Low growth temperature inhibition of photosynthesis in cotyledons of jack pine seedlings (*Pinus banksiana*) is due to impaired chloroplast development

Marianna Krol, Vaughan M. Hurry, Denis P. Maxwell, Lada Malek, Alexander G. Ivanov, and Norman P.A. Huner

**Abstract:** Cotyledons of jack pine seedlings (*Pinus banksiana* Lam.) grown from seeds were expanded at low temperature (5°C), and total Chl content per unit area of cotyledons in these seedlings was only 57% of that observed for cotyledons on 20°C-grown controls. Chl \(a/b\) ratio of 5°C-grown jack pine was about 20% lower (2.3 ± 0.1) than 20°C controls (2.8 ± 0.3). Separation of Chl–protein complexes and SDS–PAGE indicated a significant reduction in the major Chl \(a\) containing complex of PSI (CP1) and PSII (CPa) relative to LHCII 1 in 5°C compared to 20°C-grown seedlings. In addition, LHCII\(^{1}\)/LHCII\(^{3}\) ratio increased from 3.8 in control (20°C) to 5.5 in 5°C-grown cotyledons. Ultrastructurally, 5°C-grown cotyledons had chloroplasts with swollen thylakoids as well as etiochloroplasts with distinct prolamellar bodies. Based on CO\(_2\)-saturated \(O_2\) evolution and in vivo Chl \(a\) fluorescence, cotyledons of 5°C jack pine exhibited an apparent photosynthetic efficiency that was 40% lower than 20°C controls. Seedlings grown at 5°C were photoinhibited more rapidly at 5°C and 1200 \(\mu\)mol·m\(^{-2}\)·s\(^{-1}\) than controls grown at 20°C, although the final extent of photoinhibition was similar. Exposure to high light at 5°C stimulated the xanthophyll cycle in cotyledons of both controls and 5°C-grown seedlings. In contrast to winter cereals, we conclude that growth of jack pine at 5°C impairs normal chloroplast biogenesis, which leads to an inhibition of photosynthetic efficiency.

**Key words:** chloroplast, growth, temperature, photosynthesis, photoinhibition, *Pinus banksiana* Lam., ultrastructure.

**Résumé :** Les cotylédons de plantules de pin gris (*Pinus banksiana* Lam.) provenant de semences et obtenus à basse température (5°C) montrent une teneur totale en Chl par unité de surface égale à seulement 57 % de celle observée chez les cotylédons des plantules témoin cultivées à 20°C. Le rapport Chl \(a/b\) dans les cotylédons de pins gris cultivés à 5°C est environ 20 % plus bas (2.3 ± 0.1) que celui des plantes témoin cultivées à 20°C (2.8 ± 0.3). La séparation des complexes protéiques Chl–protéine et des SDS–PAGE montre une réduction significative dans les principaux complexes des PSI (CP1) et PSII (CPa) contenant la chlorophylle \(a\) par rapport au LHCII 1, lorsqu’on compare des plantules cultivées à 5°C et à 20°C. De plus, le rapport LHCII\(^{1}\)/LHCII\(^{3}\) augmente de 3.8 chez le témoins (20°C) à 5,5 chez les cotylédons obtenus à 5°C. Quant aux ultrastructures, les cotylédons obtenus à 5°C montrent des chloroplastes avec des thylakoïdes renflés ainsi que des étiochloroplastes munis de corps prolamellaires distincts. En se basant sur l’évolution du \(O_2\) en présence de \(CO_2\) saturant et sur la fluorescence in vivo de la Chl \(a\), les cotylédons du pin gris obtenus à 5°C montrent une efficacité photosynthétique apparente de 40 % plus faible que ceux des témoins obtenus à 20°C. Les plantules développées à 5°C sont plus rapidement photoinhibées à 5°C et 1200 \(\mu\)mol·m\(^{-2}\)·s\(^{-1}\) que celles...
obtained to 20°C, but it's the importance of the photoinhibition is similar to the fin. The exposure to a luminosity elevated to 5°C stimulates the cycle of the xanthophylls in the cotyledon of plants, especially well that cells obtained to 5°C. Contrary to the cereals, the authors conclude that the growth to 5°C in the pin fin affects the biogène-nese normal of the chloroplasts, which contributes to an inhibition of the efficacy photosynthétique.

