Photoprotective mechanisms in cold-acclimated and nonacclimated needles of *Picea glehnii*

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Abstract

The response of *Picea glehnii*, a cold-tolerant species in the boreal zone, to air temperature (T) was investigated for its cold-acclimated needles (*i.e.* the ones subjected to gradual decrease in T) and nonacclimated needles (*i.e.* the ones subjected to a sudden decrease in T) were compared under low temperature. Cold-acclimated needles showed a greater increase of zeaxanthin and lutein contents than nonacclimated ones, whereas the nonacclimated needles showed a greater increase of thylakoid-bound ascorbate peroxidase (tAPX) activity than cold-acclimated ones under chilling conditions (after cold acclimation). These results suggest that: (*1*) low T induces the increase of zeaxanthin and lutein content, and tAPX activity; (*2*) accumulated zeaxanthin and lutein protect needles from photooxidative stress by dissipating excess energy before the reactive oxygen species (ROS) are formed in response to a gradual decrease in T (with cold acclimation and subsequent chilling condition), and by tAPX scavenging ROS formed in the case of a sudden decrease in T (without cold acclimation and chilling condition).

Additional key words: ascorbate peroxidase; low temperature; lutein; photoprotective mechanisms; *Picea glehnii*; zeaxanthin.

Introduction

Low temperatures associated to excessive light induced photooxidative stress. Photooxidative stress is induced by reactive oxygen species (ROS) due to the excess of energy reacting on oxygen molecules, as a consequence of imbalance between absorbed and utilized light energy required for photosynthesis under stressful conditions (Aroca *et al*. 2001, Mittler 2002). The first mechanism to protect the photosynthetic apparatus from photooxidative damage or to prevent formation of ROS under stressful conditions is the thermal dissipation of excess energy *via* xanthophyll-cycle pigments (Gilmore 1997). Another important photoprotective mechanism is the development of enzymes in the antioxidative mechanism (Noctor and Foyer 1998, Asada 1999, Hansen *et al*. 2002).

During winter, evergreen coniferous trees are exposed to a photooxidative risk more directly than deciduous ones (Ottander *et al*. 1995). In the cold regions, cold acclimation acts as a decisive factor for plants to survive under severe low-temperature conditions.

Although research related to the influence of cold acclimation on the photoprotective mechanism in woody plants have been made under natural or controlled environmental conditions (Doulis *et al* 1993, Repo *et al*. 1996, Wang 1996, Li *et al*. 2002, 2004, Verhoeven *et al*. 2005, Repo *et al*. 2006) in *Pinus sylvestris, Picea rubens*, *Pinus ponderosa,* and *Betula pendula*, no information about the protective mechanism in sudden or gradual temperature-decrease conditions have previously been considered.

Picea glehnii Masters (Sakhalin spruce) is a conifer distributed in the boreal climate zone, south of Siberia and north of Japan (Kojima 1991). It is known as a cold-tolerant species that can survive at extremely low temperatures during winter. The purpose of our study was to analyse the mechanisms for preventing photooxidative stress induced by sudden or gradual temperature decrease in the current-year needles of *P. glehnii*. Therefore, we carried out an experiment using special growth chambers

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Abbreviations: A – antheraxanthin; APX – ascorbate peroxidase; C – control; CA – cold-acclimated; Chl – chlorophyll; DM – dry mass; F_m – fluorescence when all PSII reaction centres are closed in dark-exposed leaves; F_o – fluorescence of leaves in the dark when all PSII reaction centres are open; F_v – variable fluorescence ($F_v = F_m–F_o$); F_v/F_m – quantum yield of PSII photochemistry; FM – fresh mass; N – nonacclimated; PPFD – photosynthetic photon flux density; ROS – reactive oxygen species; RWC – relative water content; sAPX – stromal APX; tAPX – thylakoid-bound APX; T – temperature; TM – turgescent mass; V – violaxanthin; Z – zeaxanthin.
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where boreal winter environments of light and temperature can be reproduced. By comparing experimentally cold-acclimated (gradual decrease in T) and nonacclimated needles (sudden decrease in T), we investigated the relative water content (RWC), quantum yield of PSII photochemistry, chlorophyll (Chl) fluorescence, plant pigments, and activities of ROS-scavenging enzymes in the current-year needles of *P. glehnii* during cold acclimation and subsequent low temperature.

Material and methods

Plants and growing conditions: Several 4-year-old *P. glehnii* saplings with uniform size (shoot height 30~35 cm) from a commercial nursery (*Oji Ryokka Forestry and Landscaping Co*., Ltd., Sapporo, Japan) were selected. The saplings (75 plants) were grown for two months (23 May to 20 July 2005) at 25ºC/20ºC (day/night), relative humidity of 60%/80% (day/night) under light supplemented with artificial light (100 μmol m^{-2} s^{-1} PPFD, fluorescent tubes, *FHF32EX-N-H*) and a photoperiod of 12 h in the growth chambers (Sapporo, Japan). Nutrient solution Hyponex (N:P:K = 6:10:5) diluted 1,000 times with distilled water (300 cm^3) was regularly supplied to each sapling once a week.

