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REVERSIBLE LIGHT-DEPENDENT TRANSFORMATION OF MUTANT PLASTIDS IN VARIEGATED LEAVES OF *EUONYMUS FORTUNEI* (TURCZ.) VAR. *RADICANS* (MIQ.) REHD.

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Fine structure, pigment content and photosynthetic activity of variegated leaves of *Euonymus fortunei* var. *radicans* have been studied in detail.

The middle dark-green leaf areas contain normal chloroplasts with large grana. Their chlorophyll content is high, as is their photosynthetic activity. The marginal leaf areas with mutant plastids are highly light-sensitive. When grown in the shade they are light-green and contain chloroplasts with small grana. In sunlight they are yellow, and in their plastids, in addition to a few single thylakoids, several abnormal membrane structures appear (tubular complexes, cup-shaped stacks of thylakoids, »bridges« between thylakoids). Both the chlorophyll content and the photosynthetic activity of the mutant plastids are low, especially in the yellow tissue. Their photosynthetic efficiency (photosynthetic activity calculated on a chlorophyll basis) is, on the contrary, much higher than that of the normal dark-green tissue chloroplasts.

When the leaves are kept in the shade, the yellow leaf areas regreen in a short time and chloroplasts appear in them. This light-green tissue is able to yellow for the second time, when exposed to intense light. Plastids from this yellow tissue have only a few thylakoids.

Membrane components, which either could not be built into thylakoids, or which originate from decomposed thylakoids, accumulate in special structures (prolamellar bodies, thylakoidal bodies, plastoglobules) which, in the course of further plastid differentiation, could be used or reused for the formation of new thylakoids.

Introduction

Some plant mutants are very sensitive to strong illumination, so that full sunlight causes yellowing and eventually bleaching of their leaves. Among others, such are the s. c. aurea varieties of many garden plants, whose plastids cannot form grana and contain little chlorophyll when grown in strong sunlight. However, their photosynthetic activity is always high when calculated on a chlorophyll basis (Wrischer et al. 1976, Okabe et al. 1977).

Preliminary experiments showed that marginal parts of variegated leaves of *Euonymus fortunei* var. *radicans* behaved like aurea plants leaves. They were light-green only in the shade, but yellow in intense light. At the same time peculiar structural and functional changes appeared in their plastids. These changes are the subject of the present paper.

Material and Methods

The experiments were carried out on the ornamental plant *Euonymus fortunei* (Turcz.) var. *radicans* (Miq.) Rehd. (Krüssman 1960) having variegated leaves with yellow or light-green margins. The plant was cultivated outdoors during summer months under conditions of natural illumination. In addition to that, some twigs were shaded by semi-transparent white plastic bags allowing to pass only either about 50% or 30% of light.

The pigments were extracted in 80% acetone and the quantitative determination of chlorophylls was calculated according to Holden (1965), and of total carotenoids according to Urbach et al. (1976).

Photosynthetic activity of leaf pieces (measuring 0.25 mm^2) was determined with an O_2 electrode (Hansatech Ltd., Norfolk, England) as O_2 evolution in aqueous media (Shimazaki and Sugahara 1979). The reaction mixture contained 0.1 mol phosphate buffer (pH 8) and 0.01 mol sodium bicarbonate. The samples were illuminated with a halogen lamp giving at maximum a light intensity of 10^5 lx.

For electron microscopic investigations small pieces of leaves were fixed in 1 % glutaraldehyde in cacodylate buffer (pH 7.2) at 274 K, postfixed in 1 % OsO_4, and after dehydration embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop I.

Results

The middle areas of variegated leaves were dark-green under all conditions of illumination examined. On the contrary, the colour of peripheral leaf areas changed with the illumination intensity. When grown at low intensities (in the shade), the colour was light-green or yellow-green, but at high intensities (in the sun) more or less yellow. When the leaves had been in the sun for several weeks, the yellow leaf areas bleached, i. e. turned yellowish-white, and the leaves eventually fell off.

As can be seen in Table 1, the concentration of total chlorophyll (a + b) of the middle, dark-green leaf areas was much higher than that of the leaf margins, especially of the yellow ones. In bleached tissue the chlorophyll content dropped even more. The ratio of chlorophyll (a) to chlorophyll (b) varied much and was obviously dependent on the developmental stage of the leaves examined, a fact already known for some other aurea plants (B a u e r 1956, W r i s c h e r et al. 1975 a). A drop in the concentration of total carotenoids was also observed, although it was much less pronounced than the one of the chlorophylls (Table 1).

