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PLASTID TRANSFORMATION IN CARROT ROOTS INDUCED BY DIFFERENT LIGHTS

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Introduction

It has repeatedly been stated that, in roots and callus tissue cultures chloroplasts can be formed only in blue light, red light not being effective (Björn 1967 a, Richter 1969, Bergmann and Berger 1966, Mohr and Sitte 1971). The only exception is the statement of Bajaj and McAllan (1969), that the greening of excised potato roots is possible both in blue and in red light.

It is a well known fact that carrot roots become green if kept in white light. Ultrastructural investigations of such greening have been done recently on fully grown roots (Grönegress 1971), cultured plantlets (Israel, Mapes and Steward 1969) and callus tissue (Wrischer 1972). This author's further experiments have shown that young carrot roots (grown from seeds) also green in white light. It was thus interesting to find out what regions of the light spectrum are responsible for this phenomenon. Carrot seems to be very suitable for studies of plastid photomorphogenesis, since the following three ways of chloroplast differentiation can be studied and compared: a) the proplastid → chloroplast transformation (in roots of young plants grown up from seeds, b) the chromoplast → chloroplast transformation (in fully grown roots) and c) the chromoplast → proplastid → chloroplast transformation (in callus tissue in culture). Since it can be shown that the greening of carrot tissue is a relatively slow process, which starts only after 1 to 2 weeks of illumination with blue or red light, this experimental system appears to be especially suitable for the study of the early stages in the chloroplast transformation. Experiments were carried out on roots of young carrot plantlets (grown from seeds), on fully grown roots and on carrot callus tissue.

Material and Methods

Young carrot plants grown from seeds (*Daucus carota*, cv. "Nantes") were germinated in darkness on filter paper wetted with a diluted *Knop* solution or with tap water. After 4 to 5 days the seedlings were exposed to different light treatments. While the upper part of the seedlings was illuminated with continuous white light (mercury bulb VTF — 250 W TEŽ, illumination intensity 4000 lx), in all experiments the roots were shielded in one of the following ways: a) by colourless (transparent) plastic plate for illumination with white light, b) by black (non-transparent) plastic plate for experiments in darkness, c) by blue plexiglas filter (Röhm & Haas, GmbH, Darmstadt, GFR) No 627 (transparence maximum at 450 nm) and d) by red plexiglas filter (Röhm & Haas, GmbH, Darmstadt, GFR) No 501 (transparence maximum at 660—670 nm). To remove the dark red region of the spectrum, which was transmitted through the red and blue filters, additional filtering was provided by a 2.5 cm thick layer of 1.2% $CuSO_4$ solution in destilled water. The energy transmitted through the blue filters was up to 240 erg $\cdot$ cm^{-2} $\cdot$ sec^{-1} and the energy transmitted through the red ones was varied from 80 to 120 erg $\cdot$ cm^{-2} $\cdot$ $\cdot$ sec^{-1}. Colourless filters were combined with a 2.5 cm thick layer of destilled water.

Small pieces originating from the upper thirds of the roots, were fixed in 1% glutaraldehyde 7, 14, 21 and 28 days after the beginning of illumination. After washing in buffer the material was postfixed in 1% OsO_4 and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I (at the Institute of Biology, University of Zagreb).

Whole fully grown carrot roots (harvested in late summer or autumn) were illuminated and further prepared as already described for young roots. All observations were done on the middle portion of the root.

Carrot callus tissue, cultivated as described earlier (Wrischer 1972), was treated like the roots.

Thin hand made sections through roots and callus tissue were currently examined in the light microscope.

From fully grown carrot roots chlorophyll was extracted and measured spectrophotometrically according to the data of Anderson and Boardman (1964). The chlorophyll concentrations in roots grown in red light were expressed as relative values in regard to the control (roots grown in white or blue light).

Results

Roots of young plants grown from seeds

Light microscopic examinations have shown that cells of very young, i. e. several days old roots, grown either in darkness or in light, contain colourless proplastids. In darkness, carotene crystals begin to appear in the upper, i. e. older parts of the roots after about 10 to 14 days. This finding is in agreement with earlier reports by Steffen and Reck (1964) and Ben-Shaul and Klein (1965). If roots are grown for 10 to 14 days in light (white, blue or red) these crystals also develop but, especially in white light, their number is much lower than in dark-grown roots. Carotene crystals appear mostly in plastids of the

outer and middle cell layers of the cortex parenchyma. About 10 days after the beginning of illumination with white light some chloroplasts are already present in the root cells. They appear at the beginning in the cell layers on the border of the central cylinder, but soon the greening spreads gradually over the peripheral cell layers of the cortex. In blue and in red light the greening starts in a similar way as in white light, but is delayed, so that it begins about the 14th day in blue and about the 14th or 16th day in red light.

