The ultrastructure of chloroplasts in variegata irregulare mutants of garden petunias (*Petunia hybrida* hort. *superbissima*)

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Abstract

The ultrastructure of mutated chloroplasts in tetraploid garden petunias (*Petunia hybrida* hort. *superbissima*) was analyzed by electron microscopy. The formation of grana structure is inhibited after secondary thylacoids start forming. Rapid dezintegration of the structure is observed. It is suggested that a substance responsible for photostabilization of grana structure is lacking.

INTRODUCTION

During last years chlorophyll mutants have played an important role in the elucidation of the relation between the structure of the plastid and its genetic material (Wettstein, 1957, 1959, 1961; Sun 1963; Wettstein and Eriksson 1963; Wallis 1967).

The frequency of chlorophyll mutations is also a measure of the effectiveness of mutagenic factors. As far as chlorophyll mutations occur relatively often, mutations of the chloroplasts themselves are rather rare. The latter were observed in petunia by Terao (1929) and Muszyński (1967).

The present paper describes an analysis of the structure of mutated chloroplasts in tetraploid petunias.

MATERIAL AND METHODS

The variegata irregulare mutant was obtained after thermal neutron treatment of dry seeds of allotetraploid garden petunias (*Petunia hybrida* hort. *superbissima*). The irradiation was performed in a reactor in Brookhaven National Laboratory. The mutant was found among seedlings

of M_2 generation in 1965. Investigations of the chloroplast structure in the light microscope demonstrated that the mutated plastids are changed in shape and size and contain less chlorophyll or none at all (Muszyński 1967).

The mutated chloroplasts were studied in the electron microscope in the Medical Postgraduate Training Centre of the Polish Academy of Sciences in Warsaw. Leaf sections for analysis were taken from three different leaves: a very young one, a middle aged one and a full grown one. The latter shows distinct areas of normal and mutated tissue of irregular sizes and shapes.

For fixation of the material Caufields fixative was used with Millings buffer for the plants. The fixation time was 2 hours. The material was dehydrated with ethyl alcohol and embedded in the synthetic resin Eton.

RESULTS AND DISCUSSION

Normal chloroplasts develop grana consisting of numerous thylacoids (Figs. 1, 2). Mutated chloroplasts form a structure of parallel lamellae which becomes perforated. In this way small lamellar segments arise, forming primary thylacoids (Figs. 3, 4). Distinct osmiophilic grains are visible which probably are the site of chlorophyll pigment accumulation. Around primary thylacoids the grana start developing. They do not, however, form the full structure as in controls. Their rudiments disappear (Figs. 5, 6) and vacuoles occur instead (Fig. 7). Somewhat older chloroplasts exhibit remnants of the lamellar structure and numerous, small at first and later increasing vacuoles (Fig. 8). The chloroplast structure undergoes progressive disintegration up to complete disappearance. The chloroplast loses then its characteristic disc shape.

The fine structure of the mutated chloroplasts as visible on the photographs leads to the conclusion that the mutation inhibited the synthesis of one of the components responsible for stabilization of the lamellar structure of the chloroplast and of the chlorophyll it contains.

This is evidenced by the normal formation of the parallel lamellae structure to the moment of primary thylacoid formation. At this moment two parallel processes start in the chloroplast, namely chlorophyll synthesis and grana formation. In the mutated chloroplasts chlorophyll synthesis runs normally at first as indicated by the light green colour of young leaves and the presence of numerous minute osmiophilic bodies.

The structure thus formed is not stable, however, because of the lack of a factor which would ensure its stability in the presence of light. Probably one of the carotenoid pigments which act as photostabilizers is lacking (Griffith et al., 1955; Kandler and Schötz, 1956; Andersen et al., 1960; Bachman et al., 1967; Smith et al., 1970).

Fig. 1—2. Fragments of normal chloroplasts ($\times$ ca. 36,000 and $\times$ 40,000)

Fig. 3. Mutated chloroplasts. Structure of parallel lamellae at the moment of perforation appearance (on right upper side) and disintegration of this structure (left side; $\times$ ca. 11,000)

Fig. 4. Mutated chloroplasts. Structure of parallel lamellae with primary thylacoids and first sign of disintegration ($\times$ ca. 20,000)

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Fig. 5. Mutated chloroplasts. Distinct parallel lamellary structure with scarce primary thylacoids which start to disintegrate. Some osmiophilic bodies ($\times$ ca. 13,000)

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Fig. 6. Mutated chloroplasts. Disintegration of parallel lamellae and primary thylacoids; osmiophilic bodies ($\times$ ca. 20,000)

Fig. 7. Mutated chloroplasts. Advanced disintegration of its structure, numerous vacuoles and scarce fragments of primary thylacoids ($\times$ ca. 22.000)

Fig. 8. Far advanced disintegration of structure — numerous vacuoles ($\times$ ca. 26.000)

The other factors may also be the cause, however. It was, namely, found that the addition of amino acids (Langridge, 1955; Langridge and Brock, 1963; Walles, 1963) or of certain nutrients (Döbel and Sagromsky, 1966; Scholz and Rudolph, 1968) or growth substances (Sviesznikova et al., 1966) restores the ability of mutated chloroplasts to green. It seems, however, that the fact of formation of primary structure in mutated chloroplasts of petunia supports the concept of the lack of a stabilizing factor as the cause of ultrastructural disintegration.

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Ultrastruktura chloroplastów u mutantu variegata irregulare zawieratki ogrodowej (Petunia hybrida hort. superbissima)

Streszczenie

Mutant *variegata irregulare* zaindukowany został u zawieratki tetraploidalnej (*Petunia hybrida hort. superbissima*) działaniem neutronów termicznych na nasiona.

Badanie w mikroskopie elektronowym wykazało, że zmutowane chloroplasty wytwarzają strukturę lamelli równoległych do momentu tworzenia się thylakoidów wtórnych. W tym momencie dalszy rozwój gran ulega zahamowaniu, thylakoidy już wytworzone ulegają rozpadowi, powstają wakuole. Chloroplasty te zmieniają również swą wielkość i kształt w porównaniu do chloroplastów normalnych.