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

IV/8

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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.

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).

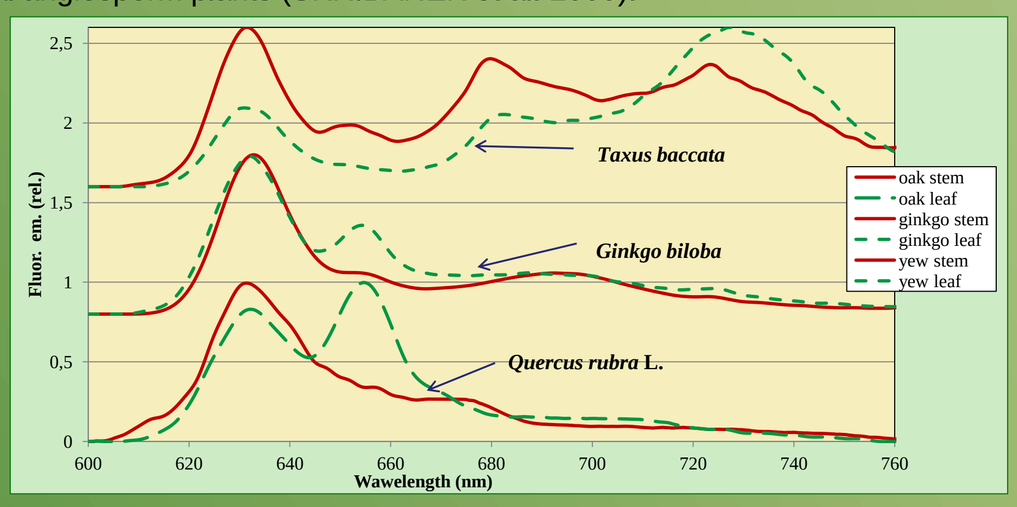


Figure 1. 77 K fluorescence emission spectra (excitation wavelength: 440 nm) of dark-germinated red oak (Quercus rubra L.) and ginkgo (Ginkgo biloba L.) seedlings and dark-forced yew (Taxus baccata L.). Solid line: stem, broken line: leaf)

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).

PFD	0,5 (µmol/s/m²)	15 (µmol/s/m²)	250 (µmol/s/m²)	0,5 (µmol/s/m²)	15 (µmol/s/m²)
Temp.	20 °C			4 °C	
1h	1,767	1,786	2,139	1,786	1,767
2h	1,814	2,707	2,930	1,674	4,656
3h	2,074	3,349	0,363	1,656	1,256
6h	2,512	7,953	0,093	1,767	0,511

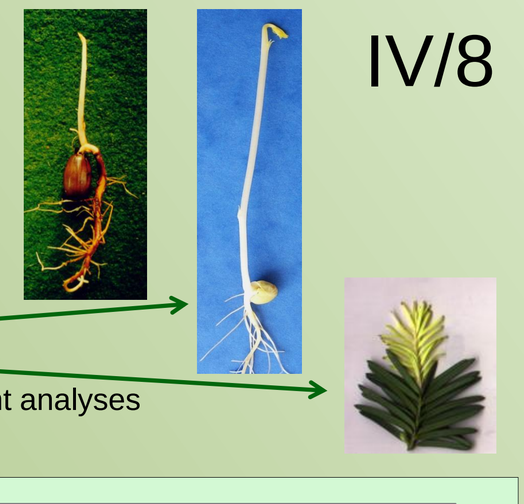
Table 1. Ratios of the amplitudes of the 77 K fluorescence emission bands at 680-682 nm (F68x) and 629-631 nm (F63y) corresponding to chlorophyllide and protochlorophyllide forms, respectively, in spectra of dark-germinated young stems of red oak (Quercus rubra L.) continuously illuminated at 20 °C and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

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 °C) 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).

Time (h)	Pchl(ide) (µg/g)		Chl(ide)a (µg/g)		Chl(ide)a+Chl(ide) b (µg/g)	
	20 °C	10 °C	20 °C	10 °C	20 °C	10 °C
0	0.142	0.142	0.15	0.15	0.152	0.152
12	0.009	0.071	8.35	3.96	8.353	3.96
24	0.001	0.060	26.79	1.69	26.788	1.69
48	0.000	0.007	69.07	0.37	84.279	0.37
72	0.000	0.000	102.48	0.20	130.516	0.20

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.

