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PLASTID DIFFERENTIATION IN *CALCEOLARIA* PETALS

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Fine structural changes and changes in pigment content of differentiating chromoplasts have been studied in *Calceolaria* petals.

At all developmental stages the plastids contained two types of membranes: thylakoids and chromoplast internal membranes (CIMs). During the plastid transformation these two membrane types appeared in different proportions. Only the thylakoids were photosynthetically active, as shown by the photooxidation of diaminobenzidine.

During the plastid differentiation the quantity of thylakoids was constantly reduced and the number of CIMs increased. Fully grown flowers contained only CIMs and no thylakoids. A direct transformation of thylakoids into CIMs was not observed.

The pigment content changed considerably in differentiating chromoplasts. The chlorophyll content dropped constantly, while the content of total carotenoids increased. At last in fully grown flowers chlorophyll was hardly detectable. About 90% of all carotenoids was lutein.

Introduction

The membranous type of chromoplasts in its typical form is very rare and has untill now been described only in the flowers of daffodil and some of its relatives (Mollenhauer and Kogut 1968, Liedvogel et al. 1976, Mesquita 1976). In fully grown flowers these chromoplasts are filled with numerous concentrically arranged membranes (CIMs; Sitte et al. 1980).

Recently, it has been found that flowers of *Calceolaria rugosa* and *Calceolaria hybrida* also contain chromoplasts of the membranous type (Falk personal comm., Wrischer and Ljubešić 1983). It has

also been observed that these chromoplasts develop from immature chloroplasts (Wrischer and Ljubešić 1983). The mode of transformation of plastid membranous systems during the differentiation of chromoplasts will be reported in this paper.

Materials and Methods

Flowers of *Calceolaria rugosa* Ruiz et Pav. at different developmental stages were used for the investigations. For some experiments, flowers of *Calceolaria hybrida* were also used. Small pieces of petal tissue were fixed in 1% glutaraldehyde in cacodylate buffer (pH 7.2 at 1° C) — usually with the addition of caffeine according to Vaughn and Wilson (1981) — and, after appropriate washing, postfixed in 1% OsO_4. Dehydrated tissue was embedded in Araldite and thin sections stained with uranyl acetate and lead citrate. For the localization of photosynthetic activity (the activity of photosystem I), after a short fixation in formaldehyde, the tissue was treated with diaminobenzidine (DAB) in phosphate buffer (1 mg/ml) as described in a previous paper (Wrischer 1978). Thin sections were examined in the electron microscope without further staining.

The pigments were extracted in 80% acetone and the quantitative determination of chlorophylls was calculated according to Holden (1965), and of total carotenoids according to Urbach et al. (1976). Carotenoids were separated by silica gel G thin layer chromatography with petrol ether — ethyl acetate — diethylamine (58 : 30 : 12) as solvent and estimated according to Stahl (1969) and Davies (1965).

Results

The colour of *Calceolaria* flowers changed from pale green, in very young flowers, to yellow, in fully grown ones.

The petals of very young flowers (only 1 mm in diameter) contained either amoeboidal proplastids or immature chloroplasts. This depends on the cell layer examined (Figs. 1, 2). In both types of plastids two

