viewed with a light microscope to analyze morphological attributes of the leaf. For this work only chloroplasts from parenchyma level were studied to avoid the background due to the use of different tissues. Therefore, these semi-thin sections were also used to select the best areas for the ultra-thin sections from parenchyma level. Ultra-thin sections cut with a diamond knife were stained on grids with uranyl acetate and lead citrate, and examined in Noran Instrument transmission electron microscope. Three fragments of three leaves from three different individuals were used in the study of the ultrastructure for each ecotype. Chloroplast ultrastructure was studied using the program ImageJ (NIH, Maryland, USA).

### Diurnal field and laboratory measurements of chlorophyll fluorescence

Chl fluorescence measurements of *C. quitensis* were performed using a portable fluorimeter (FMS 2, Hansatech

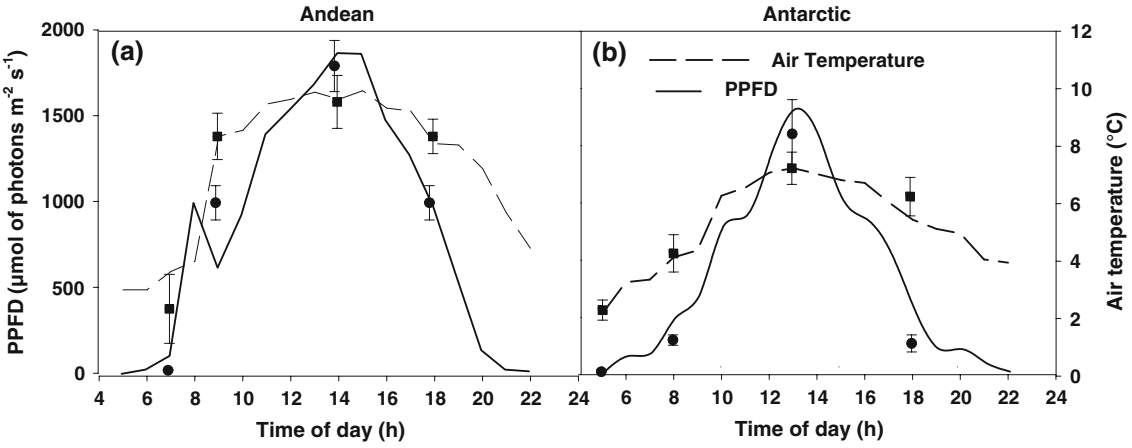


**Fig. 1** Diurnal course of light intensity and air temperature during a sunny day in the growing sites of *C. quitensis* in the Andes Mountains (**a**) and Antarctic (**b**). *Solid* and *dashed lines* represent the light intensity and air temperature, respectively, of one sunny day. *Circles* and *squares* represent the mean and ±SE of light intensity and air temperature, respectively, of individual data collected at the time of sampling and physiological measurements performed during three sunny days

Instruments Ltd., Norfolk, UK). Field measurements were done in intact plants under natural sunlight and temperature in the morning (0900 and 0800 hours in the Andean and Antarctic place), midday (1300 hours) and evening (1800 hours). Plants were first dark adapted 30 min in order to obtain the maximum photochemical efficiency of PSII (Fv/Fm), then natural light was allowed to reach the plant (field measurements) or actinic light was provided by FMSII (laboratory measurements). Laboratory measurements were taken at both 4 and 15°C at 900 μmol photons $m^{-2}$ $s^{-1}$ in detached leaves of plants collected from the field. In both cases (under field and controlled conditions), the fiber-optic was located at about 10 mm from the sample. Different light pulses were applied following the standard routines programmed as described previously by Bravo et al. (2007). NPQ components were calculated from the dark relaxation kinetic after high light treatments as described by Bravo et al. (2007).

### Pigments and xanthophyll cycle analyses

Leaves were sampled in the field at 7, 13 and 18 h and immediately frozen in liquid nitrogen and stored at −80°C until analysis. For the determination of tocopherols and photosynthetic pigments, leaf samples were extracted twice (acetone 100 and 95%) from lyophilized material and analyzed by reverse-phase HPLC following the method of García-Plazaola and Becerril (1999) with the modifications described in García-Plazaola and Becerril (2001). De-epoxidation state of the xanthophylls pigments pool was estimated as: (A + Z)/(V + A + Z).

### Western blot analysis of thylakoid proteins

Leaves of *C. quitensis* were ground at 4°C in 20 mM Tricine (pH 7.6) containing 0.4 M sorbitol and 10 mM NaCl, 5 mM EGTA, 5 mM EDTA with a mortar and pestle. To obtain thylakoids, the leaf debris was filtered through one layer of Miracloth and the filtrate was subsequently centrifuged at 3,000*g* for 5 min. The pellet was washed in 50 mM Tricine (pH 7.8), 10 mM NaCl, 5 mM MgCl and centrifuged. Then, thylakoid membranes were resuspended and solubilized in 60 mM Tris (pH 7.8) containing 12% sucrose, 1 mM EDTA, 1% DTT and 2% SDS. Chlorophyll concentrations were measured according to Arnon (1949).

