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ULTRASTRUCTURAL CHANGES OF PLASTIDS IN RIPE FRUIT OF *CUCURBITA PEPO* CV. *OVIFERA*

Mit deutscher und kroatischer Zusammefassung
Sa sadržajem na njemačkom i hrvatskom jeziku

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Introduction

Fruits of *Cucurbita pepo* cv. *ovifera* (in further text: *C. p. ovifera)* were used for some special experiments for which purpose they were also examined by electron microscopy. Since the ultrastructure of the fruit cells shows some interesting features they should be briefly reported here.

The fruit of *C. p. ovifera* has on its surface characteristic alternate dark and light meridional zones. While in the ripe fruit the light zones do not change their colour considerably (only from white yellow to yellow) the dark ones as a rule turn from dark green to intense orange after some time. The light microscope shows clearly that the plastids of these two zones are quite different. In the dark zones there are large chloroplasts (with a diameter of about 5 μm) developing to chromoplasts after some time, while in the light zones very small chromoplasts (only 2 μm in diameter) are already present in the ripe fruit. This is the reason why the dark zones of the fruit exactly keep their pattern when turning from green to orange.

Material and Methods

Three and six months stored fruit were used. The outer cell layers of the fruits were fixed in 1% glutaraldehyde, postfixed in 1% OsO_4 and embedded in araldite. The sections, made with a Reichert ultramicrotome Om U 2, were stained with lead citrate (Reynolds 1963) or both with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I.

Results

The epidermis is uniform over all parts of the fruit containing always rudimental plastids with plastoglobules and few single thylakoids.

In the green zones of freshly gathered fruits (or stored at low temperature up to three months) the chloroplasts (Fig. 1) are round shaped and still contain distinct grana. The regularity of the grana layers is however somewhat disturbed and the thylakoids (intrathylakoidal spaces respectively) somewhat dilated (Fig. 2). Between some grana stacks there are groups of plastoglobules of middle contrast (Fig. 1, 2). In the plastid stroma numerous ribosomes are present with the exception of certain regions where the dense homogeneous stroma does not contain any ribosomes. Many tubules protrude from the inner plastid membrane into the stroma.

In the yellow zones of such fruits the plastids are much smaller than in the green ones. They contain a very dense and granular stroma with big groups of plastoglobules. The tubules are abundant, but the thylakoid system is very poorly developed only single thylakoids being present (Fig. 3).

In fruits the green zones of which have already turned orange, the former chloroplasts changed their structure considerably. There still exist some plastids which contain small grana or grana-like structures (Fig. 4), but most of the thylakoids usually lie single in the stroma (Fig. 5, 6, 7). Only sometimes they are packed together to very long, mostly curved stacks resembling large grana (Fig. 6, 7, 8). One or several tubular complexes (Newcomb 1967 = thylakoid plexus Spurr and Harris 1968) are visible in each plastid (Fig. 5). From them thylakoids protrude in different directions. Sometimes stacks of thylakoids are closely packed together and show, in cross sections, a very fine striation with a periodical distance of about 12 nm (Fig. 9).

Plastoglobules are also present (Fig. 4, 6, 7). They are often oval (Fig. 6) and sometimes show very dark parts which interchange with very light ones giving the impression of crystals protruding from plastoglobules (Fig. 10).

In fruits stored for a longer time (e. g. 6 months), especially when stored at room temperature, the green zones turn (with few exceptions) thoroughly orange while the yellow ones remain yellow. In both zones the plastids have now a similar structure. They are round with dark granulated stroma and very light plastoglobules (Fig. 11). In the orange zones of such fruits in addition to that there are characteristic, elongated, needle-like formations bounded by a fine double membrane. Their content shows a very fine, regular striation consisting of thin and straight dark lines lying about 5 nm apart (Fig. 12).

