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THE FINE STRUCTURE OF PEPPER CHROMOPLASTS: THE EFFECT OF BLEACHING HERBICIDES*

BRANKA SALOPEK and *NIKOLA LJUBEŠIĆ*

(Tracer Laboratory; Laboratory of Electron Microscopy, Ruđer Bošković Institute, Zagreb)

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The fine structure of plastids and carotenoid content of the pepper fruit *(Capsicum annuum)* were studied during the various stages of ripening. Red ripe fruits contained spindle-shaped chromoplasts which originated from leuco-chloroplasts. The bleaching herbicides amitrole and SAN 9789 inhibited the biosynthesis of total carotenoids and the development of typical spindle-shaped chromoplasts. Both herbicides prevented the formation of tubules and increased the accumulation of plastoglobules. In SAN 9789 treated fruits, plastids contained few single dilated thylakoids, while amitrole caused dilatations of the retuculum which was filled with tiny grainy material. The results are discussed with regard to the already known action of these herbicides.

Introduction

Chromoplasts are photosynthetically inactive plastids which accumulate carotenoid pigments and in many species are responsible for the yellow, orange and red coloration of petals and fruits. There are five general types of chromoplasts according to the carotenoid-bearing fine structural elements (globulous, tubulous, reticulo-tubulous, membraneous and crystal type) (Sitte et al. 1980, Ljubešić et al. 1991).

Capsicum chromoplasts belong to the reticulo-tubulous group. When the fully developed, green *Capsicum* fruit begins to ripen the plastids undergo a

* Dedicated to dr. Mercedes Wrischer on the occasion of her 65[th] birthday.

number of striking structural and biochemical modifications (Spurr and Harris 1968, Suzuki 1974, Simpson and Lee 1976, Camara and Brangeon 1981, Camara et al. 1989, Oren-Shamir et al. 1993).

The relation between the formation of chromoplast structures and the biosynthesis of carotenoids is still not fully understood. A useful tool for this study are herbicides that impair carotenoid biosynthesis.

We studied the effect of two »bleaching« herbicides on the ultrastructure of plastids and composition of the pigments of *Capsicum annuum.* Both herbicides are known to directly affect the carotenoid biosynthesis in chloroplasts. Amitrole inhibits desaturation of phytan and ξ-carotene and stops the cyclization of lycopene in α– and β-carotene (Buschmann and Grumbach 1982, Barry and Pallet 1990, Wrischer et al. 1992). SAN 9789 has been reported to be a strong and direct inhibitor of carotenoid biosynthesis, which causes, more or less, a complete absence of coloured carotenoids (Laskay et al. 1983, 1986, Laskay and Lehoczki 1986).

Material and Methods

Plants of *Capsicum annuum* were grown in pots in laboratory conditions. For our observation we used control fruits at young, fully developed and ripe fruit stages.

Sublethal concentrations of water solutions of herbicides SAN 9789 (0.2 mM) and amitrole (1mM) were injected in the lumen of young, pale greenish fruits. These fruits were taken for observations 10 and 15 days after treatments.

All materials were examined in a Zeiss Axiovert 35 light microscope.

For electron microscopy, pieces of control and treated fruit tissue were fixed in 1% glutaraldehyde in cacodylate buffer (pH 7.2) and postfixed in 1% OsO_4. The dehydrated tissue was embedded in araldite and the thin sections stained with uranyl acetate and lead citrate.

The sections of the subepidermal cell layers were examined in a Zeiss EM 10 electron microscope.

For pigment analysis, total pigments were extracted in 80% acetone and measured spectrophotometrically (Specol 10 Zeiss, Jena) at wave lengths of 452.5, 645 and 663 nm.

Carotenoids were analysed by thin layer chromatography on silica gel-G plates and developed by a mixture of acetone:petrol ether 45–65°C (70:30).

Results

Macroscopic observations

Control. The young pepper fruits (2 cm long) were pale greenish in colour. During ripening they changed into pale, yellowgreenish, fully developed fruits (3 cm long), and after 15 days became intensively red ripe fruits.

