The Effect of Abiotic Stressors (Light and Temperature) on Chlorophyll Biosynthesis

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Abstract – Chlorophyll biosynthesis is a light dependent process in angiosperms, while it can proceed in most gymnosperms also in the absence of light. In this work, we have compared the temperature dependence and the light sensitivity of chlorophyll formation in dark-grown or dark-forced red oak (*Quercus rubra* L.), ginkgo (*Ginkgo biloba* L.) and yew (*Taxus baccata* L.). Chlorophyll biosynthesis is different in the stems and the leaves of red oak seedlings, and in stem-related organs; it depends strongly on the temperature and light intensity used during greening. Similarly to angiosperms, ginkgo seedlings can be fully etiolated and are unable to synthesize chlorophyll in the dark, while yew plants are only partially etiolated during dark-forcing, i.e. they accumulate both chlorophylls and the chlorophyll precursor, protochlorophyllide in the dark. The dark-forced stems and leaves of yew have similar pigment composition and greening. In addition, unlike ginkgo and red oak seedlings, the greening of dark-forced yew is not much influenced by low temperatures.

Keywords: angiosperms / chlorophyll / etioplast / greening/ gymnosperms / protochlorophyllide

1. INTRODUCTION

In photosynthetic organisms, one of the last steps of chlorophyll biosynthesis, namely, the transformation of protochlorophyllide to chlorophyllide can be catalyzed by two NADPH:protochlorophyllide oxidoreductase (POR) enzymes with completely different structures and origins (reviewed by MASUDA - TAKAMIYA 2004). One of the enzymes requires light for its activity (referred to as LPOR, light-dependent NADPH:protochlorophyllide oxidoreductase) and is characteristic for angiosperm plants, that have lost the other enzyme during evolution. In lower plants, however, besides LPOR, a dark-operative POR enzyme (DPOR) is also present, therefore, in contrast with angiosperms, lower plants are able to synthesize chlorophylls in the absence of light.

Chlorophyll biosynthesis of angiosperms is widely studied. However, almost exclusively leaves, sometimes stems or stem related organs of dark-germinated seedlings are studied in these works in which the pigments and pigment forms are characterized and/or their greening process is studied via illuminating them with flash or continuous illumination (reviewed by SOLYMOSI - SCHOEFS 2010). In the absence of light and chlorophylls, a special plastid type, the so-called etioplast differentiates instead of chloroplasts in the chlorenchymatic tissues of dark-grown angiosperms. The etioplasts have a unique inner membrane system consisting of highly regular, paracrystalline prolamellar bodies with lipids in cubic phase, and of lamellar prothylakoids. The greening process of angiosperm leaves proceeds relatively quickly, but depends on light intensity, while the greening is slower in stems.

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In most gymnosperms chlorophyll biosynthesis proceeds during their dark-development, however, there are few exceptions. There are only few data in the literature about the greening process of etiolated or partially etiolated gymnosperm seedlings (e.g. Walles and Hudák, 1975), and the possible regulatory mechanisms or interrelations between the two different enzymes (LPOR and DPOR) are not known.

Low temperature (77K) fluorescence spectroscopy is a useful tool to study chlorophyll biosynthesis both in angiosperm and gymnosperm seedlings, because protochlorophyllide and chlorophyll(ide) have distinct spectral properties, and their molecular organization also influences their spectral properties. This way it is possible to follow the alterations of the native organization of the chlorophyllous pigments during the dark growth of plants and during the subsequent greening process under illumination. For example, protochlorophyllide is present generally in three major spectral forms in etiolated angiosperm leaves, each spectral form representing a distinct molecular organization of the pigments (BÖDDI et al. 1992). Non-enzyme bound protochlorophyllide molecules located to the prothylakoids have fluorescence emission maximum at 633 nm, while the dimers and oligomers of the POR subunits (these subunits are ternary complexes of the the LPOR-protein, protochlorophyllide and NADPH) have emission maximum at 644 and 655 nm, respectively (reviewed by BÖDDI 1994). A 636 nm emitting protochlorophyllide form has been described in epicotyls of dark-grown pea seedlings (BÖDDI et al. 1994), which proved to be a monomer unit of the LPOR (BÖDDI et al. 1998), can aggregate into 644 and 655 nm emitting forms (KÓSA et al. 2006) and can transform directly into chlorophyllide (KÓSA et al. 2005).

In this work, we have compared the effect of light and temperature stress on chlorophyll biosynthesis in one angiosperm and two gymnosperm plants.

2. MATERIAL STUDIED, AREA DESCRIPTIONS, METHODS, TECHNIQUES

2.1. Plant material

Red oak (*Quercus rubra* L.) seedlings were grown and used for measurements as described by SKRIBANEK and BÖDDI (2001). Similarly, data about sampling and growth conditions of ginkgo (*Ginkgo biloba* L.) seedlings are described in SKRIBANEK et al. (2008). Yew (*Taxus baccata* L.) twigs were dark-forced as described by SKRIBANEK et al. (2011).

