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EXPRESSION OF LHC GENES AND THEIR RELATION TO PHOTO-OXIDATIVE STRESS TOLERANCE IN *Solanum lycopersicum* L. AND *Solanum chilense* (Dunal) Reiche

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Simultaneous exposition to low temperature and high light radiation cause photoinhibition of photosynthetic apparatus, affecting the productivity and geographical distribution of agricultural crops. In several Solanaceous species, tolerance to low temperature stress in combination with high light has been associated with some stimulation in non-photochemical quenching (NPQ), which involved reorganization in light-harvesting complex (LHC) proteins. To study photosynthetic performance in *Solanum lycopersicum* L. and *S. chilense* (Dunal) Reiche, and to investigate transcriptional regulation of genes encoding LHC proteins and their involvement in the NPQ, plants of both species were exposed to low temperature (4 °C) and high light radiation (1300 μmol m^{-2} s^{-1}). Lipid peroxidation, photochemical efficiency, and changes in xanthophyll cycle pigments were measured. The results presented here indicate that *S. chilense* showed higher tolerance to photoinhibition than *S. lycopersicum* under low-temperature and high light conditions, increasing light-energy consumption in photochemical processes by increasing photosynthetic capacity as indicated by photochemical quenching (qP) and relative electron transport rate (ETR) parameters. The contribution of light-harvesting chlorophyll a/b binding (LHC) protein was not related to dissipate excess excitation energy as heat (NPQ), but rather with the antioxidant function attributable to zeaxanthin as indicated by the amount of peroxidized lipids in *S. chilense*. We suggest that the differential expression of *Lhca1* transcripts, with zeaxanthin binding sites could contribute to the greater tolerance of *S. chilense* to photoxidative stress.

Key words: Lipid-peroxidation, photoinhibition, photochemical quenching, zeaxanthin.

Low temperature is one of the most important factors affecting the productivity and geographical distribution of agricultural crops (Allen and Ort, 2001). Simultaneous exposition to low temperature and high light radiation cause photoinhibition of photosynthetic apparatus (Long *et al*., 1994). Low temperature generally reduces reaction rates and therefore can limit the sinks − CO_2 fixation and photorespiration − for the absorbed excitation energy (light) (Foyer, 2002; Karpinski *et al*., 2002). The absorbed light that exceeds the photosynthetic capacities can lead to increased production of reactive oxygen species (ROS) (Ledford and Niyogi, 2005) causing photo-oxidative damage to lipids, proteins, nucleic acids and photosynthetic pigments (Mittler *et al*., 2004; Møller *et al*., 2007).

The xanthophyll cycle can play a crucial role in protecting plants from photo-oxidative damage (Demmig-Adams and Adams, 1996; Niyogi, 1999). In this cycle, the diepoxide xanthophyll violaxanthin is converted via the intermediate antheraxanthin to the epoxide-free zeaxanthin (Yamamoto, 1979). Once low light (dark) conditions are restored, zeaxanthin is epoxidized back to violaxanthin. Zeaxanthin has a role in the dissipation of excess excitation energy as heat by a process termed non-photochemical quenching (NPQ) (Holt *et al*., 2004). NPQ is important for the protection of the photosynthetic apparatus against photo-damage reducing the energy delivery to the photosystem II (PSII) reaction centre and minimizing the generation of reactive oxygen species (ROS) (Horton *et al*., 1994; Muller *et al*., 2001; Havaux *et al*., 2004). On the other hand, zeaxanthin also appears to have a role in tolerance to photo-oxidative stress protecting membranes against lipid peroxidation by a different mechanism from NPQ (Lichtenthaler and Schnidler, 1992). Carotenoids are essential components of light-harvesting chlorophyll (chl) a/b binding (LHC) protein (Wehner *et al*., 2004) and therefore the chemical transformation of the xanthophyll cycle induces a reorganization in light-harvesting complex (LHC) proteins (i.e. from a state of efficient light harvesting to a state of high thermal energy dissipation; Nelson and Yocum,