Treated fruits. Amitrole and SAN 9789 treated fruits were grown to 3 cm long, like the control. These fruits did not change in colour during the whole period of development. They remained pale greenish in colour. The majority of treated fruits dried up after three weeks. Only a few fruits survived the 40 days of the experiment.

Microscopic observations

Control. Small, pale green plastids of young pepper fruit could be observed in a light microscope. Ultrastructural examinations showed amoeboid plastids which were not fully differentiated (Fig. 1). These leuco-chloroplasts contained a few single thylakoids, small plastoglobules and large starch grains. Irregular aggregates of ferritin were often found in the plastid stroma. Many ribosomal particles were distributed throughout the stroma. In fully developed, yellow-greenish fruit, the plastids became oval in shape (Fig. 2). During ripening plastoglobules increased in number and size while the starch grains decreased. The thylakoids disappeared and a large single membrane-bound body was formed in these plastids. These plastids contained membranous invaginations of inner plastid membrane and unusual membranes of »bar-shaped structures« (Ljubešić 1977) (Fig. 3).

Light microscopy observations showed that red ripe pepper fruit contained large and intensively coloured, spindle-shaped chromoplast (Fig. 4), which appeared with birefringent and dichroitic areas under the polarizing microscope.

Electron microscopy showed that these chromoplasts were filled with numerous tubules (Fig. 5). These tubules were usually in contact with plastoglobules (Fig. 6). In most cases the plastoglobules were pierced by long straight tubules. On the cross section, these tubules showed typical light lumen (Fig. 7).

Amitrole. Ten days after treatment, all the thylakoids disappeared (Fig. 8). The plastids contained numerous plastoglobules. The membranous reticulum was poorly developed and partially dilated. These dilatations were filled with tiny grainy material.

SAN 9789. Like in amitrole treated fruit, the plastid stroma was scarce and filled with numerous plastoglobules (Fig. 9). A few single, dilated thylakoids could be observed. Poorly developed peripheral reticulum was formed by invagination of an inner plastid membrane.

Pigment analysis

Control. The pale greenish young fruit contained small quantities of chlorophyll a and b and carotenoids. In the fully developed pepper fruit the content of chlorophylls and carotenoids decreased. After 15 days, in red ripe fruit, the chlorophylls completely disappeared while the carotenoids increased about 50–fold (Table 1).

Thin layer chromatography showed that the majority of new synthesized carotenoids were β-carotene and unidentified carotenes.

Treated fruits. Amitrole and SAN 9789 affected the pigment content in a very similar way. Ten days after treatments, the chlorophylls disappeared, while the carotenoids decreased like in the control. Carotenoids slightly increased after 15 days of the experiment (Table 1).

Discussion

The ripe red fruits of different cultivars of *Capsicum annuum* contain typical spindle-shaped chromoplasts. These chromoplasts form plastoglobule-associated tubulous or fibrilar structures, as carotenoid-carrying internal

Table 1. Content of chlorophylls and carotenoids (mg/g fresh weight) during the development of untreated (control) and treated pepper fruits.

	Young fruit	Control		Amitrole		SAN 9789	
Days	0	10	15	10	15	10	15
Total chlorophylls	0.193	0.003	–	–	–	–	–
Carotenoids	0.025	0.014	0.756	0.010	0.028	0.022	0.024

elements (Deruere et al. 1994). For these structures the terms fibrils or filaments (Laborde and Spurr 1973, Simpson et al. 1977) were previously used. Recently, most authors have applied the term tubule (according to Sitte et al. 1980 – tubulous type of chromoplast). Our investigation indicated the presence of typical tubules – they exhibit an electron-dense wall and an electron-transparent core. These tubules extended out from plastoglobules during chromoplast maturation. Plastoglobules are an intermediate stage in the development of chromoplast tubules (Ljubešić 1977). The remnants of plastoglobules remain associated with a tubule during the entire chromoplast development. Morphologically, the tubules from chromoplasts of our pepper are very similar to the same structure in fruits of *Rosa rugosa* (Wuttke 1976). However, these tubules are quite different from those which are the consequence of the reorganization of materials derived from thylakoid breakdown (Falk 1976) or from ramified and anastomosing tubules from reticulo-tubulous chromoplasts (Ljubešić 1978).

