PLASTID DEVELOPMENT AND CAPACITY OF CARBOXYLATING ENZYMES IN NORMAL AND MUTANT MAIZE LEAVES UNDER DIFFERENT ILLUMINATION INTENSITY

by

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Comparative studies on chloroplasts of normal and pigment deficient mutant plants show effects of genetics factors on the development and function of chloroplasts (Maclachlan et Zalik 1963, Sun 1963, Wettstein 1961). Accordingly, the chloroplasts, which have been block in structural organization at different stages, show differences in capacity of CO_2 fixation and in photosynthetic activity (Faludi et al. 1963, Gyurján et al. 1966).

In plants with C_4-dicarboxylic acid pathway, the type of CO_2 fixation is closely connected with the anatomy of the leaf and the structure of the chloroplasts (Lactsch 1969). These plants possess an accurate regulation of activities of the carboxylating enzymes (Slack et al. 1969).

Kannangara (1969) found, that the synthesis and activation of ribulosediphosphate carboxylase are connected with structural differentiation of the chloroplasts. The activity of phosphopyruvate carboxylase was found to be influenced by the intensity of illumination (Hatch et al. 1969).

In the present work we examined the effect of different intensity of illumination on the structure of chloroplasts and on the activities of photosynthetic carboxylases in mesophyll and bundle-sheath tissue.

Material and methods

Seeds of normal and two carotenoid mutant of *Zea mays* L., accumulating lycopene or ζ-carotene, were grown at constant temperature of 25°C, in dark. 6 days old leaves were illuminated for 12 hours at an intensity of 100 and 1000 lux.

The control leaves were grown in dark.

The representative samples of control and illuminated leaves were fixed in 2% $KMnO_4$ buffered with veronal acetate (pH 7.4), embedded

in durcupan and sectioned with a Porter-Blum ultramicrotome. Preparations were examined in a KEM – 1 electron microscope.

1.0 g samples of control and illuminated leaves were used to separate the mesophyll and parenchyma-sheath cells, with the method of Björkman and Gauhl (1969). The pigment determination was carried out in ethyl ether solution with the multi-wawelength method (Faludi Dániel et al. 1970).

Activities of ribulosediphosphate carboxylase (RuDP-carboxylase EC 4.1.1.39) and phosphopyruvate carboxylase (PEP-carboxylase EC 4.1.1.31) were determined by measuring the incorporation of ^{14}C from $H^{14}CO_3$ in the presence of D-ribulose-1,5-diphosphate and phosphopyruvate, respectively (Nagy et al. 1971).

Acid-stable radioactivity was determined by liquid scintillation-counting (Nuclear Chicago 724), the samples were prepared by Bush et Hansen's (1965) method.

Results

Chlorophyll (a+b) contents of the leaves (Table 1) indicated, that illumination of etiolated seeds was favourable only for normal leaves. In this case the intensity of chlorophyll (a+b) synthesis was higher at 1000 lux, than at 100 lux. At low-intensity illumination the mutant leaves contained a small quantity of chlorophyll (a+b), but 1000 lux was damaging for them, decreasing their chlorophyll content.

Table I.

Total chlorophyll (a+b) content and CO_2 fixation by carboxylating enzymes and their distribution between two types of chloroplasts in normal and mutant maize leaves, grown under different illumination intensity

Material	Illumination lux	Chlorophyll a+b nmol/g fr.w.	% in boundle sheath	Total CO_2 fixation nmol CO_2/g fr.w	% in boundle sheath
Normal	Etiolated	...	–	376	28
	100	163	25	513	10
	1000	226	36	774	23
Lycopenic	Etiolated	...	–	770	26
	100	33	53	742	46
	1000	14	40	716	45
ζ-carotenic	Etiolated	...	–	789	35
	100	15	51	723	46
	1000	...	–	721	32

Distribution of chlorophyll between parenchyma-sheath and mesophyll seemed to be different, when comparing normal and mutant leaves. At an illumination of 100 lux the percentage of chlorophyll was lower in the parenchyma sheath of normal leaves, than in the mutant ones. In the leaves illuminated with 1000 lux, this distribution changed; it was increased in normal leaves and decreased in lycopenic ones. The ζ-carotenic mutants practically lost their chlorophyll (a+b) content.

The in vivo capacity of total CO_2 fixation considerably increased in normal leaves, in consequence of an illumination with 100 and 1000 lux. Total CO_2 fixation by the carboxylating enzymes was higher etiolated mutant leaves, than in normal ones. At different illumination intensity, this high enzyme capacity was practically unchanged both in lycopenic and ζ-carotenic leaves.

