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PLASTID DEVELOPMENT IN LEAVES OF *LIGUSTRUM OVALIFOLIUM* HASSK. VAR. *AUREUM* AT HIGH AND LOW LIGHT CONDITIONS

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The effect of high and low light conditions on the development of fine structure, pigment content and photosynthetic activity of plastids was studied on an aurea variety of privet (*Ligustrum ovalifolium* Hassk. var. *aureum*). The results obtained for the mutant were compared to those of the wild type plant.

The mutant leaves are able to green only in dim light in the shade, while those exposed to strong sunlight become yellow and finally bleach. The thylakoid system of green leaf plastids is normally developed and they are almost indistinguishable from wild type ones. Plastids from sun exposed yellow leaves lack grana and contain only longitudinal arrays of single thylakoids, or even numerous vesicles and plastoglobules. The pigment content of yellow leaves is very low and so is their photosynthetic activity. Their photosynthetic efficiency (photosynthetic activity calculated on a chlorophyll basis) is, on the contrary, 2 times higher than that of mutant green leaves, and 3.5 times higher than that of wild type leaves.

The relationship between these structural and functional changes in mutant plastids under high light conditions and the possible defect in the chlorophyll-proteins of the light harvesting complex is discussed.

Introduction

Plants are very well adapted to the light environment prevailing in their native habitat. Their adaptation to high light or low light conditions is documented by specific changes in the morphology, physiology and biochemistry of leaves and of plastids (Boardman 1977, Lichtenthaler et al. 1982).

The effect of different light intensities on plastid development is especially pronounced in some conditional mutants (H o p k i n s et al. 1980 a, b), such as aurea varieties of many plants. The leaves of aurea mutants are able to green only when grown in the shade, while those exposed to strong illumination turn yellow and eventually bleach (W r i s c h e r et al. 1975 a, b, 1976). The chlorophyll content of yellow leaves is reduced (S e y b o l d and E g l e 1938, B a u e r 1956), as well as the thylakoid system of their plastids (S c h m i d et al. 1966, W r i s c h e r et al. 1975 a, b, 1976). On the other hand, their photosynthetic rates on a chlorophyll basis under high light conditions usually become very high (W i l l s t ä t t e r and S t o l l 1918, S c h m i d 1967, W r i s c h e r et al. 1976).

The present study was initiated in order to obtain some new data about the development of mutant plastids under high and low light conditions. For this purpose the leaves of an aurea variety of privet (*Ligustrum ovalifolium* Hassk. var. *aureum*) were used. Structural and functional characteristics of wild type plastids were studied parallelly as well.

Material and Methods

The experiments were carried out during late spring and early summer on an aurea variety of privet (*Ligustrum ovalifolium* Hassk. var. *aureum*) and a wild type privet (*Ligustrum ovalifolium* Hassk.) growing in the garden of Ruđer Bošković Institute, Zagreb. The leaves exposed to different light intensities, existing in the shrub under natural conditions of illumination, were studied in detail.

Plastid pigments were extracted in 80% acetone. Chlorophylls were quantitatively determined according to H o l d e n (1965), and total carotenoids according to U r b a c h et al. (1976). Carotenoids were separated by thin-layer chromatography, dissolved in petrol ether-ethyl acetate--diethylamine (58 : 30 : 12) and estimated according to S t a h l (1969).

Photosynthetic activity of leaf pieces was measured with an O_2 electrode (Hansatech Ltd., England). The reaction mixture contained 0.1 mol phosphate buffer (pH 8) and 0.01 mol sodium bicarbonate (A n t i c a and W r i s c h e r 1982). The samples were illuminated with 5 different light intensities: 10^5lx, 55 × 10^3lx, 30 × 10^3lx, 13 × 10^3lx and 9 × 10^3lx.

For electron microscopy small leaf pieces were fixed in 1% glutaraldehyde in cacodylate buffer (pH 7.2; 1°C), postfixed in 1% OsO_4, and after dehydration embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate (R e y n o l d s 1963) and examined in a Siemens Elmiskop I.