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2006). This change is related to differential expression of genes encoding LHC proteins (Demmig-Adams *et al*., 1996; Savitch *et al*., 2002; Ensminger *et al*., 2004). It was proposed that PsbS, a distant member of the LHC family, might play a protective role within the thylakoids under photoinhibitory conditions, with a central role in NPQ (Li *et al*., 2000). A similar non-light harvesting function was proposed for ELIP proteins, another distant relative of LHC family that accumulates when the expression of Lhc proteins is downregulated and are therefore thought to play a role in photoprotection, either via a transient binding of free chlorophyll to avoid the transference to oxygen and/or in zeaxanthin-dependent photo-protection (Montané and Kloppstech, 2000; Adamska, 2000; Andersson *et al*., 2003; Hutin *et al*., 2003). Interestingly, the photosystem I (PSI) antenna complex is made up of proteins whose primary function is to collect energy. When plants are exposed to conditions that may generate photo-oxidative damage, these proteins assume a conformation able to dissipate the excess energy excitation as heat (Morosinotto *et al*., 2002; Ben-Shem *et al*., 2004).

In several Solanaceous species, tolerance to low temperature stress in combination with high light has been reported to be associated with some stimulation in NPQ (Venema *et al*., 1999; Rorat *et al*., 2001), however, there are few studies relating such tolerance to gene expression. In this context, the native species *Solanum chilense* (Dunal) Reiche, with proven tolerance to several abiotic stress conditions (Maldonado *et al*., 2003; Venema *et al*., 2005; Tapia, 2005; Yáñez *et al*., 2009) becomes as potential germplasm source.

Therefore, in an effort to compare molecular mechanisms underlying gene regulation related to low-temperature tolerance in wild and cultivated tomatoes the aim of this study was evaluate the transcriptional regulation of genes encoding LHC proteins and their involvement in the NPQ in *S. lycopersicum* and *S. chilense* under simultaneous exposition to low temperature and high light radiation.

MATERIALS AND METHODS

Plant material and growth conditions

Solanum chilense fruits were collected in the Atacama Desert of Chile, at ca. 2400 m.a.s.l. (18º28' S, 69º45' W). Seeds were extracted from fruits, washed and sterilized before sowing them in pots with a soil:perlite mix (1:1). *Solanum lycopersicum* cv. Moneymaker seeds were also germinated in pots with the same soil:perlite mixture. All plants were irrigated with a mineral nutrient solution (Murashige and Skoog, 1962, 1 g L^{-1}) and grown for 10 wk in growth chambers in a 16:8 h photoperiod regime at 20-22 ºC.

Low temperature and high-light treatment

Plants were transferred at the beginning of photoperiod to a climate room at a constant temperature of 4 °C and light intensity of 1300 μmol m^{-2} s^{-1} for 4 h. Light was provided by two high pressure sodium lights (OSRAM SON-T 400 W lamps). To prevent heating of the samples, light was filtered through a water layer of 10 cm.

Measurements of chlorophyll fluorescence parameters

Fluorescence signals were generated by a pulse-amplitude modulated fluorimeter (FMS 2, Hansatech Instruments Ltd., Norfolk, UK). Attached leaves of both species were dark adapted for 20 min with leaf-clips with a mobile shutter plate. The fiber-optic and its adapter were fixed to a ring located over the clip at about 10 mm from the sample and different light pulses (see below) were applied following the standard routines programmed within the equipment. Signal recordings and calculations were performed using the data analyses and control software provided by the manufacturers (Hansatech Instruments Ltd., Norfolk, UK). According to the terminology of van Kooten and Snel (1990), minimal or initial fluorescence (Fo) was determined applying a weak modulated light (0.4 µmol photons m^{-2} s^{-1}) and maximal fluorescence (Fm) was induced in dark adapted leaves by a short pulse (0.8 s) of saturating light (around 9000 µmol photons m^{-2} s^{-1}). Shortly after relaxation of Fm actinic light was turned on (600 µmol photons m^{-2} s^{-1}). This light intensity corresponded to the mean obtained by light response curves for both species. Both species were saturated at this light intensity, but they were not photoinhibited. Then, the same saturating pulse was applied every 20 s in order to obtain Fm', the steady state value of fluorescence immediately prior to the flash, corresponded to Fs. Finally, Fo' was measured after turning the actinic light off and applying a 2 s far red light pulse. Definitions of fluorescence parameters (qP, NPQ, Fv/Fm, and ΦPSII) were used as described by van Kooten and Snel (1990). Fv/Fm and ΦPSII = (Fm' - Fs)/Fm' are indicators of the maximum and effective quantum yield of the PSII (Genty *et al*., 1989) respectively. Photochemical quenching (qP) was calculated as: qP = (Fm' - Fs)/(Fm' - Fo'), with Fm' being the maximal fluorescence in light adapted leaves, and Fs the steady state fluorescence yield. Non photochemical quenching was calculated as: NPQ = (Fm - Fm')/Fm'. Relative electron transport rate was calculated as: ETR = PPFD × 0.5 × ΦPSII × 0.84.

