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EFFECT OF SHORT PERIODS OF LIGHT ON THE ORGANIZATION OF THE MEMBRANEOUS SYSTEM OF CORN MESOPHYLL CHLOROPLASTS

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Abstract

The effect of 16—8 h and 15—7.5 min light-dark cycles (LDC) on the lamella system of mesophyll chloroplast (Mchlp) was studied in the fourth leaves of five weeks old corn plants grown in phytotron. In the long and short LDC-s the period of light was the same (16 h/day), the light intensity was 32 Wm^{-2}.

Compared to the 16—8 h control, the 15—7.5 min LCD showed the following effects:

— The cut-surface quota (area) of the grana almost decreased to the half;
— In the case of corns 523 broader grana of lower height developed. The grana made up of 2—4 thylakoids were predominating (cc. 50%), while those standing of 14—35 thylakoids were missing;
— In the case of corns 165 and 3901 the thylakoids of the high grana became swollen, the partitions became loose, "decomposed". Stroma lamellae were frequent in these M chloroplasts.
— In the Mchlp starch occurred in lower amount and smaller size.

Introduction

It was determined by BLACKMAN in 1905 that photosynthesis has two limiting factors: photochemical reactions in case of low light intensity, and dark reactions in that of high light intensity.

The significance of the length of light periods on the development of plants and the usefulness of light was later confirmed by several authors: WARBURG (1919); GARNER and ALLARD (1931), EMERSON and ARNOLD (1932), GREGORY and PEARSE (1937), PORTSMOUTH (1937), BONDE (1955), HILLMAN (1956), WHITTINGHAM and BROWN (1953), FOGG (1968), POLLARD (1970), RAJAN et al. (1971), HORVÁTH et al. (1977, 1978) and others.

KETELLAPER (1964) holds the ratio of photoperiod and whole cycle length as an important factor. Besides the daily illumination and length of photoperiod, great importance is also attached to the length of dark periods in the short periods of light (MARÓTI et al. 1981; MARÓTI and PATAKY, 1982; MARÓTI, 1982).

It is known that the various lengths of light-dark periods have different effects on the growth of plants, however, there are only few data on its relationship to the internal membraneous system of chloroplasts.

It is assumed that the length and ratio of the light-dark period basically determine the organization of the chloroplast membraneous system. On the basis of our earlier studies (MARÓTI and GÁBOR, 1976; MARÓTI, 1982) it may also be presumed that the area and ratio of the single and stacked lamellae also have important role in the light utilization of certain plants.

It is not clear as yet in what degree the light and genotype, resp. determine the ratio of the single and stacked membranes. In fact, even the relationships between the

light intensity and granum-formation are evaluated diversely (BOARDMAN et al. 1974; VLASZOVA and OSZIPOVA, 1973).

For studies on the organization of the chloroplast membranes — STRASSER and BUTLER (1976), ARMOND et al. (1976), AKOYUNOGLOU et al. (1978) — such light periods are frequently applied, where the length varies from 0.01s to 2 min. These „flash" light experiments cannot be compared to our 15—7.5 min LDC-s, since here the light-dark ratio is 1/50 or less, and on the other hand no normal grana formation can be observed in adhered double, so-called primary thylakoids.

Compared to the 16—8h long illumination the 15—7.5 min light-dark cycle (LDC) significantly descreased the Chl a/b protein complex II. pigments on the one hand (MARÓTI, 1982), and in a degree depending on the genotype it shrinks and flattens the corn mesophyll chloroplasts on the other hand (MARÓTI and PATAKY, 1982).

The question arises whether the decrease in neoxanthin, lutein and Chl-b, and the flattening of the chloroplasts, resp. are in connection with the thylakoid number per granum.

In this paper a demonstration is given of the relationship between the afore-mentioned effects of the 15—7.5 min LDC and the shape, number and area-ratio of the grana.

Materials and methods

Zea mays L.: 165 and 523 Pioneer* inbred lines and 3901 hybrids were used for studies. The plants were grown in phytotron — HORVÁTH (1972) —, on a mixture of perlit and sand 1:1 (volume), HOAGLAND — REYSS et BOURDU (1970) — in nutriment solution with light intensity of 32 W/m² and constant temperature of 20 ± 1 °C.

