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The effect of prolonged etiolation inside the cabbage (*Brassica oleracea* L. cv. capitata) head and the greening of the different leaf layers

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ABSTRACT The outer leaves of white cabbage act as optical filters and decrease the light intensity considerably inside the cabbage head. As a consequence, the inner leaf primordia develop under prolonged etiolation conditions. The detailed analysis of plastid ultrastructure and chlorophyll biosynthesis of the different leaf layers of cabbage head was done in Solymosi et al. (2004). The innermost leaves contained multifunctional proplastids with starch grains, phytoferritin and poorly developed, concentrically arranged perforated membranes. Rarely, also prolamellar body was observed in the leaves. Photoactive and non-photoactive protochlorophyllide forms were present in the innermost primordia, their fluorescence emission spectra were similar to those of very young leaves or to slowly greening, non-leaf organs. The leaves in the 4th leaf layer contained chloroplasts with poorly differentiated grana. Their spectra indicated the presence of chlorophyll-protein complexes of both PSI and PSII. In the leaves of the 10th leaf layer proplastids were present and the leaves contained chlorophyll and protochlorophyllide, too. These leaves were illuminated during their earlier developmental stage and became etiolated only later. The greening of these three different leaf layers was compared in order to study, how plastid differentiation occurs in the differently developed leaves. The greening process of young and old (three, four month old - stored) cabbage heads was analyzed.
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KEY WORDS

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Angiosperms lack chlorophyll (Chl) when germinated in the dark, because a step of Chl biosynthesis, *i. e.* the protochlorophyllide (Pchlide) chlorophyllide transformation requires light. Many studies about Chl biosynthesis have been carried out on dark-grown seedlings in which Chl synthesis is arrested, Pchlide gets accumulated and the subsequent reaction steps can be studied in a synchronized way (for review see Ryberg and Sundqvist 1991; Sundqvist and Dahlin 1997). Plastid development is also altered in dark-grown plants: proplastids differentiate into etioplasts instead of chloroplasts (Gunning and Steer 1975). The etioplasts contain a special inner membrane system, the paracrystalline prolamellar bodies (PLBs) and prothylakoid (PT) lamellae radiating from the PLBs (Gunning and Steer 1975). Upon irradiation, the regular PLB structure is disrupted and its membrane material is used for building up chloroplast thylakoids (Henningsen 1970; Rascio et al. 1984). The studies in which dark-germinated plants are used as models of greening and of plastid development, are often criticised because conditions of constant or long lasting dark-growth are very rare in the nature.

Materials and Methods

Cabbage heads were collected from cabbage fields. The absorption spectra were measured using a Shimadzu UV-2101 PC (Japan) spectrophotometer. Fluorescence emission spectra were recorded using a Fluoromax-3 spectrofluorometer (Jobin Yvon - Horiba, France). Transmission electron microscopy was done using a Hitachi 7100 TEM (Japan). The leaf layers were irradiated with continuous white light of 20 µmol $sec^{-1}m^{-2}$ for 48 h. All other details of sample preparation and handling as well as instrumentation were as in Solymosi et al. (2004).

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Results and Discussion

The longitudinal section of cabbage heads shows the fading green colour of the leaves from outside towards the centre. The outermost leaves had absorption and 77 K fluorescence emission spectra similar to any green leaves; their Chl content was appr. 1400 µg g^{-1} fresh weight (FW). The shape of the spectra did not change up to the 8th leaf layer, only the Chl content decreased. In the 10th leaf layer a special Chl form was found with emission maximum at 682 nm, together with Pchlide forms. This can be a residual Chl synthetised at the developmental stage when it was exposed to light but covered for a long period after the cabbage head was closed. The amounts of Chl and Pchlide were similar (appr. 1 µg g^{-1} FW) in this leaf layer. The innermost leaf primordia – below the appr. 20th leaf layer – had no Chl but contained appr. 2-3 µg g^{-1}

FW Pchlide. The absorption and 77 K fluorescence emission spectra showed bands characteristic for Pchlide forms. The appearance of these forms proves that no light reaches the centre of the intact cabbage head, thus etiolation can occur in the nature. It has to be mentioned, that the developmental stage of the leaves is also different, *i.e.* the innermost leaves are young and they are in primordial stage, which is also retained for a relatively long time inside the head. Therefore, the cabbage head is suitable also for studying photomorphogenetic factors influencing plant growth and differentiation.

Electron microscopic studies showed normal or senescent chloroplasts in the outermost layers. The chloroplasts of the 4th leaf layer contained grana and only a few stroma thylakoids. In the 10th leaf layer, proplastids and amoeboid plastids were present, which usually contained (pro)thylakoids, plastoglobuli and starch grains in different ratios and distribution. The leaf primordia in the centre of the head contained small, proplastid-like plastids, however, sometimes they had etioplasts with PLBs and PTs. The presence of PLBs depended on the age, environmental and storage conditions of the studied material and was also in good correlation with the presence and accumulation of the photoactive Pchlide form (*i. e.* the 77 K fluorescence intensity of the spectral band at 653 nm) as was reported for the leaves of other, artificially dark-forced plants (Klein and Schiff 1972) and for non-leaf organs (Böddi et al. 1994; McEwen et al. 1994). The innermost leaves of freshly collected savoy cabbage (*Brassica oleracea* L. cv. capitata var. sabauda) contained many PLBs, while PLBs were rare in the innermost leaves of white cabbage (*Brassica oleracea* L. cv. capitata var. alba) heads.

Etiolated seedlings are often used for studying the greening process (for review see Ryberg and Sundqvist 1991; Sundqvist and Dahlin 1997). In their case the PLBs transform upon illumination and the formation of thylakoids starts (Henningsen 1970; Gunning and Steer 1975; Rascio et al. 1984). The greening process of the 4th, 10th and innermost leaf layers was studied via illuminating the leaves with continuous white light. Samples were collected for ultrastructural investigations, pigment determination and 77 K fluorescence spectroscopy after 3, 8, 24 and 48 h of irradiation. Chl accumulation was found in all studied leaves, but its dynamics was different in the different leaf layers. Interestingly, the innermost leaves accumulated Chl much faster than the elder leaves close to the surface of the head. After 48 h, the Chl content of the innermost leaves was 124 and 113% of the Chl contents in the 4th and 10th leaf layer, respectively. Plastid ultrastructure is not much influenced in the 4th leaf layer during irradiation, in the other leaf layers chloroplast formation started. During long storage of the heads (which occurs especially in case of commercial storage) the pigment content of the leaves decreased and the greening ability of the leaves also altered, *i.e.* the Chl biosynthesis of leaves of young, freshly collected cabbage heads was faster. This indicates that the chloroplast formation and Chl biosynthesis depends on the age and/or developmental stage of the leaves. Cabbage head is an ideal natural research material for studying the plastid differentiation stages and the symptoms of long-lasting etiolation.

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