Mots clés : chloroplaste, croissance, température, photosynthèse, photoinhibition, Pinus banksiana Lamb., ultrastructure.

[Intaduit par la Rédaction]

Introduction

Pine species become extremely cold and freezing tolerant when exposed to a combination of short days and low temperatures, conditions normally experienced during autumn in the northern temperate and boreal zones (Lindgren and Höllgren 1993; Öquist et al. 2001; Colombo et al. 2001). These conditions induce dormancy in conifers, which is essential for attaining maximum cold tolerance and winter survival. Exposure of Scots pine (Pinus sylvestris L.) or western red cedar (Thuja plicata D. Don.) to 5°C and short days under controlled environment conditions causes a significant depression in light saturated rates of CO₂ uptake (PSmaxCO₂) and photosynthetic electron transport (Oquist et al. 1980; Weger et al. 1993). Furthermore, natural overwintering of Scots pine also results in inhibition of CO₂ assimilation and depression of the quantum yield of O₂ evolution (ϕappO₂) and of the photochemical efficiency of PSII (Strand and Öquist 1985; Strand and Öquist 1988; Leverenz and Öquist 1987; Ottander and Öquist 1991; Krol et al. 1995; Öquist et al. 2001). More recently, Savitch et al. (2002) reported that Scots pine responded to cold acclimation by developing higher levels of the xanthophyll cycle pigments and a higher capacity for non-photochemical quenching as well as reduction in the daily carbon gain.

Several reports have indicated that photoinhibition of photosynthesis may occur at low temperatures under laboratory as well as field conditions in Scots pine (Öquist and Ogren 1985; Strand and Öquist 1988; Öquist and Malmberg 1989), western red cedar (Weger et al. 1993), Norway spruce (Picea abies L. Karst.) (Bohlar-Nordenkampf and Lechner 1988; Lundmark and Höllgren 1988), ivy (Hedera helix L.) (Oberhuber and Bauer 1991), holly (Ilex aquifolium L.) (Groom et al. 1991), Brassica napus (Farage and Long 1991), and wheat (Triticum aestivum L.) (Groom and Baker 1992). However, field grown pine exhibited full recovery from winter stress within several hours to days when overwintering branches were shifted to 20°C and moderate light conditions in the laboratory (Ottander and Öquist 1991; Ottander et al. 1995; Ivanov et al. 2001). Similar trends have been reported for photoinhibited holly (Groom et al. 1991) and ivy (Oberhuber and Bauer 1991). Light levels as low as 25 to 50 μmol·m⁻²·s⁻¹ are sufficient to induce significant reductions in the quantum yield of the upper, light exposed sides of needles during prolonged exposure to low temperatures (Strand and Öquist 1988; Krol et al. 1995). Öquist and Huner (1991) reported that pine exposed to 5°C and short days exhibit similar susceptibilities to low temperature photoinhibition as pine grown under summer conditions. They concluded that Scots pine is unable to adjust its sensitivity to low temperature photoinhibition upon exposure to cold hardening conditions.

Recently, it has been demonstrated that PSI is less affected and recoveries from winter stress much faster then PSII (Ivanov et al. 2001). In addition, cyclic electron transfer around PSI is active in overwintering Scots pine and that may be important in photoprotection of the photosynthetic apparatus in conifers during the winter (Ivanov et al. 2001).