In etiolated normal and mutant leaves the chloroplasts of bundle-sheath possessed about one third of the total enzyme activity. This ratio decreased in normal leaves, and increased in mutant ones, in consequence of illumination.

RuDP-carboxylase capacity was rather similar in etiolated normal and mutant leaves (Table 2). Illumination increased the activity of enzyme in normal chloroplasts. Under any experimental condition the RuDP-carboxylase capacity was unchanged in mutant leaves.

Table II.

Activity and distribution of ribulosediphosphate carboxylase in normal and mutant maize leaves, grown under different illumination intensity

Material	Illumination lux	nmol CO_2/g fr.w.	% in bundle sheath	Participation in total CO_2 fixation %
Normal	Etiolated	17	35	5
	100	15	67	3
	1000	54	56	7
Lycopenic	Etiolated	15	27	3
	100	15	47	2
	1000	18	56	3
ζ-carotenic	Etiolated	26	42	3
	100	22	45	3
	1000	26	42	4

In normal and lycopenic leaves the distribution of this enzyme was affected by the illumination. More than half of the total enzyme activity was localized in the bundle-sheath cells of normal and lycopenic leaves.

In the ζ-mutant the distribution of RuDP-carboxylase was unaffected by the illumination. During greening, the PEP-carboxylase activity of normal leaves considerably increased both at 100 lux and at 1000 lux intensity of illumination (Table 3). In etiolated mutant leaves, the level of PEP-carboxylase was much higher than in the normal ones. Under illumination of different intensity, this enzyme-capacity was unchanged or little decreased.

Table III.

Activity and distribution of phosphopyruvate carboxylase in normal and mutant maize leaves, grown under different illumination intensity

Material	Illumination lux	nmol CO_2/g fr.w.	% in bundle sheath
Normal	Etiolated	361	27
	100	498	8
	1000	720	21
Lycopenic	Etiolated	755	26
	100	727	45
	1000	698	45
ζ-carotenic	Etiolated	763	36
	100	701	46
	1000	695	31

Etioplasts of bundle-sheath cells showed a similar ratio of total PEP-carboxylase activity both in normal and in mutant leaves. The participation of these chloroplasts in the enzyme capacity decreased in normal tissues, and increased in mutant ones, as pre illumination.

Discussion

The activity of RuDP-carboxylase and PEP-carboxylase was investigated, as compared with changes in plastid structure at different intensity of illumination.

In Fig. 1, electron micrographs of normal leaves are shown. Both structure and enzyme capacity are found to be different in etioplasts of mesophyll and of bundle-sheath tissue. At 100 lux, the reduction of the prolamellar body begins, and the grana appear. This stage of differentiation is more expressed in mesophyll tissue. At the same time the intensity of chlorophyll synthesis is higher in these chloroplasts, than in bundle-sheath chloroplasts. About 90 per cent of total CO_2 fixation is also localized in the mesophyll cells. This increase of enzyme capacity seems to be risen from the activity of PEP-carboxylase. In our experi-

mental conditions, the activity of this enzyme is mainly affected by illumination.

At a higher intensity of illumination (1000 lux), thylacoid and grana formation occur both in mesophyll and in bundle-sheath cells. In this case the RuDP-carboxylase was found to be increased.

It has not been established, whether the increases in the activity of the enzyme resulted from protein synthesis or enzyme activation. Treatment with light whould appear to have induced de novo protein (enzyme) synthesis, on the other hand, RuDP-carboxylase requires an association with a structural component of the chloroplasts (B r a d b e e r 1970, K a n n a n g a r a 1969).

B r a d b e e r et al. (1970) found, that flashing light was effective to increase the chlorophyll synthesis and the activities of some enzymes (namely of RuDP-carboxylase), howewer, a little change showed in the activity of PEP-carboxylase. At the same time the fine structure of the etioplast was somewhat changed by flashing light.

During a continuous illumination of etiolated leaves, the activities of enzymes increased both inside and outside the chloroplasts (B r a d b e e r et al. 1969).