Thylakoid proteins were separated in a SDS polyacrylamide gel that consisted of a 4% (w/v) stacking gel and a 12% (w/v) separating gel containing 8 M urea. The protein profiles resolved by SDS-PAGE were transferred onto nitrocellulose and immunolabelled with LHCII and D1 antibodies (Agrisera, Vännäs, Sweden). Immuno-decorated proteins were detected by enhanced chemiluminescence (ECL) (Pierce, Rockford, USA) on X-ray film (Fuji, Tokyo, Japan). Densitometric measurements of the chemiluminescently produced bands on the X-ray films were quantified with the program ImageJ (NIH, Maryland, USA). The results were expressed in percent respect to the maximum level.

### Statistical analysis

Leaf and chloroplast micro-morphology parameters obtained from light and electron microscopy, α-tocopherol and pigments which correspond to the average of $n = 3$ were analyzed by a $t$ test ($P < 0.05$). Chlorophyll fluorescence parameters, xanthophylls cycle pigments and thylakoid protein in a diurnal cycle correspond to the mean of one measurement in three different sunny days ($n = 3$). Differences between time of the day were determined by one-way ANOVA analysis ($P < 0.05$). Chlorophyll fluorescence parameters obtained under controlled conditions ($n = 6$) were analyzed by a two-way ANOVA, where the studied factors were ecotype and temperature of measurement.

## Results

### Variations of light and temperature in a diurnal cycle

Diurnal pattern of temperature and PPDF representatives of a sunny day in the Andean and Antarctic sites were recorded while this work was performed (Fig. 1). Only sunny days were selected because they correspond with environmental conditions during the 3 days in which samples for proteins were taken and measurements of chlorophyll fluorescence were performed. Light and temperature were also measured at each time of sampling (Fig. 1).

There were no statistically significant differences ($P > 0.05$) between the maximum PPFD reached in the Andean site and the Antarctic sites during experimental days (Fig. 1a, b). However, the daily integral of photosynthetic solar radiation in the Andes was 50% higher than in the Antarctic. Air temperature during sunny days in summer was lower in the Antarctic (reached a maximum of 7°C at midday) than in the Andean site (reached a maximum about 10°C at midday). The temperature integral (area below the daily temperature curve) in the Antarctic was about 60% of the temperature integral in the Andes.

### Light and electron microscopy analyses

Mesophyll, palisade parenchyma and spongy parenchyma of the Andean ecotype were thinner than the Antarctic one (Table 1). Antarctic plants presented a higher leaf thickness than Andean plants. Chloroplasts from the Antarctic ecotype presented a higher cross-sectional area, number of grana, and area of stacked thylakoids per chloroplast than chloroplast from the Andean ecotype (Table 1). A striking

**Table 1** Comparative leaf anatomy and chloroplasts characteristics of Andean and Antarctic ecotypes of *C. quitensis*

| | Andean | Antarctic |
|---|---|---|
| Thickness of mesophyll (μm) | 386 ± 29* | 475 ± 12 |
| Thickness of palisade parenchyma (μm) | 142 ± 9* | 181 ± 7 |
| Thickness of spongy mesophyll (μm) | 229 ± 9* | 291 ± 11 |
| Chloroplast cross-sectional area ($\mu m^2$) | 4.6 ± 0.6* | 8.3 ± 0.5 |
| Grana per chloroplast | 12.9 ± 2.1* | 21.6 ± 1.9 |
| Stacking granum area ($\mu m^2$) per chloroplast | 0.85 ± 0.09* | 1.23 ± 0.01 |
| Thylakoids per granum | 6.8 ± 0.6 (n.s.) | 6.1 ± 0.2 |
| Starch size ($\mu m^2$) | 0.34 ± 0.08 (n.s.) | 0.26 ± 0.07 |
| Starch granules per chloroplast | 1.74 ± 0.05* | 3.80 ± 0.29 |
| PG size ($\mu m^2$) | 0.040 ± 0.003*** | 0.006 ± 0.001 |

Three individual plants ($n = 3$) from each ecotype were sampled at midday. Values represent average ± SE

*n.s.* not significantly different

* Mean significantly different between Andean and Antarctic ecotypes $P < 0.05$ using Tukey's test

*** Mean significantly different between Andean and Antarctic ecotypes $P < 0.01$ using Tukey's test

difference was found in chloroplast morphology among both ecotypes with respect to plastoglobules size, where the Andean ecotype exhibited bigger individual plastoglobules. In spite of the higher number of PG in the Antarctic ecotype, the area of PG per area of chloroplast was sixfold higher in the Andean ecotype (Table 1).

### Diurnal changes on photochemical and non-photochemical quenching

No differences in Fv/Fm (Fig. 2a, b) and qL (Fig. 2c, d) were observed during the day course in both ecotypes. However, ΦPSII was significantly smaller at midday than in the morning and late afternoon in both ecotypes (Fig. 2e, f). Concomitantly with the decrease in ΦPSII, there was an increase in NPQ at noon in both ecotypes (Fig. 3). The main component of NPQ in both ecotypes during daily hours of maximum light intensity was $NPQ_f$. By the end of the afternoon, there was statistically significant higher $NPQ_s$ than early hours only in the Andean ecotype (Fig. 3c).

