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ULTRASTRUCTURAL CHANGES OF PLASTIDS DURING THE YELLOWING OF THE FRUIT OF *CUCURBITA PEPO* VAR. *PYRIFORMIS*

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Introduction

A mature fruit of *Cucurbita pepo* var. *pyriformis* has two differently coloured parts. The proximal part of the fruit is yellow throughout the whole development of the fruit. In this part the proplastids develop directly into chromoplasts (Ljubešić 1970a, 1970b). The distal part of the fruit is of an intense green colour. When this green part becomes older the chlorophyll disappears, so that an old fruit becomes uniformly yellow. This yellowing is the direct consequence of the chloroplast → chromoplast transformation.

Ultrastructural changes in plastids during the process of yellowing have been the subject of a number of investigations. Grilli (1965a, 1965b, 1965c), Matienco (1964, 1969), Devidé (1970a, 1970b) used in their studies fruits of different varieties of the genus *Cucurbita*. Studies of this process are more numerous on fruits of other species (Thomson 1966, Rosso 1968, Spurr and Harris 1968, Harris and Spurr 1969a, 1969b, Harris 1970 etc.). All these investigations indicate that the process of the chloroplast → chromoplast transformation is very complex. Nevertheless, this process has not been clarified completely.

In this study an attempt has been made to investigate with greater accuracy the patterns of thylakoid-system disintegration and formation of chromoplasts of the globular type.

Material and Methods

Outer cell layers (subepidermis) of the green part of the mature fruits of *Cucurbita pepo* var. *pyriformis* were used. The plants were grown in garden conditions. Samples for investigations were taken sev-

eral times, at certain intervals (from the ripening till the decay of the fruits). This period lasted for about 3 months. The material was studied by light and electron microscopy.

For the electron microscopic investigations the material was fixed in 0.5% glutaraldehyde at pH 7.2 in cacodylate buffer for about 30 minutes. After fixation the material was washed for 1—2 hours in cacodylate buffer and postfixed in 1% OsO_4 for 2 hours. The material was dehydrated in ascending series of ethanol. All these operations were performed at a temperature of + 1° C. Finally the material was embedded in araldite. The sections were cut with glass knives on Reichert Om U2 ultramikrotome, stained with uranyl acetate and lead citrate, and observed in a Siemens Elmiskope I (at the Institute of Biology, University of Zagreb).

Results

There are numerous normal chloroplasts (Fig. 1) in the outer cell layers of the green part of the mature fruit. The thylakoid-system has the structure as in typical chloroplasts. The grana-stacks contain 3—60 thylakoids. The width of the grana (in the sections) is about 1 μm. The plastid stroma is dense and very rich in ribosomes. A number of plastoglobules with a diameter of 50 nm, which show a low osmiophilie, is situated between the grana.

The first changes of the ultrastructure can be observed in a small number of chloroplasts soon after the ripening of the fruit. The green colour of the fruit remains unchanged. The grana-stacks double their diameter (to about 2 μm) (Fig. 2). In such plastids there are 30—80 thylakoids per granum. There have been no other changes in the structures of the plastids.

About 1—2 months after the ripening of the fruits, the green part begins slowly to lose its green colour and during the next month it becomes completely yellow.

The process of chloroplast → chromoplast transformation is relatively slow and takes about a month. This phenomenon was convenient for our purposes because all the stages of the process could be carefully examined in the electron microscope.

The principle of disintegration of thylakoid systems, which is not a uniform process, is most important. It is a complex of numerous processes taking place at the same time.

The result of all these processes are chromoplasts without thylakoids but with big and numerous plastoglobules.

Generally, within one cell there is only one pattern of thylakoid disintegration. Sometimes, however, it is possible to observe several patterns of thylakoid disintegration within one and the same cell, or even in a single plastid. It is sometimes difficult or even impossible to determine whether there is only one pattern of thylakoid disintegration or several ones at work within a plastid at the same time.

All the patterns of thylakoid disintegration can be divided into two main types. The first type includes a direct thylakoid disintegration.

Contrary to this type the second one is more complex. Simultaneously with the thylakoid disintegration new unusual structures appear, which are later all disintegrated.