Sections of leaf material embedded in Araldite (about 0.5 μm thick), as well as hand-made sections of fresh leaves were also examined in the light microscope.

Results

Macroscopic observations: All the leaves of *Ligustrum ovalifolium* Hassk. var. *aureum* are light-green in early spring under natural conditions of illumination. A few weeks later the leaves growing on the outermost branches of the shrub and exposed to the sun turn golden yellow, while those growing in the shade inside the shrub become green. Yellow leaves which remain longer on the surface of the shrub soon become yellowish-white and finally bleach. On the other hand, yellow leaves which are covered by younger leaves turn greenish in a short time.

Light-microscopic observations: Yellow and yellowish-white leaves are thicker than the green ones as a result of a stronger development of the palisade mesophyll. They have two cells of palisade mesophyll across a leaf section, compared with one cell in the leaves from low light. There are also more plastids across a leaf section in sun exposed leaves, but the plastids are usually smaller than the ones in shade leaves.

Electron-microscopic observations: Wild type green leaves contain chloroplasts characterized by large grana connected with stroma thylakoids (Fig. 1). The number of thylakoids per granum depends on whether the leaves are grown in the sun (8 — 12 thylakoids) or in the shade (15 — 20 thylakoids). Starch grains are present in the stroma.

Plastids from mutant green leaves at low light levels are almost indistinguishable from those of wild type leaves. Their thylakoid system is normally developed (grana consisting of 10 — 15 thylakoids) and stroma is full of ribosomes and starch (Figs. 2, 3). The ultrastructure of plastids in yellow-green leaves is already significantly different. Plastids in young leaves usually lack grana but possess longitudinal arrays of single thylakoids, while those in older yellow-green leaves contain thylakoids dilated into vesicles, or densely packed into cup-shaped stacks (Figs. 4, 5). These cup-shaped structures appear as a result of the degeneration of grana. In yellow leaves the thylakoid system is further reduced, and is composed mostly of large vesicles and plastoglobules. The number of vesicles and plastoglobules gradually increases as yellow leaves turn yellow-white and finally completely bleach (Fig. 6). Starch grains never appear in yellow and white leaves, while ribosomes are always present. White leaf plastids usually contain phytoferritin in the stroma (Fig. 6 detail).

Pigment concentrations: The concentration of chlorophylls in mutant green leaves reaches 0.63 mg/g fresh leaf weight (43% of chlorophyll present in wild type green leaves), but with increasing light intensity there is a marked decline in the chlorophyll content. Yellow-green leaves contain 52% yellow leaves 27% and white leaves 10% of chlorophyll present in mutant green leaves (Table 1). At the same time the chloro-

Table 1. Chlorophyll and carotenoid content of aurea-privet and wild type privet leaves.

	Chlorophylls		Carotenoids		
	mg/g fr. weight	Chl a / Chl b	mg/g fr. weight	Xanthophylls / β-carotene	Chlorophylls / Carotenoids
W	0.06	1.29	0.06	—	1.12
Y	0.17	1.52	0.14	9.54	1.38
YG	0.33	3.08	0.19	—	1.89
G	0.63	2.53	0.09	2.19	7.37
DG_{su}	1.18	2.26	0.27	3.91	4.65
DG_{sh}	1.45	2.10	0.19	2.78	7.35

phyll yield from the wild type leaves drops only about 19% at high light intensities. The chlorophyll a/b ratio is the highest in mutant yellow-green leaves (3.08) and mutant green leaves (2.53), while in yellow and white leaves exposed to the sun the ratio rapidly decreases. The concentration of total carotenoids is high in the leaves under high light compared to those under low light conditions. Among carotenoids the contents of β-carotene and lutein vary considerably in sun and shade mutant leaves, while in wild type leaves this difference is less pronounced (Table 2). On the contrary, the concentrations of violaxanthin and neoxanthin remain relatively unchanged in mutant leaves, and differ in wild type ones under high and low light conditions.

Table 2. Chlorophyll and carotenoid content of aurea-privet and wild type privet leaves.