T a b l e 1. Total chlorophyll, total carotenoids and photosynthetic activity (illumination intensity: 10^5 lx) in dark-green normal tissue, light-green tissue with mutant plastids (grown in the shade), and yellow tissue with mutant plastids (grown in intense light).

	Total chlorophyll (mg/g fr. wt.)	Total carotenoids (mg/g fr. wt.)	Photosynthetic activity (μmol O_2/g fr. wt./h)	Photosynthetic efficiency (μmol O_2/mg chlorophyll/h)
Dark-green tissue	2.50	0.16	120.80	50.13
Light-green tissue	1.98	0.11	110.03	84.80
Yellow tissue	0.13	0.08	20.09	162.06

The photosynthetic activity (calculated on the fresh weight of the tissue) of light-green leaf areas was almost as high as that of dark-green ones, while the photosynthetic activity of yellow areas was very low (Table 1, Fig. 1). On the other hand, the photosynthetic efficiency (calculated on the content of the chlorophyll) of the marginal tissue containing the mutant plastids, was much higher than that of the normal, dark-green one. The highest efficiency was obtained for the yellow tissue (Table 1, Fig. 2). Measurements indicated that at the highest available light intensities applied, the saturation of photosynthesis had not yet been reached (Figs. 1, 2).

Electron microscopic investigations showed, that large chloroplasts in the dark-green central leaf area contained very large grana and large starch grains (Fig. 3).

Mutant chloroplasts in the light-green tissue had only small grana with no more than 6 — 8 thylakoids, while in the stroma there were some starch grains and small plastoglobules (Fig. 4). Mutant plastids of the yellow tissue, which had been grown in strong light, showed variations in the ultrastructure. They usually contained only single thylakoids with occasional overlappings of two thylakoids (Fig. 5). In many plastids the thylakoids were irregularly arranged and connected by triangular thylakoid dilatations ("bridges") (Fig. 6). Sometimes the thylakoids formed concentric cup-shaped structures (Figs. 5, 7). Tubular complexes were also frequent (Fig. 8). In some dilated thylakoids large dark inclusions ("thylakoidal bodies") were present, probably containing proteins (H e nry 1975, Hurkman and Kennedy 1977) (Fig. 8). In the stroma there were groups of plastoglobules, but starch was usually absent. In the plastids of bleached (yellowish-white) tissue the reduction of the membrane system progressed further, so that only short thylakoids and some vesicles remained. There were always aggregates of plastoglobules in the stroma (Fig. 9).

If the intense illumination was experimentally reduced by appropriate shading to about 50%, the yellow leaf areas regreened in a few days. The chlorophyll content rose again and new thylakoids stacked into small grana appeared. Characteristic »bridges«, i.e. triangular dilatations of some thylakoids connected neighbouring grana stacks (Fig. 10). When the illumination was reduced to about 30 %, small prolamellar bodies appeared in addition to grana (Fig. 11).

These regreened peripheral leaf areas could turn yellow again if exposed to full sunlight. This process was accompanied by a decline in the chlorophyll content and by the reduction of the number of thylakoids, which were either single or arranged in pairs. Starch grains and some plastoglobules were present in the stroma (Fig. 13). But these plastids were ultrastructurally somewhat different from those growing in intense light. No thylakoidal bodies were found here, but there were small dark inclusions lying in contact with some thylakoids (Figs. 12, 13). According to earlier investigations these inclusions were located between two thylakoids ("interthylakoidal inclusions") (Wrischer et al. 1975 b).

In other cell organelles no apparent structural changes were observed, which would be light dependent. The only unusual structures in the cells of the yellowing tissue were lipid inclusions (myelin figures) lying in the cytoplasm in the vicinity of the plastids (Fig. 13).