### Tocopherol, chlorophylls, carotenoids and diurnal changes in the xanthophylls cycle

Pigment and tocopherol pools remained stable during the day (data not shown). Thereby, the data shown in Table 2 correspond to samples collected at noon. The content of tocopherol and chlorophylls was similar in both ecotypes. Chlorophyll *a*/*b* and *β*-carotene/neoxanthin ratio were greater in the Antarctic than in the Andean ecotype. Carotenoids including lutein, neoxanthin and *β*-carotene and total carotenoids were greater in the Antarctic ecotype (the last two by a factor of 2). The pool of xanthophylls (VAZ) was also higher in the Antarctic ecotype (Fig. 4). However, the maximum de-epoxidation state was higher in Andean ecotype. On the other hand, it is remarkable that *α*-carotene, present at low concentration in the Antarctic ecotype, was absent in the Andean one.

### Thylakoid proteins: Lhcb2 and D1

In general, Antarctic plants presented higher levels of Lhcb2 and D1. No statistical differences in D1 or Lhcb2 were registered in the Antarctic ecotype during the day course (Fig. 5b, c). However, a pronounced decrease of Lhcb2 at midday and of D1 at the afternoon was observed in the Andean ecotype (Fig. 5a, b).

### Laboratory measurements of chlorophyll fluorescence

In order to assess differential sensitivity to environmental temperature in both ecotypes, functional performance of the photosynthetic apparatus was studied under controlled laboratory conditions at two different temperatures (4 and 15°C). The Fv/Fm ratio was maintained high at both measurement temperatures in both ecotypes (Fig. 6a, b). Measurements at 4°C showed a decrease in ΦPSII in the Andean ecotype and in qL in both ecotypes, with respect to 15°C (Fig. 6). ΦPSII at 4°C (Fig. 6f) and qL at both measurement temperatures (Fig. 6c, d) were higher in the Antarctic ecotype than in the Andean one. At this temperature also, the Andean ecotype showed an increase in NPQ and $NPQ_s$ (Fig. 7a, b). $NPQ_f$ was similar between both ecotypes and remained unchanged with different measurement temperatures.

## Discussion

Low temperature triggers down-regulation of photochemical efficiency (Ensminger et al. 2006), inducing a sustained NPQ characterized by dark conservation of Z (Adams et al. 1995) and limits the rate of repairing mechanisms decreasing D1 de novo synthesis (Murata et al. 2007). Therefore, simultaneous occurrences of low temperature and high light are propitious conditions that enhance photo-damage. This combination of stresses frequently occurs in polar regions and high mountain areas. Andean and Antarctic ecotypes of *C. quitensis* are exposed to two contrasting environments. In the Andes, temperature fluctuates greatly during the day with high irradiances at noon, while in Maritime Antarctic plants grow under a lower amount of total irradiance and

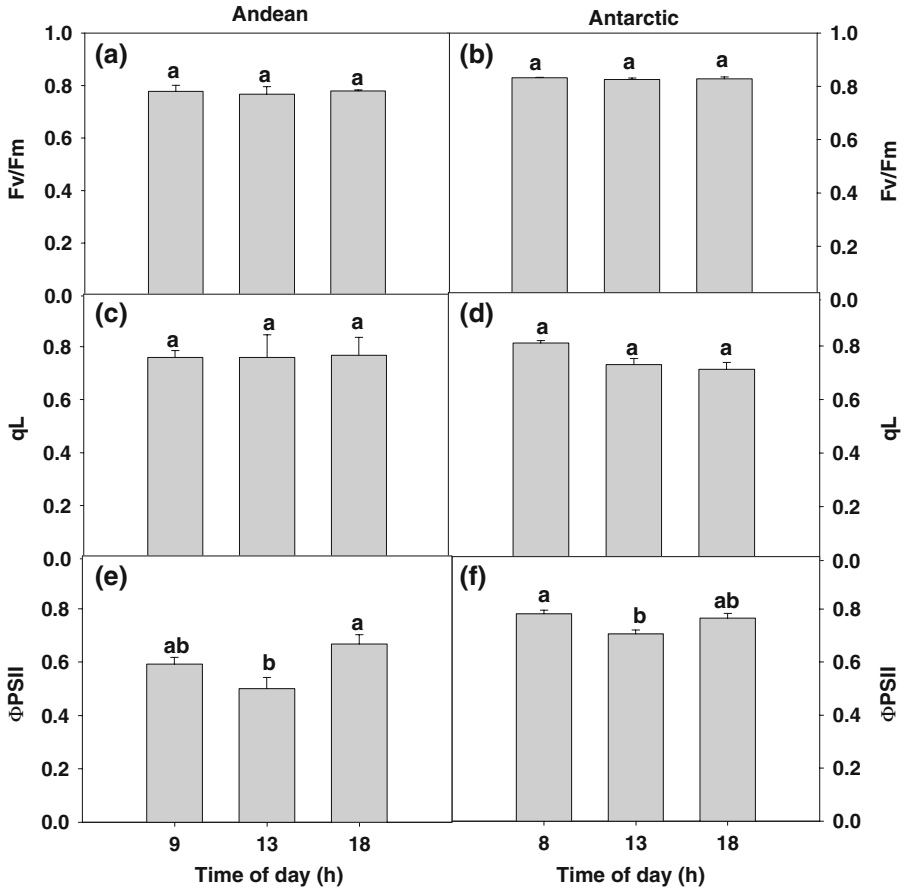


**Fig. 2** Diurnal course of photochemical efficiency under natural conditions of both ecotypes of *C. quitensis*. Fv/Fm is the maximal photochemical efficiency, qL is photochemical quenching and ΦPSII is quantum yield of PSII. Measurements were performed after dark adaptation for 30 min. *Bars* show average values ± SE calculated from three different sunny days. *Different letters* represent significant differences within the diurnal course of each ecotype, $P < 0.05$ using one-way ANOVA

sustained low temperatures (Schroeter et al. 1995; Alberdi et al. 2002; Xiong and Day 2001; Casanova-Katny et al. 2006). Both environments favor photoinhibitory conditions, but they differ in the degree and extension of such stress. Therefore, plants developed in each of these environments may have evolved different strategies to cope with it. A comparative study between both ecotypes in plants growing in the field during typical sunny days for both sites (Fig. 1) was performed in order to verify whether these two ecotypes of *C. quitensis* have been able to establish different photoprotective mechanisms in response to their contrasting environmental conditions.

