EFFECT OF ALTERNATING LIGHT-DARK CYCLES ON THE SIZE OF MAIZE CHLOROPLASTS AND ON THE ACCUMULATION OF DRY MATTER

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Abstract

We have studied the effect of the short light-dark cycles (LDC) of 30–15 and 15–7.5 min. on the size of chloroplasts and the accumulation of dry matter, on 5-week corn lines (Pioneer 165 and 523) and a hybrid (No. 3901), raised in a phytotron (32 Wm^{-2}).

Between the shrinkage of mesophyll-chloroplasts and the accumulation of dry matter, a positive connection was found in the 30–15 min. LDC.

The two corn lines (165 and 523) responded to the 30–15 min. LDC contradictorily. The size of bundle sheath chloroplasts is in a positive correlation with the dry-matter production, its volume is determined to a great extent by the size and number of grains of starch contained in it.

The 15–7.5 min. LDC considerably decreased the size of bundle sheath chloroplasts and reduced the quantity of dry matter.

We suppose that in the short LDC the frequent dark-induced proton efflux disturbs the normal formation of grana and, on the other hand, inhibits the formation of the necessary pH difference on the two sides of the thylakoid membrane.

Introduction

According to GARNER and ALLARD (1931), ALLARD and GARNER (1941), BONDE (1955), TUKEY and KETELLAPPER (1963), the equal light-dark cycles of 5 sec. each, 1, 4 and 12 hours each, are favourable for the accumulation of dry matter.

The optimum of the not equal light-dark cycles is shown, according to KETELLAPPER (1965), by the ratio between the length of light periods and that of the full cycle, the value of the so-called photofraction. The considerable inhibition of growing by the short (1–30 min.), approximately equal light-dark rhythms, and their strong reducing effect on the accumulation of dry matter were demonstrated by many researchers (GARNER and ALLARD, 1931; PORTSMOUTH, 1937; BONDE, 1955; FOGG, 1968; RAJAN et al., 1971; MARÓTI et al. 1980).

About the effects of light-dark cycles at cell level, only few data are known.

The light-induced *in vivo* shrinkage of chloroplast was demonstrated by some researchers thus KUSHIDA et al. (1964), HILGENHEGER and MENKE (1965), MAKAROV et al. (1976), NOBEL (1967), ZURZYCKI (1974). Taking the volume of chloroplast held in the dark as 100 per cent, the decrease of volume changed between 15 and 30 per cent. HELDT et al. (1973) could not observe any light-induced change in the size in isolated chloroplasts.

According to ZURZYCKI and METZHER (1977): "the shape of chloroplasts depends on two factors: the rigidity of the internal structures and mechanical effects of the surrounding cytoplasm". That's why we studied the effect of the short light-dark cycles on the size of chloroplasts. On the other hand, it was demonstrated by

MURAKAMI and PACKER (1970), HIND et al. (1974), KRAUSE (1977), DUNIC et al. (1979) that the reduction of the size of chloroplast is in connection with light-induced ion (H^+, Mg^+, K^+) exchanges, the loss of water, attenuation of the membrane The attenuation of membranes orginates from the energizing effect of the light-induced proton gradient.

After all, the degree of the shrinkage of chloroplasts depends on the intensity of the photosynthetical light reaction. Thus, it is to be supposed that from the changes in the size of chloroplasts we can draw conclusions concerning the organic matter production, as well.

According to MUSTÁRDY et al. (1976, 1981), as a result of the light and Na^+ ions, the membranes of stroma taper off in a much lower degree than the membranes of grana. The considerable attenuation of the normal membranes of grana takes place by the participation of the light-harvesting Chl a/b protein complex (LHC). The change in the size of chloroplasts depends, therefore, primarily on the number and size of grana and this may be interesting at comparing the "kind" properties. Of the nearly equal light-dark cycles induced changes in chloroplast there is only known a little information, obtained by an electron microscope (e.g. REYSS et al., 1970; HIRSCHAUER et al., 1971).

The 30–15 min. light-dark rhythms induce considerable histological and morphological changes in beans and mustard (MARÓTI et al. 1980). In our experiments in the phytotron, we have, therefore, looked for an answer to:

— What kind of effect is exerted by the 30–15 and 15–7.5 min. LDC on the size of chloroplasts and if the change in the *in vivo* sections can be measured by a light microscope?

— Is there any difference in the change of size of mesophyll and bundle sheath chloroplasts?

— What kind of conclusion may be drawn from the size of chloroplast concerning photosynthesis, the accumulation of organic matter?

Do the single corn lines respond to light treatment equally?

Materials and Methods

For the experiment, *Zea mays* L: Pioneer inbred corn lines 165 and 523, resp. Pioneer hybrid 3901 were used.* The plants were raised in a phytotron (HORVÁTH, 1972), with 400–700 nm wave-length interval, under light-tubes F_{29}, at 20 ± 1 °C daily temperature and 50–70 per cent relative moisture-content.

At identical daily illumination, light treatments were as follows:

(a) Light-dark rhythms (LDC) of identical intensity: 16–8 hrs; 30–15 resp. 15–7.5 min. light-dark periods. Light intersity: 32 Wm^{-2}.

(b) Light-light rhythm (LLC) of changing intensity: 16-8hrs. 30 min. (30.75 Wm^{-2})–15 min. (2.5 Wm^{-2}) light-light periods.

