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TRANSFORMATIONS OF PLASTIDS IN WHITE PUMPKIN FRUITS

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Introduction

The transformation of proplastids into leucoplasts occurs very frequently in the underground parts of the plant. The proplastids might have been transformed into chloroplasts but the light was missing. If these parts were exposed to light, the leucoplasts would synthesize chlorophyll and become chloroplasts (De Rezende-Pinto 1962). Colourless plastids (leucoplasts) occur also in the parts of the plant which are exposed to the sun light but have a genetic defect or are otherwise prevented from synthesizing chlorophyll (Kirk and Tilney-Bassett 1967).

The majority of the fruits of pumpkin species are pigmented, and contain many types of coloured plastids (Devidé 1970a, 1970b, Ljubešić 1970a, 1970b, 1972). However, in the stage of ripening and withering of *Cucurbita pepo* var. *patisson* fruit, colourless plastids are present showing the transformations of proplastids into leucoplasts.

Material and Methods

The plastids of the outer cell layers (subepidermis) of the *Cucurbita pepo* var. *patisson* fruits were investigated. The plants were grown in garden conditions. The samples for investigation were taken at determined intervals, i.e. immediately after the flowering, during the ripening and before decaying of the fruit.

The material was investigated by light and electron microscopy. For electron microscopy the material was fixed in 1% glutaraldehyde, postfixed in 1% OsO_4 and embedded in araldite. The sections were made on Reichert Om U2 ultramicrotome, stained with uranyl acetate and lead citrate and observed in a Siemens Elmiskop I (at the Institute of Biology, University of Zagreb).

The tissue samples taken for microscopy were also tested for pigments. The pigment compounds were examined by means of thin-layer chromatography on silica gel G (Bolliger and König 1969; solvent: petrol ether : ethyl acetate : diethylamine — 58 : 30 : 12).

Results and Discussion

The transformations of plastids in subepidermal cell layers of *Cucurbita pepo* var. *patisson* may be divided into three periods:

1. The Period of Fruit Development. This period begins with flowering and ends with the process of fruit ripening. During this period the fruits are of a pale-green colour. Thin-layer chromatography revealed small amounts of chlorophyll and carotenoids. However, since during the process of growing the fruits enlarge their volume considerably, and new amounts of pigments are not synthesized, the quantity of pigment per unit of fresh weight is diminished. In this period very small and oval plastids can be observed in the light microscope. Their colour is hardly greenish. This greenish colour is possibly a result of diffraction of light on the plastids, and not of presence of chlorophyll. No fluorescence could be noticed in the fluorescence microscope. The plastids show no visible structures. With the ripening of the fruits their volume increases, but the number of plastids per cell and their size do not change.

The presence of a small amount of chlorophyll in young fruits is evident but no chloroplasts could be observed by the electron microscope. In the subepidermis only typical proplastids could be found (Fig. 1). The plastids are oval in shape and about 2 μm in diameter. The stroma is dense and rich with ribosomes. Only a few long single thylakoids

Figs. 1. — 4. *Cucurbita pepo* var. *patisson*. Plastids from the outer cell layers of the fruit:

Sl. 1. — 4. *Cucurbita pepo* var. *patisson*. Plastidi iz subepidermskog sloja ploda:

Fig. 1. Proplastids from young fruits. Big starch grains are present. 40,000 : 1.

Sl. 1. Proplastidi iz mladog ploda. Prisutna su velika škrobna zrnca. 40 000 : 1.

Fig. 2. Leucoplast from the fruit at the beginning of ripening period. Plastoglobules are surrounded by a few long thylakoids. 30,000 : 1.

Sl. 2. Leukoplast iz ploda na početku perioda zriobe. Oko nakupine plastoglobula nalaze se dugi tilakoidi. 30 000 : 1.

Fig. 3. Leucoplast from the ripe fruit. In the central part of the leucoplast there is a large accumulation of plastoglobules. 30,000 : 1.

Sl. 3. Leukoplast zrelog ploda. U srednjem dijelu leukoplasta je velika nakupina plastoglobula. 30 000 : 1.

Fig. 4. Plastid (leucoplast) from the fruit during the decaying period. Only remnants of thylakoids are present. 32,000 : 1.

Sl. 4. Plastid (leukoplast) iz perioda propadanja ploda. Prisutni su samo ostaci tilakoida. 32 000 : 1.