The thicker leaves in the Antarctic ecotype compared with the Andean one (Table 1) seem to be a constitutive difference, since they are maintained in plants growing in a common garden under controlled laboratory conditions (Gianoli et al. 2004; Bravo et al. 2007). It has been reported that the increase of leaf thickness under cold acclimation may be beneficial for leaf survival of frost-induced cell dehydration, alleviating the mechanical stress experienced during thawing (Stefanowska et al. 1999). On the other hand, the thicker palisade parenchyma in the Antarctic ecotype may be associated with the higher $A_{max}$ values registered in the field, 8 µmol $CO_2$ $m^{-2}$ $s^{-1}$, compared with 5 µmol $CO_2$ $m^{-2}$ $s^{-1}$ for the Andean one (Xiong et al. 1999; Casanova-Katny et al. 2006).

In agreement with our hypothesis, the Andean ecotype that grows under higher total irradiance, higher daily temperature and with a longer growing season, which probably are less limiting for growth and photosynthesis, is able to decrease the probability of light capture developing smaller chloroplast with lower number of stacked granum and stacked granum area per chloroplast (Table 1). Also, it seems that this ecotype presents down-regulation of Lhcb2 at noon (Fig. 5a). This mechanism is frequently used by annual plants to cope with the excess of light (Walters and Horton 1994) and occurs through the regulation of transcription and translation of the Lhcb family of nuclear genes encoding LHCII polypeptides (Maxwell et al. 1995). On the other hand, the Antarctic ecotype which is likely more limited by temperature, light and with a shorter growing season presented a higher Chlorophyll *a*/*b* rate than Andean plants. This could be indicating that the Antarctic ecotype presents a lower antenna/core PSII proportion. The Lhcb2/D1 proportion and the dramatic difference in the index $\beta$-carotene/neoxanthin also support this result (Fig. 5; Table 2). In addition, the Antarctic ecotype presents greater levels of photoprotective pigments (Table 2). In fact,

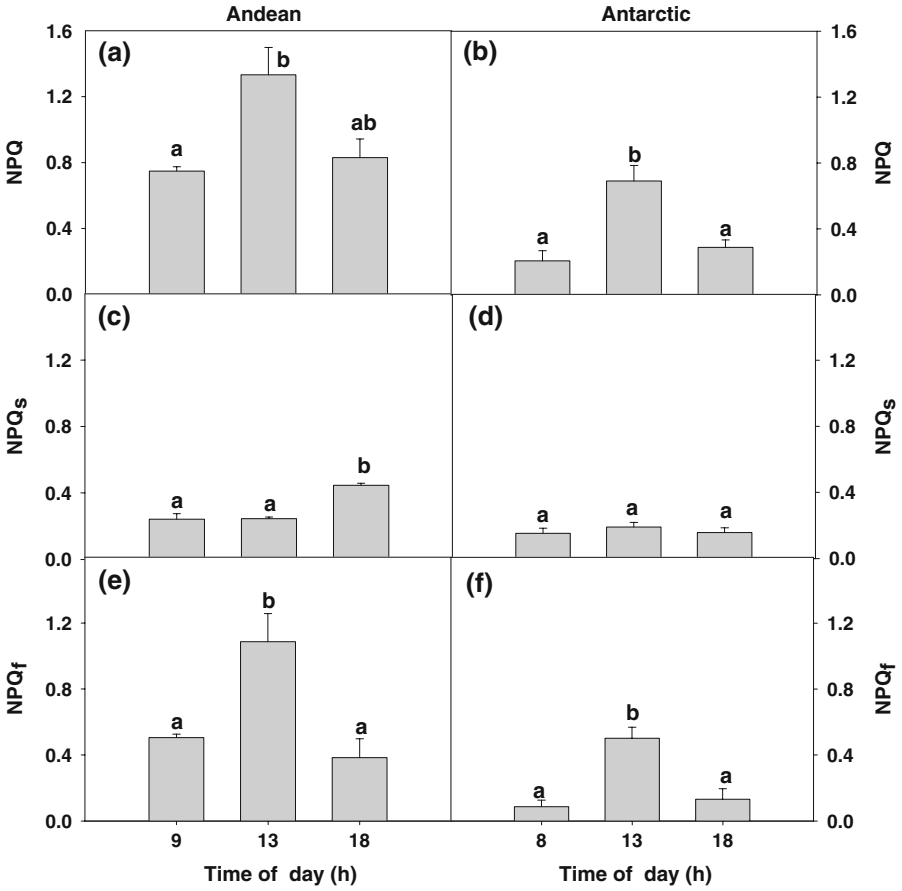


**Fig. 3** Diurnal variation of non-photochemical quenching (NPQ) (**a**, **b**), slow NPQ ($NPQ_s$) (**c**, **d**), fast NPQ ($NPQ_f$) (**e**, **f**) relaxing components of both ecotypes of *C. quitensis* under natural conditions. *Bars* show average values ± SE calculated from three different sunny days. *Different letters* represent significantly different diurnal courses of each ecotype, $P < 0.05$ using one-way ANOVA