Plants were raised in a mixture of sand-pearlite of ration 1:1, with Hoagland's nutrient solution, modified by Reyss and Bourdu (1971), till their 5-week age. Water capacity of the sand-pearlite culture was daily set with distilled water to 70 per cent. In an experiment, in four climatic chambers (with four kinds of light-treatment), in 4 times 8 pots 96 plants were raised from one "kind" of plants. The experiment was repeated 3 times in different times. The size of chloroplasts was established on the basis of two experiments and the quantity of dry matter from the means of the three experiments. The plants were elaborated at 5-week old. The dry weight was established after being fixed at 105 °C and then dehydrated at 70 °C, in each organ

* Seed-corn obtained from Dr. János Németh, Cereal Research Institute, Szeged.

separately. For investigating into the size of chloroplasts, we took samples from the middle region of the fourth leaf (counted from below) of the 5-week old plants. There were collected as controls the leaves of plants raised in 16–8 hrs LDC, in experiment I after 1hr illumination (at 8 o'clock in the morning), in experiment II at the end of an 8-hour long dark period (at 7 o'clock). From the other rhythms, leaves were taken at the end of the light period. (Three leaves in each of the experiments and light treatments.) Leaves were cut by hand, resp. with an electrical (Kryomat) refrigerating microtome of type Leitz. The 20–30μ thick sections were covered with 1.5 p.c. glutar-aldehyde, 0.5 M potassium-phosphate (pH 7.3) buffer and immediately photographed with a polerizing photomicroscope Opton. The longest diameter and thickness of the chloroplast were measured from paper pictures of known magnification (N: 1969). In each experiment: one photographic datum is the mean of 60 measurements.

Results

1. The effect of the short light-dark periods on the quantity of dry matter

The effect of the 30–15 min. LDC on the investigated dry-matter accumulation of corn lines was different. This is particularly striking in lines 523 and 165 (Table 1).

In the 30–15 min. LDC, the total dry matter of corn 523 increased by 23 per cent and that of the leaf by 38 p.c., as compared with the 16–8 hrs LDC. On the other hand, at corn line 156, the dry weight, falling on each leaf, has not changed and the total dry matter decreased by 10 p.c., as compared with 16–8 hrs LDC of the control (Table 1).

The dry-matter accumulation of hybrid 3901 corn was less stimulated by the 30–15 min. LDC than that of line 523 and was less inhibited than that of line 165.

Table 1. The effect of the light-dark periods of different length on the dry-matter quantity of 5-week old corns.

At identical daily illumination, in the 16–8 hrs, 30–15 and 15–7.5 min. LDC, light intensity was 32 Wm^{-2}; in the 30–15 min. LLC of continuous illumination, light intensity changed between 30.75 Wm^{-2} and 2.5 Wm^{-2} (30–15 min.). The following are the means of 36 plants in three experiments.
("Other" = leaf sheath, leaf and stalk primordia.)

Corns	Light treatment	Dry weight mg/plant			
		root	leaf	other	total
P 165	16–8 hrs LDC	468.9	280.6	111.9	861.4
	30–15 min. LDC	415.7	273.5	88.9	778.1
	15–7.5 min. LDC	492.2	223.5	75.3	791.0
	30–15 min. LLC	511.8	278.0	111.7	901.5
P 523	16–8 hrs LDC	472.6	222.6	97.0	792.2
	30–15 min. LDC	528.1	307.2	112.5	974.8
	15–7.5 min. LDC	419.2	206.6	75.3	701.1
	30–15 min. LLC	475.0	238.0	95.4	808.4
P 3901	16–8 hrs LDC	725.0	400.8	170.2	1296.0
	30–15 min. LDC	662.5	489.5	169.6	1321.6
	15–7.5 min.LDC	678.0	335.0	127.3	1140.3
	30–15 min. LLC	681.2	455.5	168.9	1305.6

Hybrid 3901 has outstandingly utilized the light, in spite of light intensity 32 Wm^{-2} which is low for corns. As compared with the the two lines, it gave a maximum dry-matter production in every rhythm. The 15—7.5 min. LDC considerably diminished the quantity of dry matter, both at the two lines and the hybrid. The degree of the total dry-matter decrease was the highest (12 p.c) at corn line 523 (Table 1).

The increase in the dry-matter of corn lines was favourably influenced by the continuously illuminated 30–15 min. LLC of rhythmically changing strength. As compared with the 16-8 hrs LDC, in the 30–15 min. LLC the increase in dry matter is insignificant but the difference is striking if we compare the dry-matter productions induced by the 30–15 min. LDC and the 30–15 min. LLC.

The 30–15 min. LDC increased the dry-matter quantity of corn line 523 by 23 per cent and decreased that of corn line 165 by 10 per cent. On the other hand, the 30–15 min. LLC of continuous light moderated the accumulation of the total dry matter at corn line 523 and increased it at 165, as compared with the 30–15 min. LDC.

The short rhythms of 30–15 and 15–7.5 min. caused only a slight change in the dry-matter accumulation in the organs of corn lines. The "favourable" resp. "infavourable" effect of the short light-dark periods primarily manifests itself in the dry-matter quantity of the intensively growing leaves, leaf sheaths and stalk- and leaf-primordia.

2. The effect of alternating light-dark cycles on the size of chloroplasts

Corn lines 165 and 523 considerably differ from each other in the size of chloroplasts and in their reactions given to the 30–15 min. LDC, as well.

The mesophyll and bundle sheath chloroplasts of line 165 are larger than those of corn line 523 (Table 2).

The 30–15 min. LDC hardly changed the diameter of mesophyll chloroplasts (Mchp) of corn line 165, at the same time, however, it considerably increased their thickness. Opposite to the swelling of Mchp 165, the diameter and thickness of Mchp of corn line 523 also considerably decreased (Table 2, Fig. 1).

