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ULTRASTRUCTURAL CHANGES OF PLASTIDS DURING THE RIPENING OF THE FRUIT OF *CONVALLARIA MAJALIS* L.

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Ultrastructural changes of plastids during the ripening of the fruit of *Convallaria majalis* were examined by electron microscopy. Special attention was given to the differences between development of plastids of the outer cell layer (epidermis) and the inner ones (parenchyma). The epidermis of the unripe fruit is characterized by plastids with membrane-bound inclusion while parenchyma contain chloroplasts with grana and highly developed peripheral reticulum. At a later stage, chromoplasts of the epidermis in the semi-ripe fruit still have membrane-bound inclusion and beside that, many plastoglobules. Chromoplasts of the parenchyma have numerous plastoglobules, disintegrated thylakoid system and non-chlorophyllous membranes. The epidermis and the parenchyma of the ripe fruit have a unique type of chromoplasts characterized by the presence of plastoglobules and numerous newly formed crystalloids.

Introduction

Chromoplasts are a very heterogeneous group of plastids. Sitte (1980) distinguishes five general types of chromoplasts according to the carotenoid-bearing fine structural elements prevailing in them in their final state of development. Chromoplasts of fruit of *Convallaria* belong to the globulous type.

Steffen (1964) has investigated the ontogenesis of chromoplasts in fruit of *Convallaria majalis* by light microscopy. He found that their development was different in the outer cell layer (epidermis) from the inner ones (parenchyma). The process of the chloroplast → chromoplast

transformation exists only in the parenchyma while in the epidermis chromoplasts develop directly from proplastids. The formation of chromoplasts from chloroplasts during fruit ripening of different species has been studied extensively (Spurr and Harris 1968, Simpson et al. 1975, Ljubešić 1977, Simpson et al. 1977, Kirk and Tilney-Bassett 1978).

In this study an attempt was made to investigate the ultrastructural changes of plastids in the epidermis and parenchyma cells during the ripening of the fruit of *Convallaria.*

Material and Methods

The fruits of *Convallaria majalis* L. were grown in the garden under natural conditions. Samples for investigation were taken several times at certain intervals, from the beginning of fruit formation until its decay. This period lasted for about four months and we collected white, green, light-orange, dark-orange and red fruits. The epidermis and the parenchyma of these fruits were examined by electron microscopy.

Pieces of fruit tissue were fixed in 1% glutaraldehyde in cacodylate buffer (pH = 7.2) for two hours. After fixation the material was washed in cacodylate buffer and postfixed in 1% OsO_4 for two hours. The fixed material was dehydrated in graded ethanol series and embedded in Araldite. The sections were prepared on a Reichert Om U2 ultramicrotome, stained with uranyl acetate and lead citrate and observed with an Opton EM 10.

Results

In the epidermis of the white, immature fruit there are oval proplastids which have a large membrane-bound granular inclusion. The stroma is localized at the narrow peripheral part of the organelle. There are some plastoglobules about 50 nm in diameter in the stroma. In the parenchyma of the same fruit there are plastids which mainly contain single thylakoids (Fig. 1). Sporadically they have stacked membranes (grana). The thylakoids are sometimes dilated and filled with granular material. Numerous vesicles from the peripheral reticulum and some globules are in the dense stroma.

The plastids with membrane-bound granular inclusion in the epidermis of the green fruit show very similar fine structure as in the previous stage. In the parenchyma there are elongated chloroplasts which have grana with 3—5 thylakoids. At the border of the chloroplasts there are numerous invaginations of the inner plastid membrane in the form of vesicles. The plastoglobules are tiny and rare (Fig. 2).

Significant changes of the ultrastructure can be observed in the plastids of the light-orange fruit. The plastids in the epidermis have a considerably increased number of somewhat bigger plastoglobules. Their membrane-bound granular inclusion is very dense (Fig. 3). In the parenchyma cells there are oblong chloro-chromoplasts (Fig. 4). They still contain thylakoids in grana-stacks. Numerous globules (70 nm in diameter) are arranged in rows between the thylakoids. Stroma is rich in ribosomes and without vesicles.

