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Chloroplast ultrastructure in leaves of *Cucumis sativus* chlorophyll mutant

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Abstract

The developing and young leaves of *Cucumis sativus* chlorophyll mutants are yellow, when mature they become green and do not differ in their colour from those of control plants. The mesophyll of yellow leaves contains a diversiform plastid population with a varying degree of defectiveness, which is mainly manifested in the reduction or disorganization of the typical thylakoid system. DNA areas, ribosome-like particles and aggregates of electron-dense material are preserved in the stroma of mutated plastids. Starch grains are deficient. Apart from mutated plastids, chloroplasts with a normal structure, as in control plants, were also observed.

The leaf greening process is accompanied by a reconstruction and rearrangement of the inner chloroplast lamellar system and an ability to accumulate starch. However, in the mutant chloroplasts as compared with control-plant ones, an irregular arrangement of grana and reduced number of inter-grana thylakoids can be seen.

An osmiophilic substance stored in the stroma of mutated plastids and the vesicles formed from an internal plastid membrane take part in restoration of the membrane system.

INTRODUCTION

Leaves of the chlorophyll mutant of *Cucumis sativus* were the object of this research. The results of reciprocal crosses of the mutant with a stock plant indicate that the mutant character is controled by a single recessive nuclear gene.

In the early developmental stages leaves of this mutant are yellow and become green during their further development. Initially green pigmentation appears along the vascular bundles. Mature leaves of the mutant do not differ in their colour from control plants. Plants of this mutant are capable of producing fruits and seeds.

Particular attention was devoted to the ultrastructure of the plastids in yellow, greening and greened mutant leaves and of control plants. No data are available in literature dealing with the description of *Cucumis sativus* chlorophyll mutants.

MATERIAL AND METHODS

Control plants of *Cucumis sativus* var. Borszczagowski and their chlorophyll mutants were used for this study. This mutant was obtained from seeds of *Cucumis sativus* var. Borszczagowski treated with 0.06% ethylene imine. The plants, prepared for electron microscope analysis, originated from an inbred line obtained by way of self-fertilization for 10 generations. The plants were grown in a plastic tunnel at the Experimental Station of AR-SGGW in Warsaw.

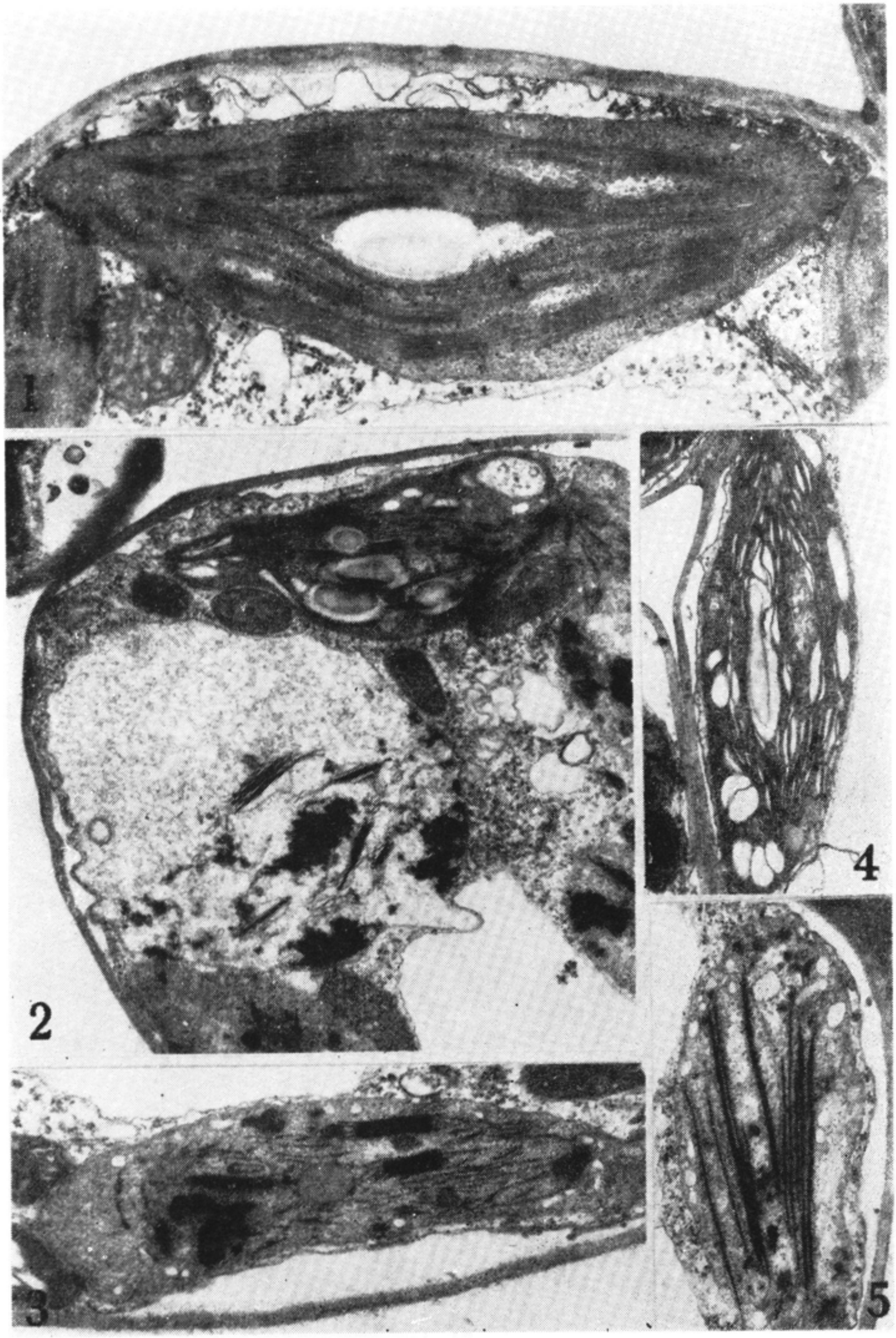
Samples 1 mm in diameter were taken from yellow, yellow-green and green leaves. They were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, at 0°C, for 3 h. After several washings with 0.1 M phosphate buffer the material was postfixed at 0°C with 1% OsO_4 in the same buffer, for 1 h. After dehydration in a graded ethanol series, the samples were prestained with alcoholic uranyl acetate, infiltrated with acetone and then embedded in Epon 812.

