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Accumulation of photosynthetic pigments in *Larix decidua* Mill. and *Picea abies* (L.) Karst. cotyledons treated with 5-aminolevulinic acid under different irradiation

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Abstract

European larch (*Larix decidua* Mill.) and Norway spruce [*Picea abies* (L.) Karst.] synthesize chlorophyll (Chl) in darkness. This paper compares Chl accumulation in 14-d-old dark-grown seedlings of *L. decidua* and *P. abies* after short-term (24 h) feeding with 5-aminolevulinic acid (ALA). We used two ALA concentrations (1 and 10 mM) fed to cotyledons of both species in darkness and in continuous light. The dark-grown seedlings of *L. decidua* accumulated Chl only in trace amounts and the seedlings remained etiolated. In contrast, *P. abies* seedlings grown in darkness were green and had significantly higher Chl content. After ALA feeding, higher protochlorophyllide (Pchlide) content was observed in *L. decidua* than in *P. abies* cotyledons incubated in darkness. Although short-term ALA feeding stimulated the synthesis of Pchlide, Chl content did not change significantly in cotyledons incubated in darkness. The Chl accumulation in cotyledons fed with ALA was similar to the rate of Chl accumulation in the controls. Higher Chl accumulation was reported in control samples after illumination: 86.9% in *L. decidua* cotyledons and 46.4% in *P. abies* cotyledons. The Chl content decreased and bleaching occurred in cotyledons incubated with ALA in light due to photooxidation. Analyses of Chl-binding proteins (D1 and LHCII*b*) by Western blotting proved differences between Chl biosynthesis in *L. decidua* and *P. abies* seedlings in the dark and in the light. No remarkable increase was found in protein accumulation (D1 and LHCII*b*) after ALA application. Our results showed interspecific difference in Chl synthesis between two gymnosperms. Short-term ALA feeding did not stimulate Chl synthesis, thus ALA synthesis was not the rate-limiting step in Chl synthesis in the dark.

Additional key words: chlorophyll biosynthesis; D1 protein; light-harvesting complex; protochlorophyllide.

Introduction

ALA represents an essential precursor in tetrapyrrole biosynthesis. In plants, ALA is formed from glutamate *via* C5-pathway consisting of three steps. ALA is assumed to be a key control point in the regulation of Chl biosynthesis (Beale 1999, Tanaka and Tanaka 2007) and is a rate-limiting step in the Chl biosynthetic pathway. Phytochrome-regulated ALA synthesis is well documented in the initial stages of greening, while in the later stages, light is thought to exert its effect on ALA formation through a feedback inhibition by Pchlide (Huang and Castelfranco 1989). Treating the leaves of dark-grown angiosperms (Granick 1959) or gymnosperms (Wolwertz and Brouers 1980, Pavlovič *et al.* 2009) with ALA caused accumulation of Pchlide, mainly in its nonphototransformable form. It has been found that all enzymes required for Pchlide synthesis were already active and present in nonlimiting amounts in etiolated plant tissue and that only the amount and activity of enzymes involved in ALA synthesis limited the synthesis rate (Granick 1959, Papenbrock and Grimm 2001). The reduction of Pchlide to chlorophyllide (Chlide)

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Abbreviations: ALA – 5-aminolevulinic acid; ALA1 – 1 mM ALA; ALA10 – 10 mM ALA; Chl – chlorophyll; ChlB – the subunit of light-independent protochlorophyllide oxidoreductase; Chlide – chlorophyllide; ChlL – the subunit of light-independent protochlorophyllide oxidoreductase; CR – control; DPOR – light-independent protochlorophyllide oxidoreductase; D1 – protein of PSII reaction centre; FM – fresh mass; LHCIIb – light-harvesting complexes associated with PSII; LPOR – light-dependent protochlorophyllide oxidoreductase; Pchlide – protochlorophyllide; PSII – photosystem II.
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is the last step of the biosynthetic pathway leading to the formation of the chlorins (Schoefs and Franck 2003). This reduction represents a major regulatory step in Chl biosynthesis (Fujita 1996). In angiosperms, Chl synthesis is strictly light-dependent and catalyzed by nuclear-encoded, light-dependent NADPH:protochlorophyllide oxidoreductase (LPOR) (Griffiths 1978, Armstrong 1998, Fujita and Bauer 2003, Masuda and Takamiya 2004).