The daily amount of light (light intensity x period) was the same. The control plants were grown in 16h continuous light and 8 h dark, while in the short cycle experiments alternating periods of 15 min light and 7.5 min dark were applied. Light tubes F_{29} served as the source of illumination.

The water capacity was 70%. The plants were watered daily with distilled water, the aliments were supplemented with 20 ml of nutriment-solution twice weekly. The dry weight of the five weeks old plants was measured according to the different organs after drying at 70 °C.

For electronmicroscopic studies samples were taken from the middle part of the fifth leaves. The leaf pieces with diameters of 0.5—1 mm were fixed in 3% glutaraldehyde, then contrasted with 2% KM_nO_4 solution, dehydrated in an ethanol series, and embedded into a Durcupan-ACM mixture. The sections prepared with a Reichert ultramicrotome were stained with Pb-citrate.

Photographs were prepared of the mesophyll chloroplasts with Tesla BS 242E and Tesla BS 50 electronmicroscopes. On the photograps of the given magnifications measurings were carried out on the planar size of the plasts (where the envelope was not observable well enough, the line of the stroma was taken as the limit of the area), the number, "width" and "height" measurements of the grana, as well as the amount of granum) thylakoid. 30—40 plasts were evaluated according to the various treatments.

Results

1. AREA-RATIO AND AMOUNT OF GRANA

In the 16—8 h LDC the area of grana amounted to cc 30% of the cut-surface (area) of the whole chloroplast in the three corn geno-types. In these chloroplasts 20—25 grana could be found per cut-surface. Regarding the number and area-ratio of granum no significant changes could be observed between the various corn genotypes.

The ratio of grana, as well as the single stroma membranes and the stroma, resp. cannot be reliably given here due to the inadequate resolution of the BS 242 E electronmicroscopic photographs.

*The corn kernels were obtained from Dr. LÁSZLÓ KÁLMÁN, Cereal Research Institute of Szeged.

In the 15—7.5 min LDC the surface quota of the grana (10—15%) decreased nearly to the half compared to the 16—18 h control. The granum number slightly decreased — 10—15 per cut surface — on the effect of the short light period. The lower decrease in the amount of grana and the higher decrease in the grana area was in connection with the fact that in the 15—7.5 min LDC the grana were made up of less thylakoid than in the 16—8 h LDC.

2. THE EFFECT OF SHORT LIGHT PERIOD ON THE SHAPE OF GRANA AND THE RATIO GRANA/THYLAKOID

During the period of continuous illumination (16—8 h LDC) grana consisting of many compartments developed by the multiple adherence of the thylakoids (Th) in the mesophyll chloroplasts (M chlp), in the case of all three genotypes of corns. To determine more exactly the grana/thylakoid ratio the grana were separated into groups, namely, those made up of 2—4, 5—8, 9—10, 11—12, 13—14, 15—20, 20—35 thylakoids. The distribution according to percentage was calculated in tese 7 groups (Fig. 1).

In all three types of corns the most frequently occurring grana were those consisting of 5—10 Th-s. Grana made up of the highest amount of Th and the lowest amount of 2—8 Th-s were found in the Mchp of the corn 165.

The number of grana consisting of 2—8 thylakoids was significant in the Mchp of the line 523, and in the hybrid corn 3901 the distribution of the low and high amount of thylakoid-containing grana was proportional.

It seemed a general rule that with the increase of thylakoid aggregation:

— the grana developed the form of narrow and high columns, their height/width ratio increased,
— the various partition lengths and the stacking surface of the various grana compartments decreased.

The 15—7.5 min LDC showed partly uniform and partly genotypdependent influence on the mesophyll chloroplast structure of the three corn types.

The general effect of the short cycle was that the intact grana consisting of 14—35 thylakoids did not develop, while those made up of 2—4 thylakoids occured in 50—70% (Fig. 1).

The differing effect of the 15—7.5 min LDC showed manifestation on the organization of the internal lamella system in two basic forms.