Ripening of pepper fruits involves conversion of leucoplasts or chloroplasts into chromoplasts. The colour of the ripe fruits is determined by four nuclear genes (three for carotenoid accumulation and one for the persistence of chlorophylls (Oren-Shamir et al. 1993). In our case the red mature fruit is a result of the presence of chromoplasts which originate from leuco-chloroplasts. During the whole process of plastid conversion only a few thylakoids are present. The tubular reticulum, which has been present in a great majority in the pepper chromoplasts described until now (Simpson et al. 1977, Carde et al. 1988), in our pepper is completely missing or exists only in a very slight form.

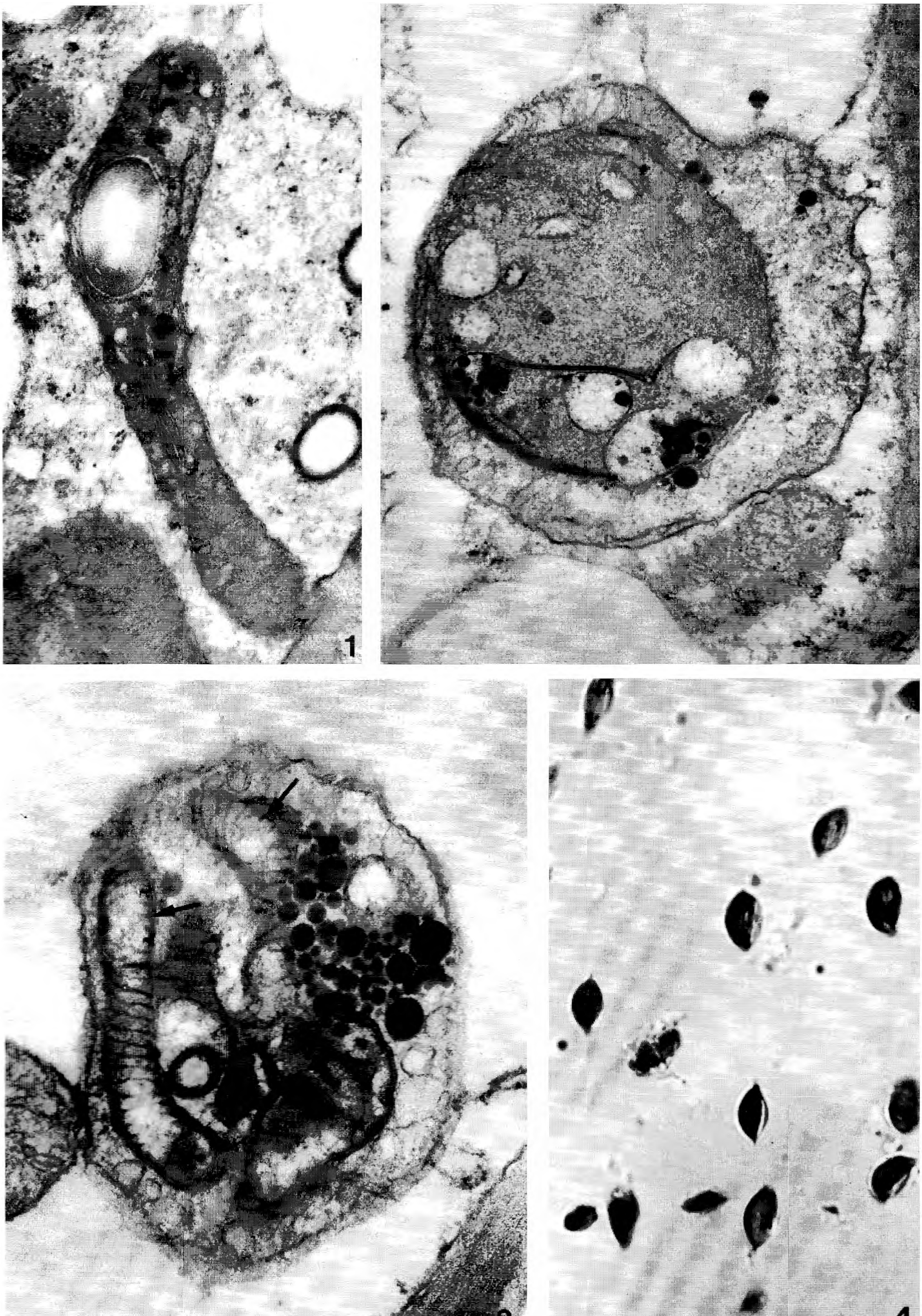
The present results show that the treatment with amitrole and SAN 9789 have very similar effects on the development of pepper chromoplasts. In both cases the effect is very drastic. The treatment inhibited the complete synthesis