On the contrary, some photosynthetic organisms, such as anoxygenic photosynthetic bacteria, cyanobacteria, many green algae, mosses, ferns, and gymnosperms are capable of synthesizing Chl and bacteriochlorophylls (Wolvertz 1980, Spano *et al*. 1992, Armstrong 1998, Dražić and Bogdanović 2000, Pavlovič *et al*. 2009) as well as to form etiochloroplasts with a developed thylakoid membrane system in the dark (Nikolić and Bogdanović 1972, Walles and Hudák 1975, Hudák *et al.* 2005, Demko *et al*. 2010). The ability to synthesize Chl in darkness is linked to the presence of light-independent protochlorophyllide oxidoreductase (DPOR) (Fujita 1996, Armstrong 1998, Fujita and Bauer 2003). DPOR consists of three protein subunits, which are products of *bchL/chlL*, *chB/chlB*, and *bchN/chlN* genes in bacteria and plants. These three protein subunits exhibit significant sequence similarity to the three subunits of nitrogenase, which catalyzes the reductive formation of ammonia from dinitrogen. *ChlLNB* homologs have not been detected neither in the chloroplast nor nucleus genome of angiosperms (Fujita and Bauer 2003). Although LPOR and DPOR catalyze the same reaction, these enzymes are completely different as far as their genes, protein structure, and catalytic mechanisms are concerned (Gabruk *et al.* 2012).

Not all conifers have the same ability to synthesise Chl. Although *Larix decidua* belongs to the gymnosperms, synthesis of Chl in the dark is very limited in this species. Reduced synthesis of Chl in the dark causes that seedlings remain etiolated in later stages, thus placing this species in the position of a transitional type between gymnosperms and angiosperms (Walles and Hudák 1975, Mariani *et al*. 1990, Demko *et al*. 2009).

Several studies have described a positive effect of ALA feeding on Chl accumulation in the light in angiosperms (Hotta *et al*. 1997, Al-Thabet 2006, Memon *et al*. 2009). On the other hand, the effect of exogenous ALA on Chl accumulation in dark-grown seedlings of gymnosperms is ambiguous.

In the present study, we compared the Chl synthesis in seedlings of *L. decidua* and *P. abies* cultivated in darkness. Wolvertz (1980), Dražić and Mihailović (1998), Dražić and Bogdanović (2000), Demko *et al*. (2009), and Pavlovič *et al*. (2009) observed the effect of long-term application of exogenous ALA on the Chl biosynthesis in cotyledons of dark-grown seedlings of gymnosperms. A missing piece of information is the short-term ALA feeding of gymnosperms. The present investigation was focused on comparison of the short-term ALA feeding (1 and 10 mM) on Chl and Pchlide accumulation in cotyledons of dark-grown *L. decidua* and *P. abies*. The presence of DPOR subunits and proteins of the photosynthetic apparatus (LHCII*b* and D1 protein) were also investigated to confirm Chl synthesis in dark-grown gymnosperms.

Materials and methods

Plant material and culture conditions: Seeds of *P. abies* (L.) Karst. (collection 2000, Zakamenné) and *L. decidua* Mill. (collection 2000, Hlachová) obtained from *Semenoles* (Liptovský Hrádok, Slovak Republic) were soaked for 24 h in distilled water and germinated in Petri dishes on moistened perlite in complete darkness at 23ºC. Samples (100 mg) of cotyledons were taken from the 14-d-old, dark-grown seedlings. They were incubated in 0.2 M phosphate buffer with pH 6.8 (control samples, CR) and in solutions with 1 and 10 mM ALA in 0.2 M phosphate buffer (ALA1 and ALA10, respectively) for 24 h in darkness and under continuous light [100 μmol(photon) m^{-2} s^{-1} of photosynthetically active radiation, PAR]. All manipulations, including the preparation of samples, were performed in a dark room under dim, green, and safelight conditions.

Pigment extraction and analysis: Cotyledon samples of 100 mg were extracted with 80% chilled acetone (4ºC) and $MgCO_3$ after the cotyledons had been ground to a powder. After centrifugation (10,000 × *g*) at 4ºC, Chl *a* and Chl *b* were quantified spectrophotometrically at 663.2 nm (Chl *a*) and 646.8 nm (Chl *b*) using a spectrophotometer (*Jenway 6400*, *Krackeler Scientific,* London, UK). The concentrations were calculated according to Lichtenthaler (1987).