	Y	G	DG_{su}	DG_{sh}
Chlorophyll a	0.095 (55.88%)	0.444 (70.82%)	0.818 (69.20%)	0.979 (67.75%)
Chlorophyll b	0.075 (44.12%)	0.183 (29.18%)	0.364 (30.80%)	0.466 (32.25%)
Chlorophyll a + b	0.170 (100 %)	0.627 (100 %)	1.182 (100 %)	1.445 (100 %)
β-carotene	0.013 (9.51%)	0.029 (31.29%)	0.054 (20.39%)	0.050 (26.51%)
Lutein	0.108 (77.27%)	0.053 (57.68%)	0.169 (63.80%)	0.091 (48.53%)
Violaxanthin	0.012 (8.69%)	0.006 (6.56%)	0.025 (9.52%)	0.023 (12.43%)
Neoxanthin	0.006 (4.53%)	0.004 (4.47%)	0.017 (6.29%)	0.024 (12.53%)
Total carotenoids	0.139 (100 %)	0.092 (100 %)	0.265 (100 %)	0.188 (100 %)

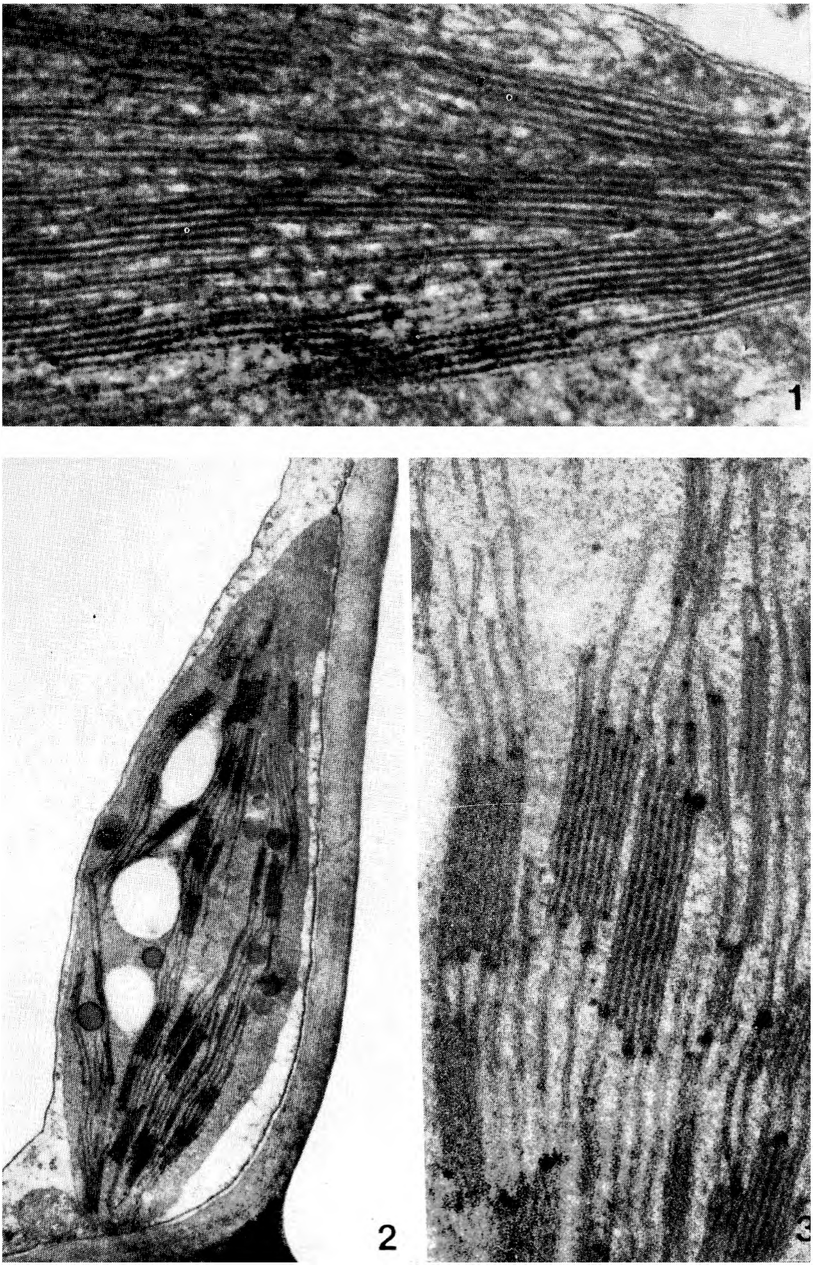
W = white leaf
Y = yellow leaf
YG = yellow-green leaf
G = green leaf
} aurea type leaves