Photosynthetic pigment determination

Leaf discs (13 mm diameter) were punched from leaves of plants which were grown at optimal or low temperature and high-light treatment conditions. Leaf discs were immersed in liquid N_2 and stored at -80 ºC until use. The frozen leaf discs were powdered with liquid N_2 and pigments were extracted with 700 μL ice-cold acetone. After centrifugation (4 °C for 6 min at 5000 g), the supernatant fractions were filtered through a 0.45 µm

syringe filter and stored at -20 °C until use. The HPLC system was a Hewlett Packard 1100 liquid chromatograph (Agilent Technologies Inc., San José, California, USA). For analysis of xanthophylls, 20 µL of extract were injected in a C18 reversed phase HPLC column (250 × 4.60 mm, 5 µm particle size). Violaxanthin, anteraxanthin and zeaxanthin were separated according to Gilmore and Yamamoto (1991) and were identified by their retention times relative to known standards. Concentrations were calculated as based on calibration-curves create with the standards. The standards were purified in our laboratory by thin layer chromatography.

Lipid peroxidation

The malondialdehyde (MDA) assay for estimating lipid peroxidation was carried out on leaf tissue as described in Hodges *et al*. (1999) with minor modifications. Briefly, leaf discs (1.33 cm^2) were ground and suspended in 1 mL of ice cold 5% w/v tricloracetic acid (TCA) and centrifuged at 4000 g for 10 min. Then, 0.5 mL of the supernatant was mixed with an equal volume of 0.67% w/v thiobarbituric acid (TBA), incubated at 100 °C for 20 min and chilled immediately. An absorbance difference of 532 nm and 600 nm was measured in a spectrophotometer (UV-1601, Shimadzu, Japan). Extinction coefficient used for MDA determination was 155 mM^{-1} cm^{-1}.

cDNA isolation from Solanum species

The cDNAs encoding *PsbS*, *Lhca1*, and *Elip* were amplified by RT-PCR with RNA extracted from *S. chilense* and *S. lycopersicum* leaves as described below. Primers used were designed based on nucleotide sequences available in the National Center for Biotechnology Information (NCBI)/GenBank Database. Primers used to amplify the homologous gene in each species was PsbSf 5´-ATG GCT CAA ACA ATG TTG TTA ACA-3', and PsbSr 5´-CCA ATC ATA GCA ACA CGG CCA AC-3', for PsbS (accession number U04336), primers Lhca1f 5´-GAG GCA TTG GGC TTA GGT AAT TGG G-3' and Lhca1r 5´-TCC CCC AAT GTT GTT GTG CCA TGG G-3´ for Lhca1 (accession number J03558) and primers Elipf 5´-ATG GCT TCA CTC TCA TCT TC-3' and Elipr 5´-AGA GAG GAA TAT CAA GGC C-3' for Elip (primers were designed by homology with *Arabidopsis thaliana* accession number AAM62880.1). For each species, three independent amplification products were cloned into the pGEMT-Easy vector (Promega) and sequenced. Nucleotide sequences were then compared to those described for tomato in the gene index of National Center for Biotechnology Information (2010). Deduced amino acid sequences were aligned using the ClustalW tool and identity between the proteins was calculated with BioEdit program (BioEdit, 2010).