Experimental design: After two months, the saplings were separated into two groups (Fig. 1). Saplings of the first group were grown in a growth chamber at 25ºC/20ºC as control (C, 25 plants) and nonacclimated group (N, 25 plants). Saplings (25 plants) of the second group (cold-acclimated group, CA) were kept at 15ºC/10ºC for 10 days and then at 5ºC/0ºC for 10 days to cold acclimation. The N and CA saplings were placed for two weeks at a low T 0ºC/0ºC (Fig. 1) for chilling treatment. Saplings of the control group were grown at 25ºC/20ºC during the entire experimental period. Current-year needles were collected during the morning (9.00–11.00 h), and were immediately frozen in liquid nitrogen and stored at –84ºC until assay.

RWC of current-year needles (n = 25) of *P. glehnii* was calculated according to the following equation: RWC (%) = (FM–DM)/(TM–DM) × 100, where FM = fresh mass, DM = dry mass, and TM = turgescent mass. The dry mass of the needles was measured after 3 days dried in an oven at 80ºC, and a turgescent mass of needles was measured after holding the samples for 12 h in distilled water at 4ºC (Cameron *et al*. 1999).

Analysis of pigments: Total Chl and carotenoid content in the current-year needles of *P. glehnii* were extracted and quantified. Needles (about 50 mg in DM) were homogenized with liquid nitrogen. Pigments were extracted with 100% (v/v) acetone and centrifuged for 3 min at 14,000 × g. Chl a and b content, zeaxanthin (Z), violaxanthin (V), antheraxanthin (A), lutein, and β-carotene were immediately analyzed with a liquid chromatography (*HPLC; LC-Vp series, Shimadzu*, Kyoto, Japan) on a *Shim-pack CLC-ODS* column (150 mm long, 6 mm in diameter; *Shimadzu*, Kyoto, Japan) with the solvent A [acetonitrile:methanol, 85:15 (v:v)] and solvent B [acetonitrile:ethylacetate, 68:32 (v:v)]. Peak identity was determined by comparison of pure standards (*DHI Water and Environment*, Horsholm, Denmark).

Chl fluorescence parameters of the current-year needles were determined with a pulse amplitude-modulated fluorometer (*PAM-2000*, *Heinz Walz*, Effeltrich, Germany). The initial fluorescence (F_o) at a 0.1 μmol m^{-2} s^{-1} PPFD and maximal fluorescence (F_m) at saturating 2,400 μmol m^{-2} s^{-1} PPFD were measured for needles dark-adapted for 15 min at 22ºC. Variable fluorescence (F_v) was calculated by subtracting F_o from F_m.

ROS-scavenging enzymes (sAPX, tAPX): For the sAPX (soluble ascorbate peroxidase), needle material was ground with liquid nitrogen and homogenized with 500 mm^3 of potassium phosphate buffer (50 mM pH 7.8) containing 5 mm^3 of 10% triton X-100 and 0.01 g myo-inositol and polyvinyl pyrrolidone (PVP), 40 mm^3 of 0.1 mM ascorbate and 20 mm^3 of 0.1mM 2Na-EDTA (ethylenediamine tetraacetic acid) (pH 8.0). The extracts were centrifuged at 15,000 × g for 10 min at 4ºC and the supernatant was used as an extract for the sAPX. For the tAPX (membrane-bound ascorbate peroxidase), 40 mm^3 of 0.1 mM ascorbate, 500 mm^3 of 50 mM potassium phosphate buffer (50 mM pH 7.8), and 5 mm^3 of 10% triton X-100 were added into the remaining sediment within the tube after extraction of sAPX, centrifuged under the same conditions. For the activity of sAPX and tAPX, the following components of the reaction mixture were added to the supernatant: 5 mm^3 of 0.1 mM ascorbate, 979 mm^3 of 50 mM potassium phosphate buffer (pH 7.0), 6 mm^3 of 0.1 mM H_2O_2 and 10 mm^3 of plant extracts (supernatant) which was obtained after centrifugation of the homogenate. All extractions were prepared at 4ºC, and enzyme assays were determined at 25ºC. The optical density in a 1 cm cuvette was recorded at 290 nm, using a spectrophotometer (*DU 7400, Beckman coulter*, CA, USA). A decrease in the optical density for 5 min of reaction was taken as a measure of the enzyme activity, which was expressed in mM min^{-1} $mg(protein)^{-1}$ (for sAPX) or mM min^{-1} $mg(Chl)^{-1}$ (for tAPX) using the molar extinction coefficients ε = 2.8 mM^{-1} cm^{-1}.