DG_{su} = dark-green leaf in the sun
DG_{sh} = dark-green leaf in the shade
} wild type leaves

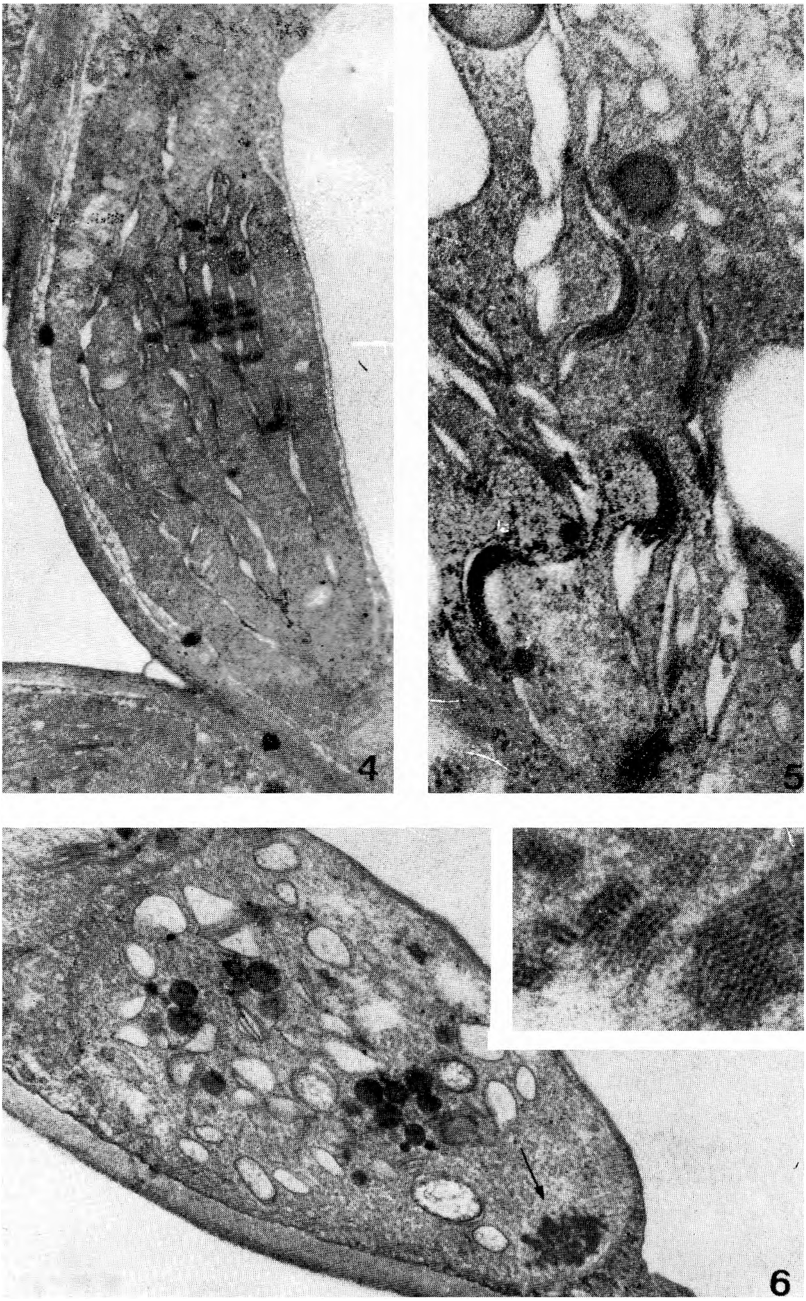
Fig. 1. Part of a chloroplast from a wild type leaf of *Ligustrum ovalifolium* Hassk. The thylakoid system is well developed. 100,000:1.