Discussion

In recent literature there are many data about the ultrastructure of chromoplasts. However, as these data refer to diferent plant materials, to different and sometimes rather undefined developmental stages as well as to different techniques (which were considerably changed in the past half decade), it seems very difficult to bring these data to a common denominator. For this reason the discussion of the results obtained should be limited only to a few most obvious findings.

The chloroplasts of the dark zones of the fruit (which are at first dark green and later intensely orange), showing impressive pictures by their dilated thylakoids, abundant stroma and numerous plastoglobules, soon undergo (especially if the fruits are stored at room temperature) along with the change of colour from green to orange, considerable changes in their ultrastructure. The grana become rare and long, single thylakoids appear, often packed together to long stacks with a charateristic striation. Such striated stacks, of exactly the same appearance, have been found by Vrhovec and Wrischer (1970) in chloroplasts of leaves treated by amitrole. These authors were able to identify this striation with a hexagonal arrangement of globular particles of an average diameter of 7 — 8 nm and distance of about 12 — 13 nm. As Vrhovec and Wrischer were succesful in inducing changes of chloroplasts with a simultaneous appearance of microscopically visible carotenoid crystals by amitrole, this treatment may perhaps become an interesting experimental model for studying and understanding the mechanism of chloroplast-chromoplast development.

In the plastids of the dark zone the plastoglobules are mostly of a low contrast (Fig. 4, 6, 7) but some are very dark. The light plastoglobules show little pecularities. They are sometimes spindle-shaped and some authors have found that tubules arise from such globules (Steffen and Walter 1958, Kirk and Juniper 1965/67). This has also been found sometimes during the present study (Fig. 6). The dark plastoglobules, which are also sometimes spindle-shaped, show often protruding, light needle-shaped formations, probably crystals of carotenoids. Such globules have been found also by other authors (e. g. by Harris and Spurr 1969a).

The so-called tubular complexes (Newcomb 1967 = thylakoid plexuses Spurr and Harris 1968) are also present in chromoplasts of the dark zones of the fruit. Harris and Spurr (1969b) stated that these formations appear in the plastids only after the chlorophyll content has been extremely lowered or zero. It is known that tubular complexes also appear in chromoplasts of different plants (Grilli 1965a, b, Spurr and Harris 1968, Ljubešić 1970).

In the yellow zones of the fruits of *C. p. ovifera* there are no noticeable changes during the senescence of the fruit. The only changes are the lowering of the contrast of the plastoglobules and the successive disappearance of the thylakoids.

In the final stage there is however little difference in the electronmicroscopic picture between the orange and yellow zones as in both the chromoplasts contain pastoglobules of very low contrast or perhaps even only cavities which were previously filled with globular substance. The only difference between the chromoplasts of the orange and yellow fruit zones, seems to be the presence of special needle-shaped inclusions, which have been found so far in the plastids of the orange zones only. These formations give an impression of solid structures, as they show staircase-like outlines. These stairs, as well as the remarkable straight striation, indicate that these formations are probably some crystals. The presence of the bounding membrane is not in contradiction with such opinion since crystals of some substances are known to develop in plastids inside a bounding membrane (Newcomb 1967, Harris and Spurr 1969b, Wrischer 1970). The chemical composition of these crystal-like inclusions is at the moment unknown.

Summary

The ripe fruit of *Cucurbita pepo* cv. *ovifera* has characteristic dark and light alternate meridional zones on its surface. The dark zones are at first dark green and turn later intense orange, while the light ones are white yellow and finally yellow.

In the dark zones the chromoplasts develop from large chloroplasts (diameter about 5 μm). During this process the thylakoid system is successively degraded and light as well as dark plastoglobules appear. From several dark plastoglobules crystals (probably carotenoids) are protruding. The thylakoid membranes are eighter single or packed together to dense long layers, some of which show a characteristic striation with a period of 12 — 13 nm.

In light zones of the ripe fruit the chromoplasts are already fully developed. They are small (diameter 2 μm) and contain few single thylakoids, numerous tubules and many ligth plastoglobules.