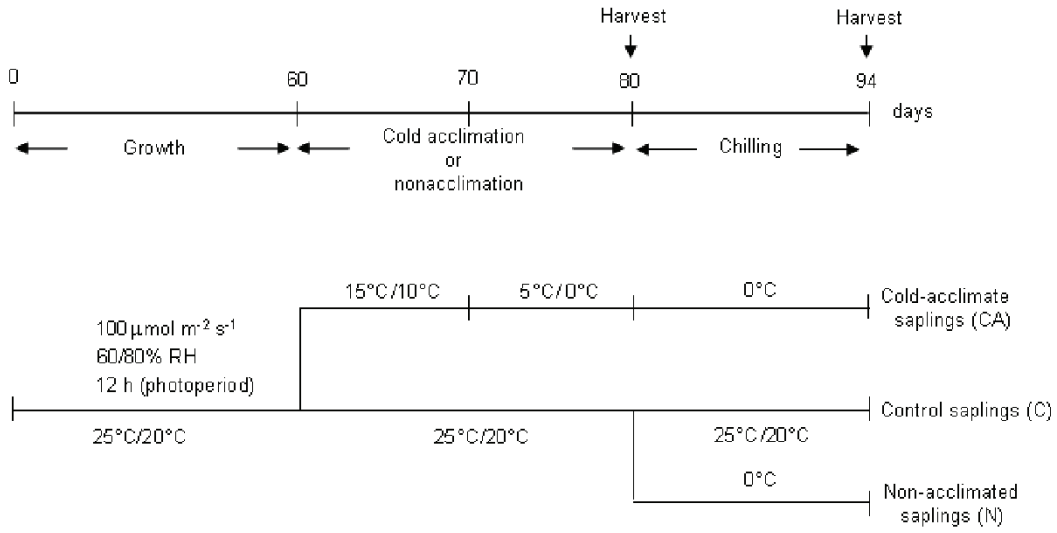

Fig. 1. Experimental design with *Picea glehnii* saplings. Each group (C, N, CA) is composed of 25 saplings.

Statistics: The results in Figs. 2–8 are shown as the mean ± SE of three (all figures except Figs. 3,5) or six (Figs. 3,5) independent replicates. Statistical analysis of control (C) data, cold-acclimated (CA) and nonacclimated (N) current-year needles were analyzed using *ANOVA*. The normality and homogeneity of variances for variables were checked and transformations of the variables such as arcsine-root were made when necessary. A *Tukey* (T) test was carried out to determine if significant differences (P<0.05) were found among the C, CA and N groups during the entire experimental period (using *SPSS 12.0 for Windows, SPSS*, Chicago, IL, USA).

Results

RWC: During cold acclimation, RWC did not decrease more than the control group. Under the subsequent low T condition (chilling), N needles had a significantly decreased RWC (P<0.05) than the control group but the CA did not show a decrease in RWC (Fig. 2).

Quantum yield of PSII photochemistry (F_v/F_m): F_v/F_m of CA decreased significantly from 0.73 to 0.32 when exposed to low T for cold acclimation (Fig. 3). Under the subsequent low T conditions (chilling), NA needles showed a significant decrease in F_v/F_m comparison to the C group, whereas there was no significant difference in F_v/F_m between CA and the C needles (Fig. 3). Therefore, low T decreases quantum yield of PSII photochemistry but the CA needles undergo less photo-oxidation at low T than N ones.

Chl content, Chl *a*/*b* ratio and fluorescence: The Chl *a*/*b* ratio of CA needles was significantly decreased ($P<0.005$) more than the N ones by low T treatment (Fig. 4). F_o (Fig. 5) and total Chl content [Chl (a+b)] remained constant during the cold acclimation and subsequent low T. F_v and F_m decreased when the needles were exposed to low T during cold acclimation and under the subsequent low T conditions (Fig. 5). Under the low T conditions, N needles showed a significant decrease ($P<0.005$) in F_v and F_m than CA needles.

Xanthophyll cycle pigments: The zeaxanthin content (Z) and the xanthophyll pool size (V+A+Z) increased in both CA and N needles by low T treatment (during cold acclimation and subsequent chilling condition) (Fig. 6). Under the subsequent low T conditions (chilling condition), CA showed significantly greater increase ($P<0.005$) in zeaxanthin than N needles.

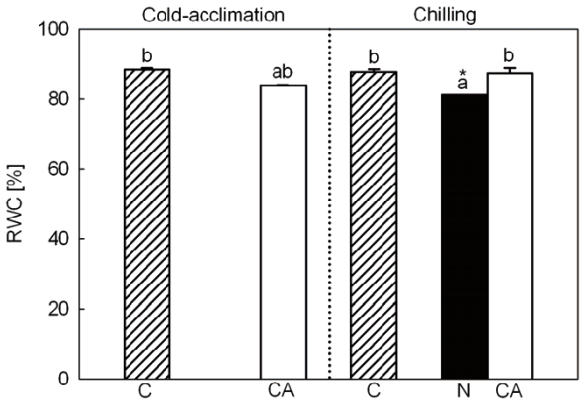

Fig. 2. Relative water content (RWC) in current-year needles of *P. glehnii*. C – control; CA – cold-acclimated; N – nonacclimated. The different letters denote significant differences by *Tukey* (T) multiple pairwise comparison (P<0.05), and the levels of significance are denoted as * (P<0.05), ** (P<0.005). (n = 3).