Fig. 2. Chloroplast from a mutant green leaf (*Ligustrum ovalifolium* Hassk. var. *aureum*) grown in deep shade. Starch and plastoglobules are present in the stroma. 15,000:1.

Fig. 3. Part of the same chloroplast as in Fig. 2 under higher magnification, showing well developed grana. 64,000:1.



Figs. 1—3.



Figs. 4—6.

Fig. 4. Plastid from a young yellow-green leaf with longitudinal arrays of single thylakoids. Plastoglobules are present in the stroma. 16,000:1.

Fig. 5. Part of a plastid from an old yellow-green leaf. Thylakoids are dilated into vesicles or densely packed into cup-shaped stacks. 62,000:1.

Fig. 6. Plastid from a white (bleached) leaf with numerous vesicles and plastoglobules. Phytoferritin inclusion is present in the stroma (arrow). 19,000:1. Detail: Phytoferritin inclusion under high magnification. 112,000:1.

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Photosynthetic activity: The photosynthetic activity expressed in μmol O_2/g fresh leaf weight/h of mutant green leaves was almost as high as that of wild type ones (Table 3), but the activity of yellow and especially white leaves was very low (as much as 4.5 times lower than that of mutant green leaves). On the other hand, the highest photosynthetic efficiency was obtained for white leaves (358.68 μmol O_2/mg Chl/h) and yellow leaves (286.25 μmol O_2/mg Chl/h; Fig. 7). The photosynthetic efficiency of white leaves is 2.5 times higher, and of yellow leaves only 2 times higher than of mutant green leaves, which is unusually low when compared to values measured for other aurea plants.

Table 3. Photosynthetic activity in aurea-privet and wild type privet leaves in μmol O_2/g fr. wt./h.

	10^5 lx	55×10^3 lx	30×10^3 lx	13×10^3 lx	9×10^3 lx
W	22.99	21.44	20.73	18.97	18.97
Y	47.39	43.98	37.37	32.55	30.24
YG	79.45	68.87	60.24	53.49	46.80
G	104.02	104.02	88.33	69.28	56.78
DG	104.92	97.54	81.30	63.54	52.90

W = white leaf
Y = yellow leaf
YG = yellow-green leaf
G = green leaf
} aurea type leaves
DG = wild type dark-green leaf

Discussion

Depending on the amounts of light available during the development, plants possess the ability to react with either a high light or a low light growth response. Plants adapted to high light exhibit, among many other characteristics, thicker leaves with more chloroplasts across a

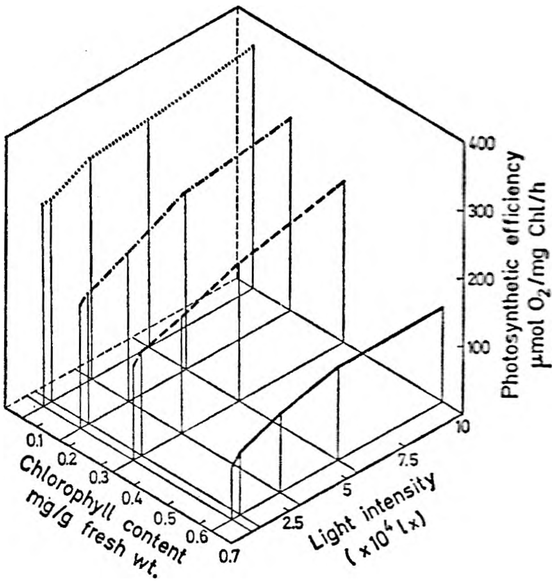

Fig. 7. Chlorophyll content and photosynthetic efficiency of aurea-privet leaves under different light intensities.
Legend: full line = green leaf; dash-line = yellow-green leaf; dash-dot-line = yellow leaf; dot-line = white leaf.

leaf section and s. c. sun-type chloroplasts with different ultrastructure, composition and activity when compared to low light plants (Boardman 1977).

