EFFECT OF SHORT LIGHT-DARK CYCLES ON THE CHLOROPHYLL AND CAROTENOID CONTENT OF MAIZE AND TOMATOES

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Abstract

We have studied changes in the pigment content induced by 30 to 15 and 15 to 7.5 min. light-dark cycles (LDC) on 5-week maize and 6-week tomato plants, raised in a phytotron.

In the short LDCs, primarily the pigments of the Chl a/b protein complex are damaged:

— The quantity of chlorophylls decreases, particularly the decomposition of Chl-b is considerable, thus the Chl a/b ratio increases.

— From among carotenoids, the decrease in neoxanthin and lutein mostly indicates the destructive effect of short rhythms. Antheraxanthin (lutein-epoxide perhaps) accumulates in the short rhythms.

Apart from the general tendencies of the change in pigments the following facts are obvious:

— The different sensitivity of the maize Pioneer lines 523 and 165, resp. of the two tomato species to the LDC.

— The much higher ratio of the decrease in violaxanthin in corn line 523, in the first two minutes of lighting, as compared with the dark control.

We suppose that the destructive effect of the short rhythm originates from the proton-efflux, induced by the frequent darkness, damaging primarily the plants, in which the proto-gradient, necessary for forming ATP, under the conditions of the given light intensity, quickly develops.

Introduction

For the development of more than one plant, the short light-dark cycles (alternating between 1–30 min.) are unfavourable. It was first observed by GARNER and ALLARD (1931) that in the minute rhythms plants became etiolated, leaves paler, resp. yellowish or greyish green. The tissues of the leaf necrotized with the occurance of so-called necrotic spots. In these cycles, more than one plant fully perished.

GARNER and ALLARD have not measured the change of chlorophylls but, even thus, they established correctly: "Destruction of chlorophyll seems to be an important feature in the unfavourable effect of these particular alternation of light and darkness".

Later on, the chlorophyll destruction, taking place as a result of the unfavourable LDC, was also observed by a number of authors: WITHROW and WITHROW (1949), HIGKIN and HANSON (1954), BONDE (1955), HILLMANN (1956), BONDE (1955) stated, for instance, that in the light green leaves of the *Xanthium*, developed in 5 to 15 min. rhythms, chlorophyll is only a quarter of the 12-hour control.

In these investigations, the single chlorophylls were not separated and carotenoids were not measured.

It is known from the literature and our investigations that, with the fine analysis of pigments, several informations may be obtained about the chloroplast:

— The chlorophyll a/b ration is — according to REYSS and BOURDU (1976), NIR and PEACE (1973), MARÓTI (1976a) and others — in connection with the degree of thylakoid aggregation. The chlorophyll a/b and carotene (chlorophyll-b ratios show, in addition — according to BOARDMAN and ANDERSON (1964), GROSS *et al.* (1966), BRIANTAIS (1968), MARÓTI (1976b), TUBA (1981) and others — the relative quantity of photosystems I and II, resp. of the light-harvesting chlorophyll a/b-protein complex (LHC).
— The violaxanthin cycle may give information on the pH state of the stroma and grana loculus — HAGER (1969).
— According to SIEFERMAN and YAMAMOTO (1975): "The xanthophyll cycle might be part of a regulatory system for photosynthesis that functions by influencing membrane properties."

In our paper we are showing the changes in pigment, induced as a result of LDC. On the basis of results, we attempt to show the destructive effect of the unfavourable LDC rhythms.

Materials and Methods

For the experiments we used three corns — *Zea mays* L.: 165 and 523 Pioneer* inbred corn lines, resp. 3901 hybrids — and two tomatoes — *Lycopersicon esculentum* MILL.: cv. "Kecskeméti merevszárú" (Km) and cv. "Kecskeméti 3" (K3).

Plants were raised, as described before — (MARÓTI and PATAKY 1981) — in a phytotron, at 32 W/m² light intensity and 20 ± 1 °C temperature.

The identical daily time of illumination (16 hrs) was divided into light-dark cycles (LDC) of 16–8 hours; 30–15 and 15–7.5 minutes. The rhythms of 30–15 and 15–7.5 minutes were called of short LDC, those of 16–8 hours LDC of long daytime (control).

For determining the pigment content of the leaf of maize, we used in experiment I the second and third leaves of the 4-week plants and in experiment II the fourth leaf of a 5-week maize.