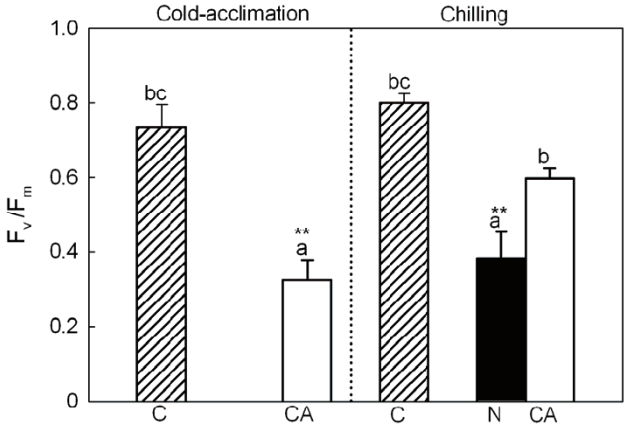

Fig. 3. Quantum yield of PSII photochemistry (F_v/F_m) in current-year needles of *P. glehnii*. C – control; CA – cold-acclimated; N – nonacclimated. The different letters denote significant differences by *Tukey* (T) multiple pairwise comparison (P<0.05), and the levels of significance are denoted as * (P<0.05), and ** (P<0.005). (n = 6).

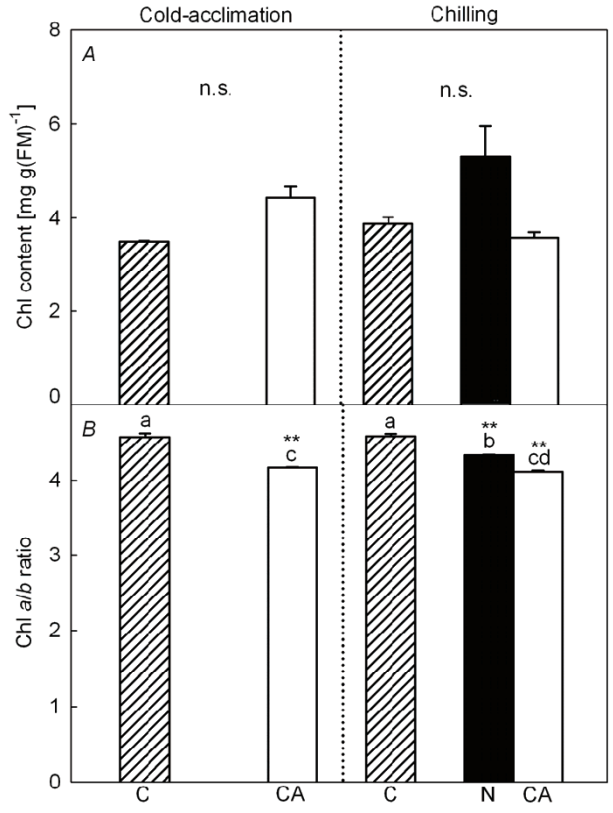

Fig. 4. Chlorophyll content [Chl (*a*+*b*)] and Chl *a*/*b* ratio in current-year needles of *P. glehnii*. C – control; CA – cold-acclimated; N – nonacclimated. The different letters denote significant differences by *Tukey* (T) multiple pairwise comparison (P<0.05), and the levels of significance are denoted as * (P<0.05), and ** (P<0.005). (n = 3).

Lutein and β-carotene contents: Lutein contents did not increase during cold acclimation in CA, but it increased under the subsequent low T conditions (chilling condition). However, there was no difference in β-carotene contents by low T treatment among groups (Fig. 6).

