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STRUCTURAL AND FUNCTIONAL CHANGES OF PLASTIDS DURING YELLOWING AND REGREENING OF LEMON FRUITS

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Photosynthetic activity, pigment content and fine structure of plastids in the rind of the lemon fruits have been studied. Lemon fruits very regularly change their colour through seasons — from green into yellow and into green again.

Photosynthetic activity has been detected only in young green and regreened fruits. In yellow fruits, although they contained small amounts of chlorophylls, phyotosynthetic activity was unmeasurable. The electron microscopic examination has shown that the changing of colour of fruits is the result of a typical transformation of chloroplasts into globular chromoplasts and vice versa.

Introduction

Lemon fruits, like most of the citrus, change the colour of their rind through seasons (Caprio 1965). In summer the fruits are green. In autumn the already developed fruits gradually lose chlorophylls and at the end of winter time they become intensely yellow. The following spring and summer they revert to green again. If we leave fruits on the tree, they may become yellow next winter.

This regularity in colour transformation offers a possibility to investigate fine structural and functional changes of plastids, and that is the main topic of this paper.

Material and Methods

The experiments were carried out on lemon fruits growing in garden conditions. During the winter period potted lemon-plants were placed in the room where temperature did not drop lower than 2° C. The samples were taken several times over the experimental period of five years. At the same time the structure of plastids was observed, and the concentration of pigments as well as the photosynthetic activity were measured.

Photosynthetic activity of small pieces of rind (not bigger than 0.5 mm in diameter) was determined by an oxygen electrode as O_2 evolution in aqueous medium (Shimazaki and Sugahara 1979, modified by Antica and Wrischer 1982). The reaction mixture contained 0.1 mol phosphate buffer (pH 8) and 0.01 mol sodium bicarbonate. The measurements were performed under the light of a halogen lamp (100,000 lux).

The pigments were extracted in 80% acetone. Chlorophylls were calculated according to Holden (1965), and total carotenoids according to Davies (1976).

For electron microscopic investigation small pieces of rind were fixed in 1% glutaraldehyde in cacodylate buffer (pH 7.2) at 1° C, postfixed in 1% OsO_4, and after dehydration embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop I.

Results

Lemon plants bloom all year round. The fruits are ready for harvesting after approximately 18 months. The colour of fruits, with the exception of very young ones, which are green, depends on the season. The fruit rind usually begins to change colour from green to yellow in November. Maximum yellow colouring is normally attained in the middle of winter. During the following spring and summer the colour of the fruits reverts to green. Next autumn the process of yellowing starts again.

Although the fruit changes its colour from intensely green to intensely yellow, the highest concentration of total carotenoids (mg/g fr. wt.) is present in young green fruits (Table 1).

Table 1. Total chlorophylls, total carotenoids and photosynthetic activity in fruit rind during yellowing and regreening.

	Total chlorophylls (mg/g fr. wt.)	Total carotenoids (mg/g fr. wt.)	Photosynthetic activity (µmol O_2/g fr. wt.)
Young green fruits	0.733	0.141	37.87
Yellow fruits	0.078	0.096	0
Regreened fruits	0.198	0.060	63.33

The concentration of chlorophylls is the highest in young green fruits (Table 1). During the fruit development the amount of chlorophylls rapidly drops and the yellow lemon (in winter) contains only 10% of chlorophylls found in the young green fruit. The process of regreening reflects the synthesis of chlorophylls. In the regreened fruit the concentration of chlorophylls never reaches that of the young green fruit. However, the maximum of photosynthetic activity does not occur in the young fruit, although there the concentration of chlorophylls is the highest; in regreening fruits it is twotimes higher than in the young ones.

Investigations of the fine structure of plastids indicate the typical transformation of chloroplasts into chromoplasts and vice versa. Young green and undeveloped fruits contain small chloroplasts in the rind cells with normal grana thylakoids and stroma thylakoids. Because of great stroma density it is hardly possible to observe the thylakoids and ribosomes. Starch grains are present in each chloroplast. Plastoglobules are rare and of low osmiophily (Fig. 1). In the process of yellowing the thylakoid system gradually disappears and the formation of structures typical of chromoplasts — big plastoglobules, long single thylakoids and small bundles of cup-shaped thylakoids — begins (Fig. 2). The peripheral reticulum is well developed and fills the greatest part of chromoplasts. Starch grains are sometimes present. The stroma is of normal density containing a lot of ribosomes. Typical grana structures do not exist. Other cell organelles are normal. Identical structures can be found in chromoplasts which originate from the chloroplasts of regreened fruits.

The process of regreening is very slow and it is possible to investigate it in detail. The thylakoids form small grana (Fig. 3). Arrays of ribosomes are often visible along the thylakoids. At the begining, the grana contain thylakoids of various length. Later they assume the typical shape. The number and dimension of the grana increase rapidly and at the end of the regreening process more than half of the chloroplasts are filled with grana. The rest of chloroplasts contains big plastoglobules. The stroma is very dense again, as in the chloroplasts of the young green fruit (Fig. 4).

