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Assimilatory pigments, photosynthetic activity and ultrastructure of chloroplasts of the variegated-leaf chimera of *Acer platanoides* L.*

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Abstract

The assimilatory pigment composition, photosynthetic activity and ultrastructure of chloroplasts were studied in the chlorophyll — deficient chimera of *Acer platanoides* L. Part of the crown of this chimera is a virescent mutant with variegated leaves.

It was found that there exists no qualitative difference in the pigment composition between normal and variegated leaves. The accumulation of chlorophyll in the mutated part is more delayed that the accumulation of carotenoids. The photosynthetic rate on a chlorophyll basis is much higher in variegated than in green leaves. This difference gradually falls off with development.

In the early spring, chloroplasts from the yellow spots of leaf blade have no lamellar system but only many vesicles are dispersed in the stroma. Occasionally also a single granum consisting of a few thylakoids occurs in the stroma. At the end of summer chloroplasts from yellow spots of variegated leaves possess a poorly developed lamellar system.

INTRODUCTION

Until now numerous types of chlorophyll-deficient mutants have been described. In some of them, the rate of photosynthesis per chlorophyll amount may be 2- to 8-fold higher than in normal plants (see: Schmid, 1967, Highkin *et al.*, 1969, Benedict and Kohel, 1968, Benedict *et al.*, 1972, and others), and the saturation of photosynthesis occurs usually at higher light intensity (Homann and Schmid, 1967, Wild and Egle, 1968).

Chloroplasts with a reduced lamellar system are formed by photosynthetically active mutants; instead of normal grana a few overlapping vesicles (thylakoids) might occur on the section. According to Homann and Schmid (1967), the mu-

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tants of higher plants having chloroplasts without double lamellae are inactive in photosynthesis.

The study of some features of the photosynthetic apparatus of the variegated-leaf chimera of *Acer platanoides* L. is the subject of the present paper. This mutant (approximately a 30-year-old tree growing in one of the pleasure gardens in Cracow) has a crown part of which is a mutant of a virescent type. In particular, this work deals with the analysis of assimilatory pigments, photosynthetic activities and the ultrastructure of chloroplasts of variegated and green leaves of this individual.

METHODS

Chlorophyll contents in variegated and green leaves were assayed by spectrophotometry (Uvispek Hilger spectrophotometer) according to the technique by Mackinney (see Holden, 1965). Carotenoid pigments were separated by thin layer chromatography and then assayed by spectrophotometric method (for details see Ficek and Więckowski, 1974).

The rates of gas exchange in the detached leaves or fragments of leaf were determined by the manometric method (Więckowski, 1966, 1971).

The procedure of the preparation of material for electron microscopy investigations has also been reported previously (Więckowski, 1969). The JEM 5Y electron microscope was used in the studies.

RESULTS AND DISCUSSION

The *Acer platanoides* individual growing in one of the pleasure gardens in Cracow mutated spontaneously and as a result, the crown of this plant consists of two kinds of branches: one forms green leaves, and on the other the leaves are non-uniformly yellowish-green coloured. This difference is particularly conspicuous in early spring. At that time the leaves of the mutated part are pale green and frequently only irregular flecks of different size are located at the base of them (Fig. 1A). The leaf blade becomes gradually green throughout development. However, the accumulation of chlorophyll takes place non-uniformly: there appear green patches which gradually enlarge and frequently become fused (Fig. 1B). It was also possible to see more intensive accumulation of chlorophyll around the veins.

All these observations indicate that part of the crown is a chlorophyll-deficient mutant of a virescent type. This paper did not concern the genetic analysis, but the morphological observations suggest that the individual is a sectorial chimera caused by back-mutation in some cells of the shoot growing-point in the early stage of plant development (see Kirk and Tilney-Bassett, 1967).