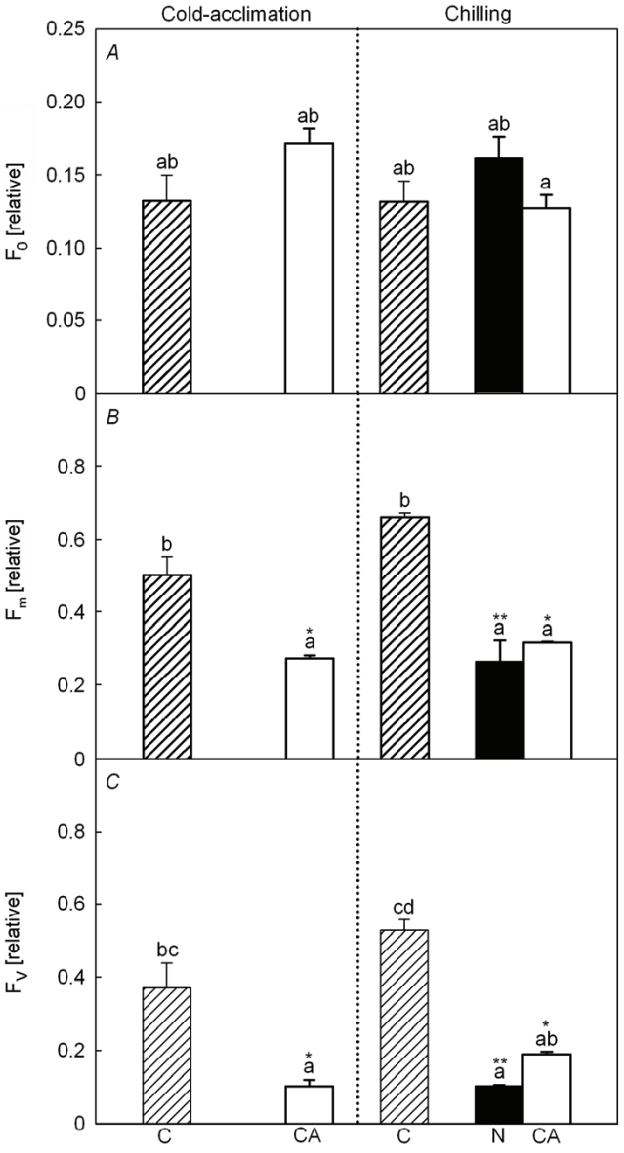

Fig. 5. Chlorophyll fluorescence (F_o – initial, F_m – maximal, F_v – variable) in current-year needles of *P. glehnii*. C – control; CA – cold-acclimated; N – nonacclimated. The different letters denote significant differences by *Tukey* (T) multiple pairwise comparison (P<0.05), and the levels of significance are denoted as * (P<0.05), and ** (P<0.005). (n = 6).

ROS-scavenging enzymes (sAPX, tAPX): The activity of the sAPX was constant in the C, CA, and N needles during cold acclimation and subsequent low T conditions. Under chilling conditions, CA needles showed the same tAPX activity as the C, whereas the N ones showed a significantly greater increase in the tAPX activity than the C and the CA ones (Fig. 7).