Differences in the rate of greening exist also between the older and the younger parts of the roots, the older ones being always the first to start greening.

Roots grown in white or in blue light contain somewhat larger chloroplasts than those grown in red light.

Electron microscopic examinations have shown that no fine structural differences exist among plastids in roots grown in light of different wavelengths. In very young roots only proplastids can be found (Fig. 1). In darkness they gradually turn into chromoplasts, containing some plastoglobules and carotene crystals, as already described by Ben-Shaul, Treffry and Klein (1968).

In white, blue or red light the changes in the fine structure of proplastids proceed as follows:

After several days of illumination plastoglobules (0.1 to 0.16 μm in diameter) are present in proplastids (Fig. 2). At the same time an increased number of cisternae, developed as invaginations of the inner plastid membrane, appears (Figs. 2, 3). Later on, detached vesicles start doubling (Fig. 4) probably by the s. c. "Überschiebung" (Wehrmeyer 1966). In addition to that, some plastids of the cortex cells also contain single thylakoids and a tubular complex (Newcomb 1967), structures common in carrot chromoplasts (Fig. 2). Vesicles filled with finely granular material also occur occasionally (Fig. 3). The vesicles multiply further, arrange themselves into stacks (Fig. 5) and gradually flatten (Fig. 6). Finally, chloroplasts with well developed grana and stroma thylakoid systems are formed (Fig. 7). Plastoglobules are still present (Figs. 5, 6). Starch granules appear in the chloroplast stroma, but carotene crystals are seldom found.

The greening of the roots progresses centrifugally. It is therefore possible to find different developmental stages of the chloroplasts in the same cross-section through the root, the most developed lying always in the deepest cell layers of the cortex.

Root cells, in which proplastids just begin to transform into chloroplasts, contain mitochondria with a well developed tubular system (Figs. 2, 3, 4).

Fully grown roots

In contrast to young roots, in fully grown ones the greening starts in the subepidermal layers of the cortex parenchyma, and then proceeds slowly into deeper regions of the cortex (Grönegress 1971). The first chloroplasts — as observed in light microscope — appear in white light 7 days after the beginning of illumination, in blue light after about 10 days, and in red light after 10 to 14 days (depending on the energy of red light used).*

* A detailed study of these relations, with more exact measurements of light energy, is in preparation and will be published elsewhere.

Fig. 1. Proplastid from a young root (7 days in white light). 24,000 : 1.

Sl. 1. Proplastid iz mladog korijena (7 dana na bijeloj svjetlosti). 24 000 : 1.

Fig. 2. Plastid from a young root (middle cell layer of the cortex; 14 days in white light) with plastoglobules, some single thylakoids, a tubular complex (tc) and numerous cisternae (arrows). Mitochondria with numerous tubules. 32,000 : 1.

Sl. 2. Plastid iz mladog korijena (srednji sloj stanica kore; 14 dana na bijeloj svjetlosti) s plastoglobulima, pojedinačnim tilakoidima, tubularnim kompleksom (tc) i brojnim cisternama (strelice). Mitohondriji s brojnim tubulima. 32 000 : 1.

Fig. 3. Plastid from a young root (middle cell layer of the cortex; 14 days in blue light — 240 erg · cm^{-2} · sec^{-1}). A large vesicle filled with dark granular substance and some cisternae (arrow) are present. Mitochondrion with numerous tubules. 21,000 : 1.

Sl. 3. Plastid iz mladog korijena (srednji sloj stanica kore; 14 dana na modroj svjetlosti — 240 erg · cm^{-2} · sek^{-1}). U stromi veliki vezikul s tamnim granuliranim sadržajem i pojedine cisterne (strelica). Mitohondrij s brojnim tubulima. 21 000 : 1.

Fig. 4. Plastid from a young root (14 days in white light) with plastoglobules and clusters of vesicles (= earliest stages of grana). Mitochondrion with numerous tubules. 32,000 : 1.

Sl. 4. Plastid iz mladog korijena (14 dana na bijeloj svjetlosti) s plastoglobulima i nakupinom vezikula (= najraniji stadiji grana). Mitohondrij s brojnim tubulima. 32 000 : 1.