2.2. Fluorescence spectroscopy and pigment analyses

77 K fluorescence emission spectra were measured and analyzed using a JOBIN YVON FLUOROMAX-3 spectrofluorimeter (Jobin Yvon Horiba, France) as described by SKRIBANEK et al. (2008). Pigment contents were determined from acetonic extracts as described by SKRIBANEK et al. (2008).

2.3. Electron microscopy

Electron microscopic samples were prepared only from dark-germinated ginkgo and dark-forced yew plants. For details about the fixation, the embedding procedure, the ultrathin sectioning and contrasting see SKRIBANEK et al. (2008). The ultrathin (70 nm) sections were visualized by a HITACHI 7100 transmission electron microscope (Hitachi, Japan).

3. RESULTS AND DISCUSSION

The leaves and stems of dark-germinated red oak seedlings do not contain chlorophylls, i.e. they are fully etiolated as all other angiosperms. LPOR bound protochlorophyllide oligomers with fluorescence emission maximum at 654-656 nm are characteristic for the leaves, while non-photoactive protochlorophyllide forms with emission maxima at shorter wavelengths are dominating in the spectra of the stems (Figure 1). Similar data have been reported for the stem-related organs of several angiosperm plants (SKRIBANEK et al. 2000).

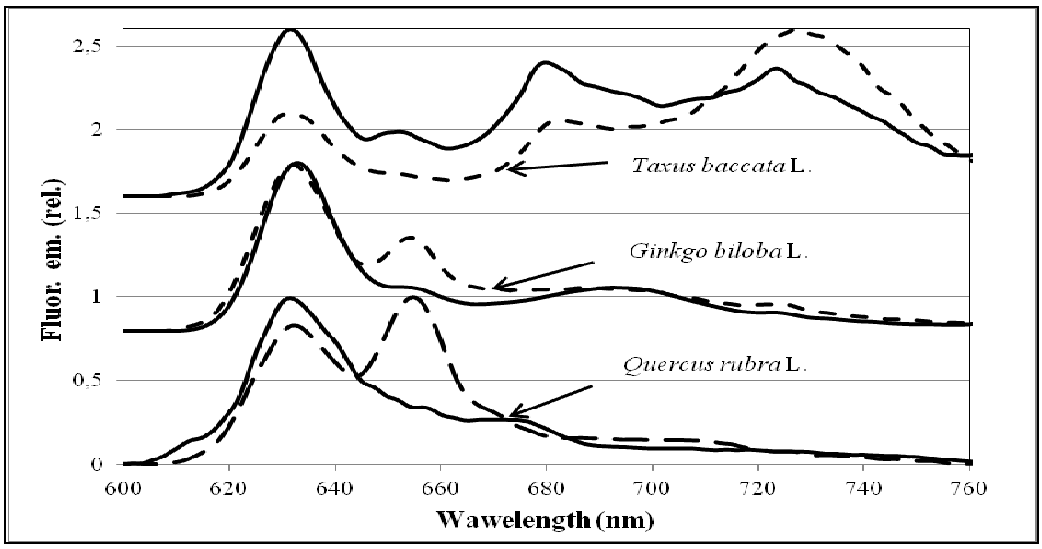

Figure 1. 77 K fluorescence emission spectra (excitation wavelength: 440 nm) of dark-germinated red oak (Quercus rubra L.) and ginkgo (Ginkgo biloba L.) seedlings and dark-forced yew (Taxus baccata L.). Solid line: stem, broken line: leaf)

Illumination with high light intensity (250 µmol s^{-1} m^{-2}) resulted in photooxidation in the stems, however, at lower light intensities this effect disappeared and the stems slowly accumulated chlorophylls during the greening process as described in SKRIBANEK and BÖDDI (2001). This is demonstrated here by the increase in the amplitude ratio of the fluorescence emission bands corresponding to chlorophyll(ides) and to non-photoactive protochlorophyllide (Table 1).

Table 1. Ratios of the amplitudes of the 77 K fluorescence emission bands at 680-682 nm (F68x) and 629-631 nm (F63y) corresponding to chlorophyllide and protochlorophyllide forms, respectively, in spectra of dark-germinated young stems of red oak (Quercus rubra L.) continuously illuminated at 20 ºC and 4 ºC for different time periods with the light intensities (given in photon flux density – PFD – units) indicated in the Table.