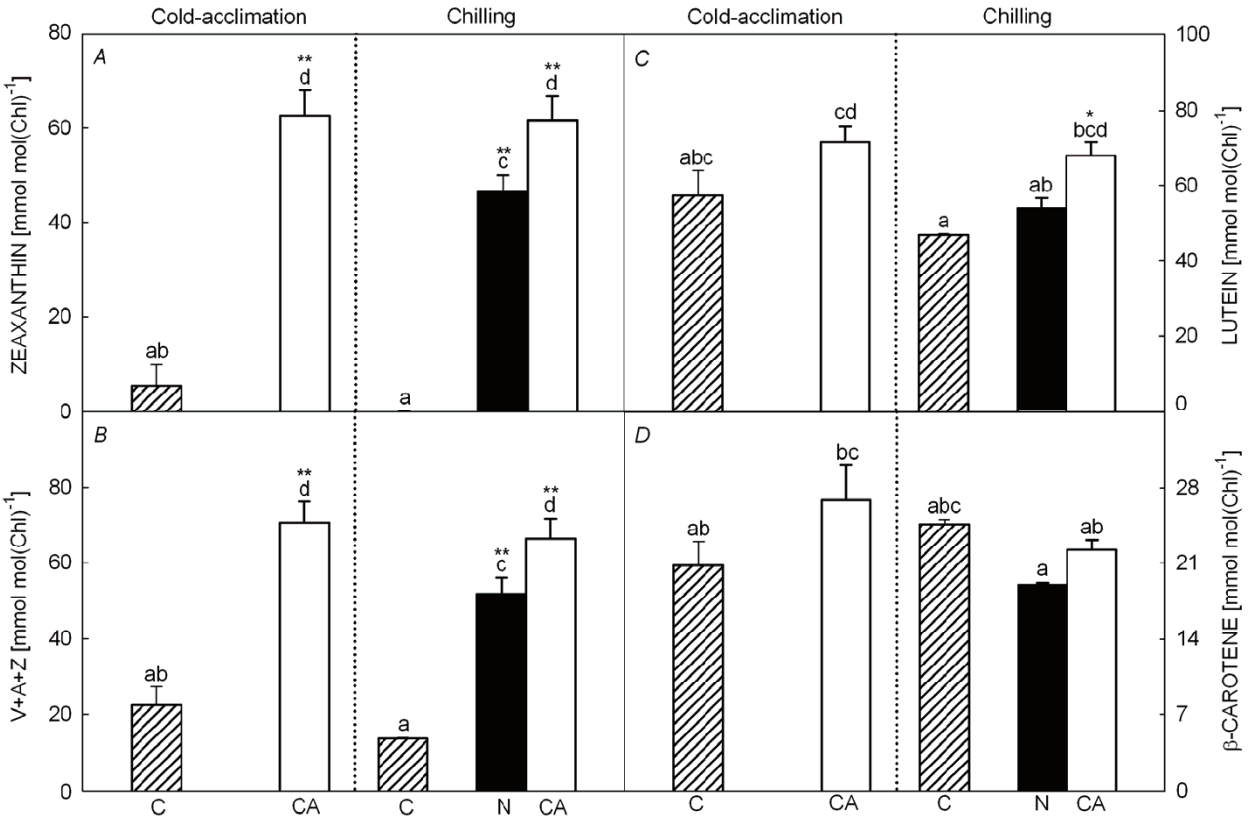

Fig. 6. The contents of zeaxanthin, xanthophyll pool size (V+A+Z), lutein and β-carotene in current-year needles of *P. glehnii*. C – control; CA – cold-acclimated; N – nonacclimated. The different letters denote significant differences by *Tukey* (T) multiple pairwise comparison (P<0.05), and the levels of significance are denoted as * (P<0.05), and ** (P<0.005) (n = 3).

Discussion

RWC: During low temperature, dehydration occurs because of imbalance between water uptake and transpiration (Bohnert *et al*. 1995). Cold-induced water deficit affects cold-sensitive plants. The results underline that chilling treatment decreases RWC in the nonacclimated current-year needles of *P. glehnii*, while the cold-acclimated needles maintained a greater RWC even under the subsequent low-temperature conditions.

F_v/F_m, Chl, and fluorescence: Plants have the ability to adjust light-harvesting antenna size in response to environmental light conditions (Murchie and Horton 1997, Tanaka and Tanaka 2000). If the light-harvesting antenna complex responds to low-temperature-induced photooxidative stress, it is expected that Chl *a*/*b* increases (*i.e*. smaller antenna size) as temperature decreases. In our results (Fig. 4), however, the light-harvesting antenna complex did not respond to low temperature-induced photooxidative stress because Chl content (main antenna pigments) was almost constant and Chl *a*/*b* decreased (*i.e*. larger antenna size) with the decrease of T. The decreases of quantum yield of PSII photochemistry (Fig. 3), F_v and F_m (Fig. 5), the constant Chl content (Fig. 4), and F_o (Fig. 5) were revealed during cold acclimation and under the subsequent chilling conditions. These results indicate that the reaction center of the photosynthetic apparatus in current-year needles of *P. glehnii* suffered more damage than the antenna complex by low-temperature-induced photooxidative stress. In our results, cold-acclimated needles showed less damage to the photosynthetic apparatus than nonacclimated ones, under the subsequent chilling conditions. It is therefore suggested that cold acclimation improves tolerance in the reaction center of photosynthetic apparatus to low-temperature-induced photooxidative stress. However, more data including analyses of core proteins of the photosystem such as D1, D2, and CP43 are needed to verify these processes (Sundby *et al*. 1993, Rintamäki *et al*. 1996, Anderson *et al*. 1997, Baena-Gonzalez *et al*. 1999).

Pigments: Our results showed increases in both zeaxanthin and lutein contents, and in xanthophyll pool size of cold-acclimated *P. glehnii* needles under low temperature (Fig. 6). These are the general characteristics of many cold-acclimated plants under low temperature, herbaceous plants (such as spinach, winter wheat, winter rye, maize) (Somersalo and Krause 1990, Hurry and Hüner 1992, Leipner *et al*. 1997, Streb *et al*. 1999), and

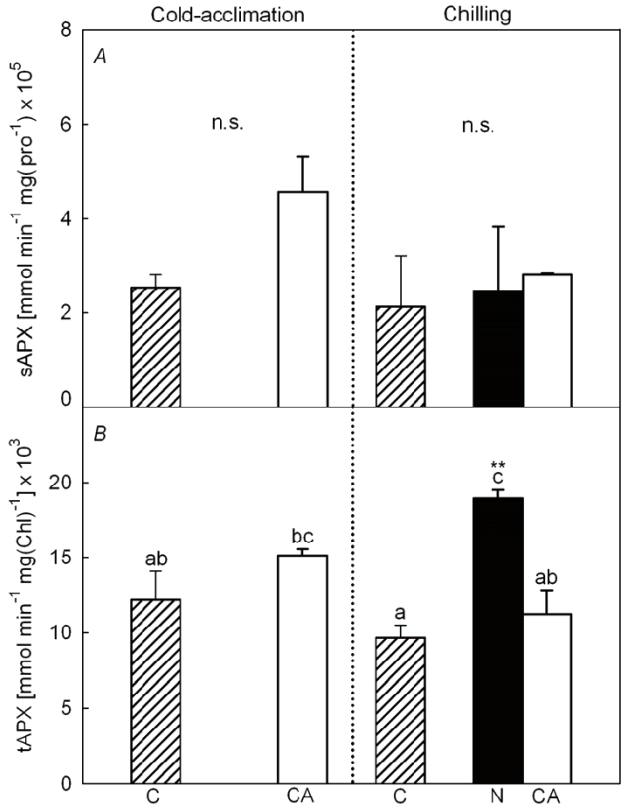

Fig. 7. The activities of sAPX and tAPX in current-year needles of *P. glehnii*. C – control; CA – cold-acclimated; N – nonacclimated. The different letters denote significant differences by *Tukey* (T) multiple pairwise comparison ($P<0.05$), and the levels of significance are denoted as * ($P<0.05$), and ** ($P<0.005$). ($n = 3$).

