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CHROMOPLASTS OF *FORSYTHIA SUSPENSA* (THUNB.) VAHL.

I. ULTRASTRUCTURE AND PIGMENT COMPOSITION

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Introduction

Chromoplasts are the organelles responsible for red, orange or yellow colour of different plant tissues. They are divided into several types according to their structures in which the carotenoid pigments are deposited (reviewed by Sitte 1974). Most chromoplasts of higher plants contain pigments in plastoglobules (globulous type), and a few flower petals and fruits contain chromoplasts rich in tubules (tubulous type).

The chromoplasts in the petals of *Forsythia suspensa* belong to the mixed type, e. g. they contain plastoglobules as well as tubules. This paper deals with some changes of their ultrastructure and pigment composition.

Material and Methods

The buds and flowers of *Forsythia suspensa* (Thunb.) Vahl. were collected from the bushes growing in the gardens of Ruđer Bošković Institute. Samples were taken several times during the developing period of the flowers (January — March).

The material was investigated by light and electron microscopy. For electron microscopy it was fixed in 1% glutaraldehyde and postfixed in 1% OsO_4 (at 1° C). After dehydration the material was embedded in Araldite. The sections were prepared on a Reichert OmU2 ultramicrotome and stained with uranyl acetate and lead citrate. The sections were observed with a Siemens Elmiskop I electron microscope.

For the pigment analysis the samples were cut in small pieces, mixed with a small amount of $BaCO_3$, quartz sand and acetone and ground in a mortar. The pigments were extracted in 85% acetone. The quantitative determination of chlorophyll *a*, chlorophyll *b* and total carotenoids was performed at 663, 644 and 452.5 nm (after Röbbelen 1957, cited by Urbach et al. 1976) on a Perkin Elmer 55 spectrophotometer. For the qualitative analysis of carotenoids the pigment mixtures were separated by thin-layer chromatography. The pigment solution was streaked onto Silica gel G thin-layer plates and developed in the mixture of petrol ether : ethyl acetate : diethylamine (58 : 30 : 12) or acetone : petrol ether (30 : 70) (Stahl 1969). The bands which contained carotenoids were eluted by acetone. Evaporated pigments were stored in refrigerator before the measurement. The criteria for individual pigment identification were: the determination of the R_f value on thin-layer plates and the position of absorption maxima in n-hexan and chloroform (absorption was measured between 350 and 500 nm with a Beckman DBGT spectrophotometer (Hager und Meyer-Bertenrath 1967).

Results

The process of flower development of *Forsythia suspensa* took about 3 weeks. The opening of buds lasted about a week. During this period the buds which contained only 2 mm long petals were constantly increasing. During the second week the buds were beginning to open. Petals were growing rapidy and in a few days reached 3 cm. At the same time the colour of petals turned at first green-yellow and afterwards golden-yellow. A week later the flowers began to wither and died in one or two days. It is possible to perform the process of flowering in experimental conditions. One year old branches immersed in tap water and kept in artificial light at about 15°C blossomed exactly in the same way as those under natural conditions.

Figs. 1.—6.

Plastids from the petals of *Forsythia suspensa* during the process of flower developing.

Fig. 1. Young chloroplast from the bud. Small grana and big starch grains are present. 39,000 : 1.