One of the types — represented by corn 523 — was characteristic of the fact that the ratio of the stacked and single lamellae did not show essential differences, nevertheless:

— such grana developed which lower height and were wider;
— the grana made up of 2—4 thylakoids were predominant (cc 50%), those standing of 5—8 Th-s were also frequent, (cc. 30%), and those having 14—35 Th-s were missing;
— there was an increase in the stacking surface of the grana disc, and in the ratio of the end granal membranes;
— the grana showed a more sporadical arrangement than in the 16—8 h LDC (Plate II, Fig. 1).

The other type of effect of the short cycle was observable in the Mchp-s of the corns 165 and 3901. Here the ratio of the length of grana and stroma lamellae showed significant alteration on the effect of the 15—7.5 min LDC, since:

— the membranes of the high grana showed "mosaic" swelling, became loose and decomposed;

— whole grana disappeared as the consequence of the destruction of the granum partitions, therefore the stroma lamellae predominated in these chloroplasts (Plate I, III).

3. Detection of starch in the mesophyll chloroplasts

Generally 40 M chloroplast sections were studied per corn. In the 16—8 h LDC the highest amount of starch was found in the hybrid 3901, 3—4 were frequent per plast. Starch was observed in about 50% of the M chloroplasts of corn 165. The

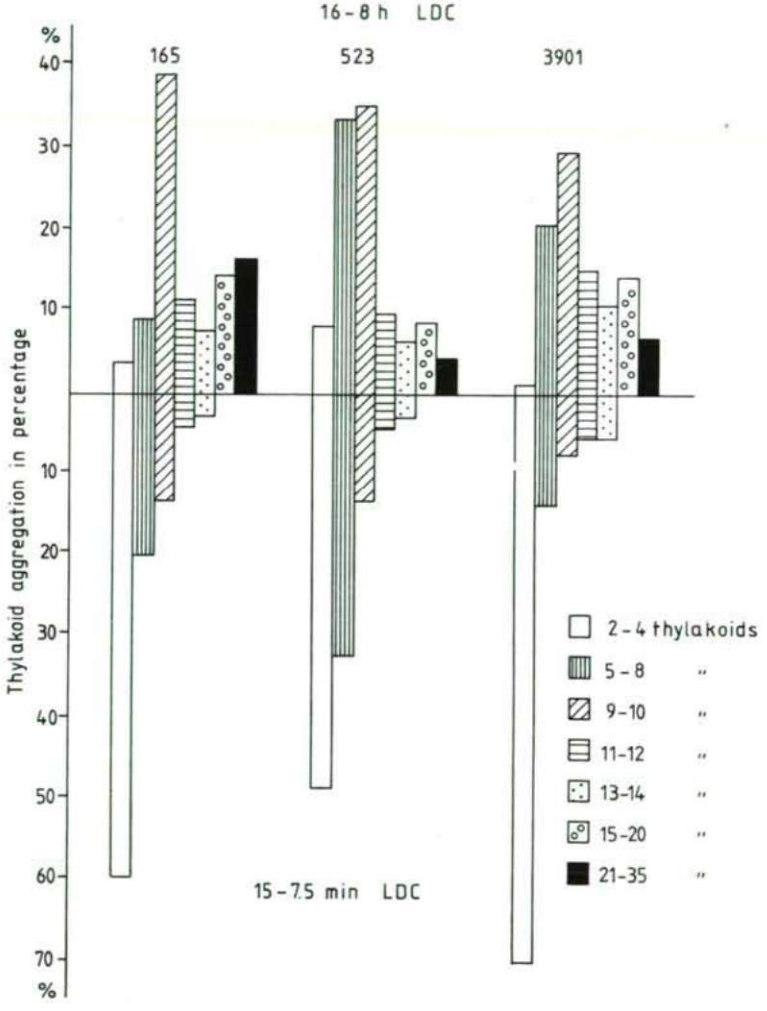
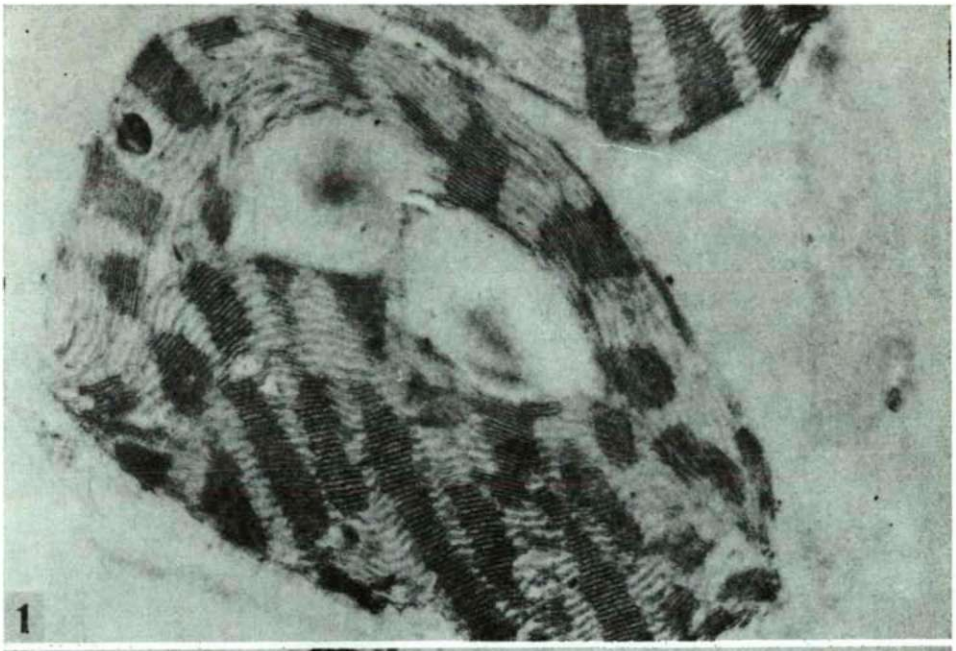