Ultrathin sections were cut on an Ultratome LKB, poststained with both uranyl acetate and lead citrate and examined in a Tesla 513 A electron microscope at 80 kV.

RESULTS

Typical, lens-shaped chloroplasts are present in the mesophyll cells of control plants (Fig. 1). Fine-grained, electron-dense stroma contains distinct DNA areas, ribosome groups and starch grains. The internal membrane system is formed of grana and stroma thylakoids. The middle-sized grana, composed of several thylakoids are arranged regularly, parallelly to the longitudinal axis of the plastid. The stroma thylakoids run also regularly, parallelly to the longitudinal axis of the chloroplasts.

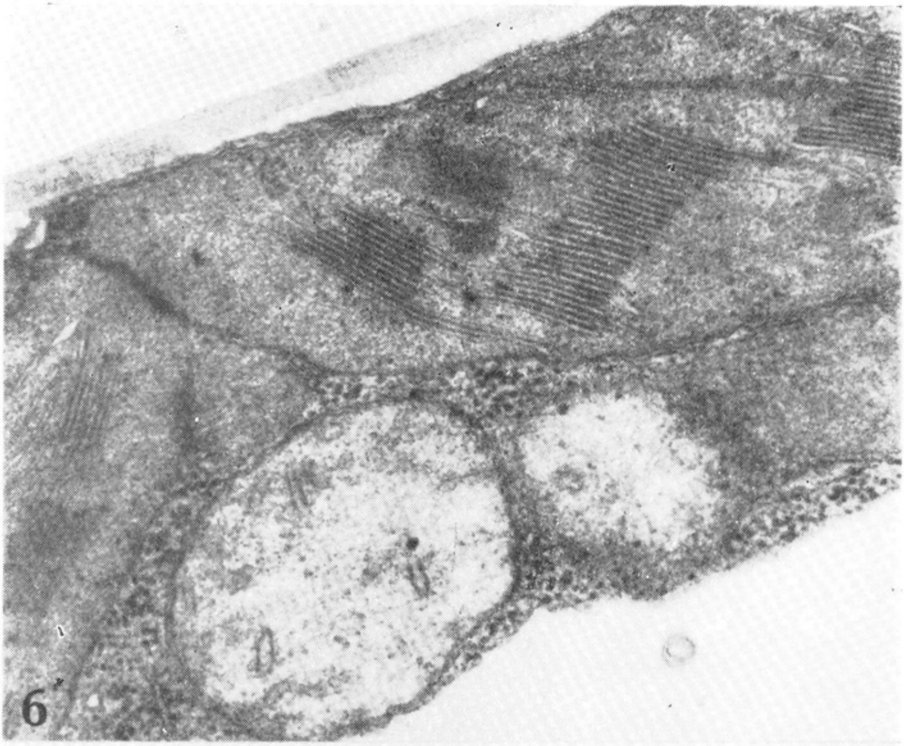
Defective chloroplasts prevail in the mesophyll cells of the yellow mutant leaves. However, aberrant and normal plastids were often present in the same cell (Fig 2). The deformation of chloroplasts is manifested in their shape, size and mainly in the disorganization of their lamellar system. More irregular in outline (Figs. 2, 11, 13), amoeboid forms are also present. Altered chloroplasts can retain a lenticular shape, if their lamellar system is partially preserved.