**Table 2** Comparative tocopherol and pigment composition of *C. quitensis* of both Andean and Antarctic ecotypes

| | Andean | Antarctic |
|---|---|---|
| Chlorophyll *a* (μmol $gFW^{-1}$) | 0.42 ± 0.11 (n.s.) | 0.30 ± 0.09 |
| Chlorophyll *b* (μmol $gFW^{-1}$) | 0.17 ± 0.05 (n.s.) | 0.10 ± 0.03 |
| Chlorophyll *a*/*b* | 2.39 ± 0.01* | 2.88 ± 0.02 |
| Neoxanthin | 27.4 ± 0.3* | 31.7 ± 0.7 |
| Lutein | 140 ± 4* | 189 ± 2 |
| α-Carotene | n.d.* | 1.64 ± 0.11 |
| β-Carotene | 51 ± 4* | 115 ± 4 |
| VAZ | 55 ± 4* | 117 ± 3 |
| Total carotenoids | 273 ± 12* | 455 ± 7 |
| β-Carotene/neoxanthin | 1.9 ± 0.1* | 3.6 ± 0.1 |
| Tocopherol | 38.58 ± 7.75 (n.s.) | 63.93 ± 27.12 |

Three individual plants ($n = 3$) from each ecotype were sampled at midday. Values represent average ± SE. Tocopherol and carotenoids data are expressed in mmol $mol^{-1}$ Chl *a* + *b*

*n.s.* not significant differences, *n.d.* no detected pigment

* Differences between Andes and Antarctic ecotypes $P < 0.05$ according to Tukey's test

carotenoids, β-carotene and VAZ levels are extremely high, even similar to plants considered extreme light tolerant such as plants from Alpine high mountains: *Homogene alpina* and *Soldanella alpina* (Streb et al. 1997). The amount of these photoprotective pigments in the Andean ecotype is much lower (about a 50%). These results are surprising considering the lower total irradiance received by the Antarctic ecotype under natural conditions compared with the Andean one. However, it can be the result of higher light acclimation response of the Antarctic ecotype under the combination of moderate total irradiance with episodes of high intensity but lower temperature than the Andes ecotype. It has been shown that even moderate light under low temperature exposure may cause a higher light acclimation response because of higher excitation pressure of PSII (reduction of PQ pool) experienced by non-acclimated plants (Huner et al. 1998). Therefore, the higher Chlorophyll *a*/*b* ratio could be a useful strategy to avoid an excess of absorbed light balancing absorbed and photochemically used energy (Ensminger et al. 2006) and the great amounts of photoprotective pigments could have an important role as antioxidants in these plants (Young et al. 1997).

Another striking morphological difference between the chloroplasts of both ecotypes was the bigger plastoglobuli size and the area occupied per chloroplast in Andean plants (Table 1). Tocopherols have been detected in all chloroplast membranes and notably in plastoglobules (Steinmüller and Tevini 1985). However, there was not a relationship

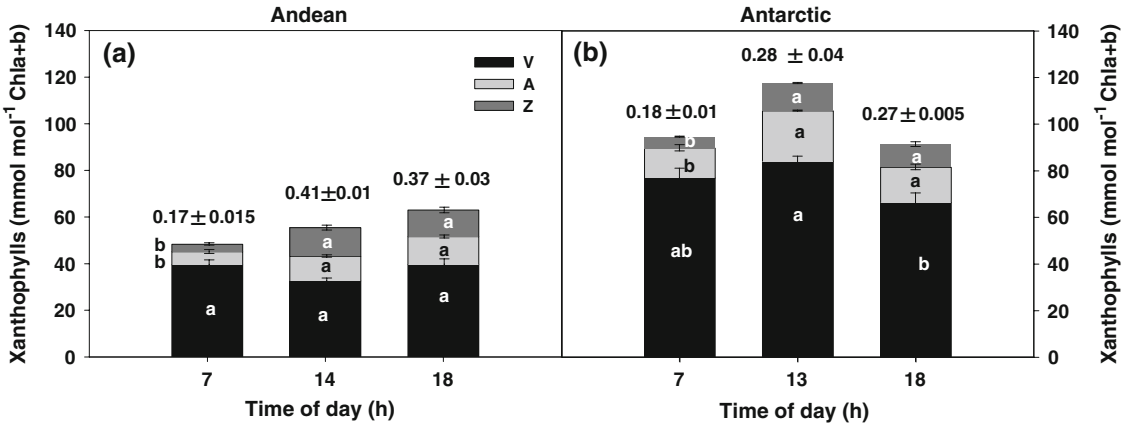


**Fig. 4** Diurnal variation of xanthophyll cycle pigments of both ecotypes of *C. quitensis* under natural conditions. The amount of each xanthophylls (*V* violaxanthin, *A* anteraxanthin, *Z* zeaxanthin) was quantitatively analyzed by HPLC and de-epoxidation states (*numbers* on *top* of the *bars*) were calculated for Andean and Antarctic ecotypes, respectively. *Bars* show mean values ± SE calculated from three different sunny days. *Different letters* represent significantly different values within the diurnal course of each ecotype, $P < 0.05$ using one-way ANOVA