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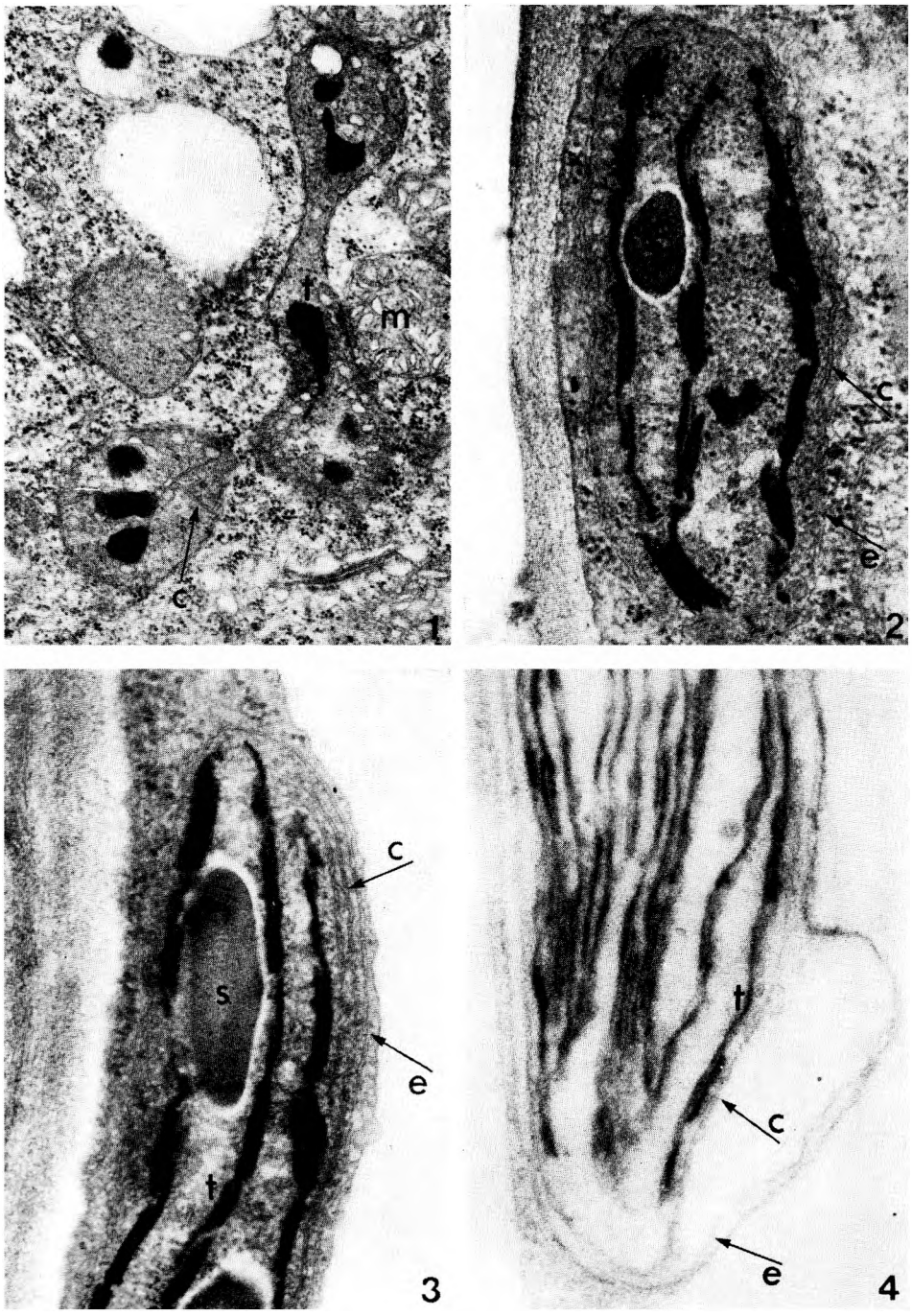
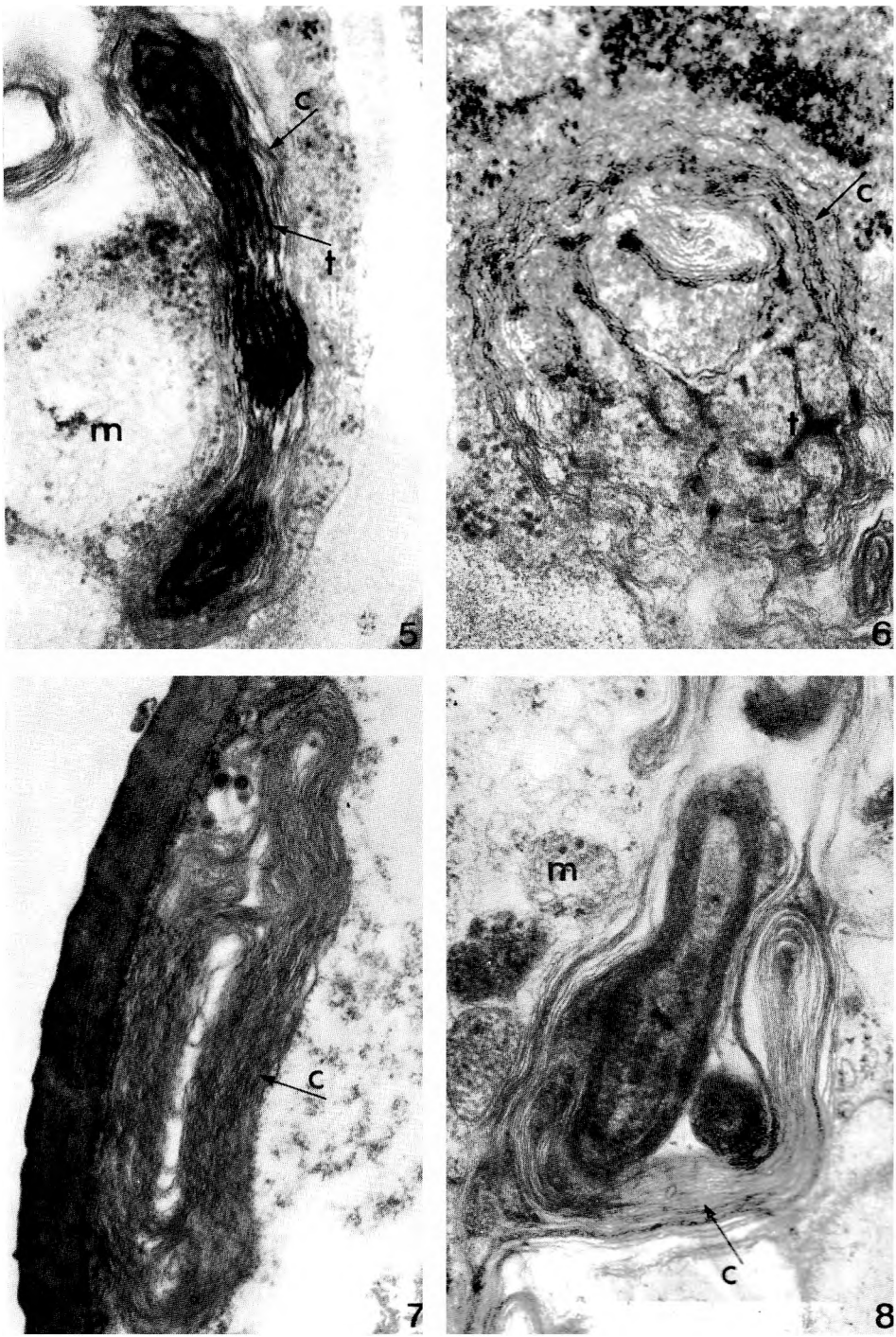
Figs. 1—3 and 5—8: plastids from the petals of *Calceolaria rugosa*