The tomatoes were during the experiment 6-week old, the pigments were extracted from the third leaf.

For measuring the pigment content, the leaves were collected immediately before the experiment at 7 in the morning, before switching on the light of the control rhythm. Each ex periment was repeated four times.

For studying the violaxanthin cycle, we collected the control 3rd and 4th LDC leaves of the 5-week old maize (the dark was followed by an hour's illumination). With the leaves colleted in the dark resp. in the light, the experiment was repeated five times. No difference was found between the leaves collected in dark and light in the violaxanthin quantity.

The disks taken from both sides of the main vessels of leaves (6–8 mm diameter) were divided into four homogeneous groups (about 1 g). One of them was a "dark" control, the others were illuminated with a strong light 900 W m^{-2} for 2, 4 and 12 minutes.

The pieces of leaves were rubbed (in a weak light) in a cold friction mortar, in the presence of a little $CaCO_3$ and sand. Pigments were extracted with acetone and petroleum ether — as published before (MARÓTI and GABNAI, 1972). Chlorophylls and carotenoids were separated with thin-layer chromatography, then measured in acetone and ethanol, respectively. The extinction coefficients, published by HAGER and MEYER-BERTENRATH (1966) were used for determination pigment quantities.

* We got the kernels of corn from Dr. J. NÉMETH (Cereal Research Institute, Szeged).

Results

1. The effect of 30–15 and 15–7.5 min. LDC on the pigment content of corn- and tomato-leaves

The formation of the pigment content is determined by the general light and dark reactions, and the structure of green pigments due to the kind of plant and the structure of chloroplast. On the basis of the change in the pigment quantity, corn line 523, then hybrid 3901 were the most sensitive, and corn line 165 the most resistant to the short light treatments (Table 1). Both tomatoes responded to 30–15 and 15–7.5 LDC with a different degree of sensitivity. At Km, the degree of the decrease in pigment is particular (Table 2).

In the effect of the short LDC, there are certain general tendencies, as well, which are only modified in a small degree by the different degrees of stability provided by the peculiarities of the "kind". These are the following:

— As a result of the short LDC, the quantity of chlorophylls decreases, particularly the decomposition of chlorophyll-b is considerable, thus the ratio of chlorophyll a/b increases (Table 1, 2).
— From among carotenoides: the destructive effect of the short rhythms is most strikingly indicated by the decrease in neoxanthin and lutein.
— Anteraxanthin accumulates in short rhythms, its de-epoxidation, resp. epoxidation is inhibited.
— Corn. leaves 2 and 3 (Table 1, experiment I) are a little more sensitive to the short LDC than leaf 4 (experiment II).
— The characteristic of the "kind" manifested itself the most in the 30–15 min. LDC, because the 15–7.5 min. LDC seems to be unambiguously harmful (Table 1).

The general tendencies of the change in pigment being given, the different sensitivity of corn lines 523 and 165, resp. of the two tomato kinds, and their different responses to the short LDC are obvious (Cf. Table 2).

This difference manifests itself in the following:

— The chlorophyll content of corn line 523 in the 15–7.5 min. LDC considerably decreases, and that of line 165 hardly changes.
— The xanthophyll cycle of corn line 165 in the 15–7.5 min. LDC is strongly inhibited.
— The 30–15 min. LDC stimulates the development of corn leaf (shoot) 523 and inhibits that of 165. Thus, the decrease of chlorophylls in 523 originates partly from the growth of the leaf, and the slight increase in chlorophyll of 165 derives from the inhibited development of the leaf (in 1 g leaf there are more cells, more chloroplast), (MARÓTI and PATAKY, 1981).
— In Km tomatoes, chlorophyll-a, chlorophyll-b, lutein and violaxanthin decompose more considerably than in Kind K_3 (Table 2). It is remarkable that the considerable differences in the chlorophyll-a, chlorophyll-b, lutein and neoxanthin quatities (16–8 min. LDC) of the two tomatoes entirely disappear as a result of 15–7.5 min. LDC (Table 2).

2. The manifestation of the "kind pecularities in the violaxanthin cycle

The different sensitivity of corn lines 523 and 165 to LDC, as well as the considerable difference in pigment destructions suggested some difference in the kinetics

of deepoxidation, resp. epoxidation in leaves raised in the control 16–8 hrs LDC, as well.