During the senescence of the fruit the differences in the plastid ultrastructure of both zones gradually disappear and after the disintegration of the thylakoid system only numerous light plastoglobules remain in the stroma. There is a difference however in the presence of needle-shaped crystal-like formations (showing a fine parallel striation and a distinct bounding membrane), which could be found so far in the chromoplasts of the dark zones only.

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ZUSAMMENFASSUNG

VERÄNDERUNGEN IM PLASTIDENFEINBAU DER REIFEN FRÜCHTE VON *CUCURBITA PEPO* CV. *OVIFERA*

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Die reifen Früchte von *Cucurbita pepo* cv. *ovifera* haben an ihrer Oberfläche abwechselnd dunkle und helle meridionale Zonen. Die dunklen Zonen sind zunächst dunkelgrün und werden später tief orange, während die hellen zuerst weiss-gelb, dann aber gelb werden.

In den dunklen Zonen entwickeln sich die Chromoplasten aus grossen Chloroplasten (Durchmesser etwa 5 µm). Während dieses Prozesses wird das Thylakoidsytem allmählich abgebaut und es bilden sich lichte und dunkle Plastoglobuli. Aus einigen der dunklen Plastoglobuli treten Kristalle (vermutlich von Carotinoiden) hervor. Die Thylakoidmembranen kommen entweder vereinzelt vor, oder werden zusammengepresst und verkleben zu langen dicken Schichten, von denen einzelne eine feine Querstreifung zeigen (Periode 12 — 13 nm).

In den hellen Zonen der reifen Frucht sind die Chromoplasten bereits voll entwickelt. Sie sind klein (etwa 2 µm im Durchmesser) und enthalten nur vereinzelte Thylakoide sowie zahlreiche Tubuli und lichte Plastoglobuli.

Während des Alterns der Frucht verschwinden allmählich die Unterschiede im Plastidenfeinbau zwischen den beiden Zonenarten und nach dem Abbau der Thylakoide bleiben im Stroma der Plastiden nur noch zahlreiche lichte Plastoglobuli zurück. Ein Unterschied besteht nur im Vorkommen von nadelförmigen kristallähnlichen Gebilden, die eine feine Längsstreifung sowie eine deutliche Grenzmembran zeigen und bisher nur in den Chromoplasten der dunklen Fruchtzonen gefunden werden konnten.

SADRŽAJ

ULTRASTRUKTURNE PROMJENE U PLASTIDIMA ZRELIH PLODOVA BUNDEVE (*CUCURBITA PEPO* CV. *OVIFERA*)

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Cucurbita pepo cv. *ovifera* ima na površini zrelog ploda karakteristične tamne i svijetle meridionalne zone koje alterniraju. Tamne zone su najprije tamnozelene, a zatim intenzivno narančaste. Svijetle zone su isprva bijeložute, a kasnije žute.

U tamnim zonama kromoplasti se stvaraju iz velikih kloroplasta (promjera 5 µm) kod čega dolazi postepeno do razgradnje tilakoidnog sistema te pojavljivanja tamnih i svijetlih plastoglobula. Iz pojedinih tamnih plastglobula izrastu kristali (vjerojatno karotinoida). Tilakoidne membrane su ili pojedinačne ili se sljepljuju u slojeve lamela od kojih neki pokazuju karakterističnu prugavost s periodom 12 — 13 nm.

U svijetlim zonama postoje u zrelom plodu već razvijeni kromoplasti (promjera svega oko 2 μm) u kojima nalazimo pojedinačne tilakoide, brojne tubule i svijetle plastoglobule.

Dok plod stari, razlike u ultrastrukturi kromoplasta tamnih i svijetlih zona postepeno se gube time što nakon razgradnje tilakoidnog sistema preostaju u gustoj stromi kromoplasta samo još brojni vrlo svijetli globuli. Jedina razlika koja je u tom stadiju mogla dosad biti uočena su tvorevine slične iglastim kristalima (s finim pruganjem i distinktnom ovojnicom), koje su dosad nađene samo u kromoplastima tamnih zona.