In contrast to conifers, cold hardened winter cereals (Huner et al. 1993; Hurry and Huner 1991; Savitch et al. 2002), spinach (Somersalo and Krause 1989, 1990; Boese and Huner 1990), and Chlorella (Maxwell et al. 1994, 1995) exhibit PSmax CO₂ and PSmax O₂ that are equal to or greater than non-hardened plants. Furthermore, growth at low temperature had no effect on either the ϕapp CO₂ or ϕapp O₂ in winter cereals (Huner et al. 1986; Hurry and Huner 1991, 1992; Öquist and Huner 1993). Growth of winter cereals (Öquist and Huner 1991; Hurry and Huner 1992; Öquist et al. 1993), spinach (Somersalo and Krause 1989; Boese and Huner 1990), and Chlorella (Maxwell et al. 1994, 1995) at low temperature and moderate irradiance results in a decreased sensitivity to light at 5°C that is five- to six-fold higher than the growth light (150–250 μmol·m⁻²·s⁻¹). This has been explained on the basis that photosynthetic acclimation to low temperature may mimic photosynthetic acclimation to high light to due the modulation of a common sensor, the relative reduction state of the thylakoid PQ pool. As a consequence plants exposed to low temperature may be exposed to a comparable excitation pressure as those exposed to high light (Huner et al. 1998).

The ability to decrease sensitivity to low temperature photoinhibition in herbaceous plants is a consequence of growth and development at low temperature (Huner et al. 1993; Gray et al. 1994, 1997). It was suggested that the reason that cold hardened pine does not exhibit a decreased sensitivity to photoinhibition is that growth at low temperatures does not occur during normal cold hardening of pine (Öquist and Huner 1991; Huner et al. 1993; Öquist et al. 2001). However, in contrast to herbaceous plants, we have reported that exposure of jack pine to a combination of low temperature stress and a short photoperiod does induce an increased tolerance to photoinhibition without significant growth. This occurs because of the accumulation of anthocyanin in the epidermal cell layer, which acts as a natural sun screen (Krol et al. 1995). In the present investigation, we examine the hypothesis that cold tolerant conifers such as jack pine acquire increased tolerance to low temperature photoinhibition; this process occurs under conditions where seedlings actually grow and develop at low temperature sim-
ilar to cold tolerant herbaceous plants (Huner et al. 1993; Öquist et al. 2001).

Materials and methods

Plant material

Jack pine seedlings were germinated and grown from seed in vermiculite in controlled environment growth chambers (Conviron, Winnipeg, Man.) maintained on a 16 h light:8 h dark photoperiod and an irradiance of 250 µmol m⁻² s⁻¹ at either 20:16°C for 4 weeks or 5:5°C for 4 months. Seedlings were supplemented with full strength Hoagland’s nutrient solution every second day as required. All experiments were carried out on fully expanded cotyledons of jack pine seedlings grown at 20°C and 5°C.

Oxygen evolution

Oxygen evolution was measured with a leaf-disc electrode (HansaTech, Model LD2, Kings Lynn, Norfolk, U.K.) at 20°C with an initial gas mixture 5% CO₂: 5% O₂: 90% N₂. Additional CO₂ was supplied in the form of sodium bicarbonate (1.0 M NaHCO₃) soaked capillary mats to ensure CO₂ saturation. Light was provided by a set of photodiodes between the lamps and the samples. In addition, two oscillating 15 cm fans provided continuous air circulation over the samples. This apparatus was maintained in a cold room set at 20°C with an initial gas mixture 5% CO₂: 5% O₂: 90% N₂. Separately, thylakoids were washed once in double-distilled water, once in EDTA (pH 8.0), and twice in 50 mM Tricine (pH 8.0). The membrane pellets were resuspended and solubilized in 0.3 M Tris (pH 8.8) containing 13% (w/v) glycerol and 1% (w/v) SDS (sodium dodecylsulfate), and then 2% (w/v) DOC (deoxycholate) in 0.3 M Tris (pH 8.8) was added immediately to give a DOC–SDS–Chl ratio of 20:10:1. The solubilized Chl–protein complexes were separated in the dark at 4°C in 7.5% (w/v) SDS polyacrylamide slab gels as described in detail elsewhere (Huner et al. 1992).