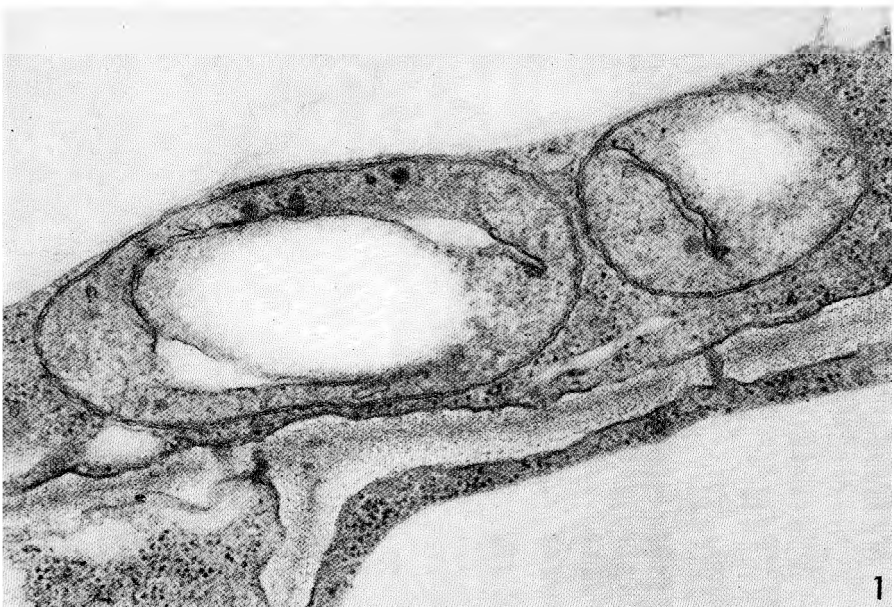
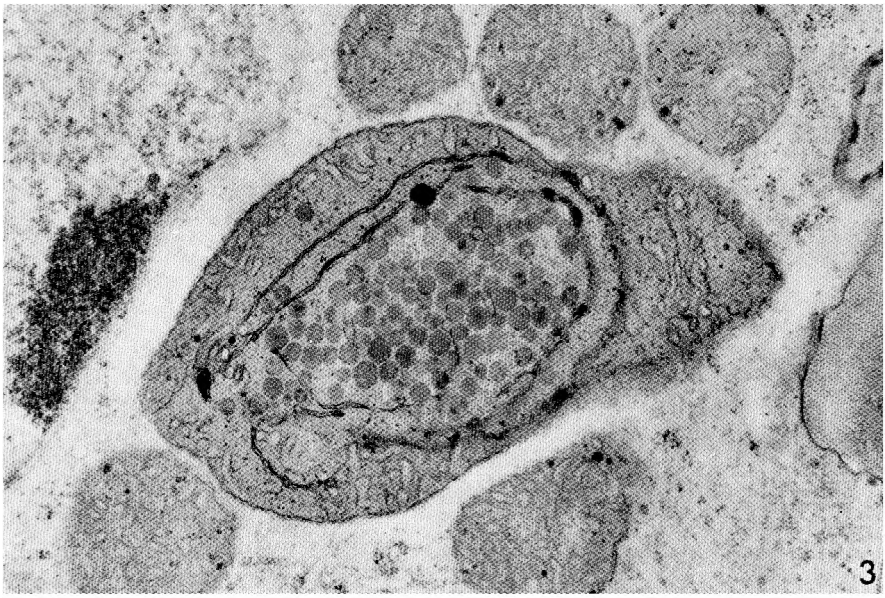


Fig. 1—2. — Sl. 1—2.