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Explanation of figures

Plate I

Fig. 1. Chloroplast of the green zone from a fruit stored 3 months. Grana (g) and groups of plastoglobules (p) are visible. 19 000 : 1.

Fig. 2. The same as in Fig. 1. Detail showing grana with dilated thylakoids. 42 000 : 1.

Fig. 3. Chromoplast from the yellow zone of the same fruit as in Fig. 1 and 2. Numerous tubules (tb), few single thylakoids (↑) and large groups of plastoglobules are visible. 35 000 : 1.

Plate II

Fig. 4. Portion of a yellowing chloroplast with grana. Green zone turning orange from a 3 months stored fruit. 35 000 : 1.

Fig. 5. The same material as in Fig. 4 showing a tubular complex (tc). 25 000 : 1.

Fig. 6. The same material as in Fig. 4 showing a closely packed thylakoid stack (ts), single thylakoids (↑) and plastoglobules (p). 25 000 : 1.

Fig. 7. The same material as in Fig. 4. Plastid showing large curved stacks of thylakoids (ts). 18 000 : 1.

Fig. 8. Detail from Fig. 7. 36 000 : 1.

Plate III

Fig. 9. The same material as in Fig. 4. Closely packed thylakoids showing fine striation. 110 000 : 1.

Fig. 10. The same material as in Fig. 4. Plastid with crystals (↑) which protrude from plastoglobules (p). 26 000 : 1.

Plate IV

Fig. 11. Plastid with very light plastoglobules (p) and dark stroma from the yellow zone of a fruit stored 6 months. 37 000 : 1.

Fig. 12. Striated inclusion in the stroma of a plastid from the orange zone of a fruit stored 6 months. 150 000 : 1.