Chlorophyll a fluorescence

Chlorophyll a fluorescence kinetics of intact cotyledons was measured at room temperature using a time resolved fluorescence instrument (PSM Chlorophyll Fluorometer, BioMonitor S.C.I. AB, Umeå, Sweden) (Öquist and Wass 1988; Bolhar-Nordenkampf et al. 1989). The samples were dark adapted at room temperature for at least 30 min before measurements were taken. Maximum photochemical efficiency of PSII was measured as the ratio of Fₜ/Fₘₚ where Fₘₚ is maximum chlorophyll fluorescence at closed PSII centres and Fₜ is the variable fluorescence. Fₚ was calculated as Fₘₚ – Fₜ where Fₜ represents instantaneous (dark) chlorophyll fluorescence at open PSI centres.

Photoinhibition

Susceptibility of jack pine seedlings to photoinhibition at low temperature was monitored as a function of time during exposure to an irradiance of 1200 µmol m⁻² s⁻¹ using a bank of three Lucalox LU-400 (CGE, Toronto, Ont.) high-pressure sodium lamps. A plexiglass heat filter containing a continuous flow of cold water, 10 cm deep, was placed between the lamps and the samples. In addition, two oscillating, 15 cm fans provided continuous air circulation over the samples. This apparatus was maintained in a cold room set for an air temperature of 5°C. Seedlings were placed in an aluminium tray and maintained on moist filter paper. This tray was set in another tray containing ice. Under these conditions, the temperature of the attached cotyledons was maintained at 5°C to 7°C during exposure to the photo-inhibitory light.

Isolation of thylakoid membranes and separation of Chl-protein complexes

Thylakoids from 20° and 5°C cotyledons were isolated at 4°C in 50 mM Tricine (pH 7.8) containing 0.4 M sorbitol, 10 mM NaCl, and 20% PEG (polyethylene glycol) 4000. Subsequently, thylakoids were washed once in double-distilled water, once in EDTA (pH 8.0), and twice in 50 mM Tricine (pH 8.0). The membrane pellets were resuspended and solubilized in 0.3 M Tris (pH 8.8) containing 13% (v/v) glycerol and 1% (w/v) SDS (sodium dodecylsulfate), and then 2% (w/v) DOC (deoxycholate) in 0.3 M Tris (pH 8.8) was added immediately to give a DOC–SDS–Chl ratio of 20:10:1. The solubilized Chl–protein complexes were separated in the dark at 4°C in 7.5% (w/v) SDS polyacrylamide slab gels as described in detail elsewhere (Huner et al. 1992).

Separation of thylakoid polypeptides

Freshly prepared thylakoid membranes were solubilized in SDS only and thylakoid polypeptides separated by polyacrylamide gel electrophoresis (SDS–PAGE). SDS–PAGE was prepared according to Laemmli (1970), using 12% (w/v) polyacrylamide and 6 M urea in the separating gel. Solubilization of thylakoid proteins was carried out at room temperature for 30 min at an SDS–Chl ratio of 20:1. Samples were centrifuged for 5 min to remove unsolubilized material. Gel slots were loaded with an equal amount of 3 to 5 µg Chl per lane.

Chloroplast ultrastructure

Sections taken from the mid-portion of jack pine cotyledons were fixed and stained for electron microscopy with uranyl acetate as described in detail elsewhere (Krol et al. 1988). Sections were viewed with a Phillips electron microscope (Model 201).

Pigment analysis

Photosynthetic pigments were separated by HPLC using a modified method of Gilmore and Yamamoto (1991). A Beckman System Gold Solvent Module (Beckman Instruments, San Ramon, Calif.) equipped with a Spherisorb ODS-1 analytical column (5 µm particle size, 250 mm x 4.6 mm I.D.) was protected by an Upchurch Perisorb A guard column (both columns from S.P.E. Inc., Concord, Ont.). Samples were injected using a Beckman 210A sample-injection valve with a 20 µL sample loop. Through modifications of the method of Gilmore and Yamamoto (1991), pigments were eluted using 100% of acetonitrile – methanol – 0.1 M Tris (pH 8.0) (74:11:3.5) followed by a 2 min linear gradient to 100% methanol–hexane (4:1), which continued isocratically until the end of the 12 min separation. The column was allowed to re-equilibrate in the initial solvent for a minimum of 10 min between injections. All solvents were of HPLC grade (Omni Solv, VWR Canlab, Ville Mont-Royal, Que.).