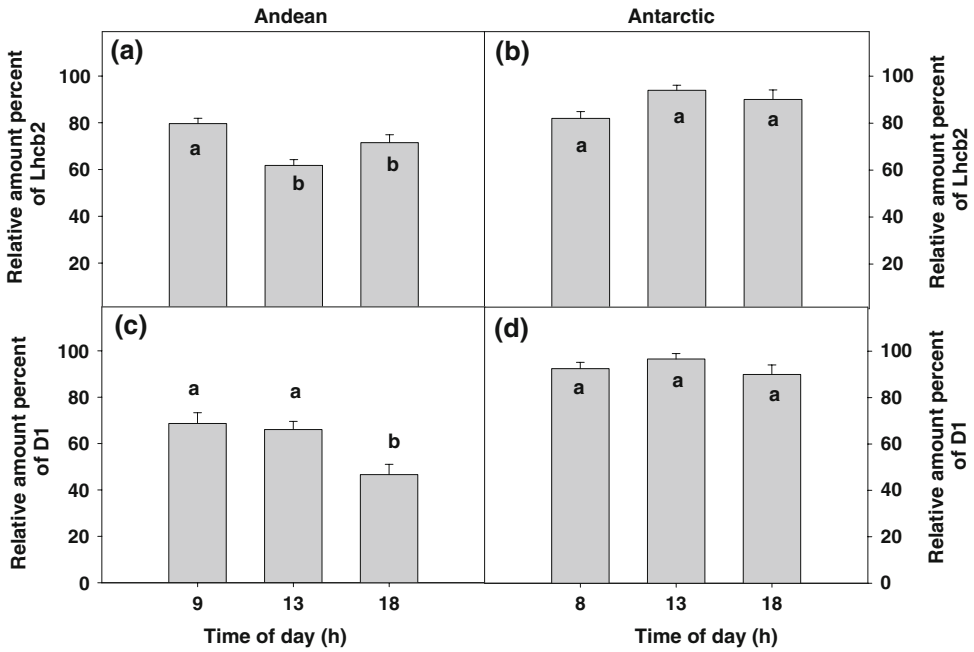


**Fig. 5** Variation of D1 and Lhcb2 in a diurnal cycle of both ecotypes of *C. quitensis*. *Bars* show mean of relative values ± SE ($n = 3$). Samples from 9 and 8 a.m. of the Andean and Antarctic ecotype, respectively, were considered as 100%. *Different letters* represent significant differences within the diurnal course of each ecotype, $P < 0.05$ using one-way ANOVA

between plastoglobuli size and tocopherol content in this study (Table 2). We could speculate that the function of plastoglobuli may be the storage of tocopherol biosynthesis pathway components and precursors; therefore, higher plastoglobuli size can be interpreted as a higher potential to synthesize instead of direct indicator of the tocopherol content. For instance, this bigger machinery to synthesize tocopherol may be a response to more elevated UV-B radiation in the Andes Mountains, which is three times higher than in the Antarctic (Cabrera et al. 1997; Xiong and Day 2001).

Physiological measurements were done under a sunny day, where the maximum light intensity at noon was similar at both study sites. However, the differential light pattern between the sites has been reported widely (Schroeter et al. 1995; Alberdi et al. 2002; Xiong and Day 2001; Casanova-Katny et al. 2006). The maintenance of high Fv/Fm ratio even at midday under high light and their respective site temperatures reflects the capacity of both ecotypes to fully recover within 30 min of dark adaptation, suggesting a high energy quenching capacity of these ecotypes at their respective natural conditions (Fig. 2). The photochemical quenching (qL) reflects the proportion of open centers that are able to accept electrons into intersystem transport chain. 1 − qL measures the relative oxidative state of $Q_A$, the first stable quinone electron acceptor of PSII (Gray et al. 1998; Huner et al. 1998). Under natural conditions, neither Andean nor Antarctic ecotype decreases its qL during the day, indicating the ability of these plants to maintain the