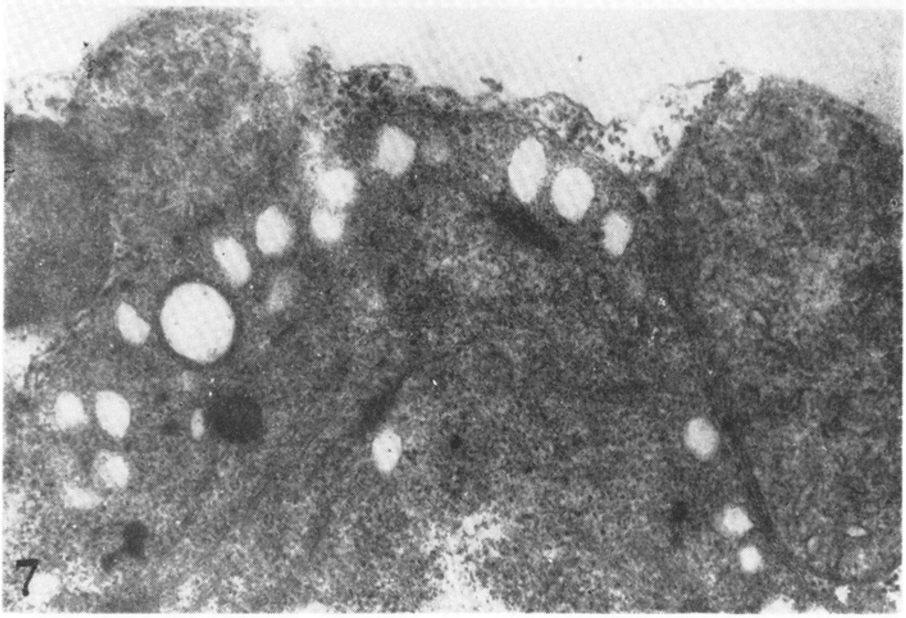


Chloroplasts of cucumber leaves

Fig. 1. Mesophyll chloroplasts of control leaf. × 21 000.