Pigments were detected with a Beckman System Gold diode array detector at 440 nm and peak areas were integrated by Beckman System Gold software. Retention times and response factors were determined by injecting known quantities of pure Chl a, Chl b, lutein, and β-carotene purchased from Sigma (St. Louis, Mo.). The retention times

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of zeaxanthin, antheraxanthin, violaxanthin, and neoxanthin were estimated by using pigments purified from barley according to Diaz et al. (1990). Epoxidation state (EPS) was calculated as $V + 0.5A / V + A + Z$, where $V$, $A$, and $Z$ represent the content of violaxanthin, antheraxanthin, and zeaxanthin, respectively (Demmig-Adams and Adams 1992).

Chl concentrations required for electrophoresis of Chl–protein complexes and thylakoid polypeptides as well as O$_2$ evolution were determined according to Arnon (1949).

Table 1. Effect of growth temperature on pigment composition of jack pine cotyledons.

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Growth temperature</th>
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<tbody>
<tr>
<td></td>
<td>20°C</td>
</tr>
<tr>
<td>Total Chl</td>
<td>1325±18</td>
</tr>
<tr>
<td>Chl $a/b$</td>
<td>2.8±0.3</td>
</tr>
<tr>
<td>Neoxanthin</td>
<td>24±3</td>
</tr>
<tr>
<td>Violaxanthin</td>
<td>25±4</td>
</tr>
<tr>
<td>Antheraxanthin</td>
<td>6±1</td>
</tr>
<tr>
<td>Lutein</td>
<td>96±5</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>4.0±0.5</td>
</tr>
<tr>
<td>$\beta$-carotene</td>
<td>87±3</td>
</tr>
<tr>
<td>Chl–carotenoid</td>
<td>4.9±0.8</td>
</tr>
<tr>
<td>EPS</td>
<td>0.800</td>
</tr>
</tbody>
</table>

Note: HL refers to attached cotyledons exposed to 1200 µmol·m$^{-2}$·s$^{-1}$ for 8 h. All data are averages of at least three experiments ± SD and are presented as µg Chl/(g fresh weight)$^{-1}$.

Results

Effects of growth temperature on pigment composition

In contrast to cotyledons of *P. banksiana* germinated and grown at 20°C and with a 16 h light : 8 h dark photoperiod, cotyledons expanded at 5°C exhibited 75% lower total Chl content per g fresh weight (Fig. 1, Table 1). This corresponded to a 42% lower total Chl content on an area basis (data not shown). In addition, 5°C cotyledons had an 18% lower ratio of Chl $a/b$ than 20°C cotyledons. Finally, fully expanded 5°C primary needles remained etiolated whereas 20°C primary needles developed normally (Fig. 1). HPLC analysis of the pigment composition revealed that all carotenoids, except antheraxanthin, were drastically reduced in 5°C grown seedlings when expressed per g fresh weight (Table 1). It should be noted, however, that the ratio of Chl/carotenoids in 5°C cotyledons was 50% lower than in 20°C cotyledons.

Electron microscopy

Chloroplast ultrastructure was examined in cross sections of cotyledons of 20°C and 5°C-grown seedlings. Figure 2A illustrates that control cotyledons exhibited chloroplast structure similar to that reported for Scots pine and Norway spruce (Soikkeli 1980). The osmiophilic bodies within the vacuole of control cotyledons may reflect the presence of tannins (Soikkeli 1980). However, cotyledons from 5°C-grown seedlings exhibited the presence of etiochloroplasts similar to those observed in etiolated leaves of rye developed under an intermittent light regime (Krol and Huner 1989). Although the 5°C seedlings developed from seed under a constant 16 h light : 8 h dark photoperiod, the plastids in 5°C jack pine exhibited a pronounced prolamellar body from which single thylakoids radiated (Fig. 2B). Moreover, some 5°C cotyledons exhibited chloroplasts with highly swollen granal stacks but no prolamellar body (Fig. 2C). In addition, 5°C cotyledons exhibited extensive vesiculation within the cytoplasm, which is typical of development at low temperature (Huner et al. 1984; Strand et al. 1999).