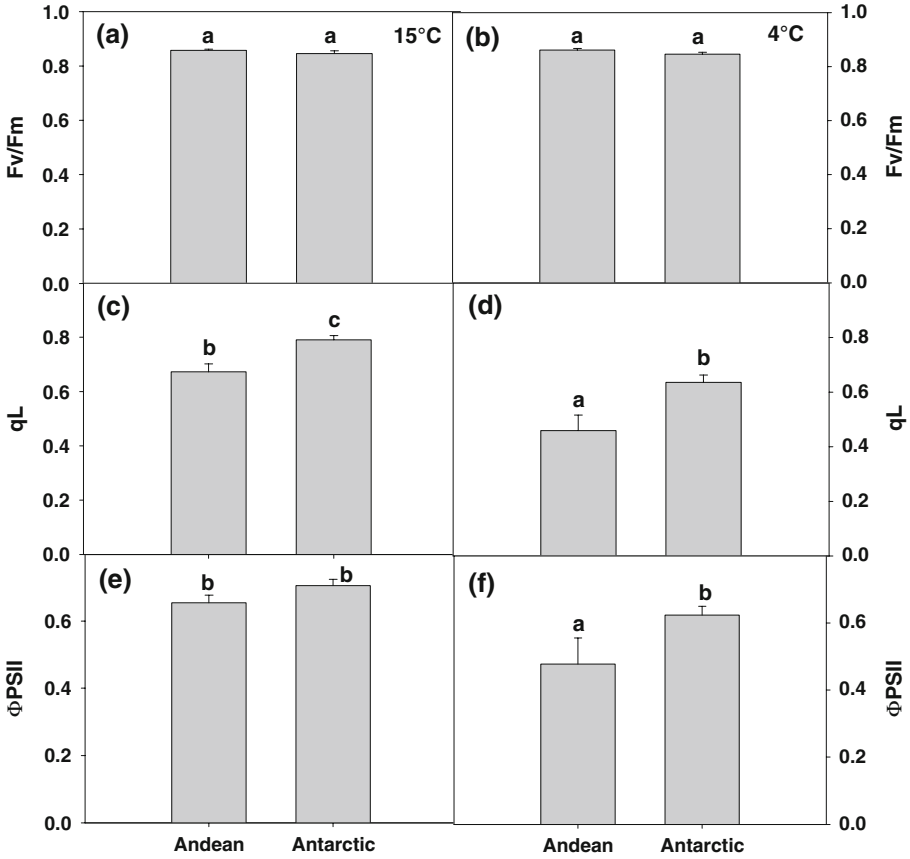


**Fig. 6** Fluorescence parameters of both ecotypes of *C. quitensis* under controlled conditions. Fv/Fm (**a**, **b**) is the maximal photochemical efficiency, qL (**c**, **d**) is photochemical quenching, and ΦPSII (**e**, **f**) is quantum yield of PSII. Measurements were done using an actinic light at 900 µmol photons $m^{-2}$ $s^{-1}$ and both 15 and 4°C. *Bars* show mean values ± SE. They were calculated from six independent measurements. *Different letters* represent significant differences between ecotypes and temperature measurement, $P < 0.05$ using two-way ANOVA

electron transport, even under high light conditions. However, the quantum yield of PSII (ΦPSII) decreases in both ecotypes at midday, reflecting a decrease in the proportion of absorbed light used in photochemistry. It was also noticed a rapid recovery of PSII in both ecotypes at the afternoon, especially in the Antarctic ecotype which is exposed at lower temperature. This latter parameter has been studied early in Antarctic plants under natural conditions with similar observations (Xiong and Day 2001). Most of these fluorescence parameters were discussed in terms of the daily pattern in each site and were not compared directly between ecotypes under natural conditions. It is critical to have exactly the same light intensity during measurements in order to be sure that responses are due to intrinsic differences in the photosynthetic apparatus of each ecotype and not merely to a reflection of different excitation pressure of PSII due to different light intensity or temperature experienced during measurements (Fig. 1). Therefore, it was necessary to conduct the fluorescence study under controlled conditions (temperature and light intensity) in the laboratory. Under these conditions, the Antarctic ecotype presents higher level of qL and ΦPSII, under low temperatures (Fig. 6), which suggest a higher degree of light acclimation in this ecotype because at low temperature the Antarctic ecotype is able to maintain a more oxidized $Q_A$ pool. These results are consistent with those of the pigment bed structure observed in the Antarctic ecotype. This could also be related with the active enzymatic system of the Calvin cycle and sucrose biosynthesis pathway that this ecotype is able to maintain under low temperature (Bascuñán-Godoy et al. 2006; Bravo et al. 2009; Pérez-Torres et al. 2006). It has been suggested that the maintenance of sucrose synthesis as a carbon sink at low temperature allows sustained positive photosynthetic rates under extreme conditions, even at 0°C, where the photosynthesis is about at 30% of its maximum (Xiong et al. 1999). The slight decrease in ΦPSII at noon in both ecotypes in the field was probably a normal down-regulation associated with an increase in NPQ, specially its component $NPQ_f$ and de-epoxidated state of xanthophylls pool (Figs. 3, 4). This cycle operated in both ecotypes principally with an increase of xanthophylls pool that favored the increase of Z and A, without changes in V levels (Fig. 4). Similarly, in plants growing under comparable conditions in growth chambers, xanthophylls pool increased under high irradiance in both ecotypes. However, in the laboratory grown plants, the xanthophylls cycle operates with the de-epoxidation and consecutive decrease of V (Bravo et al. 2007). Violaxanthin is a precursor of ABA (Schwartz et al. 1997). This plant hormone is involved in many physiological processes especially in the adaptation of plants to environmental stresses. The greater VAZ pool in Antarctic plants could be related with a stronger pathway