evergreen trees (Ottander *et al*. 1995, Gilmore and Ball 2000). They suggest that thermal dissipation of excess energy by zeaxanthin and lutein during cold acclimation (Fig. 6) plays an important role in the protection and repair of the photosynthetic apparatus against low-temperature-induced photooxidative damage in cold-acclimated *P. glehnii* needles.

ROS-scavenging enzymes (sAPX, tAPX): According to many researches, there are augmented increases in antioxidant enzymes' activities and nonenzymatic antioxidants in cold-acclimated plants at low temperature (Schöner and Krause 1990, Anderson *et al*. 1995, Leipner *et al*. 1997, Pinhero *et al*. 1997, Scebba *et al*. 1998, 1999). Research on winter injury in some coniferous needles (*Picea koraiensis*, *Pinus sylvestris*, *Pinus koraiensis*, *Pinus tabulaeformis*, *Pinus bungeana*) showed the positive correlations between their freezing tolerance and increases in ascorbate content, APX activity, or SOD activity in winter (Jin *et al*. 1989, 2003). In our results, however, *P. glehnii* did not show any increase in the activity of antioxidant enzymes of cold-acclimated needles at low temperature; it showed a significant increase only in tAPX activity (but not in the sAPX one) of nonacclimated needles under the chilling conditions. These results imply that tAPX (not sAPX) works against photooxidative stress by ROS formation due to excess energy when the current-year needles are exposed to low temperatures suddenly and without cold acclimation.

Conclusion: In conclusion, the current-year needles of *Picea glehnii* responded to low-temperature-induced photooxidative stress in two different ways depending on sudden or gradual decrease in air temperatures: both zeaxanthin and lutein increase in response to a gradual decrease in air temperature, and tAPX activity in response to a sudden decrease in air temperature.

References

Anderson, J.M., Park, Y.I., Chow, W.S.: Photoinactivation and photoprotection of photosystem II in nature. – Physiol. Plant **100**: 214-223, 1997.

Anderson, M.D., Prasad, T.K., Stewart, C.R.: Changes in isozyme profiles of catalase, peroxidase, and glutathione-reductase during acclimation to chilling in mesocotyls of maize seedlings. – Plant Physiol. **109**: 1247-1257, 1995.

Aroca, R., Irigoyen, J.J., Sánchez-Díaz, M.: Photosynthetic characteristics and protective mechanisms against oxidative stress during chilling and subsequent recovery in two maize varieties differing in chilling sensitivity. – Plant Sci. **161**: 719-726, 2001.

Asada, K.: The water-water cycle in chloroplasts: Scavenging of active oxygen and dissipation of excess photons. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **50**: 601-639, 1999.

Baena-Gonzalez, E., Barbato, R., Aro, E.M.: Role of phosphorylation in the repair cycle and oligomeric structure of photosystem II. – Planta **208**: 196-204, 1999.

Bohnert, H.J., Nelson, D.E., Jensen, R.G.: Adaptation to environmental stress. – Plant Cell **7**: 1099-1111, 1995.

Cameron, R.W.F., Harrison-Murray, R.S., Scott, M.A.: The use of controlled water stress to manipulate growth of container-grown *Rhododendron* cv. Hoppy. – J. Hort. Sci. Biotech. **74**: 161-169. 1999.

Doulis, A.G., Hauslanden, A., Mondy, B., Alscher, R.G., Chevone, B.I., Hess, J.L., Weiser, R.L.: Antioxidant response and winter hardiness in red spruce (*Picea rubens* Sarg.). – New Phytol. **123**: 365-374, 1993.

Gilmore, A.M., Ball, M.C.: Protection and storage of chlorophyll in overwintering evergreens. – Proc. Natl. Acad. Sci. USA **97**: 11098-11101, 2000.

Gilmore, A.M.: Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. – Physiol. Plant **99**: 197-209, 1997.

Hansen, U., Fiedler, B., Rank, B.: Variation of pigment composition and antioxidative systems along the canopy light gradient in a mixed beech/oak forest: a comparative study on deciduous tree species differing in shade tolerance. – Trees **16**: 354-364, 2002.

Hurry, V.M., Hüner, N.P.A.: Effect of cold hardening on sensi-

tivity of winter and spring wheat leaves to short-term photoinhibition and recovery of photosynthesis. – Plant Physiol. **100**: 1283-1290, 1992.

Jin, Y.H., Tao, D.L., Du, Y.J.: Freezing tolerance, pigments and SOD of five conifers in Shenyang. – Acta Botan. Sin. **32**: 702-706, 1989.