Fig. 5. Young chloroplast (middle cell layer of the cortex; 21 days in blue light — 240 erg · cm^{-2} · sec^{-1}) with cisternae (arrow) and dilated thylakoids arranged in stacks. Plastoglobules and numerous ribosomes in the stroma. 40,000 : 1.

Sl. 5. Mladi kloroplast (srednji sloj stanica kore; 21 dan na modroj svjetlosti — 240 erg · cm^{-2} · sek^{-1}) s cisternama (strelica) i dilatiranim tilakoidima koji su poslagani u svežnjeve. U stromi plastoglobuli i brojni ribosomi. 40 000 : 1.

Fig. 6. Chloroplast from a young root (cell layer on the border of the central cylinder; 14 days in red light — 80 erg · cm^{-2} · sec^{-1}). Dilated thylakoids are arranged in stacks. Plastoglobules are still present. 26,000 : 1.

Sl. 6. Kloroplast iz mladog korijena (sloj stanica na rubu centralnog cilindra; 14 dana na crvenoj svjetlosti — 80 erg · cm^{-2} · sek^{-1}). Dilatirani tilakoidi složeni su u svežnjeve. Plastoglobuli su još uvijek prisutni. 26 000 : 1.

Fig. 7. Chloroplast from a young root (21 days in white light) with well developed grana. 30,000 : 1.

Sl. 7. Kloroplast iz mladog korijena (21 dan na bijeloj svjetlosti) s dobro razvijenim sistemom grana. 30 000 : 1.