Discussion

There are two types of plastids in the variegated leaves of *Euonymus fortunei* var. *radicans:* normal chloroplasts in the middle dark-green leaf areas, and mutant plastids in the marginal leaf areas, which are highly light-sensitive and behave like those in aurea plants and in other light-sensitive mutants (Wrischer et al. 1976, Okabe et al. 1977, Hopkins et al. 1980 a). The mutant plastids are able to form grana in the shade, while in the sunlight they contain only a few thylakoids. In addition to single thylakoids some abnormal membrane structures also appear, which are otherwise rather common in undeveloped plastids and many chromoplasts. Another peculiarity of these mutant plastids is their high photosynthetic efficiency, connected with a low chlorohyll concentration. This characteristic is common to many mutants deficient in chlorophyll (Lemoine 1974, Wieckowski and Ficek 1974, Wrischer et al. 1976, Okabe et al. 1977, Hopkins et al. 1980 b).

Our experiments have shown, that the light-green tissue of shaded leaves is able to yellow again when exposed to intense light, a phenomenon known for some aurea plants (Sagromsky 1956). As already mentioned, there are some differences in the ultrastructure between plastids from primarily yellow tissue (grown in intense light), and those from light-green tissue which turned yellow in intense light. These differences can be explained by the fact that in the first case the thyla-

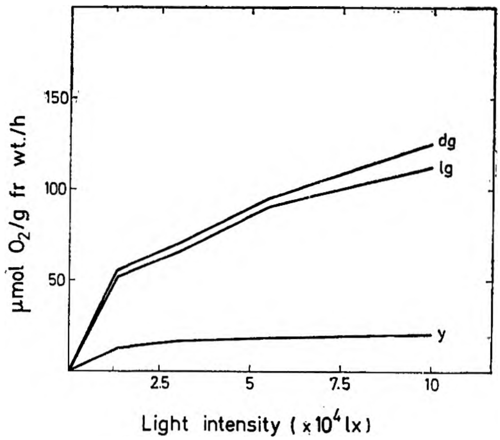


Fig. 1. Dependence of photosynthesis on the light intensity measured as O_2 evolution in µmol/g fresh weight in three types of leaf tissue. dg = dark-green normal tissue, lg = light-green tissue with mutant plastids (grown in the shade), y = yellow tissue with mutant plastids (grown in intense light).

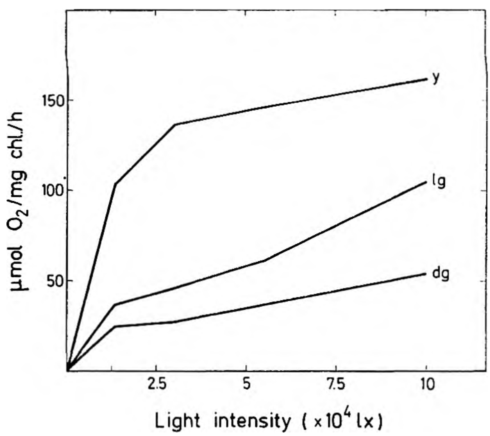


Fig. 2. Dependence of photosynthesis on the light intensity measured as O_2 evolution in µmol/mg chlorophyll in three types of leaf tissue. dg = = dark-green normal tissue, lg = light-green tissue with mutant plastids (grown in the shade), y = yellow tissue with mutant plastids (grown in intense light).

koid system is primarily undeveloped, while in the second case it is secondarily reduced.

In our mutant plastids there are two types of special structures, which indicate that the plastid development is stopped or slowed down. These are the prolamellar bodies and the thylakoidal bodies. Prolamellar bodies are found in plastids of leaves grown in very dim light. It is known that these tubular structures develop in young chloroplasts in shaded leaf areas (Wrischer 1981). They should represent structures adapted for the storage of membrane components, especially lipids, when the plastid development is stopped or slowed down by dim light (Kesselmeier and Ruppel 1979, Kesselmeier 1980).

Plastids in the yellow leaf areas grown in strong light accumulate protein material in the s. c. thylakoidal bodies. These protein inclusions often appear in very young or undeveloped plastids and probably also serve as storage material for the formation of new thylakoids (Henry 1975, Hurkman and Kennedy 1977, Casadoro et al. 1979).

Figs. 3—13. Plastids from different areas of variegated leaves of *Euonymus fortunei* var. *radicans*. Fig. 3. Normal chloroplast from the central leaf area. Figs. 4—13. Mutant plastids from the marginal leaf areas.