The full volume of bundle-sheath chloroplasts (Bchp) was considerably increased by 30–15 min. LDC in case of both corn lines (165 and 523). It is to be seen even with a light microscope that chloroplasts — with the exception of 15–7.5 min. LDC — are full of starch (Table 2, Plate I, picture 3; Plate II, pictures 2, 3; Plate IV, pictures 3, 4). At corn line 523, the dark green colour of Bchp is striking; its cells are full of chloroplasts (Plate II, picture 2).

The diamater of the Mchp-s of line 165 was a little reduced by the 15–7.5 min. LDC. It is obvious that in case of corn line 523, the change in Mchp is insignificant. At the change in Mchp-size, it can less be observed that the 15–7.5 min. LDC is unfavourable for the development of plants, for the accumulation of dry matter. The damaging effect of this rhythm is, however, shown by several facts:

- As a result of the 15–7.5 min. LDC, the size of Bchp-s strikingly decreases at both corn lines.
- In Bchp, hardly any starch can be seen.
- The Mchp-s are yellowish green, very easily burst asunder, break in to pieces.

(Table 2., Plate III, pictures 1, 2, 3.)

The diameter of Mchp-s of corn line 165 was a little reduced by the 30–15 min. LLC of continuous illumination but of rhythmically changing strength, its thickness was considerably increased by the same. The size of Mchp-s of corn line 523 was somewhat reduced in this rhythm, resp. it hardly changed (Plate IV, pictures 1, 2).

The Bchp-s are the largest in the 30–15 min. LLC, they are full of starch (Plate IV, pictures 3, 4).

The change in the thickness of Bchp-s shown an opposite tendency at the two lines. At line 165, the thickness of Bchp-s considerably increases, in contradiction to line 523, where the thickness of Bchp-s only a little changes, as compared with the control and the 30–15 min. LDC (Fig. 1).

Apart from the differences, manifestina themselves in the size of the chloroplasts of the two lines and in the tendency of chloroplasts to change their size, the tissue structure of leaves also considerably changes, as a result of treatments. In addition to phenological observations, leaf cross-sections were also measured. On the basis of some not published results, the most obvious changes are the following:

Table 2. The effect of light treatments of short rhythm on the size of chloroplast of the fourth leaf of 5-week old corn lines.

The light treatments are identical with those described in Table 1. The chloroplasts of 16–8 hrs LDC were measured in experiment I after 1 h illumination, and in experiment II at the end of an 8 hrs dark period. At the other rhythms, the chloroplasts originate from the end of the light cycle. The chloroplast diameter means the longest diameter resp. largest thickness in μ. (The data are the means of 60 measurements.)

Maize lines	Light treatment	Change in the size of chloroplasts (μ)			
		mesophyll chl. pl.		bundle sheath chl. pl.	
experiment I		diameter	thickness	diameter	thickness
P 165	16–8 hrs LDC	5.3	3.1	7.9	5.3
	30–15 min. LDC	5.3	4.4	8.5	5.6
	15–7.5 min. LDC	4.5	3.4	6.8	4.5
	30–15 min. LLC	5.0	4.1	8.6	6.1
P 523	16–8 hrs LDC	4.3	3.2	6.4	4.3
	30–15 min. LDC	3.9	3.0	6.9	4.9
	15–7.5 min. LDC	4.2	3.1	6.1	3.6
	30–15 min. LLC	4.1	3.1	7.1	4.8
experiment II					
P 165	16–8 hrs LDC	5.4	3.4	7.6	5.1
	30–15 min. LDC	5.6	4.7	8.6	5.8
	15–7.5 min. LDC	4.9	3.5	7.1	4.4
	30–15 min. LLC	5.1	4.2	8.7	6.0
P 523	16–8 hrs LDC	4.2	3.2	6.6	4.5
	30–15 min. LDC	3.8	2.8	7.3	5.3
	15–7.5 min. LDC	4.3	3.1	6.0	3.6
	30–15 min. LLC	4.2	3.0	7.2	4.6

— The thickness of cell-walls (mesophyll, epidermis) are reduced by the 15–7.5 min. LDC. The cells are "expanded". The motor cells are strikingly increased. They run to about 50 per cent of the thickness of the leaf-blade. This refers to increased water content, as well. The water quantity, falling on 1 g dry weight in the 15–7.5 min. LDC, at corn line 523, is 19 per cent and at corn line 165, 2 per cent — more in the fourth leaf, as compared with the control. The large vascular bundles are normally developed (Plate IV, picture 4; Plate V, picture 6).

— The leaves of the individuals, grown in the 30–15 min. LDC and 30–15 min. LLC, are more "massive". In case of line 523, particularly the bundle sheath cells are filled, almost fully, by chloroplasts. In case of line 165, this effect is more moderated (Plate V, pictures 3, 4, 5).

On the leaves of control individuals the changes described above cannot be observed (Plate V, pictures 1, 2; Plate I, picture 4).

Discussion

1. Dry-matter production and the length of the light-dark cycles

At establishing the optimum length of the light-dark cycles, we should take into consideration that certain plants need a different light-dark period for the generative and vegetative developments repectively. We have only investigated in our experiments the vegetative growth. Thus we consider as optimum the LDC, producing the highest dry-matter production during the time unit with identical illumination and light quantity.

According to Horváth et al. (1977, 1978), Maróti et al. (1980), the 30–15 min. and even longer LDC may have, depending upon the peculiarity of the kind, several optimum lengths, too. On the other hand, the 15–7.5 min. LDC is unambiguously unfavourable for the accumulation of dry matter. The time of daily illumination and the quantity of energy are identical in the 16–8 hrs, 30–15 min. and 15–7.5 min. LDC. So the question rises, why the 15–7.5 min. LDC is harmful.