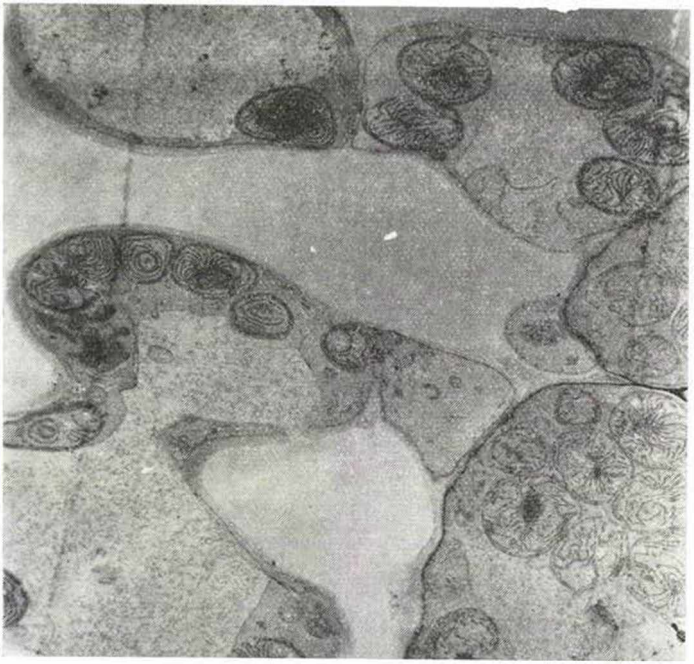

Fig. 1a Chloroplasts of etiolated normal leaves
left: mesophyll; right: bundle-sheath tissue; (1.800×)

According by the changes in the fine-structure may have been necessarry for the synthesis of certain enzymes.

In the case of mutant leaves, the etioplasts show strong heterogeneity. There are found chloroplasts with a structure similar to that of the normal ones, and on the other hand, chloroplasts with irregular structure (Fig. 2, 3). The PEP-carboxylase capacity of these leaves was much higher than that of the normal ones.

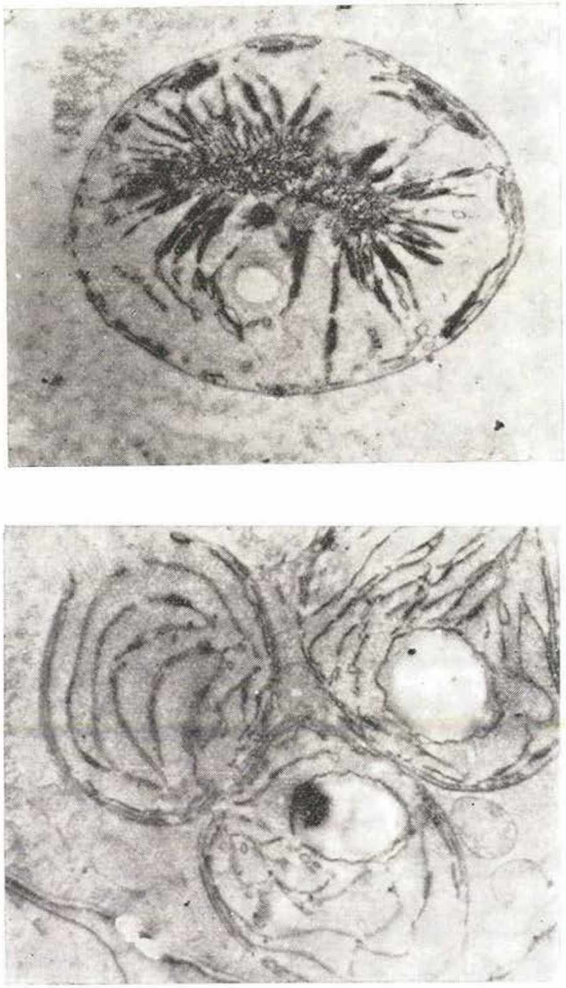

Fig. 1b Chloroplasts of normal leaf illuminated with 100 lux
left: mesophyll; right: bundle-sheath; (13.200×)

Fig. 1c Chloroplasts of normal leaf illuminated with 1000 lux left: mesophyll; right: bundle-sheath; (4.400×)

Under the illumination in the chloroplast of lycopenic leaves formation of grana was found. Howewer, the 1000 lux were very damaging for lycopenic plastids, which seemed mainly to be destroyed in mesophyll tissue. The lamellae were placed concentrically or, in other chloroplasts, they were completely destroyed. Real formation of grana could not be observed in these chloroplasts. In the mesophyll tissue decreasing the capacity of carboxylating enzymes also was found.

The structure of bundle-sheath chloroplasts was not destroyed so much. Some grana formation could be observed.