Discussion

The yellowing and regreening of citrus fruits over the seasons is a well-known phenomenon. Investigations performed on oranges (Caprio 1956) suggest that the weather may be an important factor. According to 28-year observations it was postulated that some correlations existed between the mean temperature at different stages of fruit development and the regreening process. Our investigations, however, have not confirmed this hypothesis. The observation period may not have been long enough (5 years). The direct cause of regreening is not yet known. Maybe it is the growth substances, because the treatment of fruits with potassium gibberellinate induces the regreening in oranges and lemons (Coggins et al. 1960 a, b, Thomson et al. 1967).

Pigment analysis shows a very interesting peculiarity. In the majority of examples of the yellowing process, that is during the transformation of chloroplasts into chromoplasts, a great amount of carotenoids has been synthesized. A similar situation has been observed in fruits and flowers (Simpson et al. 1975, 1977, Ljubešić 1979). In lemon fruits the concentration of carotenoids is the highest in young unripe green fruits and declines gradually during the processes of yellowing and regreening. However, it is evident that through this process the destruction of carotenoids does not occur. Some notable *de novo* synthesis is not possible. At the same time fruits grow very rapidly and enlarge their volume several times. The results of all these processes is a relative decrease in the carotenoid concentration as expressed in mg/g of fresh weight. But in spite of this fact the fruit rind changes from green to yellow. It is possible that carotenoids are masked by chlorophylls in young green fruits, and in the regreened fully developed ones. In the process of yellowing chlorophylls disappear, the carotenoids are not masked any more, and therefore the fruit rind becomes yellow.

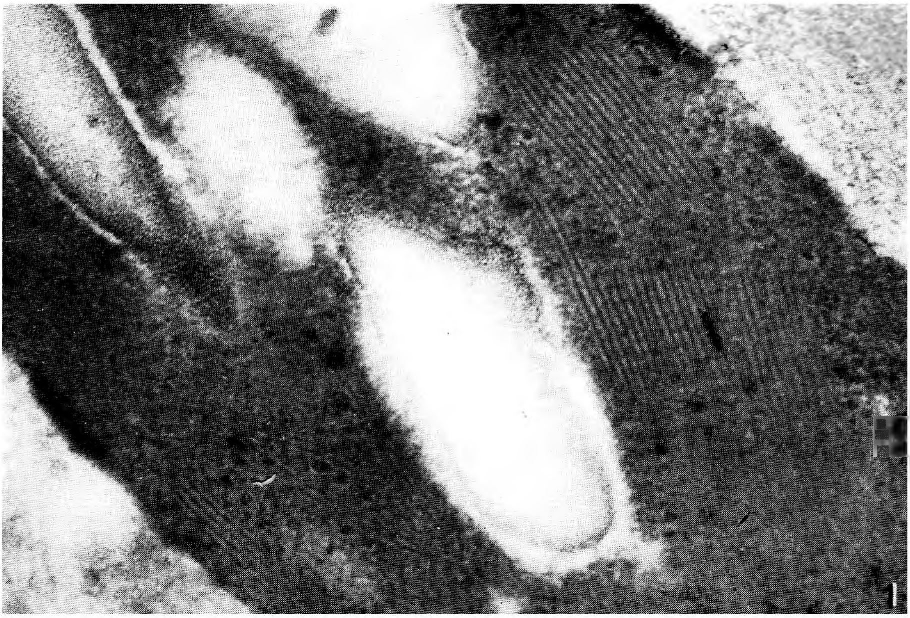
The transformation of chromoplasts to chloroplast through the process of regreening has been described many times (for literature see: Ljubešić 1981), especially in the globular type of chromoplasts (Thomson et al. 1967, Devidé and Ljubešić 1973, Ikeda 1979). The whole process is identical with the processes in orange fruits, as well as in the fruits of some species of the genus *Cucurbita*. However, in the chromoplasts of lemon fruits some thylakoids have been traced. Pigment analysis shows that they contain chlorophylls. Therefore it may be better to use the term chloro-chromoplasts. On the other hand, we are not sure that these thylakoids and chlorophylls are photosynthetically active. The method applied does not offer any proof of this activity. It is expected that further studies using cytochemical methods would solve this problem.

Fig. 1. Part of a chloroplast from the young green fruit. In very dense stroma grana and starch grains are present. 50,000 : 1.