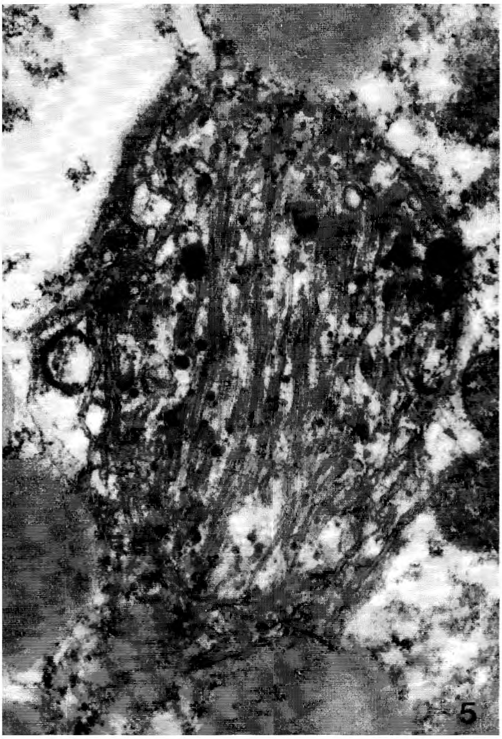
Fig. 1. Leuco-chloroplasts from young, pale greenish untreated pepper fruit. Plastids contain a few single thylakoids, small plastoglobules and starch grains. 26,000:1.

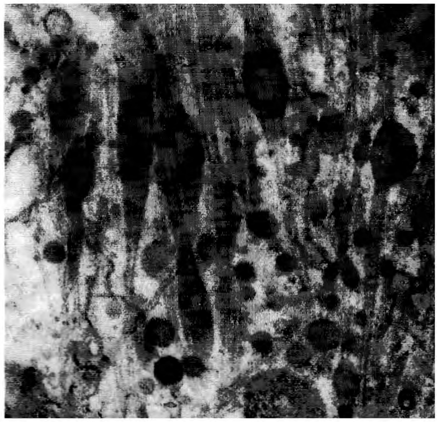
Fig. 2. Plastid from fully developed, yellow-greenish untreated pepper fruit, showing a large single membrane – bound body. 28,000:1.

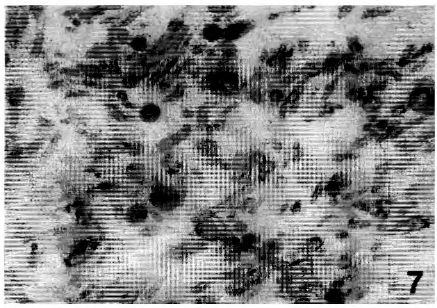
Fig. 3. Plastid from fully developed, yellow-greenish control pepper fruit with numerous plastoglobules and unusual »bar-shaped« (arrows) membranous structures. 42,000:1.