The globulous type of chromoplasts characterized by numerous plastoglobules prevail in the epidermis and the parenchyma of the dark-

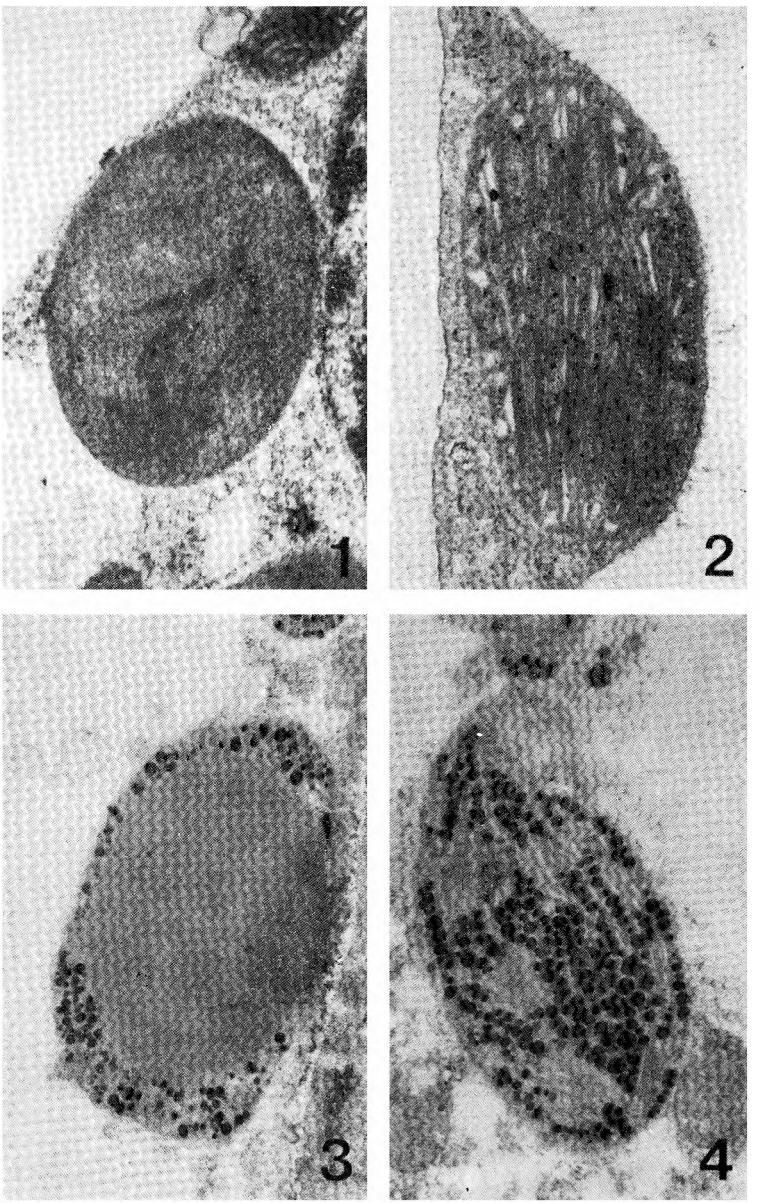
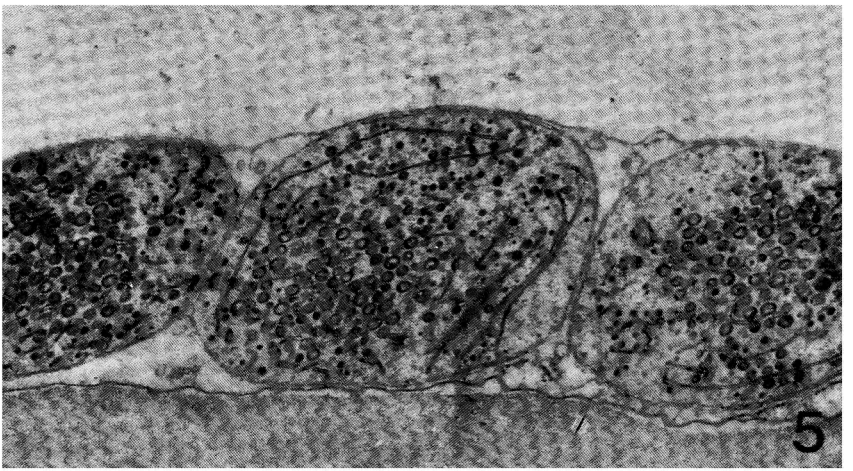


Fig. 1. Plastid from the parenchyma of the white fruit. 24,000:1.

Fig. 2. Chloroplast from the parenchyma of the green fruit. 34,000:1.

Fig. 3. Plastid from the epidermis of the light-orange fruit. 19,000:1.

Fig. 4. Chloro-chromoplast from the parenchyma of the light-orange fruit. 27,000:1.