The quantity of dry matter depends — according to Allard and Garner (1941) — primarily on the length of light periods.

The optimum length of LDC can be best illustrated — according to Tukey et al. (1963), Ketellapper (1965) — by the ratio between the length of the light period and that of full cycle. If this ratio is 5/6 (e.g.: 20–4 hrs, 30–6 and 15–3 min. LDC), then a maximum dry matter can be obtained.

According to the literary data, in our LDC the length of the light period is short. Hurd's investigation (1973) seems to support this, as well. He changed — at an identical daily illumination of 8 hrs (20 Wm^{-2}) light — 16 hrs darkness; 8 hrs (18 WM^{-2}) light + 8 hrs (2 Wm^{-2}) light — 8 hrs darkness — light intensity and the length of the LDC period. In photoperiod 8+8 light — 8 darkness he obtained 100 per cent more dry matter than in the 8–16 hrs LDC.

Despite the literary data, seemingly the unfavourable effect of short rhythms is only slightly connected with the relative length of the light-period, because:

— The ratio of the length of the light-period and that of the full cycle was identical in all LDC, i.e. 4/6.

— In the same 30–15 min. LDC, the total dry matter of corn line 523 is the most, and that of corn line 165 is the least, as compared with the other light treatments.

— In the plants investigated by the authors of this paper (maize, tomatoes, beans), the quantity of dry matter was strongly reduced by 15–7.5 min. LDC. This considerable inhibition of the short rhythms is surprising because the second- and the a-few-seconds-long LDC-s produce an outstanding organic matter (GARNER and ALLARD, 1931; FOGG, 1968).

According to our supposition, the unfavourable LDC-s primarily disturb the accumulation of thylakoid loculi and the formation of the difference of pH, necessary on the two sides of the membrane. The damaging factor originates from the often repeated dark periods, from the summarized proton emission (MARÓTI et. al, 1981). All these manifest themselves initially in the trouble of proton-transport, the swelling of the chloroplast, and later on, in the destructions of the membrane and the decrease in size of the chloroplast.

2. The volume of chloroplast and the production of organic matter

Our experiments confirm NOBEL's observation that the shrinkage of chloroplasts refers to an intensive photosynthetic functioning. The close coincidence of the smallest size of the investigated corn Mchp-s and the largest quantity of the total dry matter is striking in the 16–8 hrs and 30–15 min. LDC-s (Fig. 1).

There is an opposite change in the size of the Mchp-s of the two corn lines and the accumulation of dry matter in the 30–15 min. LDC. The size of Mchp 523 in this LDC is the smallest and largest dry matter production. The Mchp thickness of line 165 is strikingly the largest in the 30–15 min. LDC. At the same time, its dry

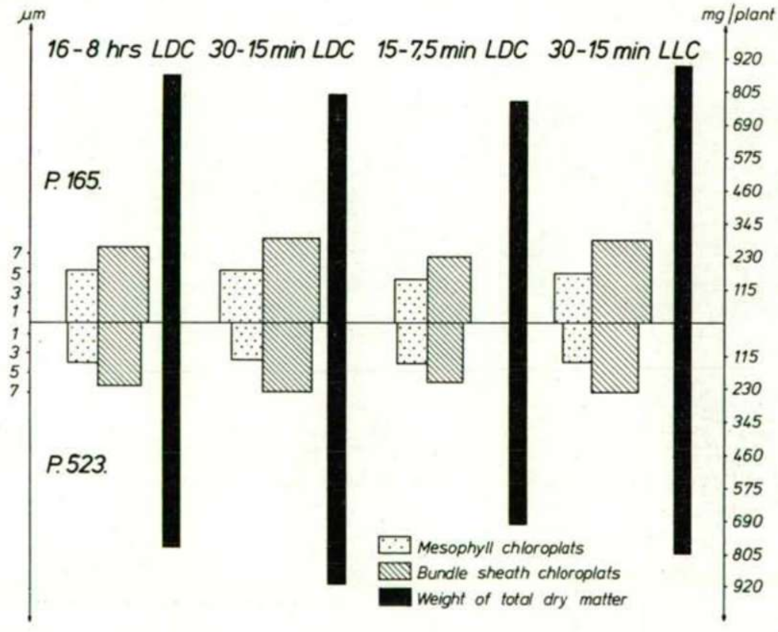
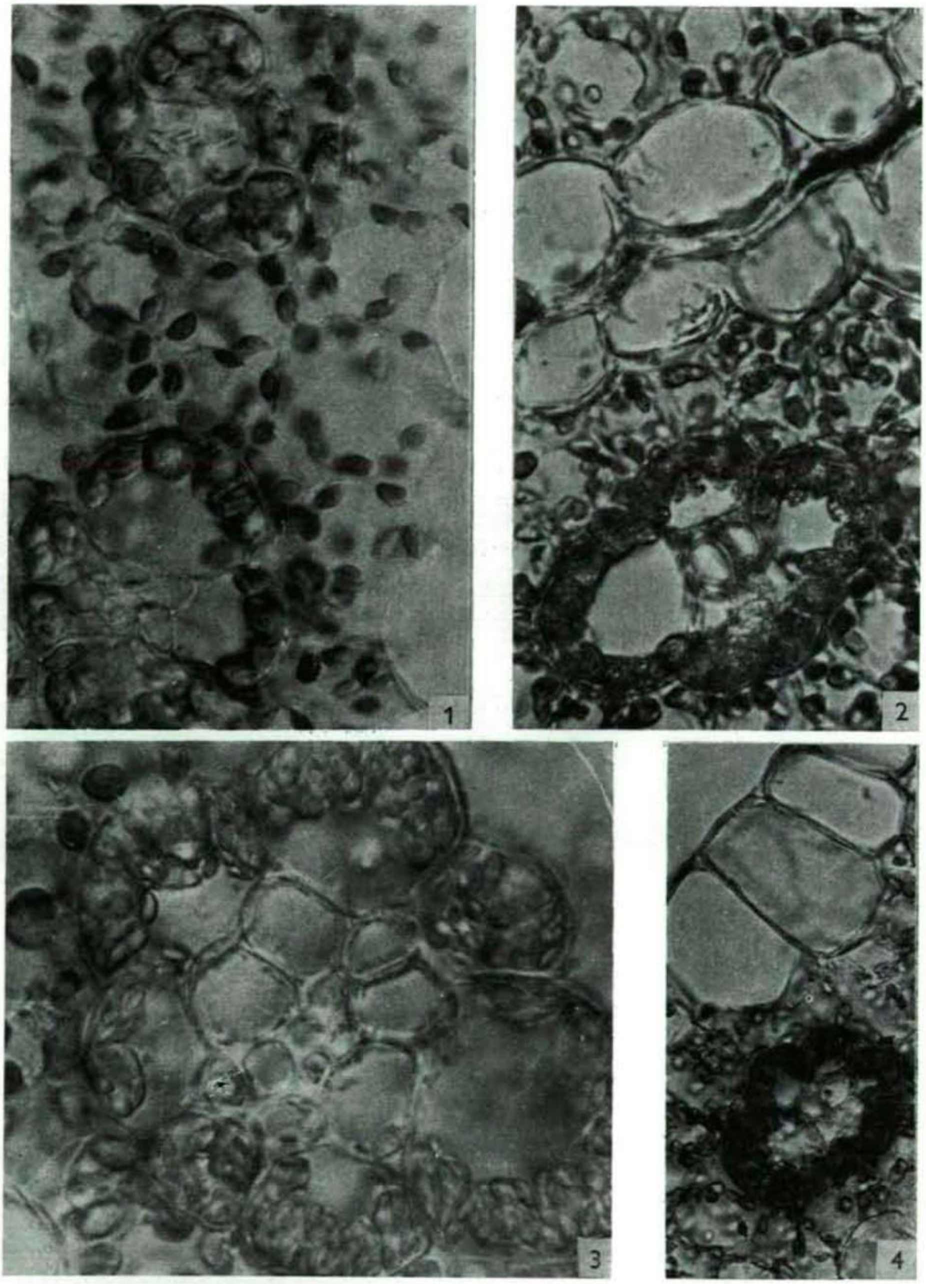