Temperature	20 ºC	20 ºC	20 ºC	4 ºC	4 ºC
PFD	0.5 µmol s^{-1} m^{-2}	15 µmol s^{-1} m^{-2}	250 µmol s^{-1} m^{-2}	0.5 µmol s^{-1} m^{-2}	15 µmol s^{-1} m^{-2}
Illumination period	Amlitude ratios of the fluoresecence emission bands F68x/F63y				
1 h	1.77	1.79	2.14	1.79	1.77
2 h	1.81	2.71	2.93	1.67	4.66
3 h	2.07	3.35	0.36	1.66	1.26
6 h	2.51	7.96	0.09	1.77	0.51

Photooxidation of the pigments is characteristic during the illumination of stem-related organs of other angiosperms, for instance it has been demonstrated in pea epicotyls that reactive oxygen species generated by both type-I and type-II sensitized reactions are involved in the process and are responsible for the wilting of the stems (ERDEI et al. 2005, HIDEG et al. 2010). At very low light intensity, chlorophyll accumulation was slow (Table 1). Illumination at low temperature (4 ºC) enhanced the photooxidation of the pigments, that is, at low temperature chlorophyll accumulation occurred only at the lowest light intensity used in this work (Table 1).

Although ginkgo is a gymnosperm, it can be fully etiolated, similarly to angiosperms. The leaves and stems or stem-related organs of dark-germinated or dark-forced ginkgo plants accumulated protochlorophyllide and contained only traces or no chlorophylls (as in SKRIBANEK et al. 2008). The stems and leaves of dark-grown ginkgo accumulated similar spectral forms of protochlorophyllide (Figure 1). These protochlorophyllide spectral forms can be transformed on a shorter (µs-ms) or longer time scale (hours) to chlorophyllide and result this way in chlorophyll accumulation upon irradiation. Both the leaves and the stems of ginkgo seedlings are light-sensitive, but can be greened at low light intensity (Figure 2), however, the greening process is very slow in them.

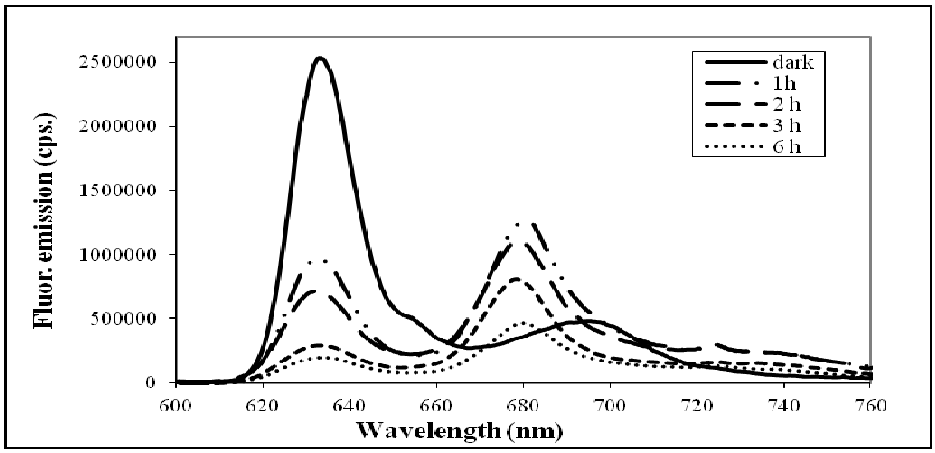

Figure 2. 77 K fluorescence emission spectra (excitation wavelength: 440 nm) of dark-germinated ginkgo (Ginkgo biloba L.) seedlings before (solid line) and after illumination with continuous low light intensity (15 µmol s^{-1} m^{-2}) for different time periods.

At low temperature, photooxidation of the pigments can be observed in the stems already after 12 h of greening (Table 2). The synthesis of chlorophyll *b* starts only after 48 h.

Table 2. Changes in the average pigment contents (n=5, data expressed in µg g^{-1} on a fresh mass basis) of dark-grown ginkgo epicotyls during greening at continous, relatively low light intensity (15 µmol s^{-1} m^{-2}) for the time periods indicated below. Abbreviations: Pchl(ide): protochlorophyllide and/or its esters, Chl(ide) a: chlorophyllide a and/or its esters, Chl(ide) b: chlorophyllide b and/or its esters.

Time after illumination	Pchl(ide)		Chl(ide) *a*		Chl(ide) *a* + Chl(ide) *b*	
	20 ºC	10 ºC	20 ºC	10 ºC	20 ºC	10 ºC
0 h	0.14	0.14	0.15	0.15	0.15	0.15
12 h	0.01	0.07	8.35	3.96	8.35	3.96
24 h	0.00	0.06	26.79	1.69	26.79	1.69
48 h	0.00	0.01	69.07	0.37	84.28	0.37
72 h	0.00	0.00	102.48	0.20	130.52	0.80

The etioplasts of dark-grown ginkgo stems show structural alterations after 1 h illumination (Figure 3). The formation of the thylakoid system of chloroplasts starts; lamellar thylakoid system is characteristic for the stems and the prolamellar bodies disappear after 24 h of greening at continuous light. At low temperatures the formation of chloroplast thylakoids is impaired (Figure 3, panel D). In angiosperm plants it is well-established, that the disruption of the prolamellar bodies and the etioplast-to-chloroplast transformation and chlorophyll accumulation is impaired at low temperatures (TREFFRY 1970). Our results indicate that ginkgo resembles angiosperm plants also in this respect.