Inside most plastids, the thylakoid-system disintegrates directly. The disintegration of the thylakoid system starts with a disruption of the grana-stacks to smaller ones (about 15 grana-thylakoids) (Fig. 3), which are rather unusual. They contain thylakoids of various lengths, the central thylakoid of the granum being the longest one. The length of the other thylakoids decreases gradually from the centre to the border of the granum. This shows that the disintegration of grana-thylakoids begins on the border of the granum. Among the small grana several single thylakoids have been found, bent in different directions. The thylakoids seem to be connected by special characteristic bridge-like connections (Fig. 3). The stroma of these plastides is usually of low electron density, and does not contain any ribosomes.

In the second type of thylakoid disintegration, the original thylakoid--system degrades and seems to transform simultaneously into the new one. This is characterized by long bundles of thylakoids, tubular complexes and long single thylakoids.

The appearance of the very long bundles of thylakoids is common (Fig. 5). Their length is 2—5 μm. The number of thylakoids in such bundles does not exceed 20. During the senescence of the fruit the thylakoids become closely packed together, so that no longitudinal stration can be observed. In cross section this structure shows characteristic, very fine cross-striation with a periodical distance of 12—14 nm (Fig. 6 and 9).

The so-called tubular complex (Newcomb 1967) has been found in the plastids with long thylakoidal bundles (Fig. 6). The number and size of the tubular complexes vary to a great extent. There is usually only one larger tubular complex per plastid. Sometimes the tubular complexes present can be more numerous, but then they are smaller.

The long (sometimes swollen) thylakoids protrude from the tubular complex. These thylakoids do not form a granum-like structure (Fig. 6). Sometimes it is possible to find plastids containing only such thylakoids (Fig. 8). The connections between these thylakoids are of great interest. In cross sections these connections appear as two small triangles. The bases of the triangles lie on the thylakoids while the tops of triangle meet (Fig. 7). Sometimes the ends of the swollen thylakoids are enlarged (in section this structure is funnel-shaped) (Fig. 7).

In a small number of plastids thylakoids are rebuilt in the shape of concentric circles, semicircles or ellipses (Fig. 4). In such a thylakoid--system there is never an evident process of formation of closely packed thylakoidal bundles.

At later stages of fruit ageing all these structures (closely packed bundles of thylakoids, tubular complexes and single long thylakoids) disintegrate gradually and numerous plastoglobules are formed.

At the last stage of yellowing of the fruit, the largest part of the chromoplasts contains only plastoglobules (0.3 μm diameter, Fig. 10). Among the plastoglobules there are only a few small fragments of thylakoids. Sometimes there is a very large vacuole in chromoplasts. The origin, the function and the contents of these vacuoles are unknown. The stroma of chromoplasts is of normal density and in a few cases contains ribosomes.

Fig. 1.—10. *Cucurbita pepo* var. *pyriformis*. Plastids from the outer cell layers of the green part of fruit:

Sl. 1.—10. *Cucurbita pepo* var. *pyriformis*. Plastidi iz subepiderme zelenog dijela ploda.

Fig. 1. Parts of normal chloroplasts from the mature fruit. 48,000 : 1.

Sl. 1. Dijelovi normalnih kloroplasta zrelog ploda. 48 000 : 1.

Fig. 2. Part of the plastid from mature fruit. Large grana-stacks are present. 48,000 : 1.

Sl. 2. Dio plastida iz zrelog ploda. Promjer grana je povećan. 48 000 : 1.

Fig. 3.—9. Plastids from the fruits during the process of yellowing of the green part.

Sl. 3.—9. Plastidi za vrijeme procesa žućenja zelenog dijela ploda.

Fig. 3. Part of the plastid with small and partially disintegrated grana. There are single, bent thylakoids in contact with bridge-like connections (arrows) among these grana. 42,000 : 1.

Sl. 3. Dio plastida s malim i djelomično razgrađenim granama između kojih se nalaze pojedinačni i svinuti tilakoidi. Ovi tilakoidi su međusobno povezani karakterističnim mostovima (strelice). 42 000 : 1.