Fig. 1. The percental change of thylakoid aggregation on the effect of long (16—8 h) and short (15—7.5 min) light periods. The sample was taken from the three corn genotypes (P 165, P 523, P 3901) at five weeks of age, from the middle part of the fifth leaves.

Plate I. *Zea mays* L. *Pioneer* 165

1. light-dark cycle 16—8 h (30 000 X)
2. light-dark cycle 15—7.5 min (30 000 X)



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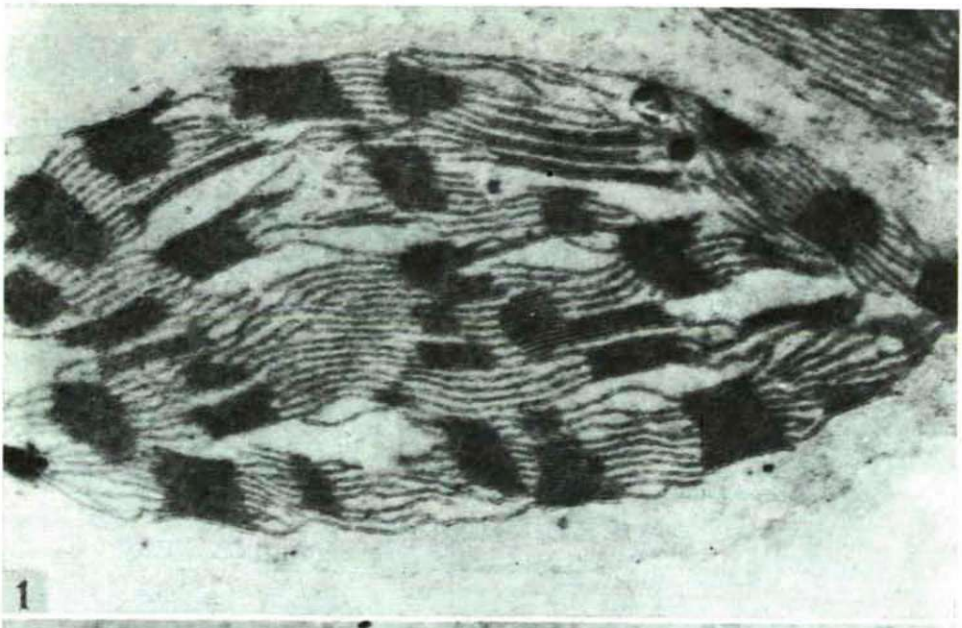