Pchlide was extracted in 3 ml of acetone:0.1 M NH_4OH (9:1, v/v). The extract was washed 3 times with equal volume of hexane. After this procedure, esterified tetrapyrroles were removed by hexane. The amount of Pchlide was quantified spectrophotometrically at 623 nm using molar extinction coefficient in diethyl ether $\varepsilon = 3.56 \times 10^4$ M^{-1} cm^{-1} (Koski and Smith 1948). Using a dilution series of Pchlide standard in acetone:0.1 M NH_4OH (9:1, v/v), calibration curve was constructed.

Protein isolation and Western blot analysis: *L. decidua* and *P. abies* cotyledons [100 mg of fresh mass (FM)] were frozen in liquid nitrogen. They were homogenized using a mortar and a pestle with liquid nitrogen and suspended in 1 ml of protein extraction buffer (28 mM dithiothreitol, 5% SDS, 175 mM sucrose, 28 mM Na_2CO_3, 10 mM EDTA). After 30 min incubation at 70°C and 10 min centrifugation (12,100 × *g*), protein concentrations were determined in supernatants using *Bichinonic Acid Kit for Protein*

Determination (*Sigma-Aldrich*, St. Louis, MO, USA). Samples (30 μg of protein) were separated by SDS-PAGE on 12% polyacrylamide gel. For immunoblotting analysis, separated proteins were transferred to nitrocellulose membrane in *Trans-Blot® SD Semi-Dry Electrophoretic Transfer Cell* (*Bio-Rad*, Hercules, CA, USA). Proteins on membrane were detected immunochemically by using specific antibodies. Antibodies against D1 and LHCII*b* were purchased from *Agrisera* (Vännäs, Sweden). Antibodies against ChlL and ChlB were provided by Y. Fujita. Goat Anti-Rabbit IgG (H+L)-HRP conjugate (*Bio-Rad*, Hercules, CA, USA) was used as the secondary antibody. Blots were visualized using *Immobilon Western* chemiluminiscent kit (*Millipore*, Bedford, MA, USA) and medical X-ray film (*Foma Biochemia*, Hradec Králové, Czech Republic). Band areas were calculated by densitometric scanning of X-ray films using *Image Station 4000MM Pro* and *Kodak Molecular Imaging Software, Version 4.5* (*Kodak*, NY, USA).

Statistical analysis: All of the measurements were performed 6 times, and the results were statistically evaluated using *Student*'s *t*-test and performed in program *Microsoft Office Excel* 2003. The means and calculated standard deviations (SD) were reported.

Results

Pigment synthesis: We measured the content of pigments in *L. decidua* and *P. abies* after the application of exogenous ALA. After 24-h incubation in darkness, Chl contents were similar in CR and ALA-treated cotyledons. In *L. decidua* cotyledons, Chl was synthesized only in trace amounts. The highest content of Chl (*a*+*b*) was found in ALA10 cotyledons, 8.8% higher than the CR. A small increase (statistically not significant) (1.5%) of Chl (*a*+*b*) was observed in ALA1 cotyledons (Table 1). In *P. abies* cotyledons, Chl (*a*+*b*) content increased by 6.6% (ALA1) and 9.1% (ALA10) compared with the CR (Table 1). Differences in the Chl content were not significant. These results indicated that ALA did not almost affect Chl accumulation in the dark-incubated cotyledons.

After 24-h incubation under continuous light, the Chl content increased significantly in CR cotyledons (in *L. decidua* by 86.9% and in *P. abies* by 46.4%) compared with CR cotyledons in darkness. Differences in the Chl content were statistically significant. The application of exogenous ALA increased amount of Chl in cotyledons incubated in light in comparison with the incubation in the dark. However, Chl content decreased with increasing ALA concentration. We observed the highest content of Chl (*a*+*b*) in both species in CR cotyledons. The lower content was observed in the ALA1 cotyledons and the lowest content was in ALA10 cotyledons (Table 2). Using given light intensity (*i.e.* PAR of 100 μmol m^{-2} s^{-1}) did not allow to distinguish the effect of ALA and the contribution of photobleaching (Fig. 1*A*).