Fig. 4. Plastids with numerous long thylakoids. 25,000 : 1.

Sl. 4. Plastidi s brojnim dugim tilakoidima. 25 000 : 1.

Fig. 5. Bundles of long thylakoids. 76,000 : 1.

Sl. 5. Dugi svežnjevi tilakoida. 76 000 : 1.

Fig. 6. Part of plastid with tubular complex and bundles of closely packed long thylakoids. 52,000 : 1.

Sl. 6. Dio plastida s klupkom tubula i dugim svežnjem slijepljenih tilakoida. 52 000 : 1.

Fig. 7. Part of the plastid with swollen thylakoids. The ends of thylakoids are funnel-shaped dilated. The thylakoids are in contact with special triangular connections in certain places (arrows). 35,000 : 1.

Sl. 7. Dio plastida s nabubrenim tilakoidima. Krajevi tilakoida su lijevkasto prošireni. Na nekim mjestima tilakoidi su međusobno povezani s karakterističnim trokutastim vezama (strelice). 35 000 : 1.

Fig. 8. Plastid with swollen single thylakoids. Among them big plastoglobules are present. 18,000 : 1.

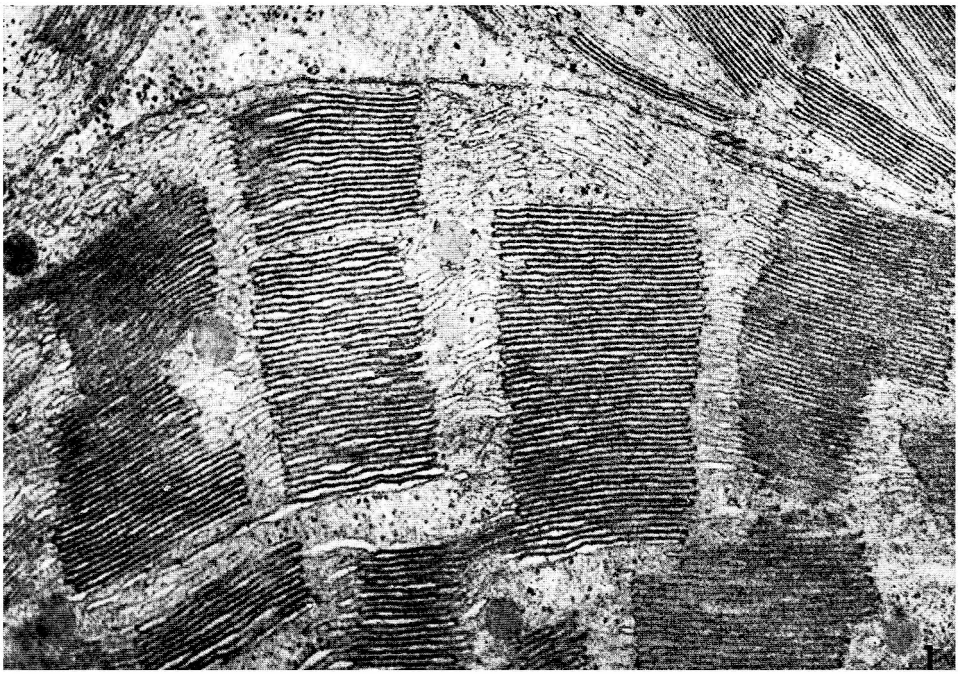
Sl. 8. Plastid s nabubrenim tilakoidima između kojih se nalaze veliki plastoglobuli. 18 000 : 1.

Fig. 9. Detail from Fig. 6. Part of closely packed thylakoids with fine cross-striation. 112,000 : 1.

Sl. 9. Detalj sl. 6. Dio svežnja slijepljenih tilakoida s finom poprečnom prugavošću. 112 000 : 1.

Fig. 10. Chromoplasts from a very old yellow fruit. Only a few short thylakoids among plastoglobules are present. A larg part of chromoplasts is filled with vacuoles. 22,000 : 1.

Sl. 10. Kromoplasti iz vrlo starog žutog ploda. Između brojnih plastoglobula nalazi se nekoliko ostataka tilakoida. Veliki dio kromoplasta ispunjava vakuola. 22 000 : 1.