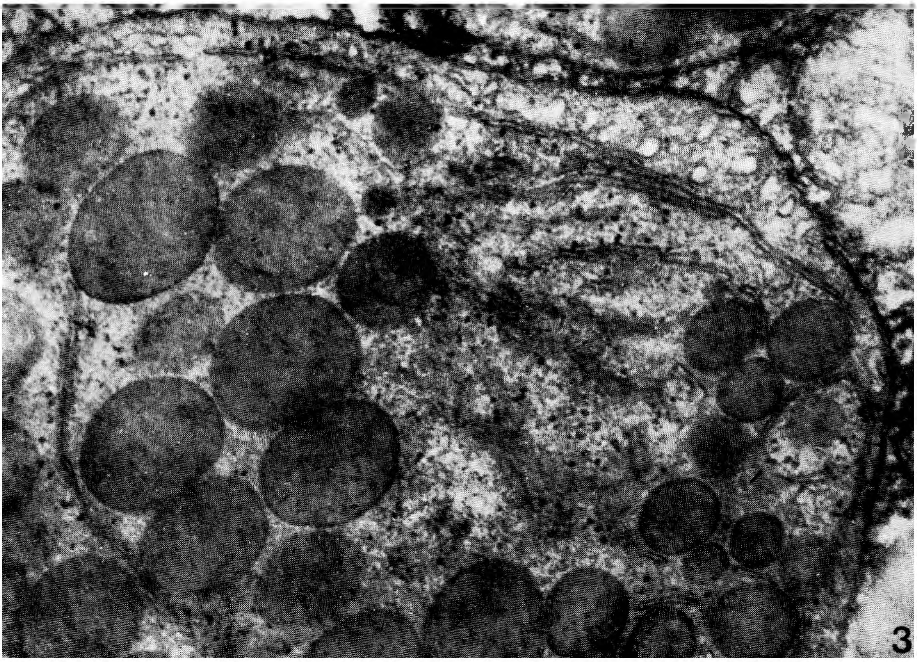
Fig. 2. Chromoplasts from the yellow fruit. Big plastoglobules and starch grains in the middle of the chromoplast. Long single thylakoids and peripheral reticulum are situated on the periphery. 30,000 : 1.

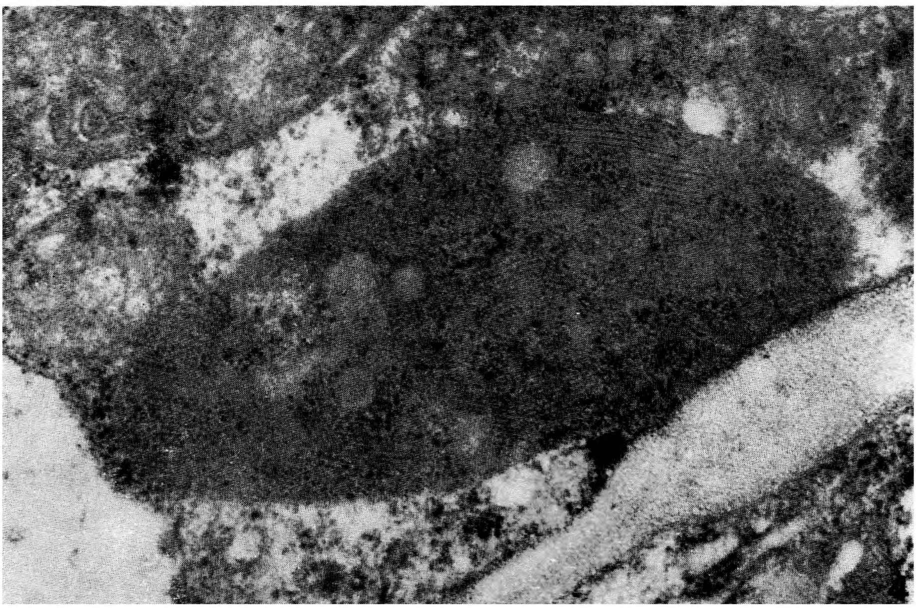
Fig. 3. Part of a chloro-chromoplast with newly formed grana at the beginning of the regreening process. Plastoglobules are big and numerous. Ribosomes form an array along the thylakoids. 52,000 : 1.

Fig. 4. Chloroplast from the regreening fruit. In very dense stroma large grana and big plastoglobules are located. 42,000 : 1.



Figs. 1—2.





Figs. 3—4.

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SAŽETAK

STRUKTURNE I FUNKCIONALNE PROMJENE PLASTIDA TIJEKOM ŽUĆENJA I OZELENJAVANJA PLODOVA LIMUNA

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Istražena je fotosintetska aktivnost, sastav pigmenata i fina građa plastida u kori plodova limuna. Plodovi limuna pravilno mijenjaju boju tijekom godišnjih doba — od zelene u žutu i ponovno u zelenu. Fotosintetska aktivnost nađena je u mladom zelenom plodu i u ozelenjelom plodu. U žutim plodovima, iako sadržavaju manje količine klorofila,

fotosinteza nije dokazana. Elektronsko-mikroskopska istraživanja pokazuju da su promjene u boji plodova rezultat tipične pretvorbe kloroplasta u kromoplaste i obrnuto.

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