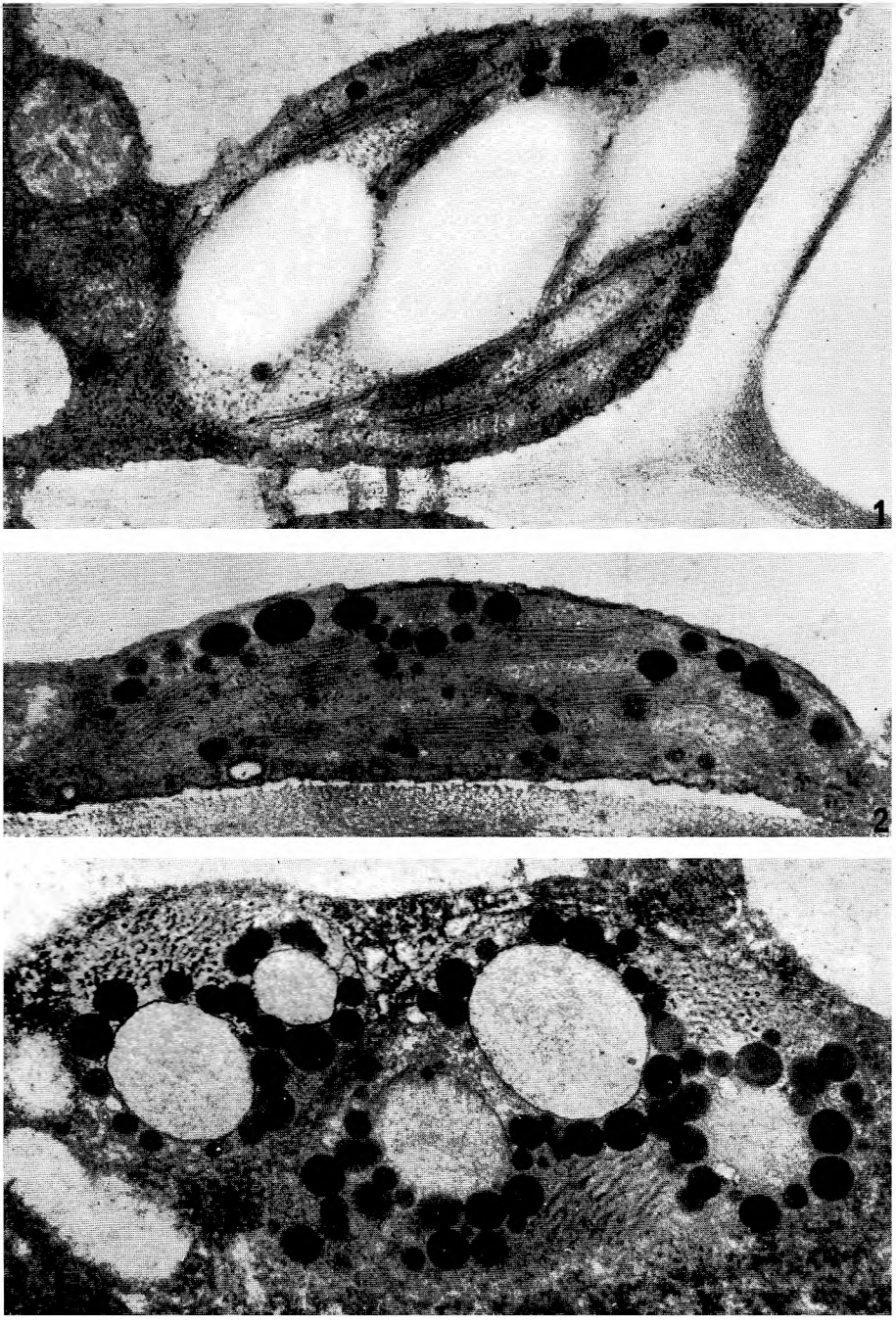
Fig. 2. Chloroplast from unfolded petals. Numerous plastoglobules occur among grana. 38,000 : 1.

Fig. 3. Chromoplasts from the fully developed flower. Tubules are arranged in bundles. Plastoglobules encircle "vacuole-like" inclusions. 39,000 : 1.

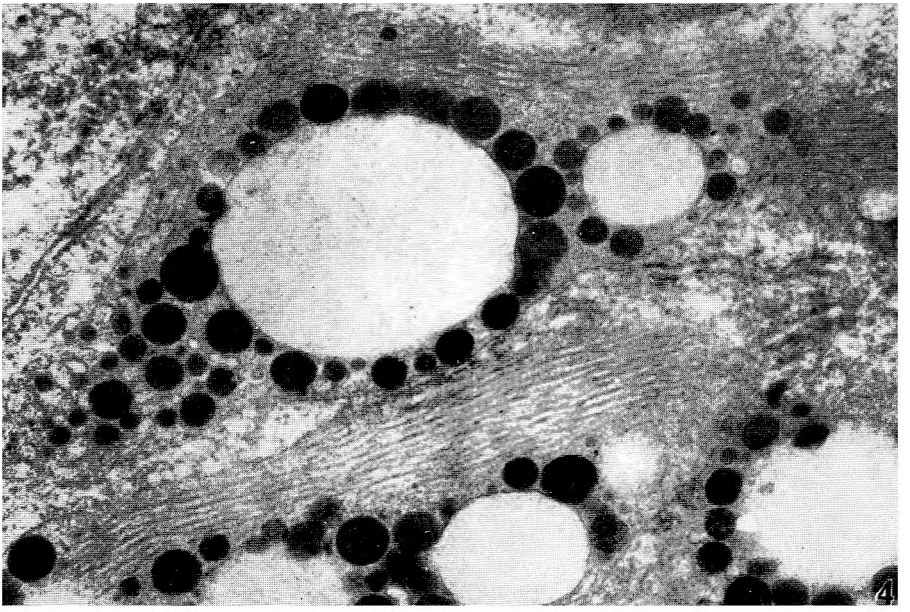
Fig. 4. Chromoplasts from the fully developed flower. Tubules form long bundles. "Vacuole-like" inclusions and plastoglobules occur in characteristic arrangement. 39,000 : 1.