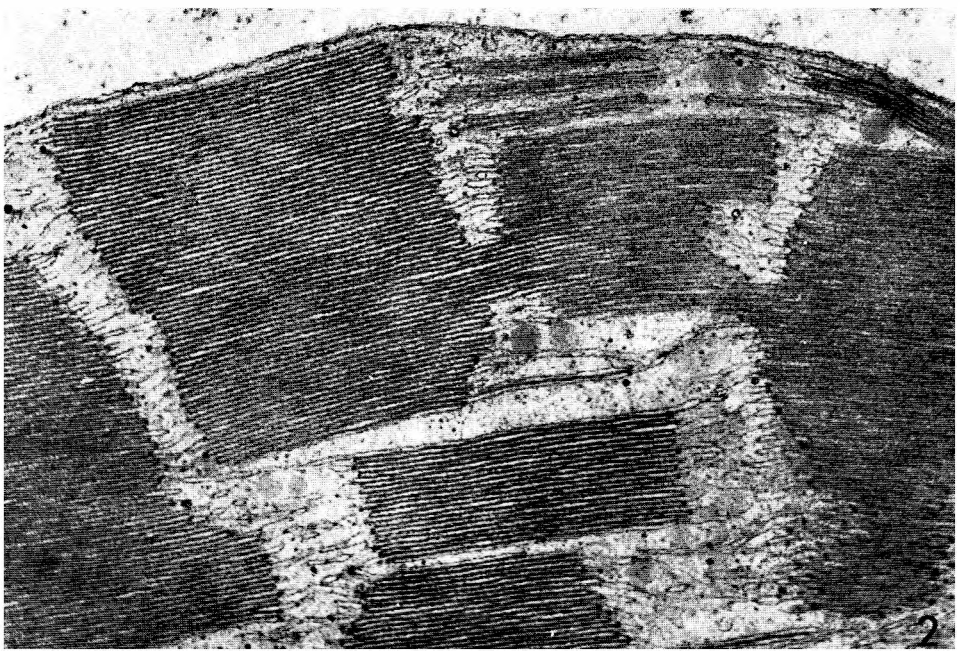
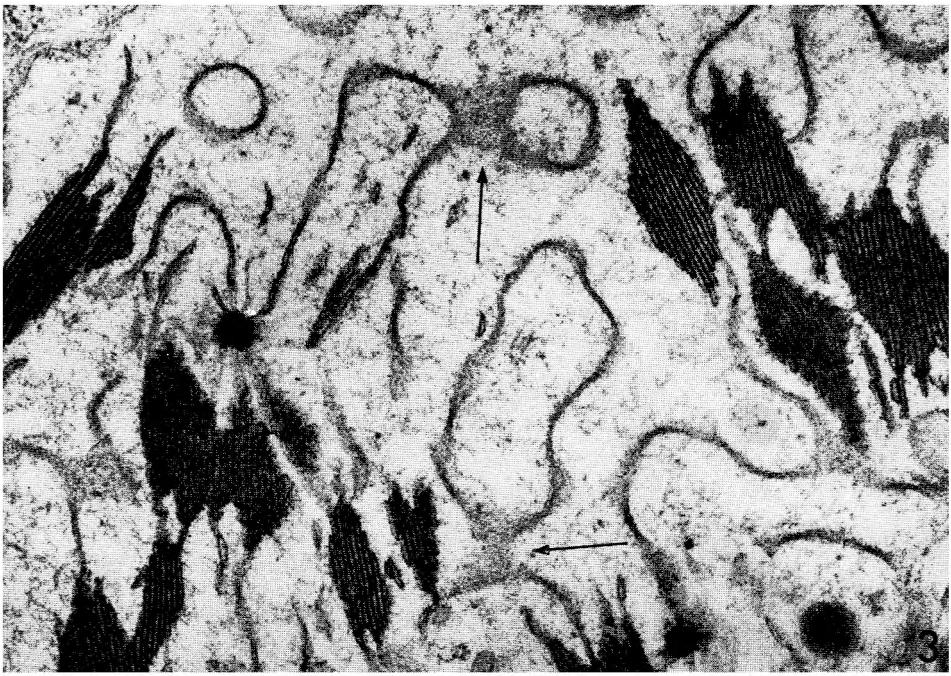


Fig. 1—2.