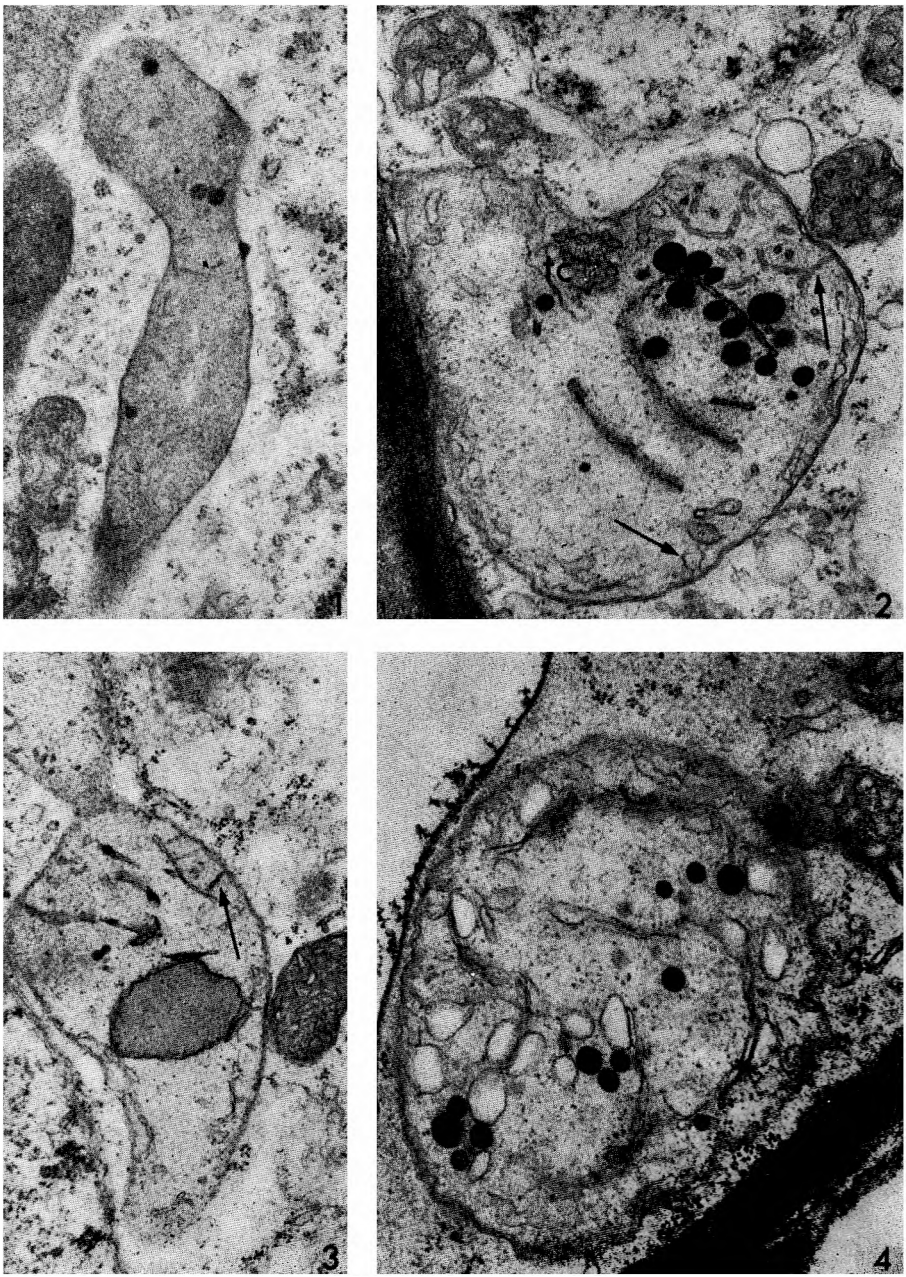
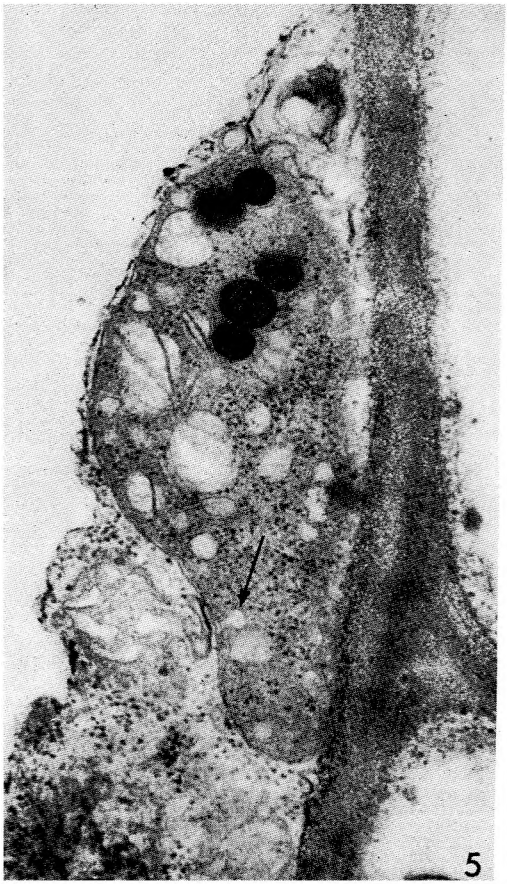
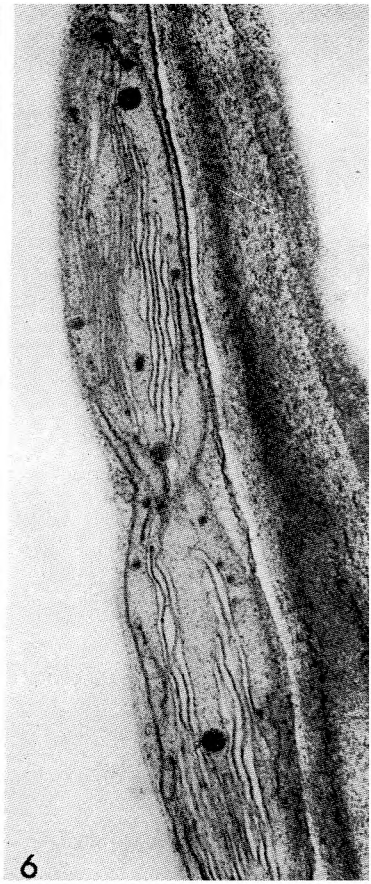


Fig. 1—4. — Sl. 1—4.





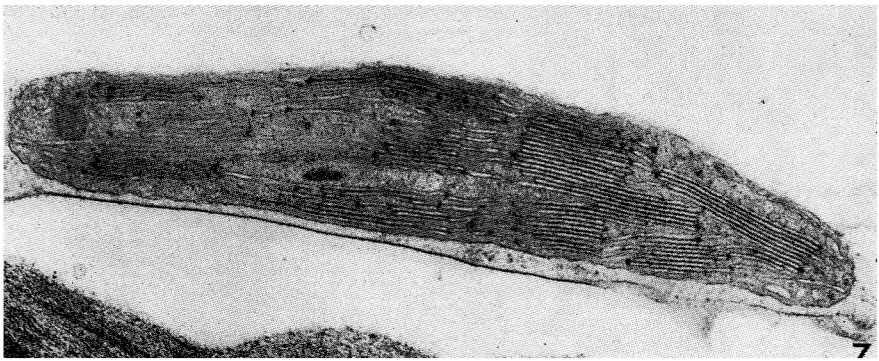


Fig. 5—7. — Sl. 5—7.