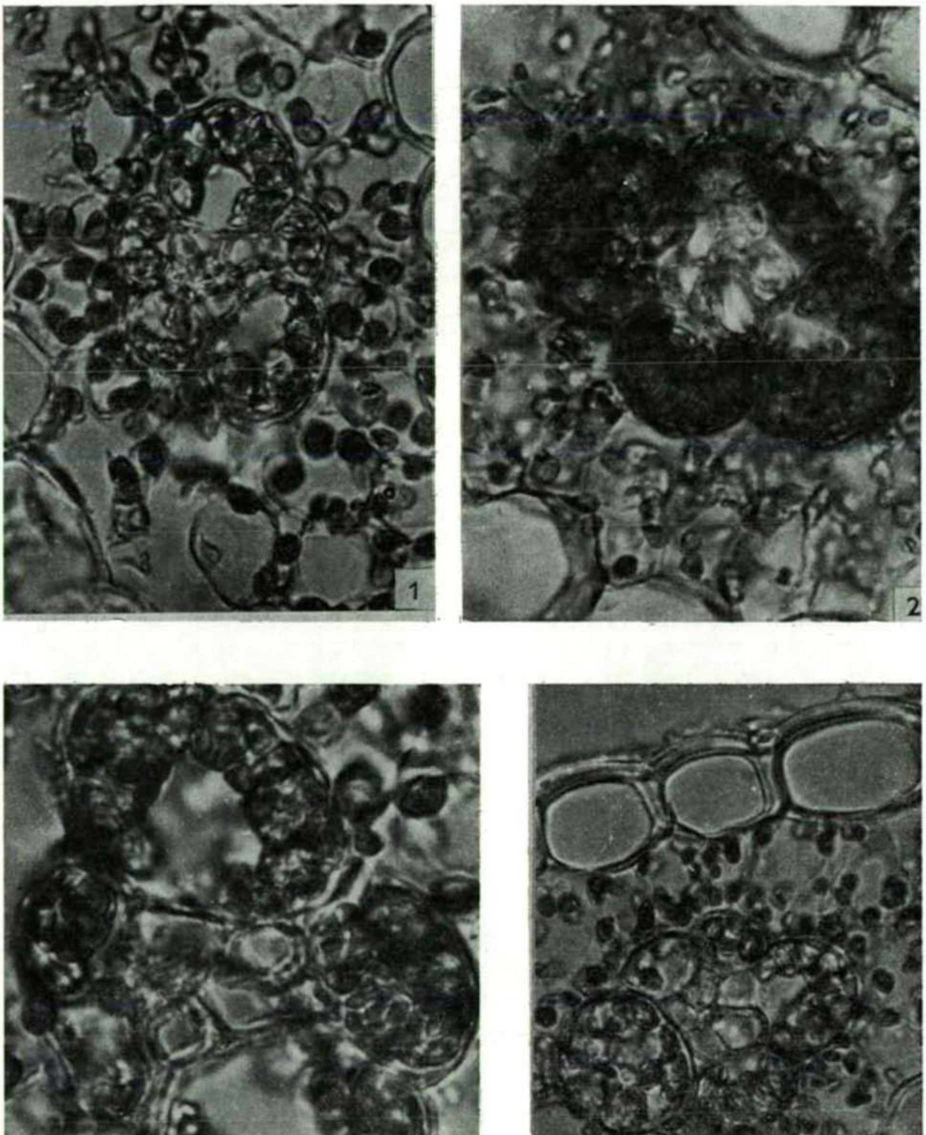
Fig. 1. Size of mesophyll and bundle sheath chloroplasts and change in the total dry weight, due to the 16–8 hrs, 30–15, 15–7.5 min. light-dark cycles (LDC) and to the 30–15 min. light-light cycle (LLC).