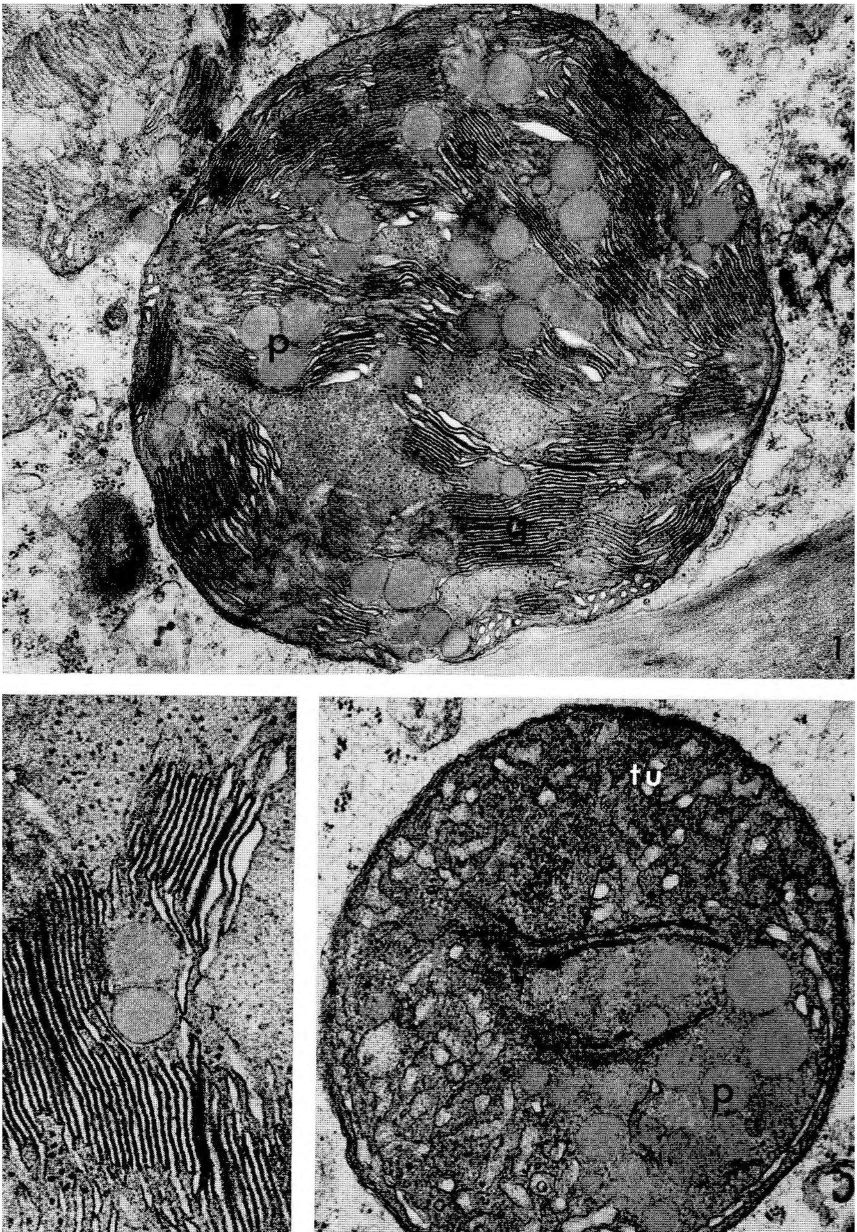

Fig. 1—3.