Gene expression analysis

For *Lhca1*, *PsbS*, and *Elip* expression analyses, three independent total RNA extractions (biological replicates) were made from leaves (100 mg) of 10 to 12-wk-old plants using the SV Total RNA Isolation System (Promega, Madison, Wisconsin, USA) following the manufacturer's instructions. Leaves were harvested, flash-frozen in liquid nitrogen and kept at -80 ºC. Total RNA integrity was verified by formaldehyde agarose gel electrophoresis and their purity by OD_{260}/OD_{280} nm absorbance ratio > 1.95. Following DNase treatment of total RNA, first-strand cDNA synthesis was carried out from 2 μg of total RNA for each sample using oligo(dT) according to the manufacturer's instructions (Thermoscript RT-PCR System, Invitrogen, Carlsbad, California, USA). Transcript levels of genes were measured by quantitative PCR (qRT-PCR) using a DNA Engine Opticon 2 Cycler System (MJ Research, USA). All reactions were performed using the Brilliant SYBR Green Master Mix (Stratagene, USA) according to the procedure described by the manufacturer. For each biological replicate, qRT-PCR reactions were carried out in triplicate (technical replicates) using 2 μL Master Mix, 0.5 μL 250 nM each primer, 1 μL diluted cDNA and nuclease-free water to a final volume of 20 μL. Controls (with no cDNA and RNA without RT) were included in each run. Fluorescence was measured at the end of each amplification cycle. Amplification was followed by a melting curve analysis with continual fluorescence data acquisition during the 65-95 ºC melt. The raw data were manually analyzed and expression was normalized to *S. lycopersicum GAPDH* gene (*GAPDH*, NCBI/GenBank Database accession number U97256) to minimize variation in cDNA template levels. For each gene, a standard curve was generated using a cDNA serial dilution, and the resultant PCR efficiency calculations were imported into relative expression data analysis. To ensure that the transcripts of single genes had been amplified, qRT-PCR amplicons were sequenced and confirmed as the expected plant DNA sequences. Primers used for each gene was, Lhca1f 5`-GAG GCA TTG GGC TTA GGT AAT TGG G-3' and qPCR-Lhca1r 5`-GCC CCA CCA GGA TAC TTC TTC-3'; ELIPf 5`-ATG GCT TCA CTC TCA TCT TC-3' and qPCR-ELIPr 5`-GAA GGA GGT CCT TTT GG-3'; PsbSf 5`-ATG GCT CAA ACA ATG TTG TTA ACA-3' and qPCR-PsbSr 5`-CCT TTT TGG GAG GAG CTT TAG C-3'; GAPDHf 5`-ACA ACT TAA CGG CAA ATT GAC TGG-3' and GAPDHr 5`-TTA CCC TCT GAT TCC TCC TTG ATT G-3'.

Experimental design and statistical analysis

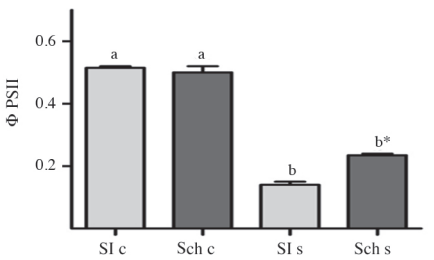
Data obtained were analyzed on the basis of a random design with three biological replicates for each treatment. Statistical analyses were performed using SigmaStat 3.1 (Systat Software, Inc. Richmond, California, USA). Differences between species and treatments were analyzed with two-way ANOVA and, where appropriate, with Tukey's test. Means were considered to be different ($P \leq 0.05$).