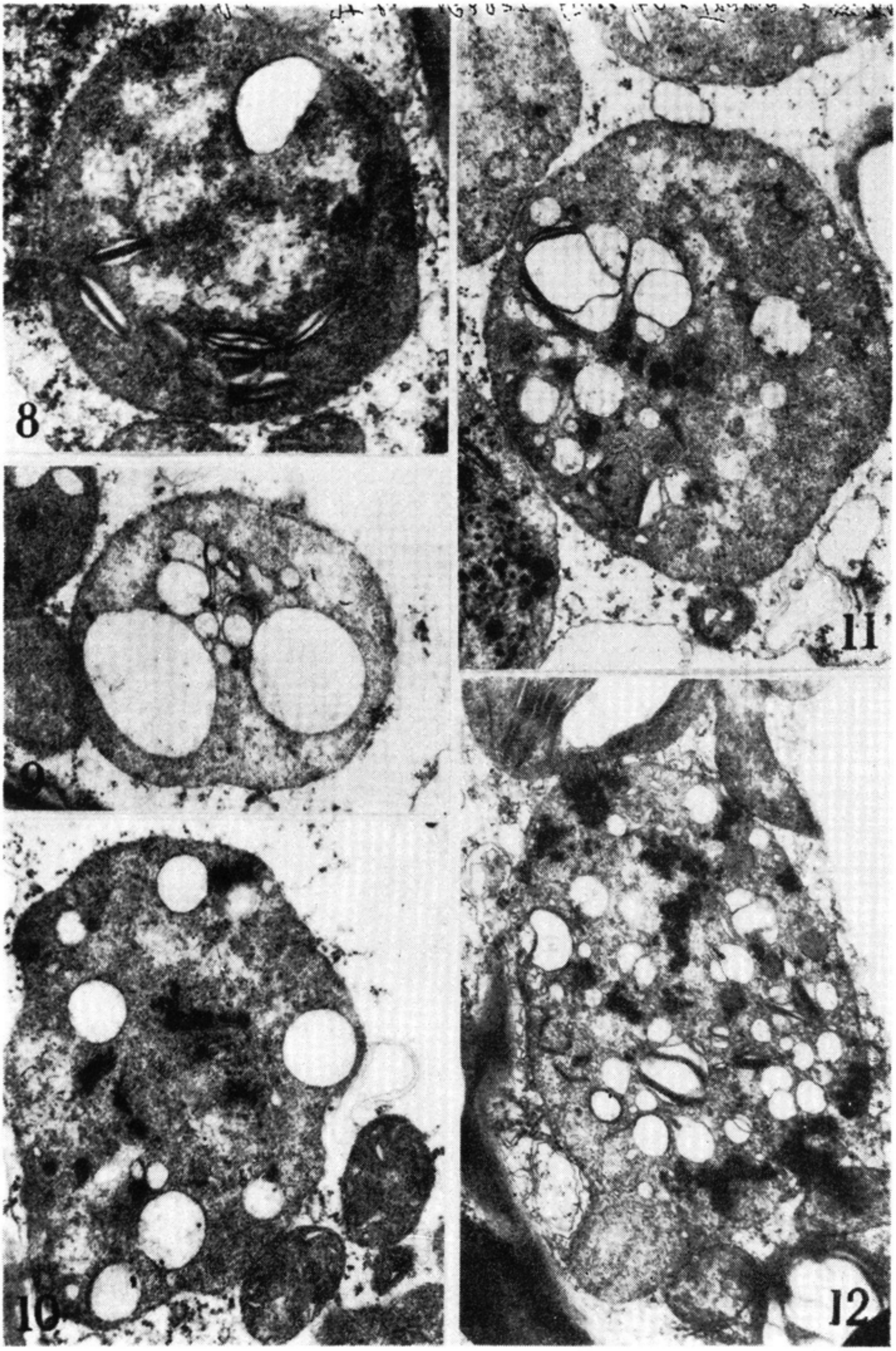
Figs. 2-5. Leaf plastids of yellow mutant. Fig. 2. Heterogeneous plastid population in the same cell. Beside the chloroplast similar to control, plastid extremely altered. × 10 800. Fig. 3. Electron-dense material visible in the stroma. × 10 800. Fig. 4. The first step of thylakoids swelling and formation of numerous vacuoles and vacuole complexes from previous grana. × 14 400. Fig. 5. Flat stacks of electron-dense, long thylakoids. × 14 000