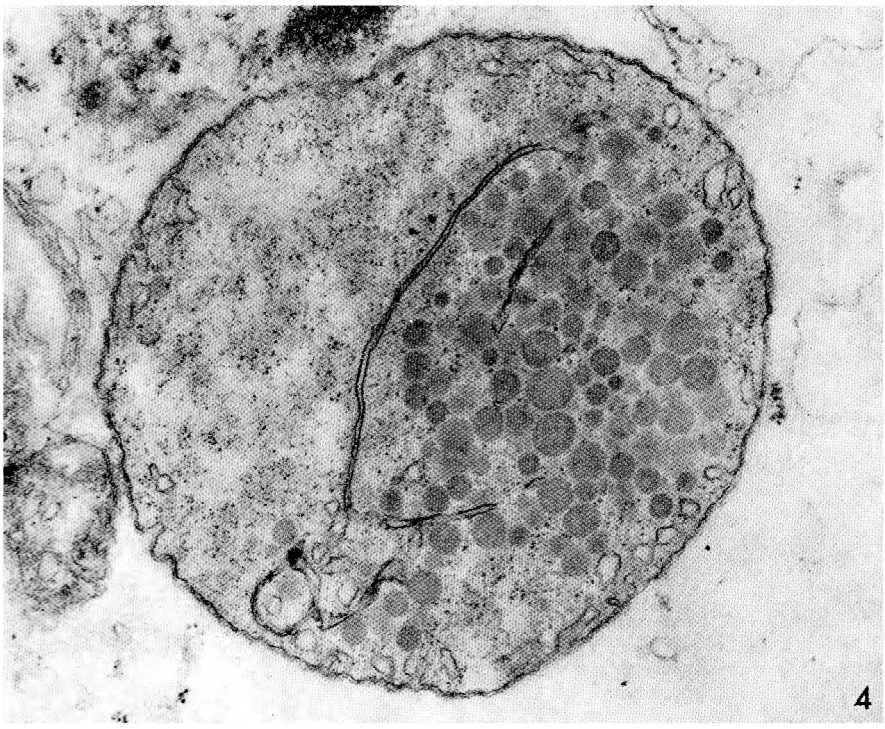


Fig. 3—4. — Sl. 3—4.

are present. Grana have never been found. Usually a big grain of starch is situated in the middle of the plastid. Such plastids are similar to amyloplasts. The plastoglobules are small and scarce.

2. The Period of Ripe Fruit. Ripe fruits are white. It was not possible to prove the presence of chlorophyll by thin-layer chromatography and UV-light. A small amount of carotenoids was found. No changes could be observed in the light microscope. These oval plastids, of about 2 μm diameter, are colourless, and they exist in mature tissue and could be called leucoplasts.

In the electron microscope these plastids (Figs. 2, 3) show a dense stroma rich with ribosomes. The central part of the plastids is filled with numerous plastoglobules (about 100 nm in diameter). The accumulated plastoglobules enlarge in volume parallelly with the ripening of the fruit (Fig. 3). The plastoglobules are surrounded by a few long single thylakoids (Figs. 2, 3). Grana or grana-like structures are not present. The majority of thylakoids is perforated. The thylakoids are dilated and form osmiophilic spots. Along the border of the plastids numerous tubules occur. In fact these tubules are the invaginations of the inner part of the plastid membrane.

There are some difficulties in respect of terminology. The ultrastructure of these leucoplasts is identical with that of chromoplasts of the globular type (Steffen and Walter 1958). In these leucoplasts numerous plastoglobules have a very low osmiophily. According to Lichtenthaler (1970) the low osmiophily of plastoglobules indicates an increased amount of carotenoids. However, these leucoplasts contain only traces of pigments and in spite of their ultrastructural similarity to chromoplasts, they are typical leucoplasts. The presence or absence of the pigments still seems to be a more important criterion for classification of plastids than morphological similarity or dissimilarity.

3. The Decaying Period of the Fruit. Signs of senescence appear in the fruits five months after ripening, and one month later the fruit decays. An increase in the volume of leucoplasts can be observed in the light microscope. The disintegration of the thylakoidal system and the appearance of numerous plastoglobules are visible under the electron microscope (Fig. 4). The remnants of thylakoids in the form of smaller or bigger vesicles are present among the plastoglobules. The composition of pigments is the same as in a ripe fruit. In spite of the evident signs of fruit degeneration (disintegration of the thylakoids), ribosomes are visible in the leucoplast stroma of normal density.

In the last stage of senescence the leucoplasts disintegrate completely. The disintegration of these leucoplasts is identical with that in chromoplasts of other pumpkin varieties (Ljubešić 1970a, 1972).

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Summary

The development of plastids in the subepidermis of fruits of *Cucurbita pepo* var. *patisson* starts with the stage of normal proplastids. In the process of fruit ripening the proplastids are transformed into

fully-grown leucoplasts, which are similar in ultrastructure to the chromoplasts of the globular type. In the process of fruit decaying the leucoplasts are completely disintegrated. The problem of plastid terminology has been touched upon.

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SADRZAJ

PRETVORBE PLASTIDA U BIJELIM PLODOVIMA BUNDEVE

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Razvoj plastida u subepidermi plodova varijeteta *Cucurbita pepo* var. *patisson* započinje sa stadijem normalno građenih proplastida. Sazrijevanjem ploda proplastidi prelaze u odrasle bezbojne plastide — leukoplaste, čija fina građa odgovara onoj globularnog tipa kromoplasta. Starenjem ploda u leukoplastima se razgrade sve membranske tvorevine i dolazi do potpune dezintegracije plastida. Istaknute su teškoće terminologije u tom slučaju.

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