RESULTS AND DISCUSSION

Photosynthetic efficiency is more affected in *S. lycopersicum* than in *S. chilense* under high light and low temperature

The photosynthetic efficiency measured as photochemical yield of PSII (ΦPSII) showed variations when *S. lycopersicum* and *S. chilense* were exposed to low temperature and high light (Figure 1), being higher in *S. chilense* (46%) than in *S. lycopersicum* (27%). Our results are in agreement with others studies (Rorat *et al*., 2001; Savitch *et al*., 2002; Hu *et al*., 2006) in which significant differences in ΦPSII were related with different tolerance to photoinhibition.

Whilst ΦPSII are related to achieve efficiency, qP and Fv/Fm provide information about underlying processes which have altered efficiency. A change in qP is due a closure of reaction centre, resulting from a saturation of photosynthesis by light. A change in Fv/Fm is due to a change in the efficiency of non-photochemical quenching (van Kooten and Snel, 1990). Photochemical quenching (qP) (Figure 2A) and photosynthetic electron transport (ETR) (Figure 2B) were significantly more affected in *S. lycopersicum* than *S. chilense* (Figure 2). On the other hand, neither Fv/Fm (Figure 2C) or NPQ (Figure 2D) were affected in both species. These results are in agreement with previous observations (Tapia, 2005; Hu *et al*., 2006) in which the depression in ΦPSII was driven primarily by qP, not Fv/Fm, suggesting that the photo-oxidation-induced decline of ΦPSII was due to down-regulation of processes such as C fixation (Hu *et al*., 2006). Thus, ours results gives an indication about the efficiency of *S. chilense* to use light for photochemistry. Interestingly, there are reports (Gray *et al*., 1994; Adams *et al*., 2001; Savitch *et al*., 2002) suggesting that tolerance of cereals to short-term photoinhibition (hours) are related to the maintenance of photosynthesis as the major quencher of excited chlorophyll.



Data represent means ± SE of three individual plants. Letters indicate differences according Tukey test ($P \leq 0.05$) inside species in both conditions. Asterisks (*) indicate significant differences between species under stress conditions. c: control condition; s: stress condition.

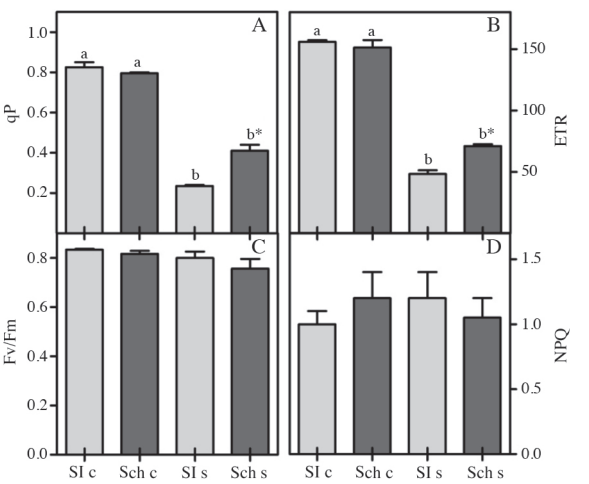
Figure 1. Changes in effective photochemical efficiency of photosystem II (ΦPSII) in leaves of *Solanum lycopersicum* cv. Moneymaker (Sl) and *S. chilense* (Sch) under low temperature (4 °C) and high light (1300 μmol m^{-2} s^{-1}).

De-epoxidized xanthophylls are slightly higher in *S. chilense* than in *S. lycopersicum* under high light and low temperature

The increase in the content of de-epoxidized (A+Z) may provide a better protection against photo-damage by increasing the capacity for xanthophyll cycle-related dissipation of excess energy in leaves exposed to high light and low temperature (Demmig-Adams and Adams, 1996). Ours results shown that in both species the conversion state of the xanthophyll cycle pool (A+Z)/(V+A+Z) increased along the stress treatment (Figure 3A) and the zeaxanthin formation was higher in *S. chilense* (44 mmol μL^{-1}) than in *S. lycopersicum* (26 mmol μL^{-1}) (Figure 3B). In spite of this, the rather constant NPQ (Figure 2C) suggest that other mechanism, different to the thermal dissipation, are involved in the major tolerance of the photosynthetic apparatus of *S. chilense* to high light and low temperatures. Ours results are in accordance with a number of cases in which the accumulation of zeaxanthin was shown to increase tolerance to photo-oxidative stress protecting thylakoid membrane lipids from oxidative damage independently from NPQ (Lichtenthaler and Schnidler, 1992; Stefanov *et al*., 1996; Havaux and Niyogi, 1999; Havaux *et al*., 2004; Johnson *et al*., 2007).