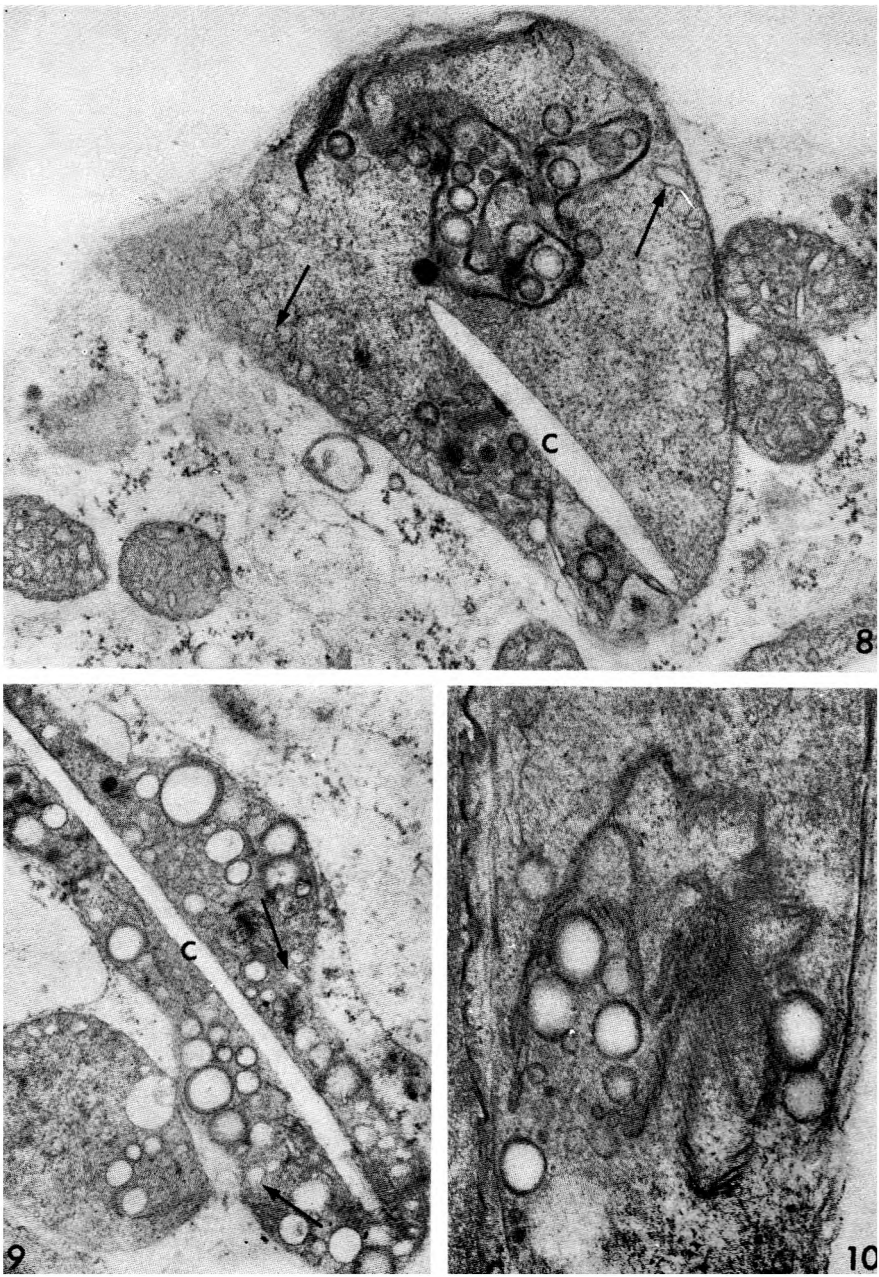


Fig. 8—10. — Sl. 8—10.