Fig. 3. Part of a chloroplast from the dark-green tissue. Granum with more than 60 thylakoids. 50,000 :1.

Fig. 4. Part of a chloroplast from the light-green tissue. Grana contain 3—6 thylakoids. A starch grain on the right. 84,000 : 1.

Fig. 5. Plastid from the yellow tissue containing single thylakoids, stacks of cup-shaped thylakoids (arrow) and some plastoglobules. 22,000 : 1.

Fig. 6. Part of a plastid from the yellow tissue. Thylakoids are connected by triangular thylakoid dilatations ("bridges"; arrow). There are plastoglobules in the stroma. 60,000 : 1.

Fig. 7. Part of a plastid from the yellow tissue with a cup-shaped stack of thylakoids. 29,000 : 1.

Fig. 8. Part of a plastid from the yellow tissue. A protein inclusion (thylakoidal body; tb) lies inside a dilated thylakoid. Single thylakoids are in connection with a tubular complex (tc). 30,000 : 1.

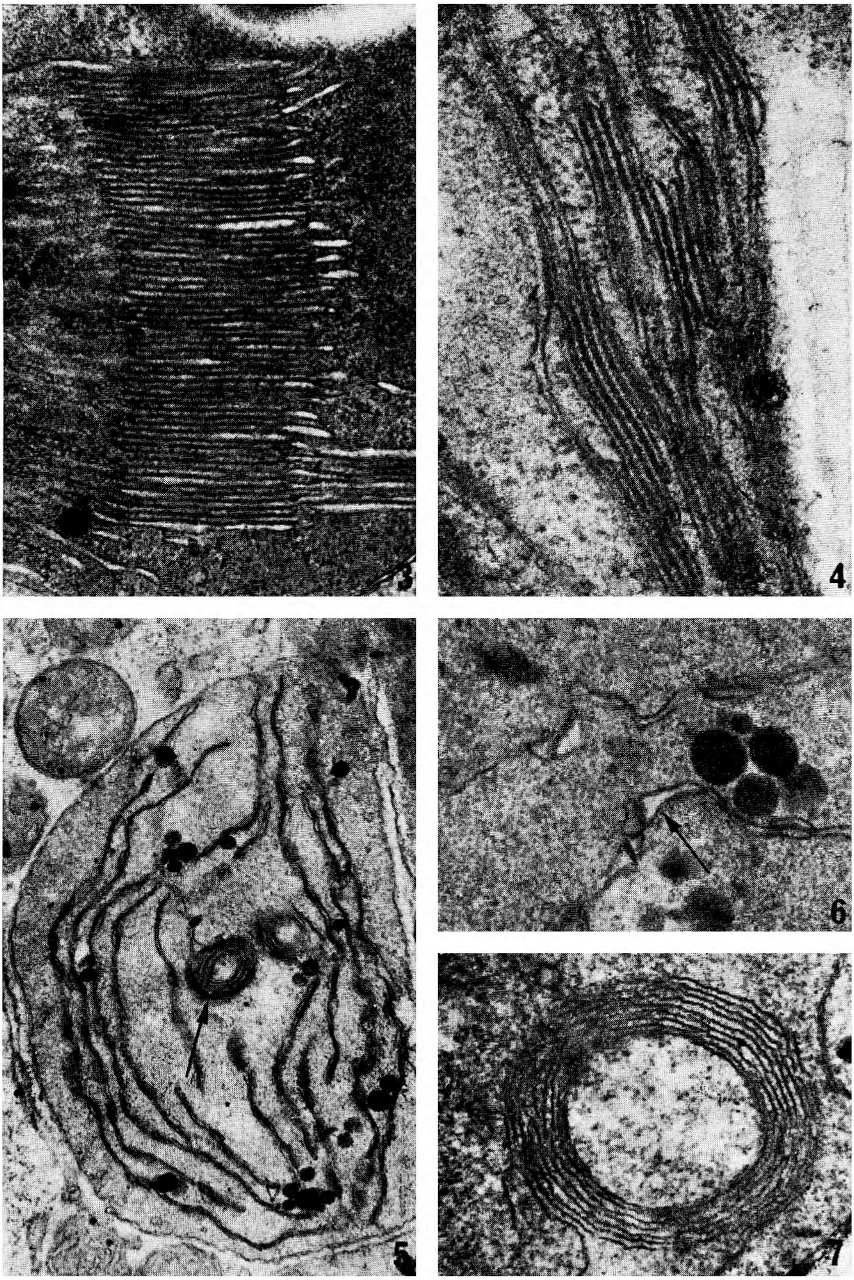
Fig. 9. Plastid from the bleached tissue containing short thylakoids, some vesicles and large aggregates of plastoglobules 8,500 : 1.