Plate I. Effect of the 16–8 hrs LDC.



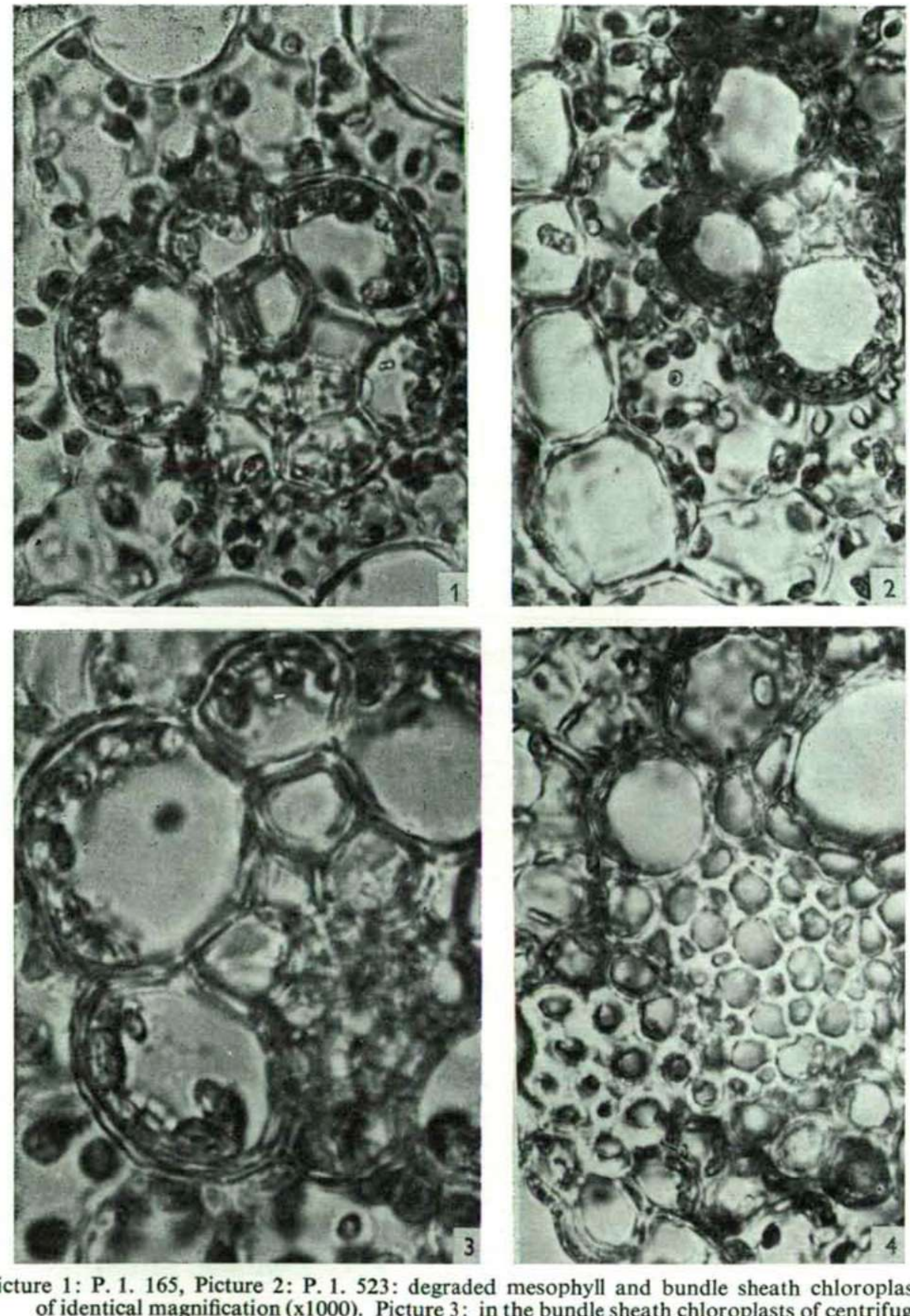
Picture 1: Pioneer line (P. 1.) 165, Picture 2: Pioneer line 523: the difference by size of mesophylls and bundle-sheath chloroplasts of identical magnification (x1000). Picture 3: Grains of starch in the bundle-sheath chloroplasts, P. 1. 165, Magnification: x1580. Picture 4: leaf cross-section, motor cells, P. 1. 165, magnification: x545.

Plate II. Effect of the 30–15 min. LDC.