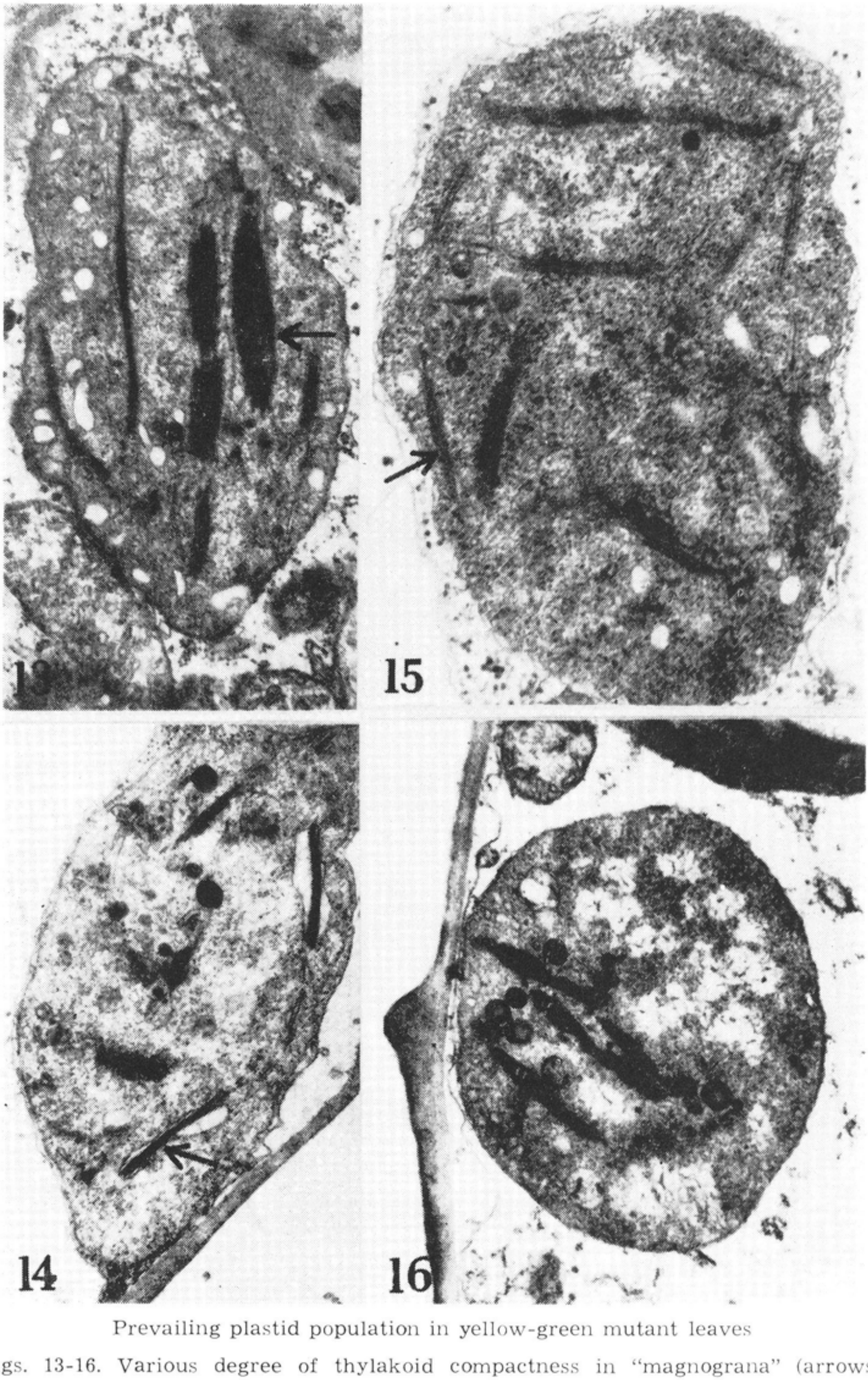
Membrane system in young yellow mutant leaves

Fig. 6. Normal stacks of grana thylakoids, reduced stroma thylakoids. × 32 400.
Fig. 7. Single perforated thylakoids arranged parallelly. Under surrounding membrane numerous vesicles. × 43 200