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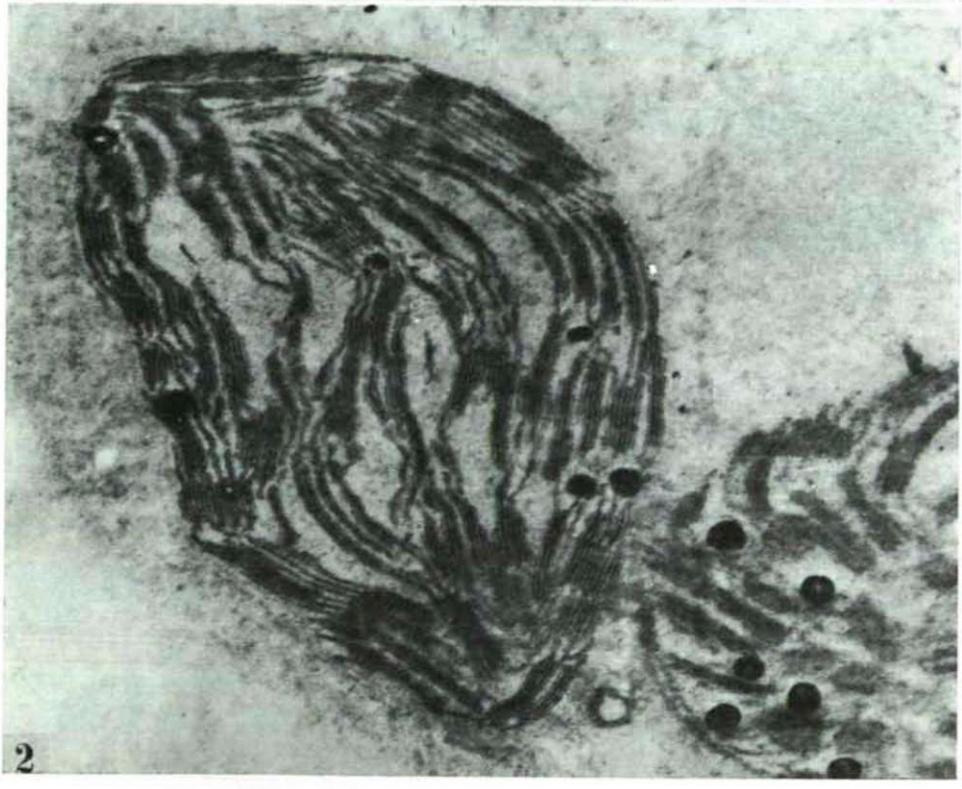

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lowest amount was found in the M chloroplasts of corn 523; in only 12 from 40 chloroplasts.

In the M chloroplasts of the leaves grown in the short 15—7.5 min LDC only a lower amount and degree of starch occurred. It was striking that only small starch granules were found in the large „starch holes". No, or only small amount of starch was observed in the granal chloroplasts of loose, swollen structure.

Discussion

The amount of internal lamella system of the chloroplasts as well as the granum formation largely depend on the light intensity.

From the examination of the light and shadow plants (ANDERSON, 1973; PRIOUL, 1973; VLASZOVA and OSZIPOVA, 1973) it can be seen that in the shadow plants the granum formation is increased, the surface of the membranes falling on a unit area is large. The dependency of granum formation on light intensity is also supported by the experiments of BOARDMAN et al. (1974) carried out on *Atriplex*. The decrease in light intensity also presumably plays a significant role in the fact that the complete length of the partitions per granum is higher in the spongy parenchymal chloroplasts, and the chlorophyll a/b ratio is lower than in the palisade parenchyma (MARÓTI and GÁBOR, 1976; MARÓTI, 1976).

The question aries that besides the same light intensity and same daily amount of light, to what extent does the length and ratio of the light-dark periods affect granum formation?