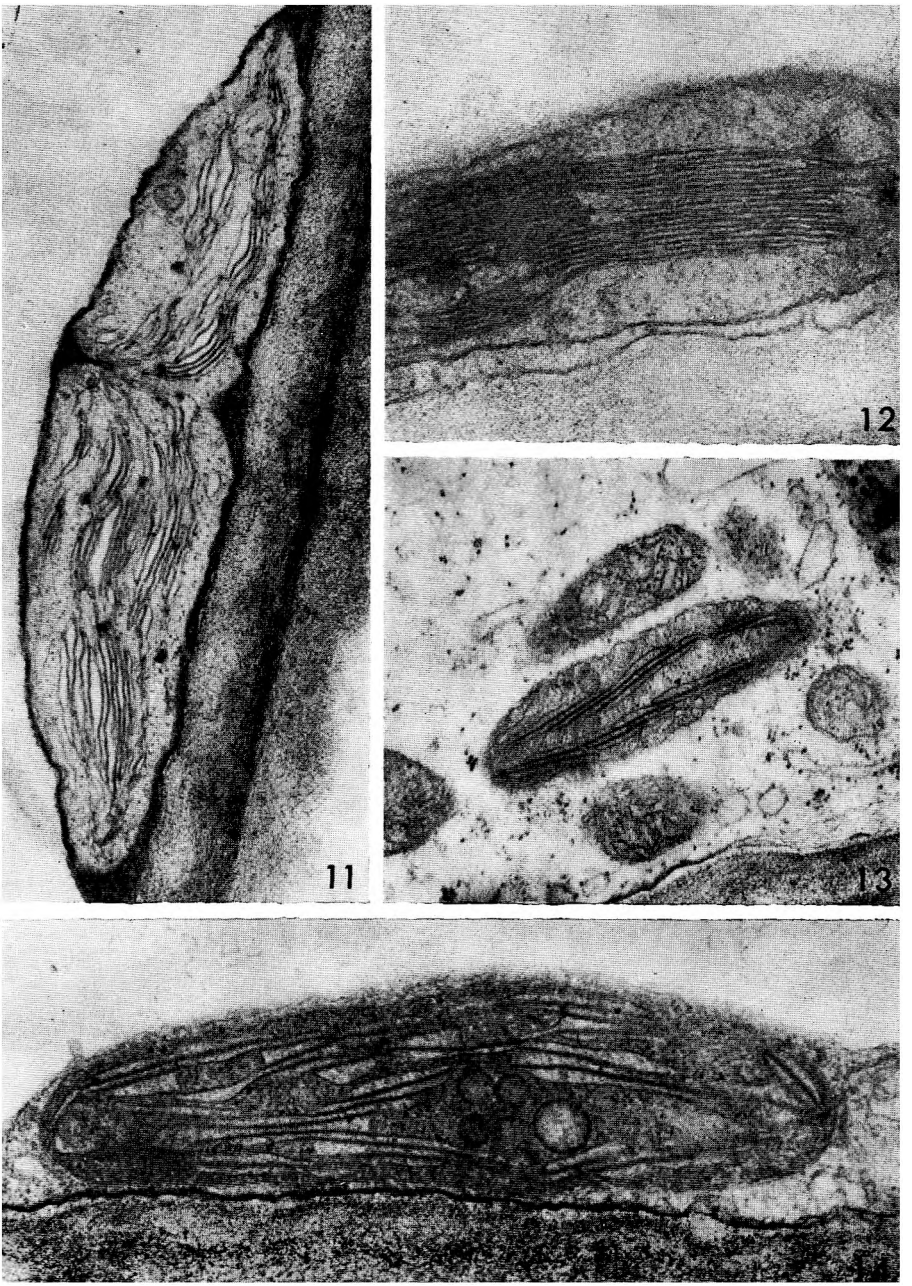


Fig. 11—14. — Sl. 11—14.

Fig. 8. Plastid from a fully grown root exposed to red light (120 erg · cm^{-2} · · sec^{-1}) for 7 days. In addition to a carotene crystal (c), some single thylakoids and plastoglobules of low electron density, numerous cisternae (arrows) appeared. Mitochondria with many tubules. 27,000 : 1.

Sl. 8. Plastid iz izraslog korijena osvjetljavanog 7 dana crvenom svjetlošću (120 erg · cm^{-2} · sek^{-1}). Osim karotenskog kristala (c), pojedinačnih tilakoida i slabo osmiofilnih plastoglobula, pojavile su se brojne cisterne (strelice). Mitohondriji s mnogo tubula. 27 000 : 1.

Fig. 9. Plastid from a fully grown root exposed to blue light (240 erg · cm^{-2} · · sec^{-1}) for 7 days with a carotene crystal (c), plastoglobules of low electron density and some cisternae (arrows). 20,000 : 1.

Sl. 9. Plastid iz izraslog korijena osvjetljavanog 7 dana modrom svjetlošću (240 erg · cm^{-2} · sek^{-1}) s karotenskim kristalom (c), slabo osmiofilnim plastoglobulima i cisternama (strelice). 20 000 : 1.