Analysis of Pchlide: The presence of Pchlide was tested in cotyledons after 24 h of ALA feeding in darkness. *L. decidua* cotyledons fed with ALA accumulated significantly higher amounts of Pchlide. The highest content of Pchlide was detected after ALA10 treatment, it increased by 83.4% compared with CR. Under ALA1, Pchlide content increased by 56.7% compared with the CR (Table 1). In *P. abies*, the highest content of Pchlide was found only after ALA10 application, it increased by 70.9% compared with CR. After ALA1, the amount of Pchlide was almost the same as in CR, only 11.1% higher than the CR (Table 1). No Pchlide was determined in the cotyledons of the illuminated seedlings (Table 2).

Protein analysis: In both species, we recorded the presence of ChlB and ChlL subunits of DPOR, LHCII*b,* and D1 protein (Fig. 2). Differences in band intensity suggested interspecific difference. In *L. decidua*, the amount of ChlB and D1 protein were the highest after ALA1 application and 24 h in darkness. Higher ALA concentration (ALA10) inhibited accumulation of these proteins. In contrast, ALA10 increased ChlL content, while ALA1 resulted in the significant reduction of this protein content. Exogenous ALA stimulated accumulation of LHCII*b* subunit (Fig. 2). Immunoblot analysis with antibody against ChlB, D1, and LHCII*b* demonstrated ALA concentration–dependent reduction in protein contents in *P. abies* cotyledons incubated in the dark. The highest amount of ChlL was found after ALA1 application and the lowest in CR cotyledons (Fig. 2).

After 24-h illumination, the highest amount of ChlB was in *L. decidua* cotyledons treated with ALA1, lower in CR, and the lowest content was found after ALA10 application. ChlL and D1 protein showed the inhibition effect of ALA on protein accumulation. The content of LHCII*b* subunits increased after application of ALA10 (Fig. 2). In *P. abies*, the highest content of ChlB and D1 subunits was detected in CR cotyledons and the lowest in cotyledons fed with ALA10. On the contrary, the highest content of ChlL subunit was detected after application of ALA10 and the lowest in CR cotyledons. The maximum increase of LHCII*b* content was found after incubation in ALA1 and the minimum one after ALA10 (Fig. 2).

Table 1. Chlorophyll (Chl) and protochlorophyllide (Pchlide) content in *Larix decidua* and *Picea abies* cotyledons after 24-h ALA feeding in darkness. Mean ± SD, $n = 6$. * – statistically significant difference at $P<0.05$ (t-test). FM – fresh mass.

Species	Incubation conditions	Chl content [μg g^{-1}(FM)] Chl *a*	Chl *b*	Chl (*a*+*b*)	Increase in Chl (*a*+*b*) [%]	Pchlide [nmol g^{-1}(FM)]	Increase in Pchlide [%]
Larix decidua	24-h darkness, control	38.2 ± 3.9	13.8 ± 3.4	52.0 ± 7.1		9.1 ± 0.1	
	24-h ALA feeding 1 mM, darkness	37.5 ± 2.6	15.3 ± 3.8	52.8 ± 6.2	1.5	21.0 ± 1.1*	56.7
	24-h ALA feeding 10 mM, darkness	34.8 ± 3.3	22.2 ± 2.7	57.0 ± 3.3	8.8	54.7 ± 0.8*	83.4
Picea abies	24-h darkness, control	523.0 ± 20.4	207.5 ± 7.1	730.5 ± 27.0		12.8 ± 0.9	
	24-h ALA feeding 1 mM, darkness	563.2 ± 14.6	218.6 ± 8.2	781.9 ± 22.6	6.6	14.4 ± 0.8	11.1
	24-h ALA feeding 10 mM, darkness	579.0 ± 17.0	224.3 ± 8.4	803.3 ± 24.8	9.1	44.0 ± 2.2*	70.9

Table 2. Chlorophyll (Chl) and protochlorophyllide (Pchlide) content in *Larix decidua* and *Picea abies* cotyledons after 24-h illumination. Mean ± SD, $n = 6$. * – statistically significant difference at $P<0.05$ (t-test). n.d. – not detected; FM – fresh mass.