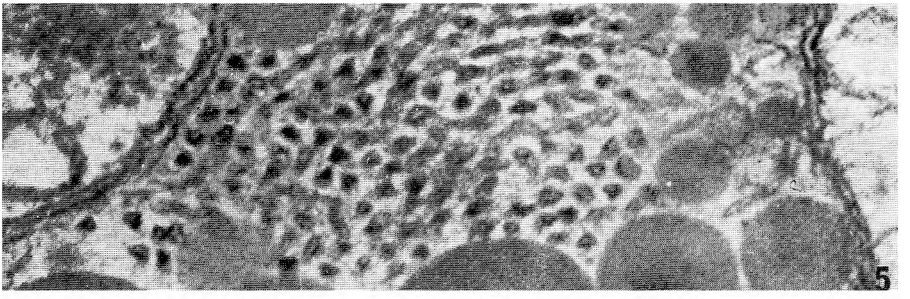
Fig. 5. High magnification of cross section of the bundle of tubules. 100,000 : 1.

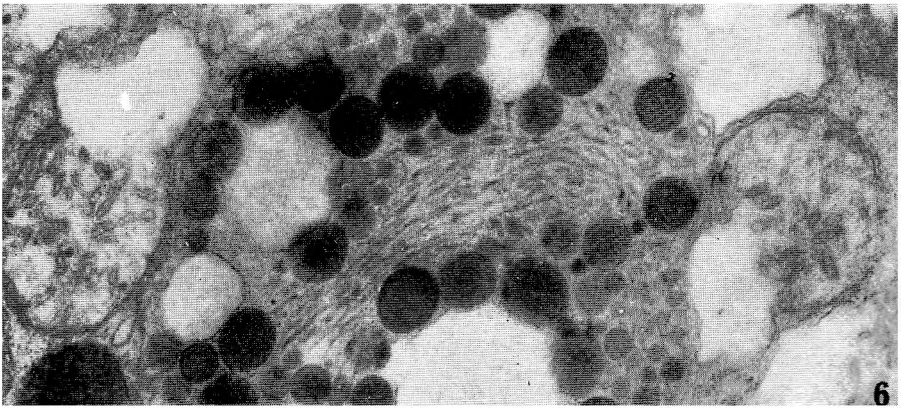
Fig. 6. Chromoplast from the flower at the beginning of withering process. All cell structures show signs of disintegration. 46,000 : 1.



Figs. 1—3.







Figs. 4—6.

Light microscopy

The petals of closed buds contained small (about 3 μm in diameter) greenish plastids oval in shape. The colour of plastids became intensively yellow during the flower development.

Electron microscopy

The cells of bud petals were small and practically without vacuoles. Small plastids occurred in dense cytoplasm (Fig. 1). According to their structure these were young chloroplasts. Stroma was dense and rich in ribosomes. Thylakoids formed small grana (about 5—10 thylakoids per granum). Plastoglobules were small and rather rare. Starch grains were frequently present.

The process of flower opening was characterized by the enlargement of chloroplasts and multiplying of grana thylakoids (Fig. 2). Stroma contained a large number of ribosomes. The number of plastoglobules began to rise. Their diameter was about 0.2 μm.

The transformation of the majority of chloroplasts was taking place during the rapid growing and yellowing of the petals (Figs. 3—6). Grana disappeared completely and characteristic tubules and numerous plastoglobules appeared simultaneously. The tubules were arranged in bundles (Figs. 3, 4). These bundles contained 20 to 60 tubules on the average. The chromoplast contained one or several bundles. It was difficult to ascertain their length, because they were very flexible and their full length was often not included in a single section (Fig. 4), but it seemed that they reached 10 μm. The outer diameter of each tubule was from 22 to 30 nm and the inner one from 9 to 20 nm. In cross section they were irregular in shape. Some of them were filled with a dense material (Fig. 5). The plastoglobules, with a diameter between 0.1 and 0.2 μm, encircled characteristic "vacuole-like" inclusions (Figs. 3, 4, 6). These inclusions were regularly round and filled with fine granular materials. Their diameter was between a half and two μm.

During the process of petal withering the chromoplasts lost the tubules. The "vacuole-like" inclusions became bigger and filled with dense material. After a short period of time the regular arrangement of plastoglobules disappeared and the cells disintegrated.