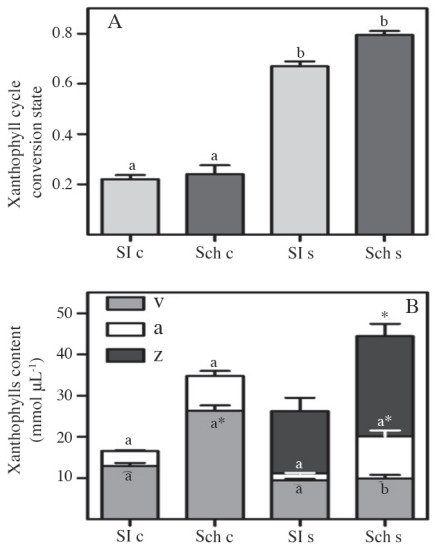
Lipid peroxidation is higher in *S. lycopersicum* than in *S. chilense*

Since the oxidative breakdown of lipid molecules yields elevated levels of malondialdehyde, the MDA content is considered a useful index of general lipid peroxidation (Hodges *et al*., 1999; Møller *et al*., 2007). In control conditions, both species contained similar amount of



A) qP: photochemical quenching; B) ETR: electron transport rate; C) Fv/Fm: maximum quantum yield ; D) NPQ: non photochemical quenching. Data represents means ± SE of three individual plants. Letters indicate differences according Tukey test ($P \leq 0.05$) inside species in both conditions. Asterisks (*) indicate significant differences between species under stress conditions. c: control condition; s: stress condition.

Figure 2. Changes in fluorescence parameters in leaves of *Solanum lycopersicum* cv. Moneymaker (Sl) and *S. chilense* (Sch) under low temperature (4º C) and high light (1300 μmol m^{-2} s^{-1}).



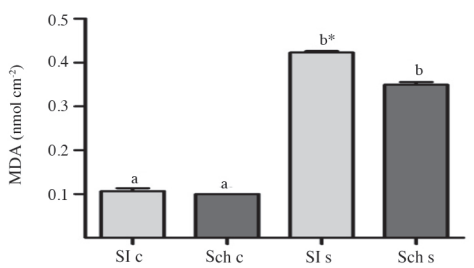
Changes in the xanthophyll cycle conversion state (a + z)/(v + a + z); B) changes in content of violaxanthin (v), anteraxanthin (a) and zeaxanthin (z) (c: control condition; s: stress condition). Data represents means ± SE of three individual plants. Letters indicate differences according Tukey test ($P \leq 0.05$) inside species in both conditions. Asterisks (*) indicate significant differences between species under stress conditions.

Figure 3. Xanthophyll variation in leaves of *Solanum lycopersicum* cv. Moneymaker (Sl) and *S. chilense* (Sch) under low temperature (4º C) and high light (1300 µmol m^{-2} s^{-1}).

lipid peroxide (Figure 4), by contrast, the MDA content in leaves of *S. lycopersicum* was significantly higher than *S. chilense* during exposition to low temperature and high light treatment. Thus, *S. chilense* seems to be more tolerant to oxidative stress, as indicated by their low levels of lipid peroxide. These results are in agreement with reports that less MDA formation is related to some mechanism of oxidative stress tolerance (Shalata and Neumann, 2001; Hutin *et al*., 2003; Golan *et al*., 2006).