The activity of PEP-carboxylase in the boundle-sheath chloroplasts was much higher than in the normal ones. In our earlier experiments we already found, that in carotenoid mutant maize leaves CO_2 might be caught by PEP-carboxylase in the bundle-sheath cells in consequence of the preponderance of its high capacity over RuDP-carboxylase activity (N a g y et al. 1970).

In the illuminated ζ-carotenic leaves, chloroplasts showed to be of blistered structure both in mesophyll and in bundle-sheath cells. At 100 lux some plastids had lamellae with concentrical structure in the bundle-

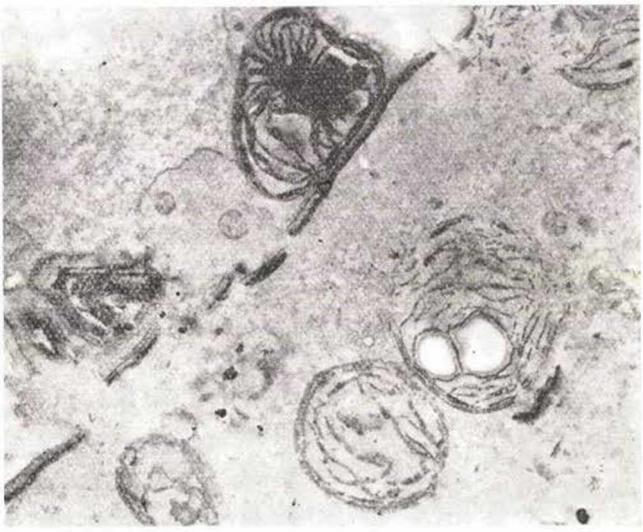

Fig. 2a Chloroplasts of etiolated lycopenic leaves
left: mesophyll; right: bundle-sheath; (8.800×)

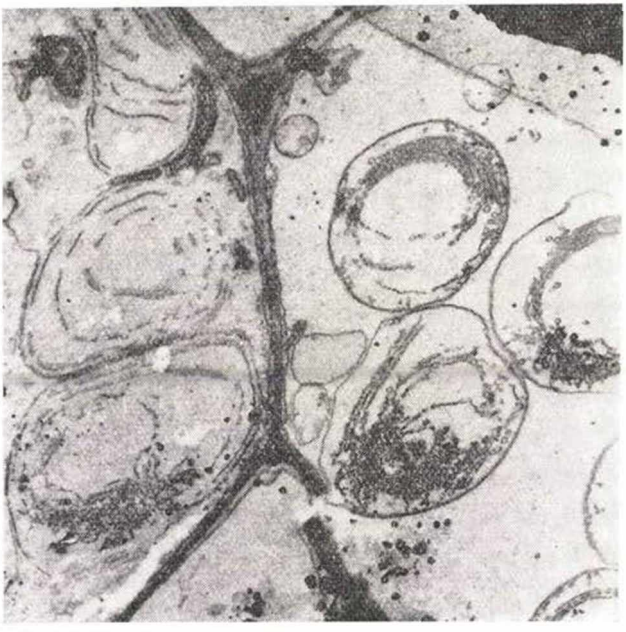

Fig. 2b Chloroplasts of lycopenic leaves illuminated with 100 lux
up: mesophyll; down: bundle-sheath; (4.400×)