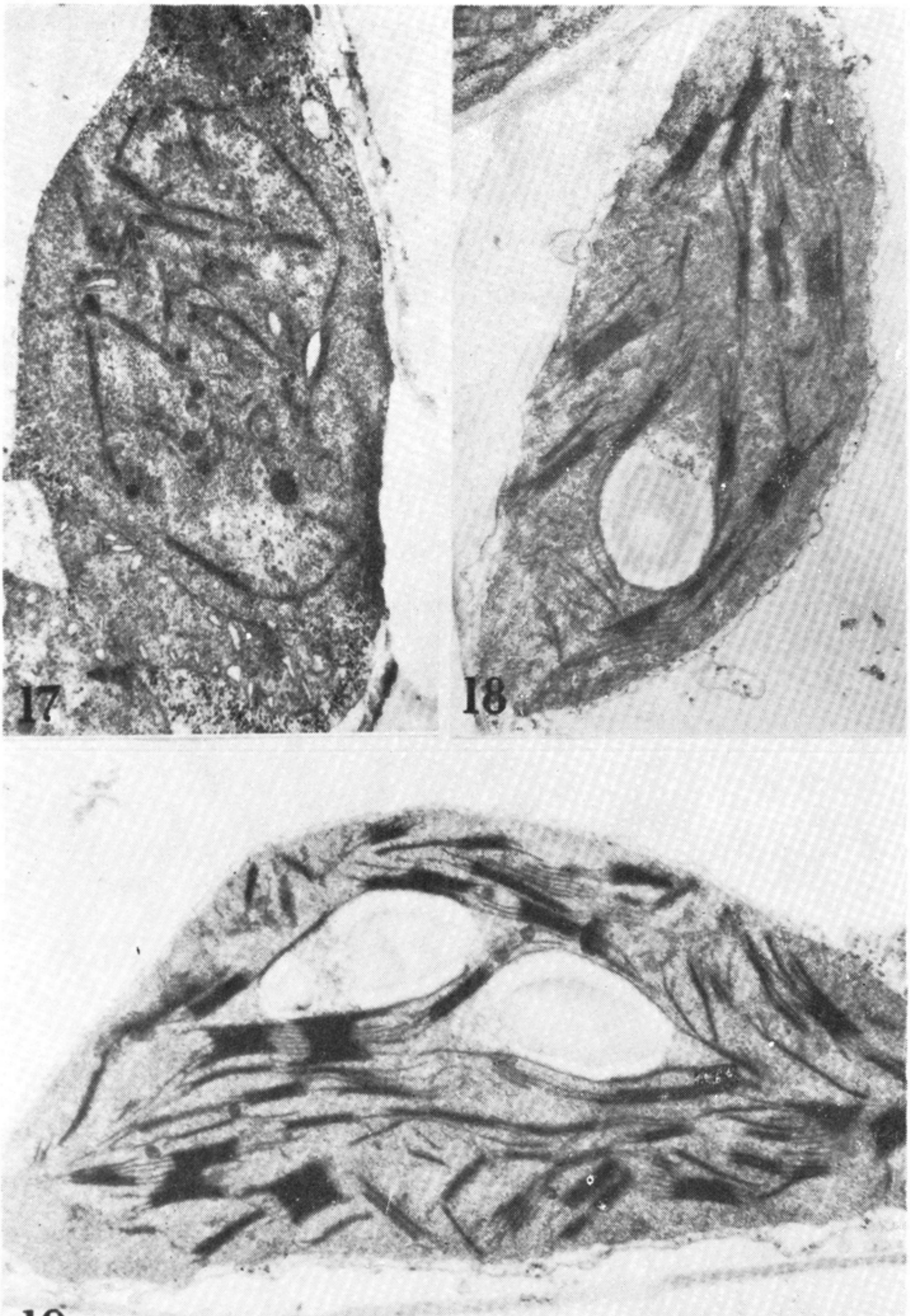
Vacuolisation of plastids in yellow-green mutant leaves

Fig. 8. Swelling outer thylakoids in low grana. × 18 000. Figs. 9, 11. Membranes of swollen thylakoids are interrupted. Groups of vacuoles and great vacuoles are finally formed. Small vesicles are also visible. × 18 000. Figs. 10, 12. Plastids with irregular shapes, caused by the presense of larger vacuoles. Electron-dense material visible in the stroma (10 — × 14 000, 12 — × 14 400)



Prevailing plastid population in yellow-green mutant leaves

Figs. 13-16. Various degree of thylakoid compactness in "magnograna" (arrows). Single thylakoids and low thylakoid stacks of various length, DNA-containing areas, ribosome-like particles, osmiophilic material and globules are visible (13, 14, 16 — × 18 000, 15 — × 27 000)



Reconstruction of thylakoid system in chloroplasts of leaves of mutated plants turning green

Figs. 17-19. Irregular arragement of grana and reduced number of stroma thylakoids as compared with leaf chloroplast of control plants are visible (Fig. 1). × 18 000

The stroma of some observed plastids is electron-transparent (Fig. 2). Large electron-dense bodies, irregularly shaped, composed of a great number of amorphous granules of variable electron density are present in the plastid matrix (Figs. 2, 3, 5, 7). Such an electron-dense material can also be observed in other, below described, chloroplast types.

Derangement of the lameller system in the cucumber defective plastids in mainly manifested by the lack of typical grana and stroma thylakoids.