Species	Incubation conditions	Chl content [μg g^{-1}(FM)] Chl *a*	Chl *b*	Chl (*a*+*b*)	Pchlide [nmol g^{-1}(FM)]
Larix decidua	24-h illumination, control	262.1 ± 21.4	133.8 ± 7.8	395.9 ± 21.0	n.d.
	24-h ALA feeding 1 mM, light	280.5 ± 18.4	113.7 ± 6.5	394.2 ± 24.8	n.d.
	24-h ALA feeding 10 mM, light	65.2 ± 7.3*	45.5 ± 8.9*	110.7 ± 13.7*	n.d.
Picea abies	24-h illumination, control	939.3 ± 18.5	422.5 ± 12.9	1,361.8 ± 31.1	n.d.
	24-h ALA feeding 1 mM, light	843.4 ± 26.1*	374.3 ± 13.6	1,217.7 ± 39.5	n.d.
	24-h ALA feeding 10 mM, light	484.8 ± 26.1*	217.9 ± 11.7*	702.8 ± 37.6*	n.d.

Discussion

In our experiment, we used *L. decidua* and *P. abies* seedlings, which differ in their ability to synthesise Chl in complete darkness. In 14-d-old dark-grown seedlings, the difference in Chl content could be distinguished visually: *P. abies* cotyledons were green, while those of *L. decidua* appeared etiolated. Stems were long, thin, and brittle as a result of poorly developed, mechanical tissues. Similarly to our study, Mukai *et al*. (1992) observed that dark-grown cotyledons of *Larix kaempferi* were unable to synthesize significant amounts of Chl. Chl synthesis in the dark-grown seedlings of *L. decidua* is restricted only to the early stages of development. With prolonged skotomorphogenesis Chl content declines. Mature larch seedlings resemble angiosperm plants in their behaviour (Demko *et al*. 2009). Chl synthesis in the dark is very low in the larch seedlings. In contrast, *P. abies* is considered as the species with the highest accumulation of Chl of all Pinaceae during skotomorphogenesis (Fujita and Bauer 2003).

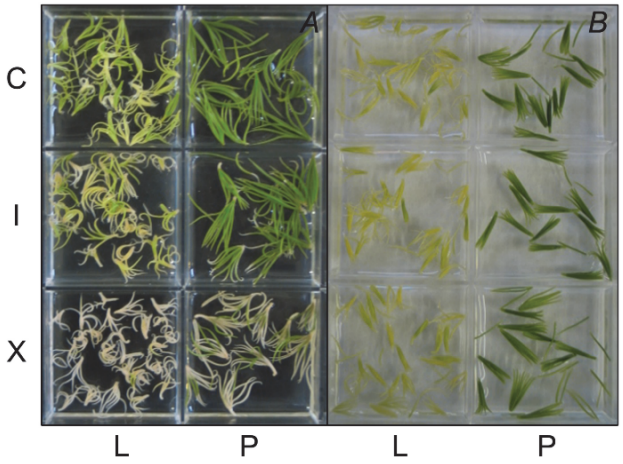

Fig. 1. Isolated *Picea abies* (P) and *Larix decidua* (L) cotyledons incubated in solution of 1 mM ALA (I), 10 mM ALA (X), and 0.2 M phosphate buffer (C) for 24 h in continuous light (*A*) and in darkness (*B*).

The Chl biosynthesis is enabled by DPOR activity in dark-grown, gymnosperm seedlings (Fujita 1996, Armstrong 1998, Fujita and Bauer 2003). Although most gymnosperms possess this ancient enzyme, not all species have the same ability to synthesise Chl in the dark. Pchlide reduction is an important step in the biosynthetic pathway of Chl. DPOR activity depends on the presence of 3 subunits, such as ChlL, ChlN, and ChlB. In our experiment, Western blot analyses confirmed the presence of ChlL and ChlB subunits in darkness and after 24-h illumination. Even though *L. decidua* contained DPOR subunits, long term, dark-grown seedlings remained etiolated. In dark-grown larch seedlings, the reduced capability of Chl synthesis is probably caused by inappropriate processing of *chl*LN polycistronic transcripts or synthesis of partially dysfunctional ChlB polypeptide (Karpinska *et al*. 1997). Demko *et al*. (2009) observed that efficient *chlB* RNA-editing was attenuated in 14-d-old larch seedlings resulting in decrease in the amount of ChlB subunit. The explanations imply that enough Pchlide is available, but DPOR is not acting sufficiently. Differential expression of the DPOR subunits represents an important species-specific regulatory mechanism that controls DPOR function in response to developmental and environmental stimuli (Demko *et al*. 2009).