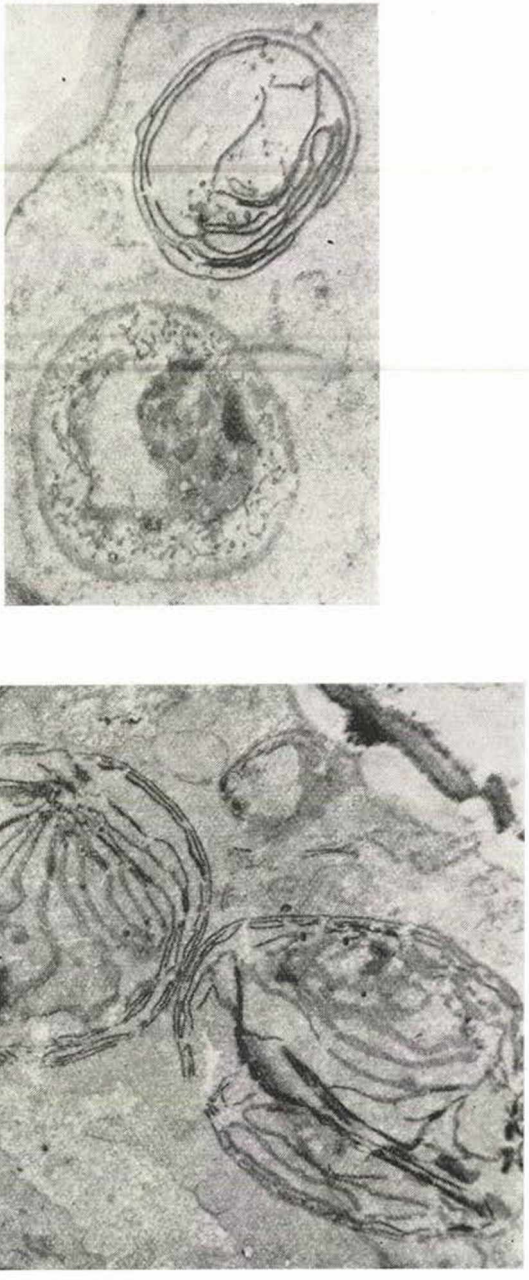

Fig. 2c Chloroplasts of lycopenic leaves illuminated with 1000 lux
up: mesophyll; down: bundle-sheath; (8.800×)

sheath tissue. The enzyme capacity of the illuminated leaves was similar to that of the etiolated ones. Both the activity and the distribution of enzymes were practically unchanged at different intensities of illumination.

Destroying the structure of mutant plastids resulted in decreasing the chlorophyll content. This process overtakes destruction of enzyme capacity.

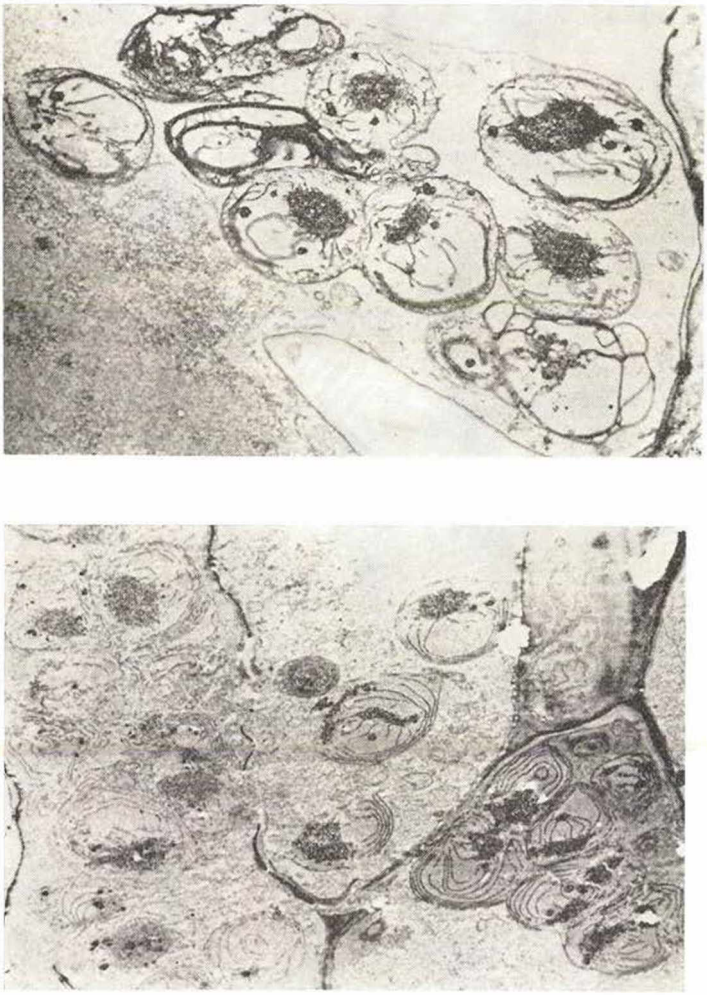

Fig. 3a Chloroplasts of etiolated ζ-carotenic leaves
up: mesophyll; down: bundle-sheath; (4.400 : 1; 2.200×)