Pigment analysis

Through all the stages of flower development it was possible to find chlorophyll (Table I), but its concentration was constantly decreasing

Table I. The concentration of chlorophyll and total carotenoids (mg/gr fresh wt.) in petals during the development of flowers after 0 (bud), 7 and 14 days.

	0 day	7 days	14 days
Chlorophyll a + b	0.4189	0.1860	0.2256
Total carotenoids	0.2863	0.5560	0.6704

with the opening of flowers. Simultaneously the amount of carotenoids increased rapidly. The process of withering (14 days after the beginning of flower development) was characterized by a small increase in the relative concentration of chlorophyll and total carotenoids (mg/gr fresh wt.), caused by a rapid decrease in the flower fresh weight (Radić 1978). Thin-layer chromatography showed that the amount of xanthophylls was not notably changed during the flower development. As for the amount of carotenes in total carotenoids, they increased significantly. Preliminary investigations indicated a very high percentage of α-carotene and ζ-carotene in the petals of fully developed flowers.

Discussion

The existence of more than one type of pigment-bearing structures in chromoplasts is quite rare (Matienko and Chebanu 1973, Ljubešić 1977). The chromoplasts of *Forsythia suspensa* contain at the same time tubules and plastoglobules as pigment-bearing structures. These tubules are morphologically different from similar structures which occur in the chromoplasts of other plants (Smith and Butler 1971, Brandão and Salema 1974, Falk 1976, Wuttke 1976 etc.). The chromoplast tubules of *Forsythia suspensa* are very irregular in cross section and of various diameters. But according to their origin these tubules show the same characteristics as those in the chromoplasts of *Cucumis sativus* (Smith and Butler 1971) and *Tropaeolus majus* flowers (Falk 1976), which are formed without any previous accumulation of plastoglobules. The formation of tubules in *Forsythia suspensa* seems to be connected with the reorganization (disintegration) of the thylakoid system. The material from the disappearing thylakoids may thus be utilized directly in the production of tubules.

The plastoglobules are present through all stages of chromoplast development. Their shape is globular, and they are never transformed into fibrils, tubules or crystalloids. These facts indicate the low concentration of carotenoids in plastglobules.

Typical chromoplasts of *Forsythia suspensa* contain characteristic "vacuole-like" inclusions which resemble some similar structures earlier described in various types of plastids (Marty 1973, Casadoro and Rascio 1977, Martin and Larbalestier 1977). These authors have suggested that such inclusions might contain storage material — the protein for the synthesis of thylakoids. In our material these inclusions appear side by side with the thylakoid disintegration. They may contain proteins originating from disintegrated thylakoids.

We suppose that the tubules and the plastoglobules are the place of carotenoid localization in the chromoplasts of *Forsythia suspensa.* The reason for this suggestion is the homogeneously coloured chromoplasts in light microscope. The tubules and plastoglobules are arranged in large separated groups. If only one structure (either tubules or plastoglobules) contained carotenoids, the result would be an unhomogeneous colour of chromoplasts. The isolation and analysis of tubules and plastoglobules performed separately can solve this problem.

Summary

The ultrastructure and pigment composition of chromoplasts in petals of *Forsythia suspensa* were investigated at different stages of development. The buds contained chloroplasts. During the flowering period thylakoids disappeared and numerous tubules and plastoglobules were formed. The tubules, arranged in bundles, were heterogeneous in shape and size. The plastoglobules encircled the characteristic "vacuole--like" inclusions. The tubules and plastoglobules were probably the pigment-bearing structures of chromoplasts. The changes of pigment composition were investigated during the flower development.

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SAŽETAK

KROMOPLASTI KOD VRSTE *FORSYTHIA SUSPENSA* (THUNB.) VAHL.
I. FINA GRAĐA I SASTAV PIGMENATA

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Istražene su promjene u finoj građi kromoplasta iz latica vrste *Forsythia suspensa* u različitim stadijima razvitka. Istovremeno su praćeni sastav i količina pigmenata. Latice pupa sadržavaju tipične kloroplaste. Tijekom procesa razvoja cvijeta dolazi do razgradnje tilakoida i formiranja kromoplasta s mnogobrojnim tubulima i plastoglobulima. Tubuli, koji su nepravilnog oblika, složeni su u snopiće. Plastoglobuli okružuju karakteristične »vakuolama slične« uklopine. Pigmenti kromoplasta vjerojatno se nalaze u tubulima i plastoglobulima.

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