In dark-grown seedlings, Pchlide represents well measurable intermediate in the Chl biosynthetic pathway. It has been documented that Pchlide was present in the same range as in dark-grown wheat [10–15.8 nmol g^{-1}(FM)] in dark-grown seedlings of *L. decidua, P. abies* (Demko *et al.* 2009), and Scots or Jeffrey pine (Selstam *et al.* 1987). In our study, we found similar concentrations of Pchlide in cotyledons of control samples. Several authors have proven that exogenous application of ALA increases the accumulation of Pchlide in etioplasts (Granick 1959, Wolwertz and Brouers 1980, Schendel *et al*. 1996, Domanskii *et al*. 2003, Pavlovič *et al*. 2009). In our study, Pchlide concentration increased after 24 h incubation in ALA10 by 83.4% and 67.3% in *L. decidua* and in *P. abies*

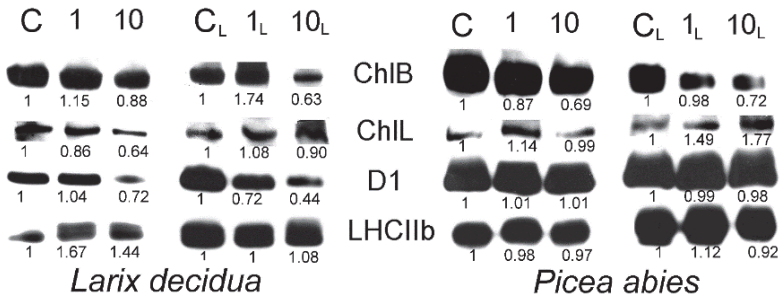

Fig. 2. Western-blot analysis of ChlB, ChlL, D1, and LHCII*b* proteins in *Picea abies* and *Larix decidua* cotyledons after 24-h ALA feeding in darkness and continuous light. Cotyledons incubated in solution of 0.2 M phosphate buffer for 24 h in darkness (*lane* C) and in continuous light (*lane* C_L), cotyledons incubated in 1 mM ALA solution for 24 h in darkness (*lane* 1) and in continuous light (*lane* 1_L) and cotyledons incubated in 10 mM ALA solution for 24 h in darkness (*lane* 10) and in continuous light (*lane* 10_L). Band areas were calculated by densitometric scanning of X-ray films and expressed as arbitrary units (area of bands in pixels related to the control).

seedlings, respectively, compared with their control in the dark. Overall, more Pchlide accumulated in *L. decidua* seedlings than in *P. abies* after ALA feeding. Newly synthesized Pchlide was not transformed to Chlide due to dysfunctional DPOR enzyme, but it accumulated in the dark. Pchlide content was concentration-dependent in cotyledons treated with exogenous ALA.

No statistically significant relationship was found between ALA and Chl accumulation in the dark. Despite the higher Pchlide concentration, Chl content did not change significantly compared with the control. This indicated that ALA formation was not a rate-limiting step for Chl accumulation in the dark. Dražić and Mihailović (1998) observed that dark-grown seedling of *Pinus nigra* decreased the accumulation rate of Chl after ALA feeding (0.1, 1, and 10 mM). Pavlovič *et al.* (2009) consider that DPOR is able to cope only with Pchlide supplied natively. Moreover, Fujita and Bauer (2003) suggested that a large pool of Pchlide bound to LPOR suppressed the DPOR synthesis in *Pinus jeffreyi* after ALA feeding. ALA is accumulated in the dark but, rather than being utilized for Chl, it is used for the synthesis of some other products, especially protoporphyrin IX and protochlorophylide, or it is rapidly degraded (Dražić and Bogdanović 2000). On the other hand, Pavlovič *et al*. (2009) found that long-term ALA feeding (20 mM) can stimulate Chl synthesis in the dark in intact *P. abies* seedlings.