It apparently follows from Table 1 that only essential quantitative and not qualitative differences exist in the pigment composition between green and variegated leaves. It is also evident that the delay in chlorophyll accumulation exceeds the delay

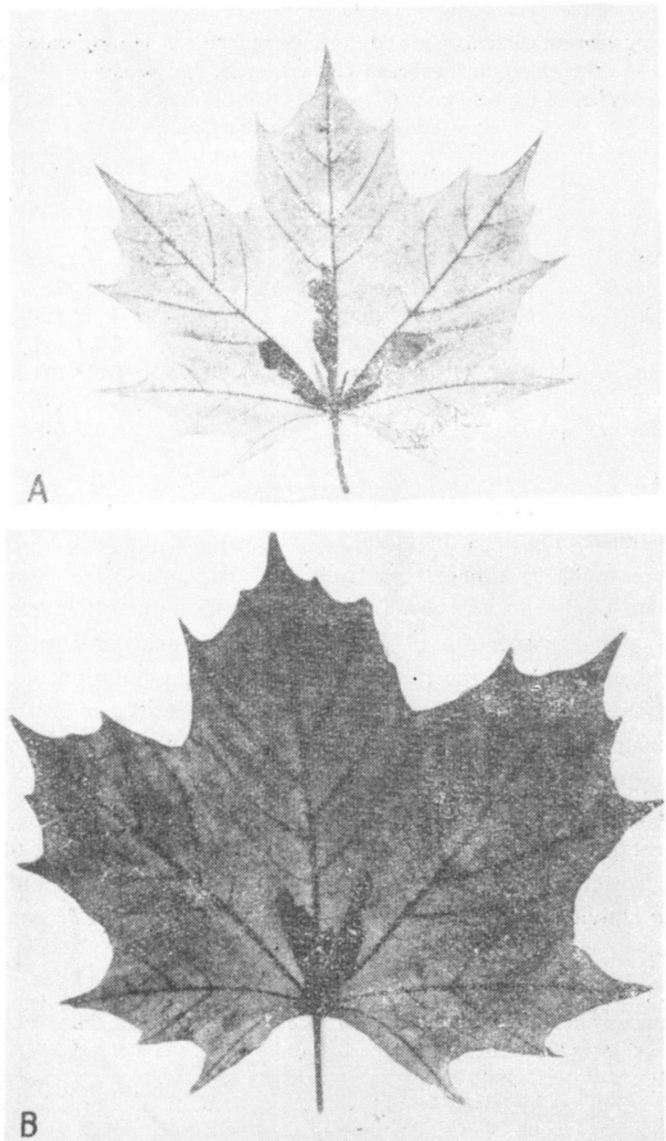

Fig. 1. Typical variegated leaves of the chimera of *Acer platanoides* in spring (A) and at the end of summer (B). Dark spots are due to chlorophyll accumulation.

in the accumulation of carotenoid pigments. For instance, at the spring time there occurred 10- and 2- to 5-fold higher concentration of chlorophylls and carotenoids, respectively, in green leaves than in variegated ones. These differences markedly fell off with development: in autumn the average values of chlorophyll and carotenoid concentrations in mutant leaves reached one-third and one-second, respectively, of the values of pigment amounts in normal leaves.

Table 1

Assimilatory pigment content (in mg dm^{-2}) in green (control) and variegated leaves of the chlorophyll-deficient chimera of *A. platanoides L.*
Procentage values of pigment content in variegated leaves compared with normal green ones are given in brackets.

	April, 23		September, 3	
	control	mutant	control	mutant
chlorophyll *a*	1.38	0.12 (8.7)	2.99	0.96 (32.1)
chlorophyll *b*	0.41	0.02 (4.9)	1.33	0.31 (23.3)
β-carotene	0.083	0.017 (20.5)	0.219	0.077 (35.1)
lutein	0.139	0.024 (17.3)	0.199	0.081 (40.7)
zeaxanthin	0.025	0.011 (44.0)	0.036	0.018 (50.0)
violaxanthin	0.038	0.012 (31.7)	0.072	0.033 (45.8)
neoxanthin	0.033	0.007 (21.2)	0.073	0.033 (45.2)

More intensive accumulation of the pigments near the veins suggests that a substance (or substances) required for pigment synthesis, the production of which is repressed in the mutant, is transported from the green tissue by vascular bundles. However, we are unable to give any indications of its nature; the treatment of the mutant leaves with the solution of δ-aminolaevulinic acid or aqueous extract of green tissue did not enhance the rate of chlorophyll accumulation.

At the applied light intensity, the rate of photosynthesis per unit area in variegated leaves was almost equal with that in green ones (Tab. 2). But as was expected, the rate of photosynthesis on a chlorophyll basis was much higher in variegated than in green leaves.

The demonstrated results are in good agreement with the data of other authors. For instance, Benedict and Kohel (1968) also claim to have found a very high photosynthetic activity in the chloroplast mutant of *Gossypium hirsutum*. The high

Table 2

Photosynthetic rate in two types of leaves of chlorophyll-deficient chimera of *A. platanoides* at the spring time and at the end of summer time.