Fig. 4: plastid from the petal of *Calceolaria hybrida*
t = thylakoid, c = chromoplast internal membrane, s = starch, e = plastid envelope, m = mitochondrion

Fig. 1. Amoeboidal proplastid from a flower 1 mm in diameter. 27,000 : 1.
Fig. 2. Immature chloroplast from a flower 1 mm in diameter. 56,000 : 1.
Fig. 3. Part of a chloro-chromoplast from a flower 3 mm in diameter. 53,000 : 1.
Fig. 4. Part of a chloro-chromoplast — DAB reaction. 54,000 : 1.
Fig. 5. Chloro-chromoplast from a flower 5 mm in diameter. 53,000 : 1.
Fig. 6. Chloro-chromoplast from a flower 7 mm in diameter. 56,000 : 1.
Fig. 7. Chromoplast from a fully grown flower. 26,000 : 1.
Fig. 8. Chromoplast from a fully grown flower. 42,000 : 1.



Figs. 1—4.



Figs. 5—8.

types of membranes could be found. The first type were vesicles or thylakoids filled with a dark (osmiophilic) substance*; the second type were vesicles or tubules with lightly stained content, which were often in contact with the inner membrane of the plastid envelope (Figs. 1, 2). The stroma was filled with ribosomes and starch grains were observed only in immature chloroplasts (Fig. 2). The thylakoids reacted positively with DAB, while the vesicles, tubules and plastid envelopes were always DAB negative.

Flowers 3 mm in diameter contained chloroplasts whose thylakoids were filled with osmiophilic substance and were arranged into small grana. Besides that, there were one or two double layers of membranes with lightly stained content (CIMs), which were usually arranged at the periphery of the plastid. Starch grains were often in the stroma, but plastoglobules were rare (Fig. 3). After DAB treatment only thylakoids reacted positively and contained osmiophilic DAB polymers, while CIMs remained without DAB deposits (Fig. 4).

In the next two developmental stages examined (in flowers 5 and 7 mm in diameter) the quantity of thylakoids was progressively reduced, while at the same time the number of CIMs increased. These were usually arranged concentrically in several layers arround the remaining thylakoids, which could be detected by their dark content (Figs. 5, 6).