Effects of growth temperature on the Chl–protein complexes

The relative amount of the Chl–protein complexes in thylakoid membranes isolated from pine seedlings developed at 20°C and 5°C, respectively, were estimated from non-denaturating SDS–PAGE profiles. As seen from a typical scan of an electrophoretic separation of Chl–protein complexes, seven major green bands corresponding to the Chl–protein complexes of LHC1, CP1, LHCII$^1$, LHCII$^2$, CPa, LHCII$^3$, and FP in order of increasing electrophoretic mobility were observed (Fig. 3). The LHCII, which is functionally coupled to PSII, is represented by three bands:

![Fig. 1. Jack pine seedlings grown at either 20°C (A) or 5°C (B) and at an irradiance of 250 µmol photons·m$^{-2}$·s$^{-1}$. The etiolated structures in B illustrate primary needles of jack pine grown at 5°C and 250 µmol photons·m$^{-2}$·s$^{-1}$ for 4 months.](image-url)
LHCCI\textsuperscript{1} (oligomeric), LHCCI\textsuperscript{2} (dimeric), and LHCCI\textsuperscript{3} (monomeric). Although thylakoids from seedlings grown at 5°C exhibited a lower proportion of the Chl–protein complexes associated with PSI (LHCI and CPI) than cotyledons developed at 20°C, the ratio of Chl–protein complexes representing the reaction centers of PSI and PSII (CPI and CPa) was similar at both temperatures. The ratio of LHCCI\textsuperscript{1}/LHCCI\textsuperscript{3} was about 45% lower in 20°C cotyledons (3.8) than in cotyledons expanded at 5°C (5.5). Differences in thylakoid polypeptide composition between two types of seedlings were also found (Fig. 4). A major reduction of PSI related polypeptides within the range of 60–50 kD was observed in 5°C cotyledons.

Effects of growth temperature on photosynthesis

Jack pine seedlings grown at 5°C exhibited approximately a 50% lower \( \varphi_{\text{appO}_2} \) (0.035 mol O\(_2\)/mol photons) compared to seedlings grown at 20°C (0.080 mol O\(_2\)/mol photons). In addition, low growth temperature resulted in a 55% lower light saturated rate of CO\(_2\) saturated, O\(_2\) evolution (Fig. 5A). However, since the Chl content per area was reduced in 5°C seedlings, the data were recalculated on a per mg Chl basis (Fig. 5B). These results confirm a lower photosynthetic efficiency in 5°C than 20°C cotyledons as indicated by a 40% lower initial slope but comparable light saturated rates of CO\(_2\) saturated O\(_2\) evolution. The decreased photosynthetic efficiency is consistent with a 38% lower F\(_{\text{V}}\)/F\(_{\text{M}}\) in cotyledons developed at 5°C (0.49 ± 0.05) relative to those grown at 20°C (0.78 ± 0.01). Thus, chloroplast biogenesis in jack pine at low temperature appears to cause a significant depression in photosynthetic efficiency, whereas photosynthetic capacity on a Chl basis is not affected.

This depression in photosynthetic efficiency was reversible as indicated by the recovery of \( \varphi_{\text{appO}_2} \) upon shifting seedlings grown at 5°C to 20°C (Fig. 6). After only 18 h at 20°C, 5°C grown jack pine exhibited an \( \varphi_{\text{appO}_2} \) (0.063) that was 85% of the control 20°C grown seedlings. After 66 h at 20°C, both the \( \varphi_{\text{appO}_2} \) (0.072) and the F\(_{\text{V}}\)/F\(_{\text{M}}\) (0.70) had recovered to about 90% of that observed for 20°C seedlings.
Effect of growth temperature on susceptibility to photoinhibition