	Respiration	Total photosynthesis	
	μl O_2 h^{-1} dm^{-2}	μl O_2 h^{-1} dm^{-2}	μl $O_2 h^{-1}$ mg $chl.^{-1}$
May, 3-7			
control	179 ± 28	215 ± 23	103 ± 7
mutant	169 ± 19	154 ± 28	682 ± 154
August, 30 to September, 8			
control	26.8 ± 84.7	149.7 ± 15.6	25.0 ± 1.9
mutant	36.2 ± 6.8	149.3 ± 21.8	69.1 ± 25.1

The presented data indicate only relative photosynthetic activities of the two types of leaves because the photosynthetic rates were measured at the light intensity below the saturation point (at about 42 W m^{-2} of photosynthetically active radiation) and dark respiration instead of photorespiration was taken into consideration.

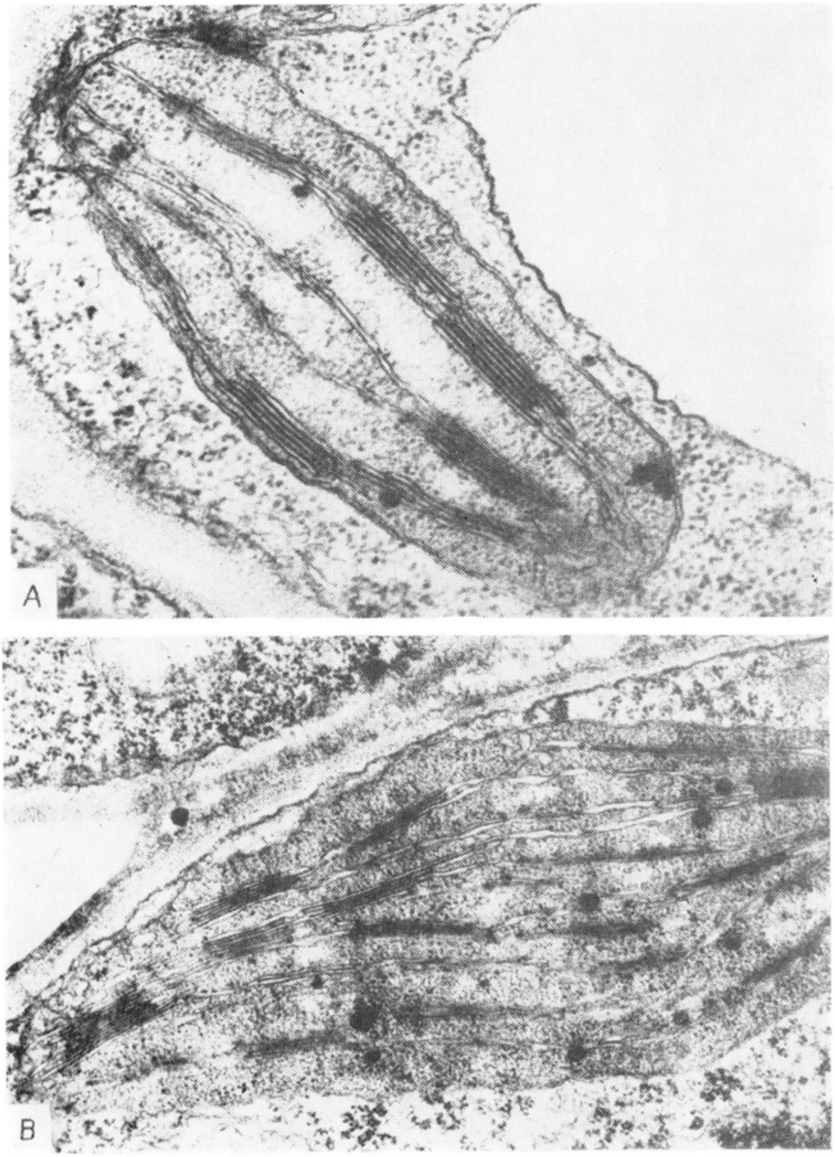

Fig. 2. Chloroplast ultrastructure of the chlorophyll-deficient chimera of *A. platanoides* at the spring time.

A — chloroplast of the unmutated part of the crown, 34 600 ×;
B — chloroplast of the green flecks of variegated leaves, 24 500 ×.