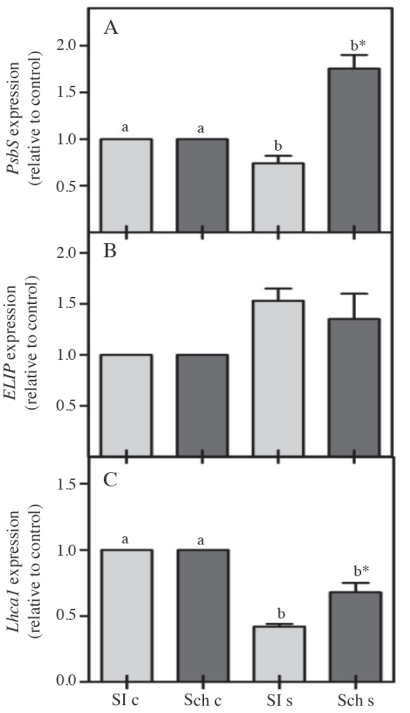
Expression of *PsbS* and *Lhca1* is higher in *S. chilense* than in *S. lycopersicum* under high light and low temperature

Isolated cDNAs for *PsbS*, *Elip* and *Lhca1* proved homologous in both species (data not shown). As a first approach to ascertain the role of this gene in *S. chilense* plants exposed to low temperature and high light, its expression pattern was analyzed (Figure 5). qRT-PCR analysis of *PsbS* showed an increase in transcript levels in *S. chilense* while no significative change was observed in *S. lycopersicum* (Figure 5A). This result could be consistent with others reports (Niyogi, 1999; Li *et al*., 2000), which suggest that plant tolerance to photo-oxidative stress induced by high light involve an increased NPQ capacity through adjustment of PsbS abundance. However, our results also showed that the mechanism of NPQ was not induced (Figure 2C), suggesting, as in Rorat *et al*. (2001) and Norén *et al*. (2003), that there is no increase in *PsbS* translation at low temperature, and that post-transcriptional regulation plays a fundamental role in *PsbS* expression. However as shown in Figure 3B, there is an increase in zeaxanthin content, accordingly there are reports that indicate that zeaxanthin could be bound by other members of the LHC family, the ELIP proteins (Havaux *et al*., 2004; Demmig-Adams *et al*., 2006; Johnson *et al*., 2007). ELIP protein has been associated with photoprotection in tolerant species (Montané *et al*., 1999; Adamska, 2000; Hutin *et al*., 2003). An increase in ELIP transcript levels in *S. chilense* was expected, however no significant differences between both species was observed upon low temperature and high light treatment (Figure 5B), suggesting, as in Hutin *et al*.



Data represents means ± SE of three individual plants. Letters indicate differences according Tukey test ($P \leq 0.05$) inside species in both conditions. Asterisks (*) indicate significant differences between species under stress conditions. c: control condition; s: stress condition.

Figure 4. Malondialdehyde (MDA) variation in leaves of *Solanum lycopersicum* cv. Moneymaker (Sl) and *S. chilense* (Sch) under low temperature (4º C) and high light (1300 µmol m^{-2} s^{-1}).



The expression levels of genes were normalized against that of Sl*GAPDH* (c: control condition; s: stress condition). Data represents means ± SE of three individual plants. Letters indicate differences according Tukey test ($P \leq 0.05$) inside species in both conditions. Asterisks (*) indicate significant differences between species under stress conditions.

Figure 5. Expression analysis of *PsbS* (A), *ELIP* (B) and *Lhca1* (C) in leaves of *Solanum lycopersicum* cv. Moneymaker (Sl) and *S. chilense* (Sch) under low temperature (4º C) and high light (1300 µmol m^{-2} s^{-1}).

(2003), that ELIP accumulation did not increase thermal energy dissipation (NPQ), but that it might be involved some antioxidative mechanism related to zeaxanthin. Because proteins for light harvesting are thought to have evolved from photoprotective ELIPs (Green, 2003), it seems logical to propose that the apparent ability of these proteins to prevent lipid peroxidation has been retained in the LHC proteins of both photosystems. It was believed that PSI was tolerant to environmental stresses, however was shown to be photoinhibited under chilling conditions and when the linear electron transport chain is unbalanced (Terashima *et al*., 1994; Sonoike and Terashima, 1994; Tjus *et al*., 1998; Ivanov *et al*., 1998; Scheller and Haldrup, 2005). PSI photoprotection has been suggested to be mainly mediated by oxygen scavenging enzymes (Asada, 1999), although recent evidences, suggested that PSI antenna proteins might play a relevant role in photoprotection against excess energy (Melkozernov and Blankenship, 2005; Alboresi *et al*., 2009), which could be regulated through carotenoids presents in the LHCI complex (Morosinotto *et al*., 2003). Taking that into account, we analyzed the transcript levels of the *Lhca1* gen, which encodes for one of the major polypeptide that form the antenna complex of PSI (Ben-Shem *et al*., 2004).