In the varying light-dark cycles where the L/D ratio is 1/50 or less (STRASSER and SIRONVAL, 1972; STRASSER and BUTLER, 1976) there is no normal granum formation. In the single and long-streched adhered double, so-called primary thylakoids developed in such a way the synthesis of the pigments, lipids and proteins, and their integration into functional units, resp. is restrained (AKOYUNOGLON, 1977; AKOYUNOGLON and ARGYRONDI-AKOYUNOGLON, 1978).

HORVÁTH and MIHALIK (1978) cultivated mustard in continuous, 3—21 h long LDC, and in such a rhythmic illumination where the 30 min of light was followed by increasing: 30, 60, 120, 240, 360, 480 min. long dark periods. In the 15 days old plants, due to the rhythmic illumination the number of grana and stroma thylakoids increased compared to the 3 h long continuous light. This result shows that in the case of a low daily amount of light and 1/7 light-dark ratio the periodical light stimulates the formation of grana and stroma thylakoids better than the continuous illumination.

On the contrary, one of the characteristic effects of our 15—7.5 min LDC experiments was that in the mesophyll chloroplasts of corns 165 and 3901 the number of grana thylakoids decreased and the intact grana consisting of many (14—35) thylakoids were missing (Fig. 1). One of the characteristic unfavourable effects of the 15—7.5 min LDC was that compared to the 16—8 h LDC — it decreased the dry matter content in the studied corns (MARÓTI and MIHALIK, 1982). The question is whether this is in connection with the daily amount of illumination, light intensity, or the length of the light-dark period?

In the 16—8 h and 15—7.5 min LDC-s the daily illumination was 16 hours, the light-dark ratio was 2/1, nevertheless, in the short cycle the leaves of the plants were

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Plate II. *Zea mays* L. *Pioneer* 523
1. light-dark cycle 16—8 h (30 000 X)
2. light-dark cycle 15—7.5 min (30 000 X)