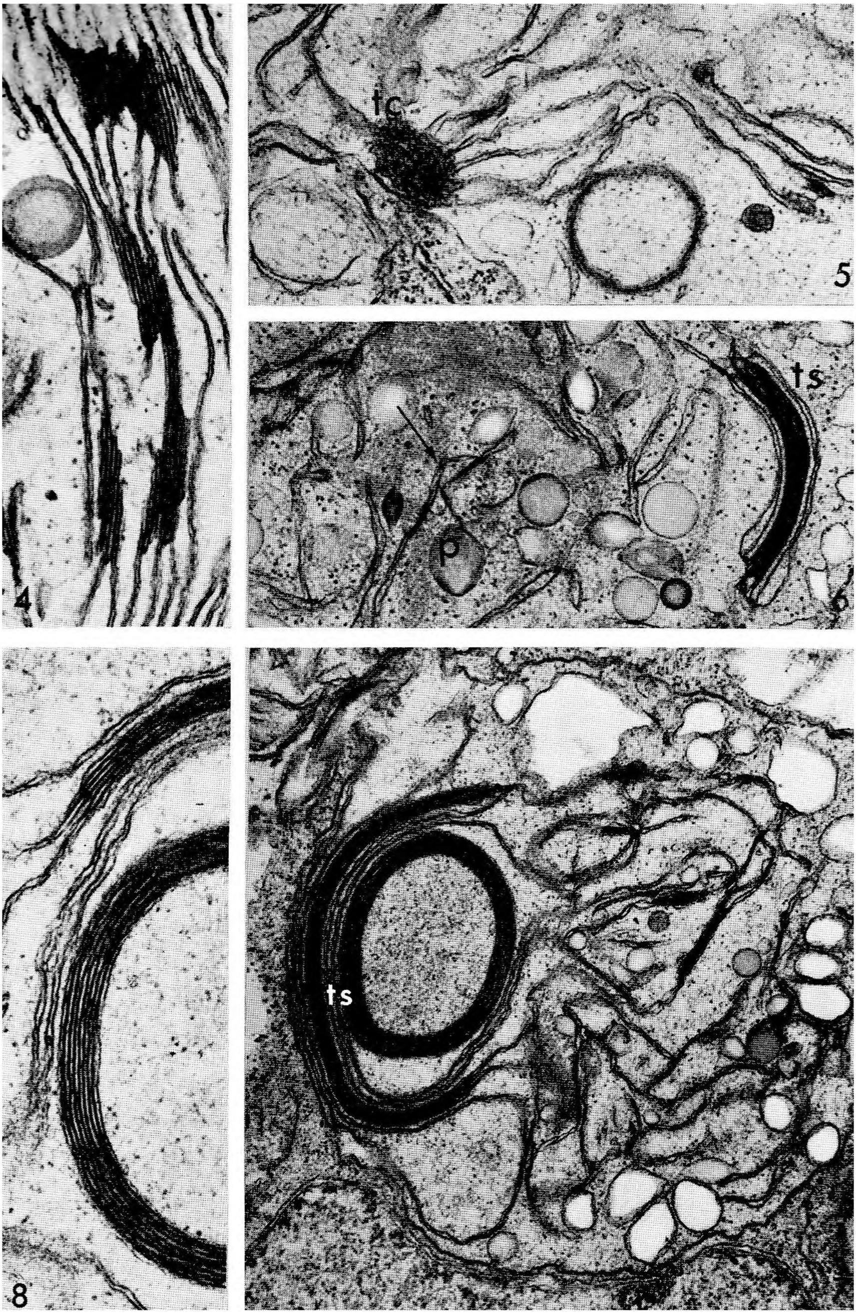

Fig. 4—8.

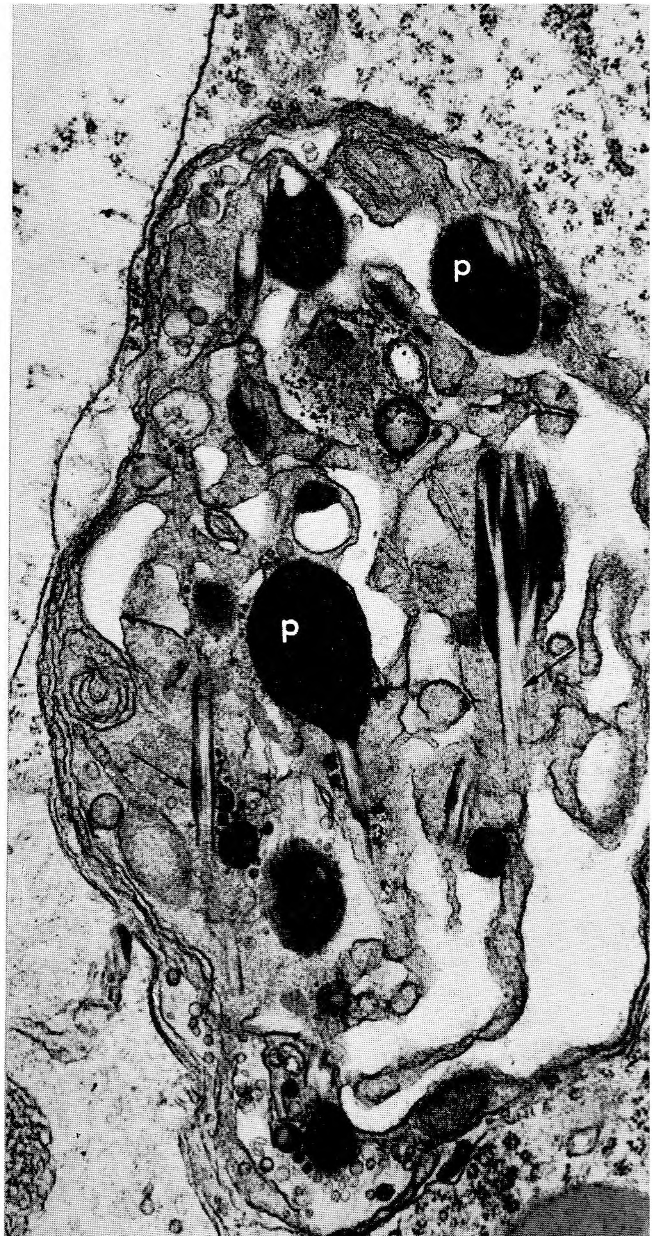

Fig. 9—10.