Fig. 10. Part of a chloroplast from the regreened tissue (14 days in sunlight reduced to 50%). Peripheral thylakoids of the neighbouring grana are connected by tringular thylakoid dilatations ("bridges"; arrows). 55,000 : 1.

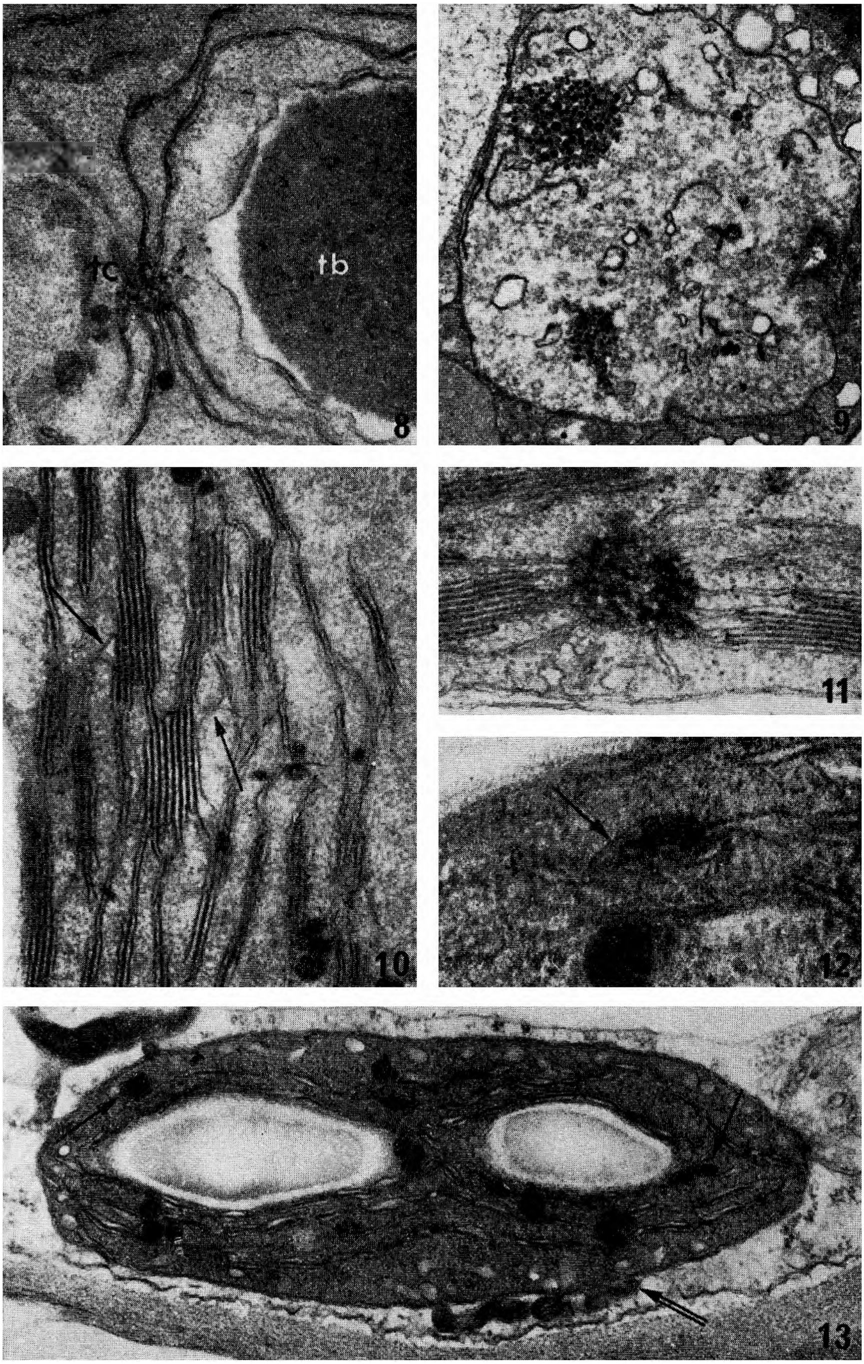
Fig. 11. Part of a chloroplast from the regreened tissue (26 days in sunlight reduced to 30%). Between grana there is a prolamellar body. 65,000 : 1.