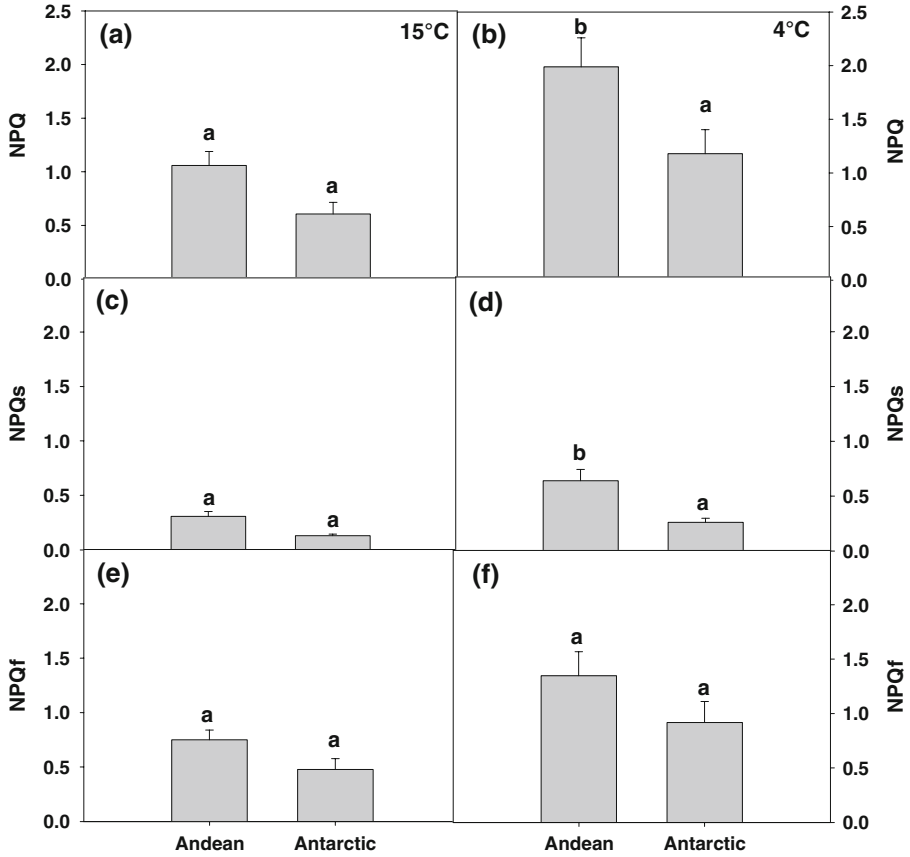


**Fig. 7** Non-photochemical quenching (NPQ) (**a**, **b**), slow NPQ ($NPQ_s$) (**c**, **d**) and fast NPQ ($NPQ_f$) (**e**, **f**) relaxing components measured in plants of both ecotypes of *C. quitensis* under controlled conditions. Measurements were done using an actinic light at 900 μmol photons $m^{-2}$ $s^{-1}$ and both 15 and 4°C. *Bars* show mean values ± SE. They were calculated from six independent measurements. *Different letters* represent significant differences between ecotypes and temperature measurement, $P < 0.05$ using two-way ANOVA

of ABA synthesis under conditions of permanent cold and associated environmental constrains (Bravo et al. 2009). It has been suggested that V would have to convert either to ABA or to Z or both depending on environmental factors (Sharma et al. 2002).

The tendency of the Andean ecotype to dissipate excess energy by a non-photochemical way is not only a phenotypic response to different environments. Measurement under control conditions of plants from the field also supports this tendency (Figs. 3, 7). However, NPQ and its component $NPQ_f$ seem not to be a constitutive difference between the ecotypes, since when both populations grow in chambers under comparable conditions Antarctic plants present higher levels than the Andean one (Bravo et al. 2007). Therefore, this may reflect different plasticity to respond to growing conditions, more than an ecotypic attribute.

On the other hand, the increase of $NPQ_s$ observed in the Andean ecotype in the late afternoon when plants still exposed to high irradiance (over a 1,000 μmol photons $m^{-2}$ $s^{-1}$) and relatively low temperatures (about 8°C) is related with the tendency to decrease the level of D1 (Fig. 5a) and evidences higher irreversible photo-damage than in Antarctic plants. These results in Andean plants are likely due to a higher sensitivity of this ecotype to low temperature induced photoinhibition. These results are supported by measurements under controlled conditions at 4°C (Fig. 7d) and are also consistent in plants growing under grow chamber conditions (Bravo et al. 2007). The highest environmental variability (wider range of daily temperature and irradiance) in the Andean environment may have imposed a lower PSII excitation pressure response and hence less acclimation capacity than the Antarctic one.

Summarizing, this work shows that both ecotypes are highly efficient under its respective natural conditions; during a sunny day: (1) both are able to maintain its photochemical process, and (2) both can respond with an increment in the de-epoxidation state of VAZ pool that allows an increase of the thermal dissipation of excess absorbed energy. Also, this work shows that *C. quitensis* from contrasting climatic origins have different photoprotective attributes that reflect a different degree of photo-oxidative stress protection. The strategy of the Andean ecotype is to decrease the probability of light capture by smaller chloroplasts, stacked granum area and down-regulation of proteins from the antenna and also exhibits a greater de-epoxidation state of xanthophylls cycle that allows higher level of thermal dissipation. On the other hand, the Antarctic ecotype shows a lower antenna/core PSII ratio, higher level of photochemical utilization of absorbed energy with a lower sensitivity to low temperatures and greater levels of photoprotective pigments.

More than representing responses to a single stress, the plasticity of biochemical mechanisms reported here allows a fine and precise adjustment of metabolism to a wide range of environmental constraints such as those encountered by *C. quitensis* in Antarctica and in the Andes. Dynamics of photoprotective strategies could be the key for plants growing under highly variable conditions, such as experienced by plants in the Andes of Central Chile. Also, the combination of a high level of cold acclimation which probably allows active energy sinks and the photoprotection supplied by carotenoids could be an important strategy for survival and reproduction in areas that allow only a very short growing season such as Antarctic.

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