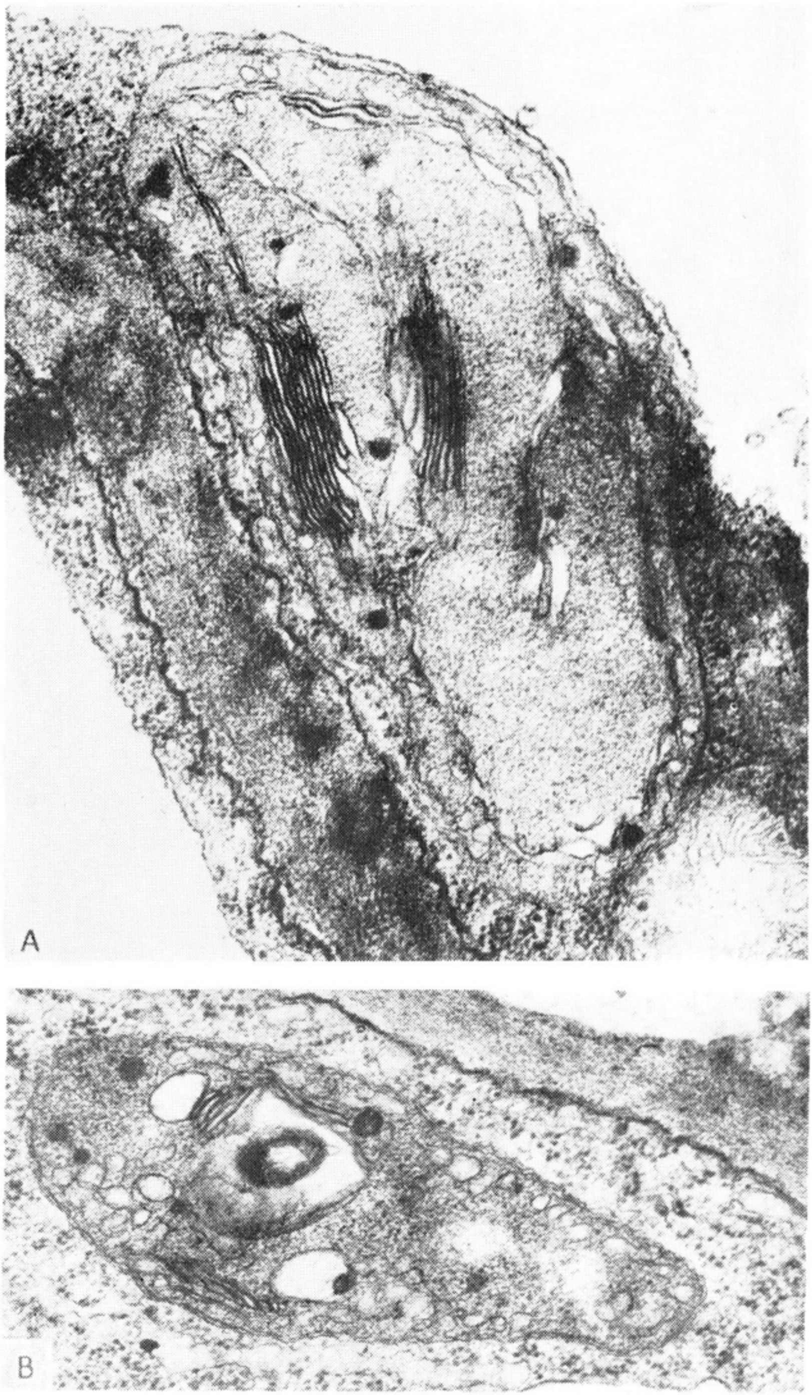

Fig. 3. A, B — Ultrastructure of chloroplasts from yellow spots of the variegated leaves of *A. platanoides* at the spring time. Numerous vesicles and rarely also single granum may occur in the stroma. A — 35000 ×, B — 39200 ×.

photosynthetic rate on a chlorophyll basis has been also discovered in a tobacco mutant referred to as Su/su (Schmid and Gaffron, 1967, Schmid *et al.*, 1966), *Chlorella* mutant (Wild and Egle, 1968), and many others. It is commonly regarded that the high photosynthetic activity per unit amount of chlorophyll may probably be caused by relatively (in comparison with chlorophyll amount) high activities of some Calvin cycle enzymes. For example, in the above mentioned mutant of *Gossypium hirsutum*, the activities (per unit of fresh weight) of ribulose-1,5-diphosphate carboxylase (Benedict and Kohel, 1968), glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglyceric acid kinase, and fructose-1,5-diphosphate aldolase (Benedict *et al.* 1972) are as high as in normal plants in spite of great difference in chlorophyll content. It is also well known that the synthesis of ribulose-1,5-diphosphate carboxylase is not correlated with that of chlorophyll (Benedict and Kohel, 1969).