Fig. 12. Part of a plastid form the yellow-green tissue (2nd yellowing) with an interthylakoidal inclusion (arrow). 112,000 : 1.

Fig. 13. Plastid from the yellow-green tissue (2nd yellowing). Thylakoids arranged into groups of two contain interthylakoidal inclusions (arrows). Peripheral reticulum is well developed. In the stroma starch grains and plastoglobules. Myelin figures in the cytoplasm (double arrow). 32,000 : 1.



Figs. 3—7.



Figs. 8—13.

On the other hand, the two structures, interthylakoidal inclusions and plastoglobules, indicate a degradation of thylakoids. Interthylakoidal inclusions in plastids of yellowing tissue probably represent damaged membranes or their components lying between structurally normal thylakoids (W r i s c h e r et al. 1975 a, 1976).

In mutant plastids of the yellow, and especially of the bleached tissue, lipids accumulate in numerous plastoglobules. It is a well established fact that plastoglobules originate from degraded thylakoids, and that they serve for the formation of new thylakoids during regreening (L j u b e š i ć 1968, D e v i d é and L j u b e š i ć 1974, L j u b e š i ć 1976, I k e d a 1979), a phenomenon observed in our mutant plastids as well. During the regreening thylakoidal bodies also disappear. Therefore we assume that both plastoglobules and thylakoidal bodies might serve as storage material, which for some reason accumulates in plastids exposed to strong light, and which, during their regreening, might participate in the formation of new thylakoids.

The significance of lipid inclusions (myelin figures) found in the cytoplasm of yellowing tissue, is for the moment unknown, although they seem to be somehow in connection with degradational processes taking place in the plastids. Perhaps they represent the last step in the degradation of thylakoids and their elimination out of the plastids.

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SAŽETAK

REVERZIBILNA TRANSFORMACIJA PLASTIDA U PANAŠIRANIM LISTOVIMA VRSTE *EUONYMUS FORTUNEI* VAR. *RADICANS*

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Istraženi su ultrastruktura, sadržaj pigmenata i fotosintetska aktivnost plastida panaširanih listova vrste *Euonymus fortunei* var. *radicans*. Normalni kloroplasti središnjih tamnozelenih dijelova lista imaju velika grana, mnogo klorofila i visoku fotosintetsku aktivnost. Rubni dijelovi lista, koji sadrže mutirane plastide, vrlo su osjetljivi na osvjetljenje, te mijenjaju boju ovisno o intenzitetu svjetlosti. Ako rastu u sjeni, oni su svjetlozeleni i sadrže kloroplaste s malo grana. Na jakoj sunčevoj svjetlosti ti rubni dijelovi požute, a u njihovim plastidima uz pojedinačne tilakoide pojavljuju se tubularni kompleksi, čaškasto svinuti svežnjevi tilakoida i »mostovi« među tilakoidima. Sadržaj klorofila i fotosintetska aktivnost, posebno žutih dijelova lista, vrlo su niski. Fotosintetska efikasnost (fotosintetska aktivnost izražena po jedinici klorofila), naprotiv, mnogo je viša nego u normalnim tamnozelenim dijelovima lista.

Ako se listovi drže u sjeni, žuti dijelovi mogu ozelenjeti, te opet sadrže kloroplaste. Takvo svjetlozeleno tkivo može na suncu ponovno požutjeti, a plastidi u njima sadrže ponovno vrlo malo tilakoida.

Komponente membrana, koje se iz bilo kojih razloga ne mogu ugraditi u tilakoide ili koje potječu od razgrađenih tilakoida, mogu se nagomilati u posebnim strukturama (prolamelarnim tjelešcima, tilakoidnim tjelešcima, plastoglobulima). Te se strukture tijekom dalje diferencijacije plastida mogu po potrebi iskoristiti za izgradnju novih tilakoida.

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