Jin, Y.H., Tao, D.L., Hao, Z.Q., Ye, J., Du, Y.J., Liu, H.L., Zhou, Y.B.: Environmental stresses and redox status of ascorbate. – Acta Botan. Sin. **45**: 795-801, 2003.

Kojima, S.: Classification and ecological characterization of coniferous forest phytogeocoenoses of Hokkaido, Japan. – Vegetatio **96**: 25-42, 1991.

Leipner, J., Fracheboud, Y., Stamp, P.: Acclimation by suboptimal growth temperature diminishes photooxidative damage in maize leaves. – Plant Cell Environ. **20**: 366-372, 1997.

Li, C.Y., Junttila, O., Heino, P., Palva, E.T.: Low temp. sensing in silver birch (*Betula pendula* Roth) ecotypes. – Plant Sci. **167**: 165-171, 2004.

Li, C.Y., Puhakainen, T., Welling, A., Viherä-Aarnio, A., Ernstsen, A., Junttila, O., Heino, P., Palva, E.T.: Cold acclimation in silver birch (*Betula pendula*). Development of freezing tolerance in different tissues and climatic ecotypes. – Physiol. Plant **116**: 478-488, 2002.

Mittler, R.: Oxidative stress, antioxidants and stress tolerance. – Trends Plant Sci. **7**: 405-410, 2002.

Murchie, E.H., Horton, P.: Acclimation of photosynthesis to irradiance and spectral quality in British plant species: chlorophyll content, photosynthetic capacity and habitat preference. – Plant Cell Environ. **20**: 438-448, 1997.

Noctor, G., Foyer, C.: Ascorbate and glutathione: Keeping active oxygen under control. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **49**: 249-279, 1998.

Ottander, C., Campbell, D., Öquist, G.: Seasonal changes in photosystem II organisation and pigment composition in *Pinus sylvestris*. – Planta **197**: 176-183, 1995.

Pinhero, R.G., Rao, M.V., Paliyath, G., Murr, D.P., Fletcher, R.A.: Changes in activities of antioxidant enzymes and their relationship to genetic and paclobutrazol-induced chilling tolerance of maize seedlings. – Plant Physiol. **114**: 695-704, 1997.

Repo, T., Leinonen, I., Wang, K.Y., Hänninen, H.: Relation between photosynthetic capacity and cold hardiness in Scot pine. – Physiol. Plant **126**: 224-231, 2006.

Repo, T., Hänninen, H., Kellomäki, S.: The effects of long-term elevation of air temp. and CO_2 on the frost hardiness of Scots pine. – Plant Cell Environ. **19**: 209-216, 1996.

Rintamäki, E., Kettunen, R., Aro, E.M.: Differential D1 dephosphorylation in functional and photodamaged photosystem II centers. Dephosphorylation is a prerequisite for degradation of damaged D1. – J. Biol. Chem. **271**: 14870-14875, 1996.

Scebba, F., Sebustiani, L., Vitagliano, C.: Protective enzymes against activated oxygen species in wheat (*Triticum aestivum* L.) seedlings: Responses to cold acclimation. – J. Plant Physiol. **155**: 762-768, 1999.

Scebba, F., Sebustiani, L., Vitagliano, C.: Changes in activity of antioxidant enzymes in wheat (*Triticum aestivum*) seedlings under cold acclimation. – Physiol. Plant **104**: 747-752, 1998.

Schöner, S., Krause, G.H.: Protective systems against active oxygen species in spinach: response to cold acclimation in excess light. – Planta **180**: 383-389, 1990.

Somersalo, S., Krause, G.H.: Reversible photoinhibition of unhardened and cold-acclimated spinach leaves at chill
ing temperatures. – Planta **180**: 181-187, 1990.

Streb, P., Shang, W., Feierabend, J.: Resistance of cold-hardened winter rye leaves (*Secale cereale* L.) to photooxidative stress. – Plant Cell Environ. **22**: 1211-1223, 1999.

Sundby, C., McCaffery, S., Anderson, J.M.: Turnover of the photosystem II D1 protein in higher plants under photoinhibitory and nonphotoinhibitory irradiance. – J. Biol. Chem. **268**: 25476-25482, 1993.

Tanaka, R., Tanaka, A.: Chlorophyll *b* is not just an accessory pigment but a regulator of the photosynthetic antenna. – Porphyrins **9**: 240-245, 2000.

Verhoeven, A.S., Swanberg, A., Thao, M., Whiteman, J.: Seasonal changes in leaf antioxidant systems and xanthophyll cycle characteristics in *Taxus x media* growing in sun and shade environments. – Physiol. Plant **123**: 428-434, 2005.

Wang, K.Y., Canopy CO_2 exchange of Scots pine and its seasonal variation after four-year exposure to elevated CO_2 and temperature. – Agri. Forest Meteorol. **82**: 1-27, 1996.