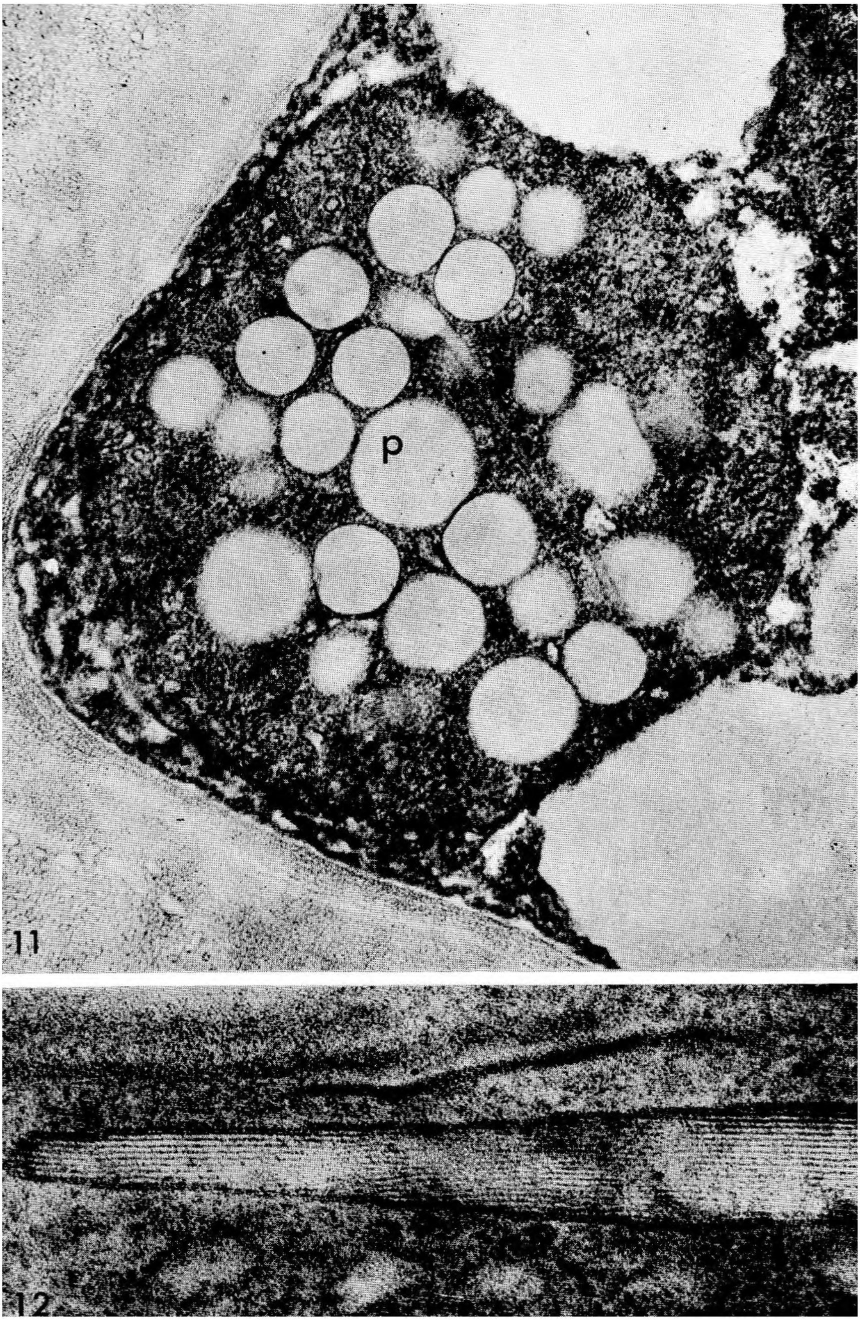

Fig. 11—12.