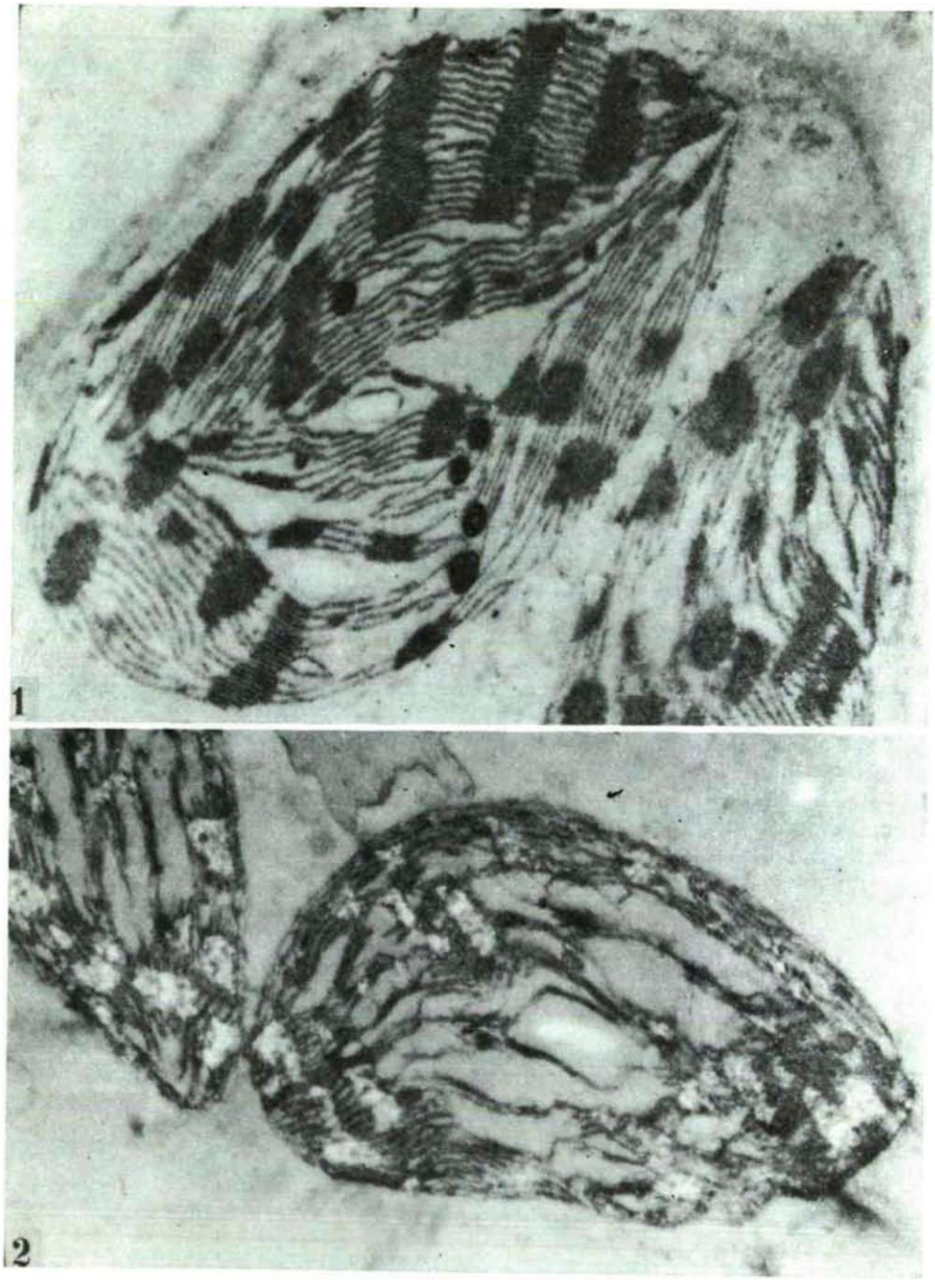

Plate III. *Zea mays* L. *Pioneer* 3901
1. light-dark cycle 16—8 h (30 000 X)
2. light-dark cycle 15—7.5 min (30 000 X)

pale green, yellowish-green. The assumption arises that the amount of light is moderate in the 15—7.5 min LDC, the leaves become etiolated, therefore normal granules do not develop.

As a matter of fact, the light intensity of 32 Wm^{-2} and the 512 Wm^{-2} amount of light is not sufficient for the normal development of the corns, however, this cannot be the cause of the unfavourable effect of the 15—7.5 min LDC, since they grew under the same amount of light as the control plants.

It has been demonstrated (MARÓTI, 1981) that the decrease in pigment-content is not due to the inhibition of synthesis, but to the destructive effect of the short cycles. In the short LCD-s firstly the pigments of the Chl-a/b protein complex become damaged:

— the amount of chlorophyll decreases, the decomposition of Chl-b is particularly significant, thus the ratio of Chl a/b increases,
— from the carotenoids, the decrease of neoxanthin and lutein indicates the destructive effect of the short cycles the best.

It can be concluded from the non-complete destruction of neoxanthin and Chl-b that only one component (that of 25—30 Daltons?) of the light-harvesting Chl a/b-protein complex (LHC) becomes deficient. It is also probable that only the amount of pigments become fewer.

On the basis of the results of THORNBER (1975) ahd SIEFERMANN-HARMS (1980), as well as our comparative pigment and electronmicroscopic studies, resp., it is assumed that the stacked membrane surface is proportional to the amount of neoxanthin. The fact is striking that relatively more becomes decomposed of the content of Chl-b and neoxanthin, lower in corn 523, the species accommodating better to the short cycle.

It is also assumed that due to the frequent dark periods in the 15—7.5 min LDC-s the tight adhesion of the grana thylakoids (MURAKAMI and PACKER, 1970; BARBER, 1976; BARBER and CROW, 1979) becomes loose and deficient, resp., because of the repeated proton efflux. Due to the deficient and looser adhesion, the neoxanthin, lutein and Chl-b found on the surface of the LDC-s become free and start to decompose. Therefore, the H^+/Mg^{2+} exchange between the interthylacoid stacking surface and the loculus may be one of the important regulators of the grana aggregation.

The observed significant increase in the amount of grana consisting of 2—4 thylakoids during the course of the 15—7.5 min LDC is brought into connection with foregoing.

The mosaic-like loosening, "decomposing" of the partition of grana containing many thylakoids can be explained by the frequently recurring dark-induced proton efflux, and the complete destruction of certain light-harvesting Chl a/b-protein complexes.

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