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Special features of the greening of *Ginkgo biloba* L.

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ABSTRACT Most studies on chlorophyll biosynthesis and greening are carried out on angiosperm leaves. Unlike angiosperms, most gymnosperms can synthesize chlorophyll in the dark and cannot be etiolated. Ginkgo (*Ginkgo biloba* L.) is an exception. In this work, the greening of the stems and the leaves of ginkgo were analyzed. Pigment content changes, changes in the 77 K fluorescence emission spectra and plastid ultrastructure were studied and compared at different temperatures.

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KEY WORDS

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The maidenhair tree or ginkgo (*Ginkgo biloba* L.) is a living fossil belonging to gymnosperms. It has both primitive and advanced features. Some features of its life cycle resemble ferns and cycads others resemble conifers.

The transformation of protochlorophyllide (pchlide) into chlorophyllide (chlide) is a key step of chlorophyll (chl) biosynthesis. This reaction is catalyzed by the NADPH: protochlorophyllide oxidoreductase (POR) enzyme. POR is light dependent in angiosperms (referred to as LPOR); in lower plants a light-independent enzyme (referred to as DPOR) is responsible for pchlide transformation (Masuda and Takamiya 2004). Therefore, chl biosynthesis is generally light independent in angiosperms, but in some conifers both enzymes (LPOR, DPOR) are present, and the greening of the seedlings still requires light (e.g. *Pinus jeffreyi* L., *Pinus sylvestris* L.; Schoefs et al. 1998). The ginkgo is unique, because the formation of chl requires light both in seedlings and in mature shoots (Chinn et al. 1993). The pchlide content of dark-germinated ginkgo leaves is low, and – similarly to etiolated angiosperms - they contain etioplasts with prolamellar body and prothylakoids (Mariani et al. 1982). Since etiolated gingko seedlings can be grown in which chl biosynthesis is synchronized, it was interesting to study the first steps of its greening process and to compare it with similar processes in higher plants. The light-dependent steps of chl biosynthesis have been mainly analyzed in angiosperm leaves; data of this process in non-leaf organs, in epi- and hypocotyls of woody plant species are very scarce in the literature (Skribanek et al. 2000; Skribanek and Böddi 2001).

Materials and Methods

The greening of leaves and stems of 14-20-day old dark-germinated Ginkgo seedlings was studied. The strong, natural light intensities induce photodegradation, thus continuous, low (10 μmol $sec^{-1}m^{-2}$) light intensity was used for irradiation of the plants. Samples were irradiated at 5, 10, 20, 35 and 40ºC to study the effect of temperature. The pchlide content, pchlide/protochloropyll ratio, the chl-a and chl-b content of the leaves were determined from pigment extractions. Spectral changes were measured with 77 K fluorescence spectroscopy using a Fluoromax-3 spectrofluorimeter (Jobin Yvon-Horiba, France). Plastid ultrastructure was studied by TEM (Hitachi 7100 TEM, Japan). For other details of sample preparation, measurements and instrumentation see Solymosi et al. (2004).

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Results and Discussion

The 77 K fluorescence emission spectra of the leaves and the stems of ginkgo were similar. The short-wavelength pchlide bands were dominating in both spectra. This is similar to stems of dark-germinated or dark-forced woody angiosperm plants (Skribanek et al. 2000). Therefore, the chl synthesis of ginkgo was compared with that of the stems of red oak (*Quercus rubra* L.; Skribanek and Böddi 2001). High light causes bleaching of the pigments in both plants (Skribanek and Böddi 2001). Irradiation with low light intensity results in significant chl accumulation already after few hours; however, the extent of chl accumulation is temperature dependent. At low (5 and 10ºC) and high (35 and 40ºC) temperatures pigment degradation occurs similarly to the stems of red oak (Skribanek and Böddi 2001). However, the pigments and the whole plant regenerates under natural conditions, while the red oak seedlings did not recover after photodegradation. Similar etioplasts - with well-developed prolamellar bodies (PLBs) - were present in the leaves and the stems. Disintegration of the PLBs took place upon irradiation, but grana formation could not be observed even after 48 h of irradiation. Reformation of PLBs and re-accumulation of pchlide occured during re-darkening of the irradiated plants. In addition to basic research problems, these results have some practical relation; e.g. the light and temperature sensitivity of the seedlings should be considered in the propagation and cultivation procedure.

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