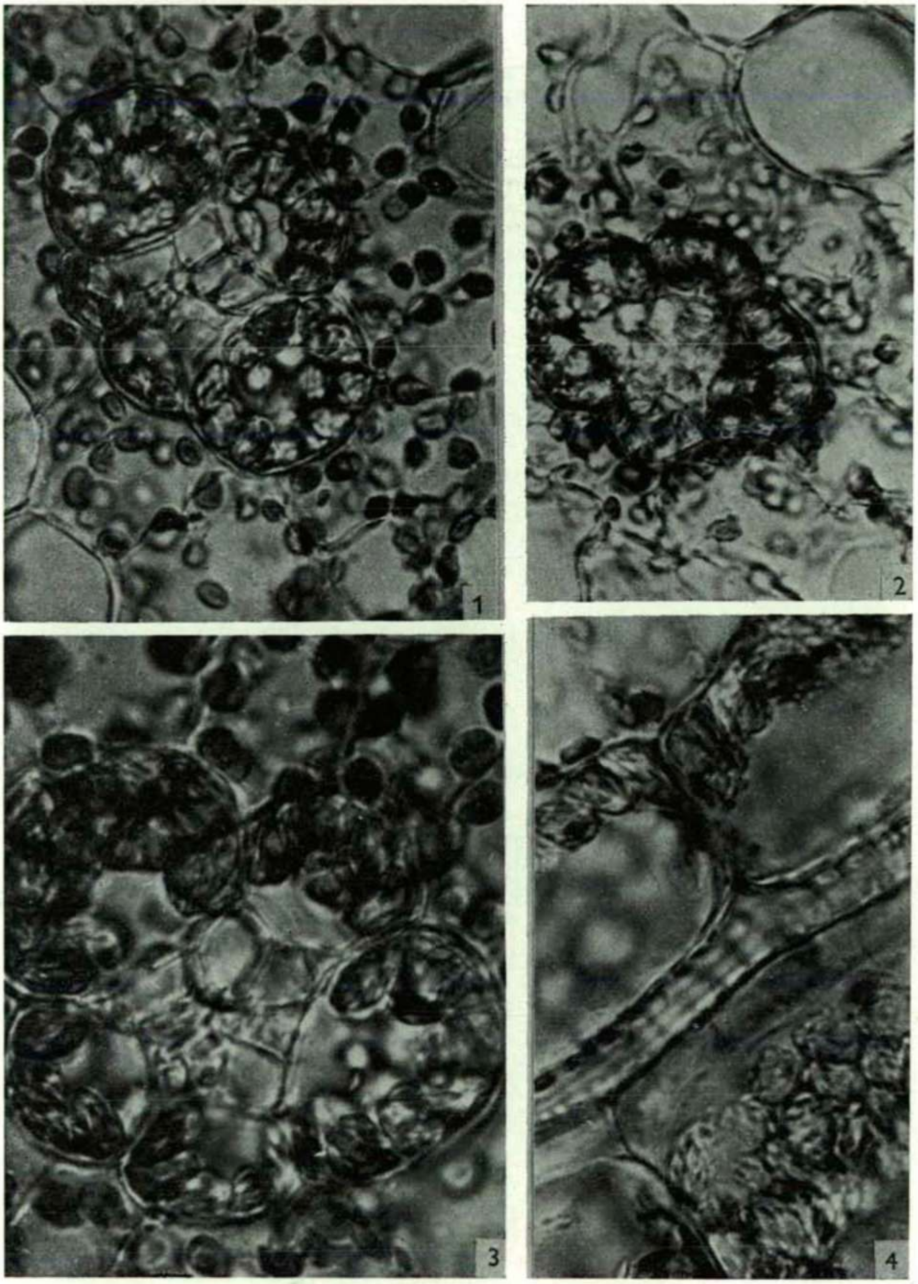
Pictures 1, 3: Mesophyll and bundle sheath chloroplasts of P. 1. 165, magnification: x1000 (picture 1), x1580 (picture 3). Picture 2: mesophyll and bundle sheath chloroplasts of P. 1. 523, magnification: x1000. Picture 4: Leaf cross-section of P. 1. 523, magnification: x810.

Plate III. Effect of the 15–7.5 min. LDC.