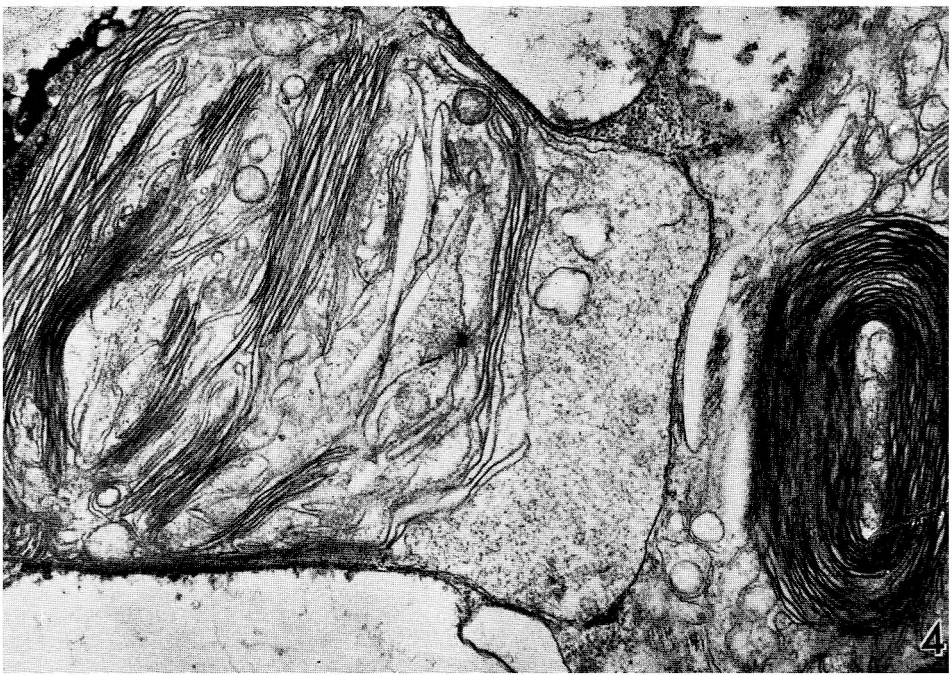
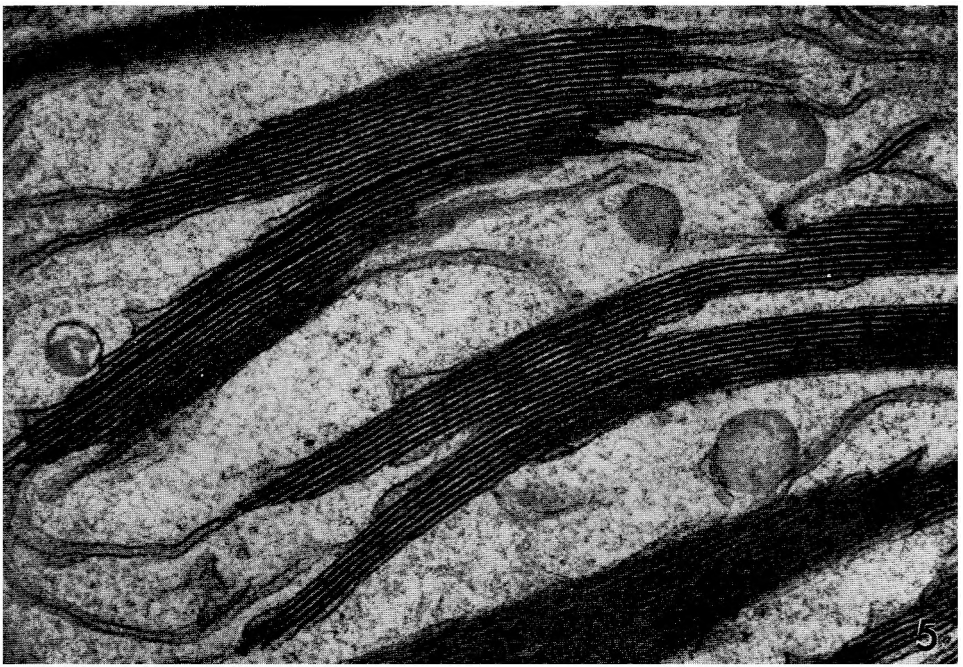


Fig. 3—4.