Although our results indicate that in both species there is a down-regulation of the *Lhca1* transcripts levels, it is important to point out that it remains higher in *S. chilense* than in *S. lycopersicum* (Figure 5C). Interestingly, there are reports that under certain conditions (e.g. high-light) there is a differential expression of LHCI genes (Bailey *et al*., 2001; Ensminger *et al*., 2004; Gáspár *et al*., 2006). On the other hand, Wehner *et al*. (2004) suggested that the zeaxanthin formed in LHCI proteins is involved in an antioxidative mechanism implicated in suppression of ROS-triggered lipid peroxidation rather than heat energy dissipation (NPQ), which could be consistent with our observations on the accumulation of zeaxanthin (Figure 3B) which can function as antioxidant, as evidenced by the amount of peroxidized lipids (Figure 4) and the invariable value of NPQ (Figure 2C).

CONCLUSIONS

The results presented here indicate that *S. chilense* showed higher tolerance to photoinhibition than *S. lycopersicum* under low-temperature and high light conditions, increasing light-energy consumption in photochemical processes by increasing photosynthetic capacity as indicate by qP and ETR parameters. The contribution of light-harvesting chlorophyll a/b binding (LHC) protein was not related to dissipate excess excitation energy as heat (NPQ), but rather with the antioxidant function attributable to zeaxanthin as indicated by less MDA content in *S. chilense*. We suggest that the differential expression of *Lhca1* transcripts could contribute to the greater tolerance of *S. chilense* to photoxidative stress.

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Expresión de genes LHC y su relación con la tolerancia al estrés foto-oxidativo en *Solanum lycopersicum* L. y *Solanum chilense* (Dunal) Reiche. La exposición simultánea a las bajas temperaturas y alta intensidad lumínica causa la foto-inhibición del aparato fotosintético, fenómeno que afecta la productividad y la distribución geográfica de los cultivos. En varias especies de solanáceas, la tolerancia a este tipo de estrés se ha asociado con un cierto estímulo en el apagamiento no fotoquímico (NPQ), lo que implica una reorganización en las proteínas pertenecientes a los complejos colectores de luz (LHC). Para evaluar el rendimiento fotosintético de *Solanum lycopersicum* L. y *S. chilense* (Dunal) Reiche y evaluar la regulación transcripcional de genes que codifican para proteínas del LHC y su participación en el NPQ, plantas de ambas especies fueron expuestas a 4 °C y 1300 μmol m^{-2} s^{-1} y se realizaron evaluaciones sobre la peroxidación lipídica, la eficiencia fotosintética y los cambios en los pigmentos del ciclo de las xantófilas. Al utilizar el exceso de energía de excitación en el proceso fotoquímico, según lo indicado por los parámetros qP y ETR, los resultados indican que *S. chilense* posee una mayor tolerancia a la foto-inhibición que *S. lycopersicum*. La contribución de las proteínas LHC no estaría relacionada con disipar el exceso de energía en forma de calor (NPQ), sino más bien con la función antioxidante atribuida a la zeaxantina, como se indica por la cantidad de lípidos peroxidados observados en *S. chilense*. Se sugiere que la expresión diferencial de los transcritos *Lhca1*, con sitios de unión para zeaxantina, podría contribuir a la mayor tolerancia al estrés foto-oxidativo por parte de *S. chilense*.

Palabras clave: Fotoinhibición, apagamiento fotoquímico, zeaxantina, lipoperoxidación.

LITERATURE CITED

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