Fig. 10. Plastid from a fully grown root exposed to red light (120 erg · cm^{-2} · · sec^{-1}) for 14 days. Beside plastoglobules, rows of thylakoids, which are in connection with a tubular complex, are present. 40,000 : 1.

Sl. 10. Plastid iz izraslog korijena osvjetljavanog 14 dana crvenom svjetlošću (120 erg · cm^{-2} · sek^{-1}). Osim plastoglobula postoje nizovi tilakoida koji su u vezi s tubularnim kompleksom. 40 000 : 1.

Fig. 11. Chloroplast from a fully grown root after 14 days in blue light (240 erg · cm^{-2} · sec^{-1}). Partly dilated thylakoids are arranged into grana. 32,000 : 1.

Sl. 11. Kloroplast iz izraslog korijena osvjetljavanog 14 dana modrom svjetlošću (240 erg · cm^{-2} · sek^{-1}). Djelomično dilatirani tilakoidi složeni su u grana. 32 000 : 1.

Fig. 12. Part of a chloroplast from a fully grown root (outer cell layer of the cortex) exposed to red light (120 erg · cm^{-2} · sec^{-1}) for 14 days. Well developed grana with numerous thylakoids are present. 54,000 : 1.

Sl. 12. Dio kloroplasta izraslog korijena (vanjski sloj stanica kore) osvjetljavanog 14 dana crvenom svjetlošću (120 erg · cm^{-2} · sek^{-1}). Postoje dobro razvijena grana s brojnim tilakoidima. 54 000 : 1.

Fig. 13. Young chloroplast from a 21 days old callus grown in red light (120 erg · cm^{-2} · sec^{-1}). Dilated thylakoids filled with a granular substance are arranged in rows. 32,000 : 1.

Sl. 13. Mladi kloroplast iz 21 dan starog kalusa izraslog na crvenoj svjetlosti (120 erg · cm^{-2} · sek^{-1}). Dilatirani tilakoidi s granuliranim sadržajem složeni su u nizove. 32 000 : 1.

Fig. 14. Young chloroplast from a 21 days old callus grown in blue light (240 erg · cm^{-2} · sec^{-1}). Dilated thylakoids are filled with granular material and are arranged in stacks. 40,000 : 1.

Sl. 14. Mladi kloroplast iz 21 dan starog kalusa izraslog na modroj svjetlosti (240 erg · cm^{-2} · sek^{-1}). Dilatirani tilakoidi s granuliranim sadržajem složeni su u svežnjeve. 40 000 : 1.

In the beginning, the green parts of the chloroplasts are usually attached to the carotene crystals of the original chromoplasts, but later on, chloroplasts without crystals are often found.

Electron microscopic examinations of the tissue show that, after seven days of illumination with blue or red light, the usual fine structure of the root chromoplasts (e. g. Wrischer 1972, Fig. 1) is changed. Structures known to appear in chromoplasts — i. e. empty, sharp-edged vacuoles (which *in vivo* contained carotene crystals), numerous plastoglobules of low electron density and a tubular complex — are still present. But in addition to that, numerous invaginations of the inner plastid membrane appear in the form of cisternae (Figs. 8, 9). The vesicles, deriving probably from these structures, begin to multiply and arrange themselves into stacks (Fig. 10). The next stage in the differentiation are chloroplasts with grana, whose lumina are still somewhat dilated (Fig. 11). Still later normally formed chloroplasts with well developed grana appear (Fig. 12).

The chloroplasts in roots illuminated with white light are always the largest, with blue light they are somewhat smaller, while those with red light are the smallest. The chlorophyll content of these tissues is in agreement with this finding. After 14 days of illumination the chlorophyll concentration in roots kept in red light is e. g. 40 to 60% of those exposed to blue light.

Callus tissue

Fine structural changes of plastids in callus tissue, exposed to blue or red light, progress in the same way as those found for callus grown in white light (Wrischer 1972). In blue light the greening of the tissue is again somewhat delayed in comparison to that in white light, and in red light this delay is still more pronounced, being dependent in addition on the energy of the red light used. Forteen days after the beginning of the illumination in blue or red light chloroplasts are already present. Very often transitional stages between proplastids and chloroplasts contain dilated thylakoids, whose lumina are filled with a finely granular material (Figs. 13, 14), probably of proteinaceus nature (Wrischer 1972 and literature cited therein). In fully developed chloroplasts these dilatations and inclusions disappear.