High photosynthetic activity of some chloroplast mutants is perhaps also due to high efficiency of the electron transport system, e.g. Homann and Schmid (1967) found that the photoreduction of ferricyanide and $NADP^+$ is much higher by the Su/su tobacco mutant than by control plants. The high activities of Hill reaction and photophosphorylation have also been established in the chloroplast mutant of soybean (Keck *et al.*, 1970).

Some authors have found smaller photosynthetic units in the photosynthetically very active mutants than in normal plants. For example, according to Schmid and Gaffron (1969), the photosynthetic unit in their tobacco mutant consists of approximately 300 molecules of chlorophyll whereas in normal plant it consists of approximately 2700 molecules. Likewise Wild (1969) calculated that approximately 2000-2400 and 800 chlorophyll molecules were involved in the reduction of one CO_2 molecules in normal and in his mutant cells of *Chlorella*, respectively. It would seem that a smaller unit is working more efficiently than a bigger one. It may be due, among others, to relative high concentration of other than chlorophyll electron carriers in the mutated leaves.

Probably, high photosynthetic activity in some chloroplast mutants might be related to the high rate of photosynthesis in the leaves in the early stage of their development. For instance, it is reported (Więckowski, 1966, 1969) that the rate of photosynthesis on a chlorophyll basis is higher in very young than in older bean leaves. It seems likely that in our mutant, as well as in many others described in the literature, the development of photosynthetic units is stopped at an early stage and high chlorophyll efficiency is related to this phenomenon.

It appears that in the green flecks of variegated leaves the lamellar system of chloroplasts developed similarly as in the normal green leaves (Fig. 2). At the end of April each granum consists of about ten thylakoids. At the same time the yellow parts of the variegated leaf blade possess chloroplasts with untypical structure; usually numerous free vesicles and single lamellae are dispersed in the stroma. Occasionally a single granum consisting of a few thylakoids is also observable in the stroma (Fig. 3).

At the end of summer some poorly differentiated grana can be observed in the yellow parts of variegated leaves (Fig. 4). At that time there occur high electron-dense globuli in the stroma of chloroplasts isolated from the leaves of variegated and green parts of the crown. Presumably the lipids liberated from the lamellae in the senescent leaves are storaged in these bodies (see also Stearns and Wagebaar, 1971).

The presented observations do not permit the assumption that all chloroplasts which occur in the variegated leaves are active in photosynthesis. Nevertheless the demonstrated data seem to support the view that the occurrence of double lamellae in the chloroplasts of higher plants is required for photosynthetic ability.

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Barwniki asymilacyjne, aktywność fotosyntetyczna i ultrastruktura chloroplastów chimery Acer platanoides L. o liściach plamistych

Streszczenie

Celem pracy było zbadanie składu barwników asymilacyjnych, aktywności fotosyntetycznej i ultrastruktury chloroplastów liści chimery *A. platanoides* L.. Część korony tego okazu uległa spontanicznej mutacji typu *virescent*. Akumulacja chlorofilu jest jednak nie tylko opóźniona, ale odbywa się nierównomiernie w całej blaszce przez co liście są plamiste.

Stwierdzono, że nie występują jakościowe różnice pomiędzy składem barwników w liściach normalnie zielonych i plamistych. W części zmutowanej korony akumulacja chlorofilu jest bardziej opóźniona niż akumulacja karotenoidów. Natężenie fotosyntezy, w przeliczeniu na mg chlorofilu $a+b$, jest kilkakrotnie wyższe w liściach plamistych niż normalnie zielonych. Różnice te stopniowo zmniejszają się w miarę upływu wegetacji.

Wiosną chloroplasty z żółtych fragmentów liści mutanta nie posiadają wykształconego systemu lamellarnego; na terenie stromy występują jedynie liczne pęcherzyki. Czasem spotyka się także pojedyncze grana zbudowane z kilku tylakoidów. Pod koniec lata chloroplasty z żółtych fragmentów liści plamistych posiadają słabo wykształcony system lamellarny.