MATERIALS AND METHODS:
Plant material – dark-forced
Red oak (*Quercus rubra* L.) seedlings
Ginkgo (*Ginkgo biloba* L.) seedlings
Yew (*Taxus baccata* L.) twigs
Fluorescence spectroscopy and pigment analyses
Electron microscopy



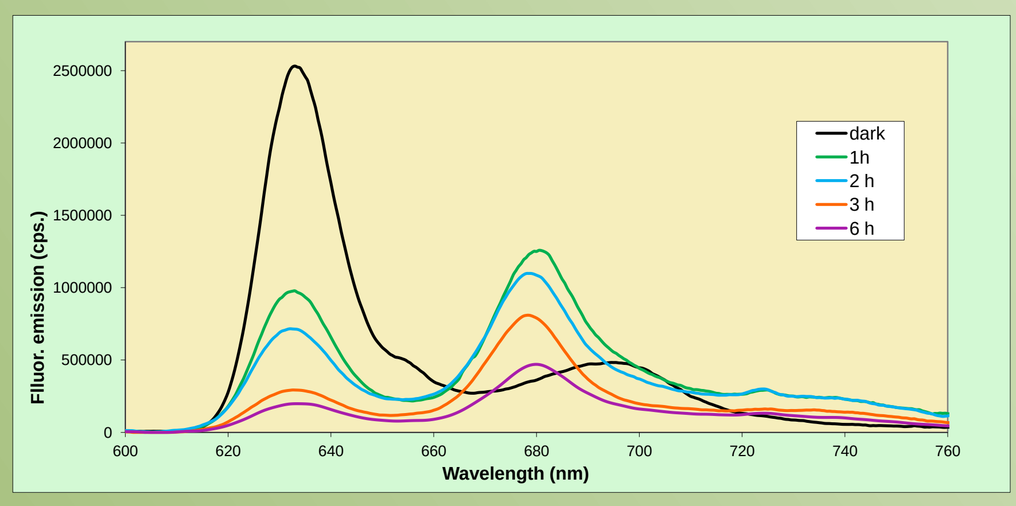


Figure 2. 77 K fluorescence emission spectra (excitation wavelength: 440 nm) of dark-germinated ginkgo (Ginkgo biloba L.) seedlings before (solid line) and after illumination with continuous low light intensity (15 µmol s^{-1} m^{-2}) for different time periods.

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.

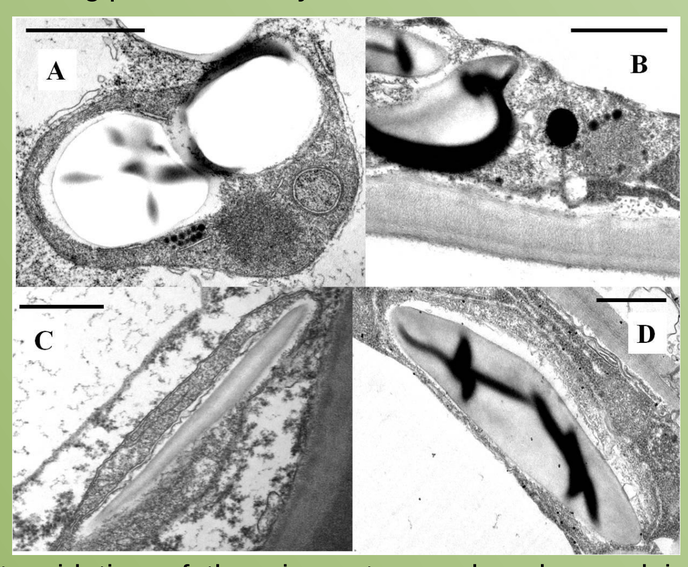


Figure 3. Plastid ultrastructure in the epicotyls of dark-grown ginkgo (Ginkgo biloba L.) seedlings before (A) and after continuous illumination with white light of low intensity (15 µmol s^{-1} m^{-2}) (B-D). B: 1 h illumination at 20 °C, C: 24 h illumination at 20 °C, D: 24 h illumination at 10 °C.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

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.

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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