Susceptibility of the seedlings grown at 20°C and 5°C to low temperature induced photoinhibition was assessed by Chl α fluorescence (Fig. 7). Seedlings grown at 5°C exhibited a 40% lower initial Fv/FM than seedlings developed at 20°C, which is consistent with the lower ϕapp O2 exhibited by the pine grown at 5°C (Fig. 5A). Although the extent of the depression of Fv/FM was similar for both 5°C and 20°C cotyledons after 12 h at 1200 μmol·m−2·s−1 and 5°C, the maximum depression of Fv/FM in seedlings grown at 5°C occurred after 3 h at 5°C and 1200 μmol·m−2·s−1, whereas that of seedlings grown at 20°C required 12 h of exposure to 5°C and 1200 μmol·m−2·s−1. Incubation in the dark at 5°C for 12 h did not have any significant effect on Fv/FM (data not shown).

Zeaxanthin and antheraxanthin have been implicated in protection of the photosynthetic apparatus under high light stress through non-radiative dissipation of excitation energy (Demmig-Adams and Adams 1992). From the results in Table 1, we calculated an EPS of 0.800 for 20°C seedlings prior to exposure to HL, which decreased to 0.407 after exposure to HL. Similarly, the 5°C seedlings exhibited an EPS of 0.714 and 0.450 before and after exposure to HL, respectively. These results were consistent whether calculated on a per g fresh weight or on a per μg Chl basis (data not shown). Thus, it appears that the xanthophyll cycle is fully operative in seedlings grown at both 20°C and 5°C. However, jack pine grown at 5°C exhibited a 53% loss in the total xanthophyll cycle pool after exposure to HL (Table 1). In contrast, such a decrease in xanthophyll cycle pool size was not observed when jack pine seedlings grown at 20°C were exposed to HL.

Discussion

Previous reports have indicated that exposure to damaging frost causes chlorosis and abnormal growth in young needles of Scots pine seedlings (Repo et al. 2001; Rikala and Repo 1987). It has also been demonstrated that exposure to frost at the end of the growing season could result in various reversible ultrastructural alterations in mesophyll cells without...
affecting photosynthesis (Ryppö et al. 1997). Our pigment and ultrastructural analyses of jack pine seedlings indicate that germination and growth at low, nonfreezing temperatures also may result in abnormal chloroplast development (Fig. 1, Figs. 2B and 2C). The impairment of normal chloroplast biogenesis in jack pine seedlings under our experimental conditions is accentuated by the fact that, although primary needles expand, they remain etiolated and are unable to green during exposure to 5°C and a 16 h light:8 h dark photoperiod at an irradiance of 250 µmol·m⁻²·s⁻¹ (Fig. 1). In addition, the presence of etiochloroplasts with distinct prolamellar bodies and abnormal grana stacks in the cotyledons (Figs. 2B and 2C) clearly demonstrate that chloroplast development is highly disturbed, even though the seedlings are exposed to a 16 h light:8 h dark photoperiod. Generally, prolamellar bodies are not considered to be structures involved in normal chloroplast development and are thought to be present only in etioplasts of seedlings developed in the dark (Gunning and Steer 1975). Etioplasts are thought to represent an intermediate stage in the transition from etioplast to chloroplast and can be induced by exposing etiolated rye seedlings to intermittent light (2 min of 15 µmol·m⁻²·s⁻¹ every 2 h) at low temperature (Krol and Huner 1989). However, the etiochloroplasts present in cotyledons of jack pine seedlings grown at 5°C (Fig. 2) developed from seed under a normal 16 h light:8 h dark photoperiod, albeit at low temperature. We are unaware of previous published reports illustrating the presence of etiochloroplasts in leaves of higher plants or conifers exposed to a normal 16 h light:8 h dark photoperiod during development from seed. Gymnosperms can accumulate chlorophyll and develop chloroplasts in the dark (Krol 1978; Forreiter and Apel 1993; Mukai et al. 1992; Oster et al. 1996). It has been demonstrated that the light-independent Chl synthesis is temperature sensitive and pine cotyledons are able to accumulate Chl in the dark when seeds are germinated at temperatures higher than 8°C (Karpinska et al. 1995; Muramatsu et al. 2001). At temperatures below 8°C, Chl does not accumulate and chloroplast biogenesis is arrested in an early developmental stage. This is in close correlation with the observation that plastid DNA levels gradually increase with increasing the temperature from 5 to 25°C (Karpinska et al. 1995). More recent observations indicated that the transcription level of nuclear encoded cab genes was high at low temperatures, but the accumulation of the light-harvesting Chl a/b-binding apoproteins was restricted because of limited synthesis of Chl (Muramatsu et al. 2001). Thus, the lower total amount of Chl (Table 1) and the electron microscopic observation of prolamellar-body-like structures (Figs. 2B and 2C) in cotyledons of 5°C pine seedlings compared to those of 20°C pine seedlings clearly indicate that normal chloroplast development is disrupted by low temperature in P. banksiana seedlings. Analysis of the Chl–protein complexes and polypeptide composition indicated that PSI-related proteins are more affected by low temperature than those of PSII. In agreement with earlier reports, LHCII polypeptides were also reduced in 5°C grown cotyledons (Ottander et al. 1995).