In cotyledons of dark-grown *L. decidua* seedlings, Chl content increased by 86.9% after 24-h illumination. This dramatic change did not occur in *P. abies* cotyledons, where Chl content increased only by 46.4%. Considering that mature larch seedlings resemble angiosperms in their behaviour, our results agreed with Reinbothe and Reinbothe (1996). They observed that the synthesis of ALA increased upon illumination in etiolated angiosperm seedlings. The same effect was documented by Demko *et al.* (2009) in *L. decidua* cotyledons. They observed rapid stimulation of ALA and Chl synthesis after illumination of dark-grown seedlings. Light-stimulated synthesis of ALA is mediated by phytochrome (Huang and Castelfranco 1989, Huang *et al*. 1989). Although light had a stimulating effect on Chl accumulation, the Chl content declined with increasing concentrations of ALA due to photobleaching (Fig. 1*A*). This part of experiment can be only on the level of descriptive observation. In fact, two parallel processes could take place in this case. ALA causes the stimulation of Chl biosynthesis and light could cause bleaching. To find out the effect of exogenous ALA on Chl accumulation in light, further experiments will be needed, employing illumination with lower PFD values.

Chl is essential for the stabilisation of proteins of the light-harvesting antennas and photosystem reaction centres required for the assembly of the photosynthetic apparatus. Accumulation of these proteins is light-dependent in angiosperms, but gymnosperms do not require light (Canovas *et al*. 1993, Peer *et al*. 1996). Our data suggested that the protein synthesis of the LHCII*b* complex proceeded even in the absence of light in both dark-grown species. The expression of *Lhcb* genes has been shown to be independent of light in many gymnosperms (Peer *et al*. 1996). The accumulation of LHCII*b* polypeptides has been also observed in *P. menziesii* (Alosi *et al*. 1990) and *P. thunbergii* seedlings (Yamamoto *et al*. 1991) grown in the dark. Demko *et al*. (2009) observed that the Chl content decreased and thylakoid membranes were poorly developed in 14-d-old, dark-grown, *L. decidua* seedlings. Decreased content of Chl in *L. decidua* seedlings might cause reduced accumulation of light-harvesting complex (LHC) subunits. Although both species can synthesize LHC polypeptides in the dark, the most pronounced accumulation of LHC subunits occurred after illumination. In gymnosperms, most components of photosynthetic apparatus do not require light for induction of their synthesis or the assembly of the LHC. However, light is obviously required for the final form of the subunits in the light-growing seedlings (Canovas *et al*. 1993). We also detected the synthesis of D1 protein in the dark in both studied species. The expression of the *psbA* gene encoding D1 protein has been observed in *P. thunbergii* (Yamamoto *et al*. 1991, Mukai *et al*. 1992) and *P. menziesii* seedlings (Alosi *et al*. 1990) grown in the dark. D1 protein is stable in the dark, but it is subject to rapid turnover in light. Chl and its precursors are highly reactive molecules in their free forms. These molecules can transfer excitation energy to oxygen, which leads to the formation of singlet oxygen and photooxidative damage of plants upon illumination (Hideg *et al*. 1998). D1 protein, due to rapid turnover, represents the primary site of PSII damage (Aro *et al*. 1993). In the present study, cotyledons of both species were photobleached after ALA feeding. Exogenous ALA increased free Pchlide that became toxic after exposure to continuous light (Papenbrock *et al*. 2001).

In conclusion, *L. decidua* and *P. abies* seedlings were found to synthesize Chl in the dark. Although both species belong to the gymnosperms, there was a noticeable interspecific difference in Chl biosynthesis. Short-term ALA feeding (24 h) stimulated the synthesis of Pchlide, however, it had no effect on Chl accumulation in cotyledons incubated in complete darkness. Thus, ALA synthesis was not the rate-limiting step in Chl synthesis in the dark. In cotyledons of *L. decidua*, exogenous ALA stimulated Pchlide accumulation more than in *P. abies*. Even though *L. decidua* contained DPOR subunits, long-term, dark-grown seedlings remained almost etiolated. In Chl synthesis, mature larch seedlings resemble angiosperms in their behaviour. Upon illumination of dark-grown seedlings, Chl biosynthesis was highly activated, especially in etiolated *L. decidua* seedlings. Short-term ALA feeding in light caused a significant decrease of Chl content in cotyledons of both species due to photobleaching. This was caused by the accumulation of free Pchlide, resulting in photooxidative damage mainly of D1 protein. No effect of exogenous ALA on accumulation of LHCII*b* proteins was observed.

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