Differences in the dimensions of the chloroplasts, grown in light of different wavelengths (red, blue), occur also in these tissues.

Discussion

The differentiation of chloroplasts in cells of carrot roots and callus tissue takes place in light of different wavelengths (blue, red). The transformation of proplastids or chromoplasts into chloroplasts in all three examined systems was somewhat slower in red light than in blue light. Besides that, the chloroplasts, which had developed in red light, were smaller than those in blue or in white light, and the concentration of the chlorophyll was in accordance with these findings. This fact could perhaps be brought in connection with the higher protein synthesis

happening in chloroplasts of some plant materials in blue light, as proved e. g. by Bergfeld (1970). But this could also be the consequence of different energies transmitted through the blue and red filters used. These two possibilities will now be further analysed in detail.

An only slightly lower chlorophyll content in red light than in the blue one was described by Bajaj and McAllan (1969) for cultured potato roots. On the other hand Björn and Odhelius (1966) quoted that the roots of cucumber, grown in blue light, contained 16 times more chlorophyll than those grown in red light. In pea and in wheat roots this difference was still greater (Björn 1965, Björn and Odhelius 1966). Richter (1969) has also stated that in isolated cultured pea roots in red light almost no chlorophyll was synthesized. The dependence of chloroplast differentiation on blue light was stated also for some callus tissue in culture (Bergmann and Berger 1966). The experiments of the author with attached (i. e. young) and detached (i. e. fully grown) carrot roots and callus tissue do not confirm these findings. It is possible that there are different ways of light responses in roots of different plants.

In all three systems studied the course of the chloroplast transformation was practically independent of the wavelengths of the light used for illumination. The fine structure of chloroplasts formed in red and in blue light was morphologically undistinguishable from those found earlier in fully grown carrot roots (Grönegress 1971) and in carrot callus tissue (Wrischer 1972), both illuminated with white light.

The stages of plastid differentiation found in young carrot roots — except for some peculiarities typical of developing chromoplasts — resembled those found by Heltne and Bonnet (1970) in Convolvulus roots greened in white light. They differed considerably from those of green roots of wheat and rye (Salema 1971, Fadeel and Al-Sani 1972), where during the chloroplast development in white light a stage containing a prolamellar-body could always be observed.

It has been claimed (Björn 1967 b) that the greening of cultured roots and callus tissue is probably not regulated by the phytochrome system, but by a yet unknown flavoprotein, which should have its absorbance in the blue region of the light spectrum. On the other hand Bajaj and McAllan (1969) discuss the possibility that the fairly similar greening of potato roots in blue and in red light should be in connection with the "preillumination history of the potato tuber". In carrot this possibility could be taken into consideration only in the case of fully grown roots and callus tissue, but not in the case of young roots developing from seeds. Further experimental work on carrot is necessary to clarify this problem.

Summary

The effect of red (660—670 nm), blue (450 nm) and white light on the development of chloroplasts in carrot roots and callus tissue has been investigated.

The experiments indicate, that the rate of the greening of the tissue and the size of the chloroplasts are dependent on the wavelength of the light used, because the chloroplasts develop somewhat later and are smaller in red than in blue or in white light.

The electron microscope has, however, shown that there is no significant difference in the effect of different lights on the ultrastructural changes, in which the inner plastid membrane plays a dominant role in the formation of membrane material of the newly formed thylakoids.

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SADRŽAJ

TRANSFORMACIJA PLASTIDA U KORIJENU MRKVE IZAZVANA SVJETLOŠĆU RAZLIČITIH VALNIH DUŽINA

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Ispitivan je utjecaj crvene (660—670 nm), modre (450 nm) i bijele svjetlosti na razvitak kloroplasta u korijenu i kalusnom tkivu mrkve.

Istraživanja ukazuju na to da su brzina ozelenjavanja tkiva i dimenzije nastalih kloroplasta ovisne o dužini vala korištene svjetlosti. Na crvenoj svjetlosti kloroplasti nastaju nešto kasnije i manjih su dimenzija od onih na modroj ili bijeloj svjetlosti.

Elektronsko-mikroskopska istraživanja pokazuju međutim da među ispitanim svjetlostima različitih valnih dužina nema bitne razlike u načinu njihovog djelovanja na ultrastrukturne promjene plastida. Kod svih istraženih objekata unutrašnja membrana plastida igra dominantnu ulogu u formiranju membranskog materijala novo stvorenih tilakoida.

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