In young, yellow leaves the plastids with a well developed thylakoid system, forming grana of normal height, may occur. The stroma thylakoids, however, are weakly developed and they may be discontinuous (Fig. 6). The lamellar system in some plastids can occur in the form of single thylakoids of various length, oriented sometimes parallelly (Fig. 7). The presence of numerous vesicles, derived from an internal plastid membrane is evident in the plastids of yellow leaves (Figs. 3, 5, 7). The thylakoids may be arranged in very irregular atypical stacks of various length, sometimes nearly equal to the plastid length (Fig. 5). The stacks are usually flat, composed of tightly aggregated thylakoids. The aggregation is closer than in normal grana, giving a thinner layer between the discs, which may be slightly visible or even invisible (Figs. 3, 5). The lamellae are also more electron-dense. These structures resemble some "magnograna" described in the literature concerning mutants.

A swelling of intra-thylakoidal spaces occurs in some plastids of mesophyll cells of both yellow and yellow-green leaves. The thylakoids of relatively slightly altered grana (Fig. 4) and also of those occurring in the stacks characteristic for the above described mutated plastids are capable of swelling (Figs. 2, 8, 15). Usually only outer thylakoids are swollen. This swelling leads to vacuolisation of the plastids (Figs. 9, 10, 11, 12) and gives them an amoeboid appearance.

The groups of thylakoids in the form of flat stacks, already described in the yellow mutant leaves, occur in the greater part of a plastid population in the greening leaves. These stacks are sometimes very long (Figs. 13, 14, 15, 16). Besides them, single thylakoids (Figs. 14, 15, 16, 17) and two-folded thylakoids (Figs. 14, 15, 17) are visible in the plastid sections.

A great number of DNA areas in some plastids of the mutant as compared with the control ones should be emphasized (Figs. 8, 10, 11, 13). Such areas occur also in the plastids of yellow leaves. The mutant plastids contain ribosome-like particles and osmiophilic globules (Figs. 14, 15).

The stroma of defective plastids was lacking in starch grains, while the control chloroplasts contained sometimes great amounts of starch.

It should be noted that electron-dense material was also present in yellow-green leaf plastids (Figs. 10, 12, 16), similarly as in the yellow ones.

The leaf greening process is accompanied by plastid lamellar system reconstruction and the appearance of starch grains. Chloroplasts become elongated, the stroma contains numerous grana composed of several thylakoids: in addition many short grana, each composed of two-folded thylakoids are seen (Fig. 17). DNA regions, plastoglobules and slight amounts of electron-dense material are present in the stroma as well.

The fully greened leaves of the mutant contained mostly normal plastids. The difference is revealed in an irregular arrangement of grana and in a poor development of stroma thylakoids (Figs. 18, 19). It should be emphasized that grana formation occurred before that of stroma lamellae which are reduced in number. The restituted chloroplasts accumulate starch grains.

DISCUSSION

The *Cucumis sativus* chlorophyll mutant under examination represents a unique type, to the authors knowledge, not recorded in literature. Owing to the greening ability of its initially yellow leaves characteristic for the young part of the plant the mature mutant has yellow, yellow--green and green leaves. Spotted leaves may occur as well. Therefore, as genotype it is characterised by chlorophyll deficiency at the early developmental stage, which is corrected during plant maturation. Thus, this mutant could be classified as virescens but the lack of biochemical data makes difficult its classification to a definite group.

Investigations of the ultrastructure of this mutant have demonstrated the occurrence of a heterogeneous population of defective plastids beside those with normal structure. The distinguished types of plastids can be found in both yellow and yellow-green leaves, the preponderance of a definite plastid type decides of the colour of leaves.

We did not succeed in finding distinct transitional forms in the *Cucumis* mutant plastid restoration, therefore, interpretation of the results is rather difficult. Therefore, a discussion of this type of aberrant plastids structure must include references to studies on other types of plastid mutants.

Comparison of defective cucumber plastids with those derived from the yellow or yellow-green leaves of various mutant types does not show any analogy (Wettstein 1961, Röbbelen 1966, Walles 1967, Andersen et al. 1974). Particular structure elements may be similar as in the case of stacks composed of compactly arranged lamellae, usually of great length, described in the mutant as "magnograna". They are described in tomato as lamellae forming lower and more compact discs (Andersen et al. 1974), large grana-like structures in *Oenothera xantha* (Wettstein 1961, Turischeva et al. 1980), "magnograna" in *Arabidopsis xantha* type (Röbbelen 1966). It should be noted that "magnograna" occur also in otherwise coloured mutants.