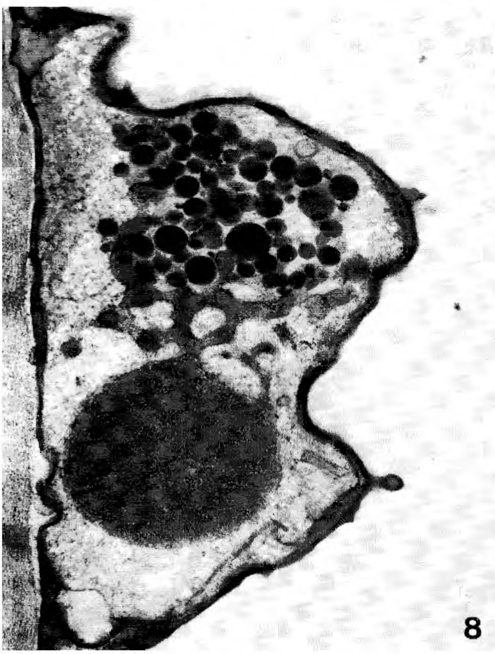
Fig. 4. Light microscopy micrograph of spindle-shaped chromoplasts from untreated, red ripe fruit. 1,000:1.











of chromoplast carotenoids and prevented the development of typical chromoplast structures. Only scarce, small and nontypical tubular reticulum is present.

This is partly in disaccordance with the study on daffodil flowers (Hloušek-Radojčić and Ljubešić 1988), *Calceolaria* flowers (Wrischer et al. 1991) and on tulip tree flowers (Hloušek-Radojčić and Ljubešić 1985, Ljubešić and Matijević 1992). In all these cases amitrole and SAN 9789 only partially change the carotenoid synthesis and the formation of chromoplasts and their characteristic structures. We suppose that the concentrations of herbicides applied were somewhat too high for our pepper fruits. It is also possible that the fruits were too young at the time of treatment. On the other hand, none of the treated fruits have ever shown any signs of damage. They grew to normal dimensions and remained on the plant longer than the control ones.

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Fig. 5. Chromoplast from untreated, red ripe fruit. Numerous tubules are present in stroma. 20,000:1.

Fig. 6. Part of chromoplast from a control, red ripe fruit, showing tubules in contact with plastoglobules. 41,600:1.

Fig. 7. Cross section of tubules in chromoplast of control, red ripe fruit. 54,400:1.

Fig. 8. Plastid from pepper fruit treated with amitrole (1 mM). The thylakoids disappeared and numerous plastoglobules and dilated reticulum are present in stroma. 33,600:1.

Fig. 9. Plastid from pepper fruit treated with SAN 9789 (0.2 mM), showing few dilated thylakoids and numerous plastoglobules. 21,250:1.

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SAŽETAK

FINA GRAĐA KROMOPLASTA PAPRIKE: UTJECAJ HERBICIDA SAN 9789 I AMITROLA

Branka Salopek i *Nikola Ljubešić*

(Radioizotopni laboratorij; Laboratorij za elektronsku mikroskopiju, Institut »Ruđer Bošković«, Zagreb)

Istraženi su fina građa plastida i sadržaj karotenoida tijekom više stadija zoridbe plodova paprike *(Capsicum annuum)*. Zreli crveni plod sadrži vretenaste kromoplaste koji nastaju iz leukokloroplasta. Herbicidi amitrol i SAN

9789 koče sintezu karotenoida i razvitak tipičnih vretenastih kromoplasta. Oba herbicida sprečavaju stvaranje tubula, a uzrokuju nagomilavanje plastoglobula. Plastidi tretirani herbicidom SAN 9789 sadrže samo nekoliko proširenih tilakoida, dok amitrolom tretirani plastidi sadrže proširenja tubularnog retikuluma, koja su ispunjena finom zrnatom supstancom. Raspravljeni su nađeni rezultati u odnosu na poznata djelovanja korištenih herbicida.

Branka Salopek, dipl. inž.
Dr. Nikola Ljubešić
Institut »Ruđer Bošković«
POB 1016
41 001 Zagreb, Hrvatska (Croatia)