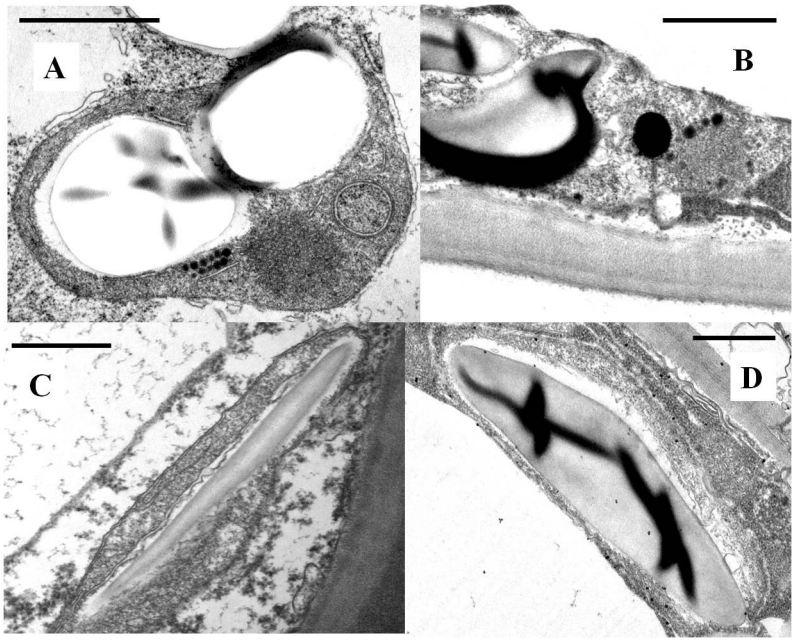

Figure 3. Plastid ultrastructure in the epicotyls of dark-grown ginkgo (Ginkgo biloba L.) seedlings before (A) and after continuous illumination with white light of low intensity (15 µmol s^{-1} m^{-2}) (B-D). B: 1 h illumination at 20 ºC, C: 24 h illumination at 20 ºC, D: 24 h illumination at 10 ºC. (Bar:1 µm.)

The stems and leaves of dark-forced yew contain low amounts of chlorophylls, but accumulate at the same time protochlorophyllide as well. This latter pigment is organized in various pigment complexes (Figure 1). The plants show etiolation symptoms, i.e. the dark-forced shoots are pale yellowish and have more elongated stems when compared with light-grown shoots. The simultaneous presence (and accumulation) of chlorophylls and protochlorophyllide in dark-grown shoots indicates that both LPOR and DPOR are present and active in the shoots of yew. There are slight differences in the protochlorophyllide forms of the stems and leaves of dark-forced yew, therefore, the greening of the two organs is also slightly different. Similarly, the spectral forms of the leaves and stem-related organs is different in etiolated angiosperm seedlings, for instance in pea (BÖDDI et al. 1994), red oak (SKRIBANEK – BÖDDI 2001).

High light intensity causes bleaching in yew stems (not shown). Plastid differentiation is very slow during greening, prolamellar body like structures can be detected even after 48 h illumination at low light intensities as described in SKRIBANEK et al. (2011). Yew is less sensitive to low temperature stress during greening than red oak and ginkgo seedlings. The pigment synthesis is reduced only by 15-20% when the greening is carried out at 10 ºC instead of 20 ºC. However, the different temperature sensitivities of the studied three species may be also related to their different natural habitats and different optimal growth temperatures.

4. CONCLUSIONS

All the three studied species can be (at least partially) etiolated and can accumulate protochlorophyllide when grown in the dark, however, yew accumulates simultaneously significant amounts of chlorophylls. All studied species are light sensitive, but yew is less sensitive to high light, and it is also less sensitive to low temperature stress than etiolated red oak and ginkgo seedlings. The greening process is relatively slow in the stems, compared to the leaves as in case of yew. All three studied species contain LPOR enzyme, and yew is the only species that has functionally active DPOR as well. The fact that protochlorophyllide accumulated in dark-forced yew indicates that DPOR cannot transform all precursors into chlorophyllide, i.e. a still unknown regulation or labour distribution must exist between the DPOR and LPOR enzymes.

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