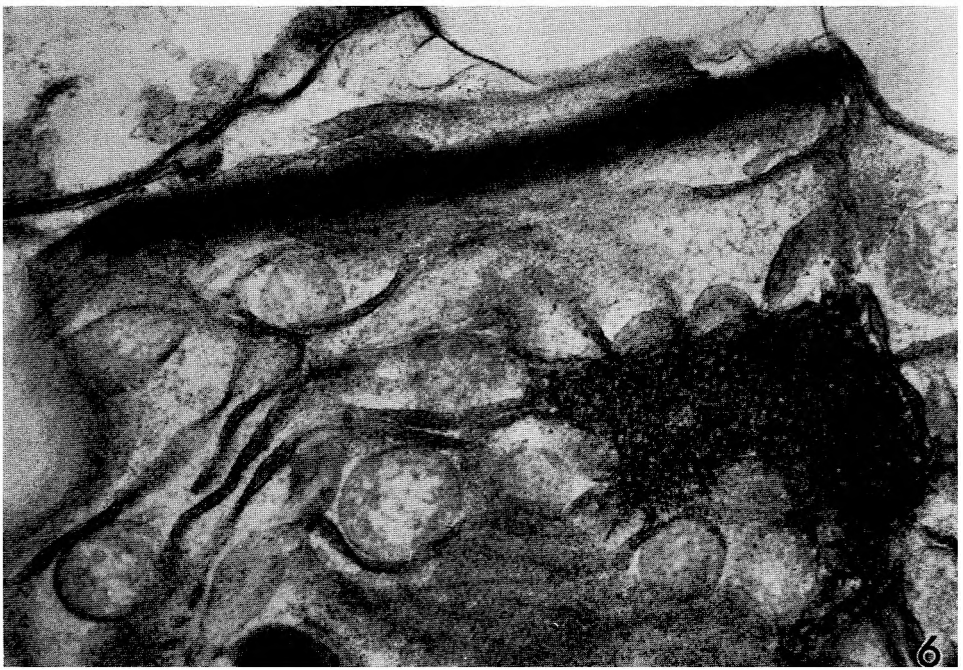


Fig. 5—6.

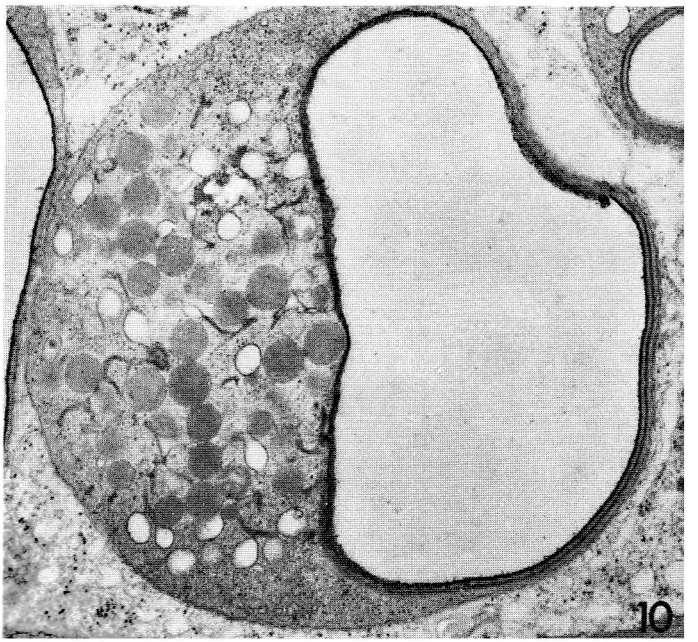


Fig. 7—10.