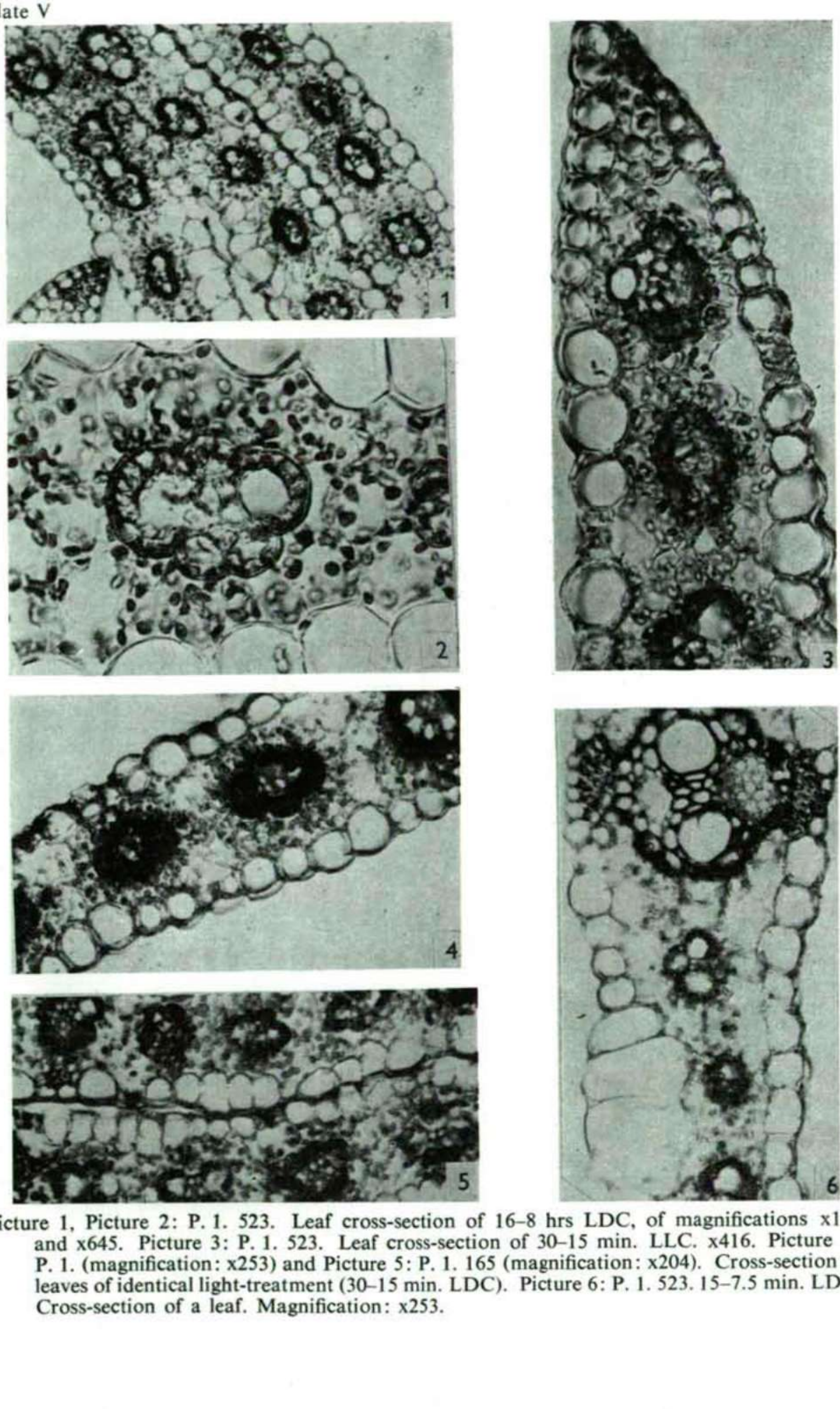
Picture 1: P. 1. 165, Picture 2: P. 1. 523: degraded mesophyll and bundle sheath chloroplasts of identical magnification (x1000). Picture 3: in the bundle sheath chloroplasts of centrifugal arrangement there can hardly be seen any grains of starch. P. 1. 165, magnification: x1580. Picture 4: the sclerenchyma-, phloem-xylem-part- and bundle sheath-cells of the cross-section of large veins. P. 1. 523, magnification: x1000.

Plate IV. Effect of the 30–15 min. LLC.



Picture 1: P. 1. 165, Picture 2: P. 1. 523, strongly increased bundle sheath chloroplasts of identical magnification (x1000). Pictures of the cross-section (Picture 3: P. 1. 165) and of the longitudinal section (Picture 4: P. 1. 523) of bundle sheath chloroplasts of centrifugal arrangement, full of starch, of identical magnification (x1580).

Plate V



Picture 1, Picture 2: P. 1. 523. Leaf cross-section of 16–8 hrs LDC, of magnifications x163 and x645. Picture 3: P. 1. 523. Leaf cross-section of 30–15 min. LLC. x416. Picture 4: P. 1. (magnification: x253) and Picture 5: P. 1. 165 (magnification: x204). Cross-section of leaves of identical light-treatment (30–15 min. LDC). Picture 6: P. 1. 523. 15–7.5 min. LDC. Cross-section of a leaf. Magnification: x253.

matter quantity is the smallest. According to the literature — NOBEL, 1967; MURAKAMI and PACKER, 1970b, MUSTÁRDY, 1981 — the authors suppose that the considerable swelling of the Mchp of line 165 (in 30–15 min. LDC) originates from the thickening of granum membranes. Owing to the often repeated dark periods:

— On the two sides of thylakoid, the necessary proton gradient is not formed. The ATP formation decreases (HIND and JAGENDORF, 1963; PICK et al., 1973).

— The Mg^{2+}/H^{+} exchange between the loculus and partition is defective. According to BARBER (1976), BARBER and CROW (1972), the Mg^{2+} bond between the light. harvesting complex carboxyl groups, which is important from the view point of membrane-adhesion, does not take place, resp. it is but partial.

— According to MURAKAMI et PACKER (1970b), the grana are swelling because the carboxyl groups in the membranes (R-COO-) are hydrated. The question rises, why corn line 523 responds just opposite to the 30–15 min. LDC.

According to our supposition, in the Mchp of line 523, at the applied 32 Wm^{-2} light intensity, the necessary proton gradient is earlier formed. Our hypothesis (MARÓTI et. al., 1981) is supported by the following facts:

a) The — loculus pH-depending — xantophyll cycle of chloroplasts 523 is faster than that of line 165.

b) On the basis of the quantity piaments (chlorophyll b, lutein, neoxanthin) of the light-harvesting Chl a/b-protein complex (LHC), the volume of a chloroplast granum-loculus is, in case of corn line 165, much larger than that of line 523. Therefore, the determined pH difference (5–9) between the closed inner and outer membrane-spaces of Mchp 165 is formed comparatively slower.

c) The shrinkage of the Mchp, induced by the 30–15 min. LLC of continuous illumination, as well as the high value of the dry matter production seem to support our exception that the damaging effect of short rhythms originates from the proton emission of the frequently repeated dark period (the intrathylakoid space).

But the final decision of these questions, requires further many-sided investigations.

The uniform decrease in the size of Mchp in the 15–7.5 min. LDC originates from the — supposedly more increased — membrane destruction. It is shown by our pigment investigations (MARÓTI et al., 1981) that primarily the light-harvesting Chl a/b-protein complex is damaged.

The size of Bchp shows a positive connection with the quantity of dry matter. It is considerably determined by the number and size of the grains of starch in it.

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