Parallel systems of single lamellae described in literature (Crang and Noble 1974), characteristic rather for the alba mutants, are rare in the cucumber. The phenomenon of vesiculation of thylakoids leading to plastid vacuolisation observed in certain groups of cucumber plastids was noticed in numerous mutants (Maclachlan and Zalik 1963, Chollet and Paolillo 1972, Vallane and Vallane 1972, Knoth 1975, Kutzelnigg et al. 1975b, Knoth and Hagemann 1977, Kirk and Tilney-Bassett 1978, Turischeva et al. 1980). It is characteristic particularly in white mutants. The meaning and the mechanism of this process are widely discussed. They are frequently considered as a symptom of plastid degeneration. This phenomenon was also noticed in chlorophyll mutants produced by a recessive nuclear gene (Crang and Noble 1974).

Greening of the cucumber mutant leaves is accompanied by restitution of the lamellar system and accumulation of starch grains. The grana thylakoids and stacks of grana are formed first, then a few stroma thylakoids appear. This information has also been reported for other virescent mutants of maize (Chollet and Paolillo 1972), barley (Jhamb and Zalik 1975) and *Arabidopsis* (Röbbelen 1966).

As regards submicroscopic structure the plastids described in the literature show a varied appearance. In the virescent mutant, with young leaves of yellow (Paolillo and Reighard 1968, Benedict et al. 1970, Chollet and Paolillo 1972) or yellow-green colour (Maclachlan and Zalik 1963), several types of altered plastids occur similarly as in cucumber (Paolillo and Reighard 1968, Jhamb and Zalik 1975).

In the group of single nuclear gene mutants capable of greening, the restituted plastids in fully green leaves, similarly as in the cucumber mutants under examination, do not generally attain the form of the control leaf chloroplasts (Paolillo and Reighard 1968, Jhamb and Zalik 1975). However, the above authors did not elucidate definitively the restitution process at the ultrastructural level.

The presence of electron-dense material in defective cucumber plastids is striking. Some pictures seem to indicate that it constitutes a part of the material of the plastid lamellar system. Its density is often the same as that of "magnograna" characteristic for this mutant. This material can also be seen in sections deprived of "magnograna". However, its amount decreases in the plastids of greening and greened leaves in which a part of normal grana is already formed. Thus, it seems that electron-dense material constitutes a reserve of lamellar building material.

In some papers attention is called to the presence of electron-dense material (Röbbelen 1966, Kutzelnigg et al. 1975a, b, Turischeva et al. 1980). Electron-dense material was found by Kutzelnigg et al. (1975a, b) in numerous *Oenothera* mutants. Contrary to our observations he did not find any spatial connection of this material with thylakoids. He supposes that this material is of protein character, and might be used for thylakoid building. He is ready to consider the presence of such a kind of inclusions as characteristic for plastom mutants but not for nuclear mutants. Turischeva et al. (1980) investigating plastid morphogenesis in the revertant plastom mutant of *Lycopersicon* found electron-dense material in all the green spots both in plastids deprived of the lamellar system and plastids containing this system at various developmental stages.

According to Kutzelnigg et al. (1975a, b) the electron-dense inclusions are an expression of definite functional injury which will be eliminated in the course of development.

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Ultrastruktura chloroplastów liści chlorofilowego mutanta Cucumis sativus

Streszczenie

Rozwijające się i młode liście chlorofilowego mutanta *Cucumis sativus* są żółte, dojrzałe stają się zielone i nie różnią się zabarwieniem od liści roślin kontrolnych. Mezofil liści żółtych zawiera różnorodną populację plastydów o różnym stopniu defektywności, która przejawia się głównie w redukcji lub dezorganizacji typowego systemu tylakoidowego. W stromie zmutowanych plastydów zachowują się obszary DNA, rybosomopodobne cząstki, agregaty elektronowo-gęstego materiału. Brak jest ziaren skrobi. Obserwowano też obok zmutowanych plastydów chloroplasty o normalnej strukturze, jak w roślinach kontrolnych. Procesowi zielenienia liści towarzyszy odbudowa i uporządkowanie wewnętrznego systemu lamelarnego w chloroplastach oraz zdolność akumulowania skrobi. Jednak w chloroplastach mutantów w porównaniu z chloroplastami roślin kontrolnych zauważa się nieregularny układ gran i zredukowaną liczbę tylakoidów międzygranowych. W odtwarzaniu systemu membranowego bierze udział osmofilna substancja zmagazynowana w stromie zmutowanych plastydów oraz pęcherzyki utworzone z wewnętrznej membrany plastydów.