In fully grown flowers (10 to 12 mm in diameter) the thylakoids completely disappeared and the CIMs occupied the greatest part of the chromoplast. The stroma contained only a few plastoglobules and no crystalloids (Figs. 7, 8). The DAB reaction remained completely negative in these chromoplasts.

Table 1. Content of chlorophyll and total carotenoids in *Calceolaria* petals during the flower development.

Diameter of flowers (mm)	Chlorophyll (mg/g fr. wt.)	Total carotenoids (mg/g fr. wt.)	Chlorophyll/ /carotenoids
1	0.13	0.12	1.12
3	0.13	0.34	0.37
5	0.05	0.48	0.09
7	0.04	0.49	0.07
10	0.03	0.73	0.04

In very young flowers (1 mm in diameter) there was a small, but still detectable quantity of chlorophyll (Table 1). It remained practically unchanged in flowers 3 mm in diameter. In older flowers (5 and 7 mm in diameter) the chlorophyll content dropped considerably. At last in fully grown flowers it was hardly detectable. On the other hand, the concentration of carotenoids increased constantly during the flowers' growth. In fully developed flowers it was about 6 times higher than in

* In *Calceolaria* hybrida the thylakoid compartments were not osmiophilic. Therefore these plants were preferred for cytochemical studies.

the youngest ones examined. Thin layer pigment analysis has shown that in fully grown flowers 90% of all carotenoids is lutein and the rest β-carotene (5%) and an unidentified carotene (5%).

Discussion

In chloroplasts and chloro-chromoplasts of *Calceolaria rugosa* electron dense (osmiophilic) intrathylakoidal inclusions make the discrimination between thylakoids and CIM possible, as well as the observation of the process of thylakoid degradation and disappearance. These dark inclusions should be a characteristic of the incompletely organized thylakoid membranes in their early ontogenesis (Casadoro and Rascio 1978, 1979). It is supposed that they may be polyphenolic (Sitte 1977), since polyphenoloxidase could be detected in thylakoids (Olah and Mueller 1981). According to their different reactions with DAB the two types of membranes found in chloro-chromoplasts are functionally different. Only the thylakoids can be considered as photosynthetically active.

In the youngest flowers examined CIMs are formed by infoldings of the inner membrane of the plastid envelope. A similar mode of formation of CIMs is found in the corona chromoplasts of daffodils (Liedvogel et al. 1976, Mesquita 1976). The plastid envelope plays an important role in plastid biogenesis; it is also the origin of the first thylakoids in proplastids (Wellburn 1982).

The formation of thylakoids and CIMs in *Calceolaria* starts simultaneously, although the growth of thylakoids soon stops. On the other hand CIMs develop slowly at the beginning, but later, after the thylakoids begin to degrade, much faster. A direct transformation of the thylakoids into CIMs could not be observed, and the same has also been stated for daffodil chromoplasts (Mollenhauer and Kogut 1968, Liedvogel et al. 1976, Mesquita 1976).

During the development of Calceolaria chromoplasts the quantity of plastoglobules remains low. As crystalloids have never been observed either, it is probable that the carotenoids, mainly lutein, are incorporated into the CIMs. The same conclusion can be drawn from the investigations by Liedvogel et al. (1976) who have shown that most of the pigments in the daffodil are located in CIMs. Further studies in this direction would be desirable.

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SAŽETAK

DIFERENCIJACIJA PLASTIDA U CVJETOVIMA KALCEOLARIJE

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Istražene su promjene u ultrastrukturi plastida i sastavu njihovih pigmenata tijekom procesa diferencijacije kromoplasta u cvjetovima kalceolarije.

Plastidi svih istraženih razvojnih stadija sadržavaju dva tipa membrana: tilakoide i tzv. unutrašnje membrane kromoplasta. Ta su dva tipa membrana tijekom diferencijacije kromoplasta zastupana u različitim omjerima. Samo su tilakoidi fotosintetski aktivni, jer mogu fotooksidirati diaminobenzidin.

Za vrijeme transformacije plastida količina tilakoida stalno se smanjuje, dok se količina unutrašnjih membrana kromoplasta povećava. Izravna pretvorba tilakoida u unutrašnje membrane kromoplasta nije zapažena.

Sadržaj pigmenata također se znatno mijenja tijekom transformacije plastida. Količina klorofila stalno pada, a količina se ukupnih karotenoida povećava. U potpuno razvijenom cvijetu klorofil se, konačno, jedva može dokazati, a 90% svih karotenoida čini lutein.

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