Studies of mutant plants have revealed similar adaptational patterns although more extreme in expression. The aurea variety of privet is a conditional mutant whose expression can be controlled by light intensity. Under low light conditions it is characterized by green leaves, which are comparable in colour to young wild type ones, while the leaves growing in the sun become golden-yellow (Wrischer et al. 1975 a,b, 1976). The carotenoid content of young yellow leaves is 36% higher than of mutant green ones, but in older yellow and white leaves the carotenoid level also gradually declines. Especially rapid is the degradation of β-carotene, as can be seen from the increase of xanthophylls/β-carotene ratio.

In order to increase light absorption and overcome the light shortage, low light leaves contain chloroplasts with large grana stacks and high chlorophyll concentration. In contrast to that, plastids from yellow leaves grown in the sun possess no grana, and contain only single thylakoids or thylakoids dilated into vesicles. Their chlorophyll content is reduced to 27% of that present in green leaves.

Despite their chlorophyll deficiency and changes in plastid ultrastructure, yellow leaves exhibit maximal photosynthetic rates on a chlorophyll basis which are two times higher than those of mutant green leaves, and 3.5 times higher than those of wild type green ones. This increased photosynthetic efficiency of sun exposed mutant plastids reflects their smaller photosynthetic unit size, caused by the loss of antenna chlorophyll under high light intensities (Okabe et al. 1977, Hopkins et al. 1980 b).

All these differences in ultrastructure, pigment composition and photosynthetic efficiency between high light and low light wild type plastids, and especially mutant plastids, are due to differences in the levels of the various chlorophyll-proteins of the photosynthetic apparatus. The low light plastids are characterized by the high percentage of chlorophyll-a/b-proteins of the light harvesting complex (LHCP) and the reduced amount of a/β-carotene proteins CPI and CPIa of photosystem I complex, as well as CPa of photosystem II complex. On the other hand, increased CPI, CPIa and CPa levels and low levels of light harvesting chlorophyll-proteins correspond to sun type plastids (Lichtenthaler et al. 1982). An analysis of chlorophyll-protein complexes of some light dependent mutants has shown that with increasing light intensity the proportion of light harvesting chlorophyll-proteins not only declines, but that two polypeptides, identified as light harvesting chlorophyll--proteins, are actually completely missing (Hopkins et al. 1980 a, Koivuniemi et al. 1981). It seems that all the structural and functional changes in mutant plastids are the consequence of the defect in light harvesting chlorophyll-protein complex. Possibly this concerns the aurea privet as well.

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SAŽETAK

RAZVOJ PLASTIDA U LISTOVIMA SVOJTE *LIGUSTRUM OVALIFOLIUM* HASSK. VAR. *AUREUM* NA SVJETLOSTI RAZLIČITIH INTENZITETA

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Istražen je utjecaj intenziteta svjetlosti na ultrastrukturu, sadržaj pigmenata i fotosintetsku aktivnost plastida kaline tipa aurea (*Ligustrum ovalifolium* Hassk. var *aureum*). Rezultati su uspoređeni s onima dobivenim za plastide kaline divljeg tipa.

Listovi tipa aurea mogu normalno ozelenjeti samo na slaboj svjetlosti u sjeni, dok na intenzivnoj sunčevoj svjetlosti postaju zlatnožuti i na kraju izblijede. Plastidi zelenih listova imaju normalno razvijen tilakoidni sustav i gotovo se ne razlikuju od plastida divljeg tipa. U plastidima žutih listova izloženih suncu postoje samo pojedinačni tilakoidi, ili čak brojne vezikule i plastoglobuli. Koncentracija pigmenata u žutim listovima je vrlo niska, a također i njihova fotosintetska aktivnost. Fotosintetska efikasnost (fotosintetska aktivnost izražena na jedinicu klorofila) u njih je, naprotiv, 2 puta viša od one u zelenim listovima tipa aurea i 3,5 puta viša od one u listovima divljeg tipa.

Strukturne i funkcionalne osobine plastida tipa aurea na jakoj sunčevoj svjetlosti vjerojatno su posljedica promjena u sastavu antenskih kompleksa klorofila i proteina (light harvesting complex).

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