Jack pine grown at low temperatures exhibited both a depressed photosynthetic efficiency as well as reduced light saturated rates of CO₂ saturated O₂ evolution relative to controls grown at 20°C when calculated on an area basis. However, on a per µg Chl basis, cotyledons of 5°C seedlings exhibit comparable light saturated rates of photosynthesis but a lower photosynthetic efficiency (qₚₜₜ, O₂) and photophysical efficiency (Fₚ/Fₘ) than cotyledons of 20°C seedlings. This indicates that low growth temperature inhibits photosynthetic efficiency to a greater extent than photosynthetic capacity. Thus, we conclude that impaired chloroplast development at low temperature in jack pine seedlings is reflected primarily at the level of PSII and the thylakoid membrane components rather than at the level of

![Graph](image-url)
the Calvin cycle enzymes. Although normal chloroplast development is impaired at low temperature, xanthophyll cycle activity (estimated as a decrease in EPS upon exposure to high light) is not inhibited and appears to be fully active. This should provide protection from high light through non-photochemical quenching (Demmig-Adams and Adams 1992). In addition, the increased aggregation of LHCII as indicated by the ratio of LHCII\textsubscript{1}/LHCII\textsubscript{3} (Fig. 3) may also reflect increased capacity for photoprotection through nonphotochemical quenching of excess light as previously suggested (Otlander et al. 1995; Gilmore and Ball 2000).

It is interesting to note that although jack pine is extremely cold tolerant, it appears unable to grow and develop normally at low temperature in contrast to cold-tolerant herbaceous plants such as wheat, rye, and spinach (Huner et al. 1993), as well as green algae (Maxwell et al. 1994). Furthermore, growth of herbaceous plants and algae at low temperature results in increased tolerance to photoinhibition (Savitch et al. 1989, 1990; Boese and Huner 1992; Hurry and Huner 1992; Òquist and Huner 1993; Òquist et al. 1993; Huner et al. 1993). Clearly, our initial hypothesis is incorrect, since growth of jack pine at low temperature does not impart significant tolerance to photoinhibition (Fig. 5) because of a low temperature limitation of normal chloroplast development. It thus appears that pine seedlings could develop cold hardiness and enhanced resistance to photoinhibition only when the photosynthetic apparatus is fully developed.

We conclude that low growth temperature limits photosynthesis indirectly in jack pine through its effects on chloroplast development. Conifers attain maximum cold tolerance through the induction of a photoperiod- and low temperature-dependent dormant state. A dormancy-induced inhibition of growth is a prerequisite for attaining maximum cold tolerance in conifers (Óquist et al. 2001). Thus, conifers would not require a capacity for active photosynthesis at low temperature (Savitch et al. 2002). In contrast, cold-tolerant cereals and spinach require growth, and as a consequence photosynthetic capacity, at low temperature to acquire maximum cold tolerance (Savitch et al. 2002). These contrasting strategies for the development of cold tolerance may have a significant impact on the structure, function, and development of the photosynthetic apparatus.

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