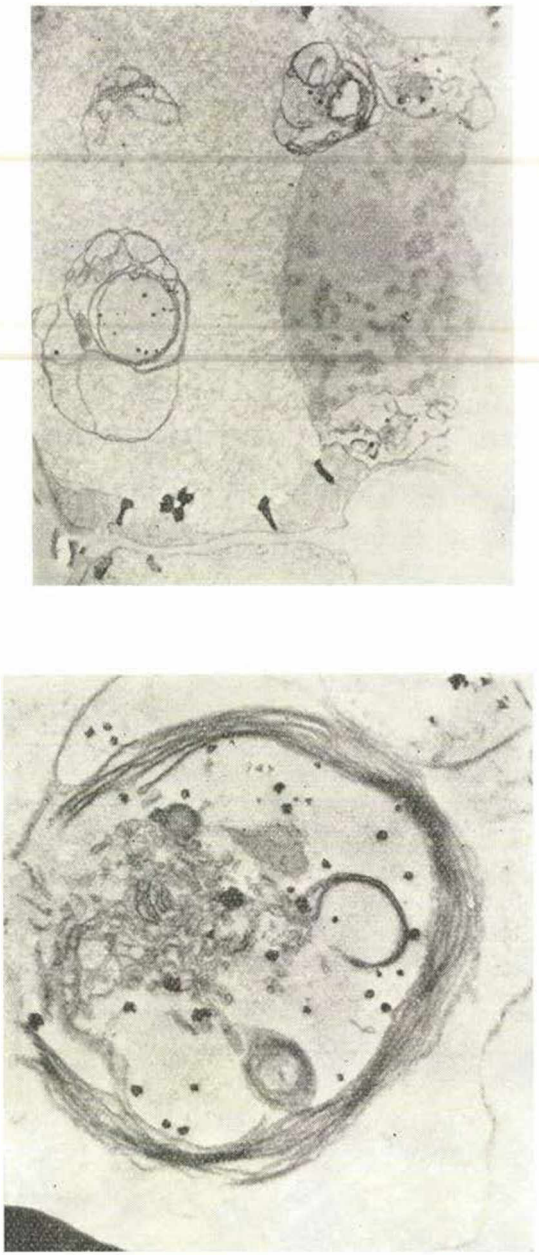

Fig. 3b Cloroplasts of ζ-carotenic leaves illuminated with 100 lux
up: mesophyll; down: bundle-sheath; (4.400 : 1; 13.200×)

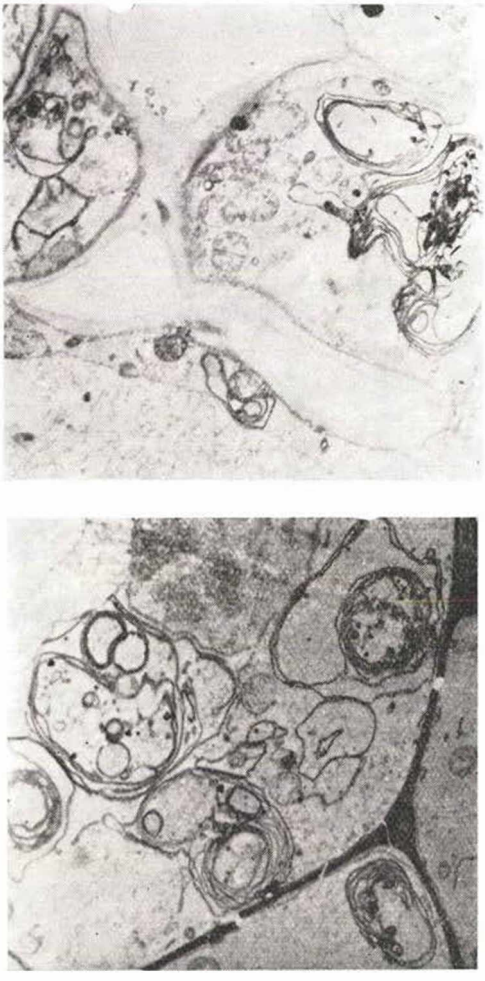

Fig. 3c Chloroplast of ζ-carotenic leaves illuminated with 1000 lux
up: mesophyll; down: bundle-sheath; (4.400×)

Summary

At different intensities of illumination characteristic stages of differentation were found in normal and carotenoid mutant maize leaves, both in mesophyll and in bundle-sheath chloroplasts. These structural changes were in accordance with the changes in the activities and with the distribution of the two carboxylating enzymes.

In consequence of illumination, the activity of RuDP-carboxylase and PEP-carboxylase increased, depending on the intensity of illumination.

Etiolated mutant leaves had much higher PEP-carboxylase capacity, than the normal ones. This was not changed in illuminated mutant leaves.

Mesophyll and bundle-sheath chloroplasts were affected by illumination in different ways.

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