Discussion

The first type of grana-disintegration (direct disintegration pattern) has recently been studied by Spurr and Harris (1968). The transformation process of chloroplasts into chromoplasts is not present in a typical form. Spurr and Harris (1968) have found that grana disintegrate as a whole. In the present study a gradual grana-disintegration, i.e. from the border to the centre was observed in plastids of *Cucurbita pepo* var. *pyriformis*.

The second pattern of thylakoidal disintegration (indirect disintegration pattern) is more interesting than the first one. A different process of rebuilding the new form of thylakoids takes place during the thylakoid-disintegration.

For the first time long bundles of thylakoids were observed on chlorophyll-defficient plants (Schötz and Diers 1968, Bachmann, Robertson and Bowen 1969 etc.). Later the same structure was studied in plastids during the senescence of pumpkin fruits (Devidé 1970a, 1970b) and in plants treated with amitrol (Vrhovec and Wrischer 1970).

On closely packed bundles of thylakoids Devidé (1970a, 1970b) and Vrhovec and Wrischer (1970) observed fine striation with a periodical distance of about 12 nm in cross section, and hexagonally arranged globular particles (7—8 nm in diameter) in tangential section. The distance between the particles was also 12—13 nm. In plastids of the fruit of *Cucurbita pepo* var. *pyriforms* long bundles of closely packed thylakoids with fine cross-striation were found in cross sections, but no globular particles could be noticed in tangential sections. The existence of this structure in *Cucurbita pepo* var. *pyriformis* should be proved by further investigations.

Tubular complexes (Newcomb 1967) or thylakoid-plexuses (Spurr and Harris 1968) are always present in plastids during the thylakoid-disintegration (Harris and Spurr 1969a, Devidé 1970a, 1970b). Tubular complexes rarely appear in mature chromoplasts (Grilli 1965a, 1965b, 1965c). A three-dimensional view and an explanation of the function of this structure have not been attempted as yet.

In the chromoplasts of the yellow part of *Cucurbita pepo* var. *pyriformis* the so-called "bridge-like connections" have been found (Ljubešić 1970a, 1970b). They seem to be characteristically perforated. In spite of this, bridge-like connections in plastids of the green part (during the yellowing) of the same pumpkin do not show any perforations. Schötz, Diers and Bathelt (1968) have found such connections between the thylakoids in some mutant of *Oenothera*. They have given a model of their three-dimensional view.

The three-dimensional structure of the connections between the long and swollen thylakoids in the shape of triangles (fig. 7) is unknown at present.

Summary

The transformation of chloroplasts to chromoplasts in the green part of the fruit of *Cucurbita pepo* var. *pyriformis* was investigated at the ultrastructural level during the process of yellowing. Two main patterns of thylakoid disintegration have been found.

1. The thylakoidal-system disintegrated and numerous plastoglobules are formed.

2. Grana-thylakoids and stroma-thylakoids gradually disappear and are transformed at the same time into long bundles of thylakoids, tubular complexes and long single thylakoids. Later on these thylakoidal-structures form closely packed bundles of thylakoids. Finally all these structures disintegrate with simultaneous appearance of a great number of plastoglobules.

In both cases the final result of the thylakoid-disintegration is exactly the same, namely chromoplasts of a globular type.

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SADRŽAJ

Praćene su promjene u finoj građi plastida tijekom procesa žućenja zelenog dijela ploda vrste ***Cucurbita pepo*** var. ***pyriformis***. Nađena su dva osnovna tipa razgradnje tilakoidnoga sistema.

1. Tilakoidni sistem plastida direktno se razgrađuje i formiraju se brojni plastoglobuli.

2. Grana-tilakoidi i stroma-tilakoidi postupno se razgrađuju uz istovremeno formiranje novih struktura u obliku dugih snopova tilakoida (koji se tijekom procesa žućenja ploda postupno međusobno sljepljuju), klupka tubula i dugih pojedinačnih tilakoida. Na kraju se sve ove strukture razgrade i formira se veliki broj plastoglobula.

U oba slučaja razviju se kao konačni rezultat kromplasti globularnog tipa.

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