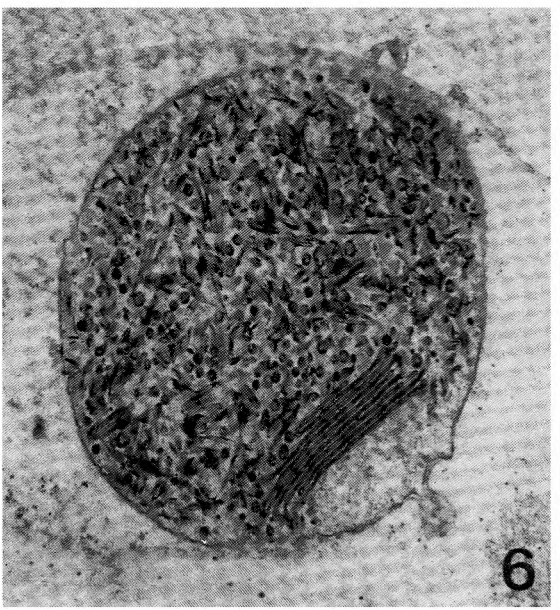


Fig. 5. Globulous type of chromoplasts of the dark-orange fruit. 21,000:1.

Fig. 6. Chromoplast of the ripe dark-orange fruit. 24,000:1.

Fig. 7. Part of the chromoplast with crystalloids from the over-ripe fruit. 69,000:1.

-orange fruit. Most plastoglobules have electron-translucent centres inside an electron dense circular periphery (Fig. 5). Somewhere among these globules there are remnants of thylakoids. Beside that, chromoplast internal membranes are sometimes arranged in a specific group (Fig. 6). Some chromoplasts of the same fruit, probably from the riper part, contain rodlike crystalloids about 15 nm wide and 200 nm long.

Chromoplasts of the ripe red fruit have dark globules and numerous electron-translucent, rodlike crystalloids (Fig. 7). Chromoplasts of the over-ripe fruit show signs of tissue decay. They are swelled, their stroma is light and the plastid envelope is damaged so that crystalloids flow out.

Discussion

During the ripening of *Convallaria fruit* the differentiation of chromoplasts is similar in the epidermis and in the parenchyma. The main difference is that chromoplasts of the epidermis develop from plastids which have never contained thylakoids. The plastids in the epidermis of the immature fruit contain membrane-bound granular inclusion which is a common characteristic of this type of tissue. These inclusions are gradually reabsorbed during the maturation of plastids (Casadoro et al. 1977). It has been established by cytochemical methods that they are proteinous (Hurkman and Kennedy 1977). Their function is not exactly known but probably they serve as a storage of enzymes. The chromoplasts of parenchyma in the semi-ripe fruit have membranes which are sometimes arranged in a specific group (Gross et al. 1983). These membranes do not originate from the photosynthetic membranes. They are either newly formed or derived from the inner plastid envelope membrane. They represent the non-chlorophyllous membranes (Camara ad Brangeon 1981). Most of the plastoglobules in chromoplasts of the ripe fruit are osmiophylic and uniformly electron dense. Some of the globules have electron-translucent centres, which is the first sign of pigment crystallization that begins in them (Simpson et al. 1978a). The plastoglobules which give rise to crystalloids are chemically different from the globules resulting from granal disintegration (Simpson et al. 1978b). The number of the globules that are source of crystallization decreases parallelly with the formation of new crystalloids. The chemical nature of the crystalloids in *Convallaria* chromoplasts has to be determined.

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SAŽETAK

PROMJENE U FINOJ GRAĐI PLASTIDA TIJEKOM SAZRIJEVANJA PLODA ĐURĐICE (*CONVALLARIA MAJALIS* L.)

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Elektronsko mikroskopskim metodama praćene su promjene u finoj građi plastida tijekom sazrijevanja ploda đurđice (*Convallaria majalis* L.) Posebno je istražena razlika u diferencijaciji plastida epiderme i parenhima. U nezrelim plodovima plastidi epiderme sadrže velike proteinske uklopine, dok u stanicama parenhima dolaze kloroplasti s grana strukturama i jako razvijenim perifernim retikulumom. U epidermi i u parenhimu zrelih plodova dolazi globularni tip kromoplasta. Kromoplasti epiderme uz mnogobrojne globule još uvijek imaju proteinske uklopine. Kromoplasti parenhima osim globula sadržavaju ostatke tilakoidnog sustava te pojedinačne nefotosintetske membrane. Kromoplasti jedinstvene strukture, koju karakterizira prisutnost globula i veliki broj novo stvorenih kristaloida, dolaze u svim stanicama vrlo zrelih plodova.

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