According to the facts, described in the methodical part, the transformation of violaxanthin, induced by a strong light (900 W/m²), was measured in the leaves, collected from 16–8 hrs LDC (Fig. 1). This experiment with the third and fourth leaves was repeated five times, and the result was in every case that the percentile decrease of violaxanthin, as compared with the dark control, was initially much higher in corn line 523 than that of line 165. After being illuminated for 12 minutes, the percentile transformation of violaxanthin was approximately identical in the two lines (55–57 p.c.) (Fig. 1).

After the illuminations for 4, resp. 12 minutes, in leaf-disks, held in the dark for 30 minutes, the epoxidation was faster in maize 165 (Fig. 2).

Table 1. The effect of the dark-light periods on the pigment content of the corn-leaf. In experiment I, the 2nd and 3rd leaves of 4-week plants were used for the pigment analysis. In experiment II, the 4th leaf of the 5-week plants was used. At identical daily illumination, in the ligh-dark cycles (LDC), light intensity was 32 Wm^{-2}. One pigment datum is the mean of four measurements. Chl-a = chlorophyll-a; Chl-b = chlorophyll-b; Car = β-carotene; Lut = lutein + zeaxanthin; Ant = antheraxanthin + lutein-epoxide; Viol = Violaxanthin; Neo = Neoxanthin.

Corn lines	Light-treatment	mg pigment/100 g fresh weight							
		Chl-a	Chl-b	a/b	Car	Lut	Ant	Viol	Neo
Experiment I									
P 165	16–8 hrs LDC	176	44,2	3.9	13.2	17.4	1.2	8.7	5.1
	30–15 min. LDC	132	31.9	4.1	9.1	14.7	1.8	7.6	3.7
	15–7.5 min. LDC	151	35.9	4.2	9.5	14.6	1.6	8.6	4.1
P 523	16–8 hrs LDC	138	33.5	4.1	9.8	11.6	0.8	7.1	3.5
	30–15 min. LDC	92	20.6	4.4	6.7	10.2	1.7	4.4	2.7
	15–7.5 min. LDC	90	19.5	4.6	6.0	9.6	1.8	4.3	2.7
P 3901	16–8 hrs LDC	134	33.6	3.9	11.3	13.3	0.9	7.2	3.4
	30–15 min. LDC	100	21.8	4.5	7.6	10.8	2.0	4.9	2.7
	15–7.5 min. LDC	115	21.2	5.4	8.1	12.0	1.8	5.4	2.4
Experiment II									
P 165	16–8 hrs LDC	133	30.3	4.3	10.9	13.8	1.6	7.9	4.5
	30–15 min. LDC	142	33.0	4.3	11.0	17.3	2.5	8.2	3.9
	15–7.5 min. LDC	140	32.6	4.3	11.4	15.2	2.2	8.5	4.0
P 523	16–8 hrs LDC	122	29.0	4.2	8.8	11.2	0.9	5.8	3.5
	30–15 min. LDC	103	22.1	4.6	7.9	11.0	1.8	5.4	2.7
	15–7.5 min. LDC	80	17.3	4.6	5.5	9.7	1.7	5.2	2.6
P 3901	16–8 hrs LDC	115	25.0	4.6	8.9	12.6	1.0	4.9	3.3
	30–15 min. LDC	130	28.7	4.5	9.2	14.0	1.6	7.3	3.4
	15–7.5 min. LDC	107	22.8	4.7	8.7	12.2	1.8	6.2	2.5

Table 2. The effect of the 30–15 and 15–7.5 min. LDC on the pigment content of the 3rd leaf of 6-week tomatoes. The experimental conditions, abbreviations are to be found in Table 1.

Toma-toes	Light-treatment	mg pigment/100 g fresh weight							
		Chl-a	Chl-b	a/b	Car	Lut	Ant	Viol	Neo
K_3	16–8 hrs LDC	90	28.6	3.1	6.8	9.8	1.0	3.9	3.0
	30–15 min. LDC	60	17.2	3.5	5.1	7.5	1.5	3.3	2.7
	15–7.5 min. LDC	58	14.8	3.9	4.4	6.7	1.3	3.6	1.7
Km	16–8 hrs LDC	109	34.9	3.1	7.7	12.0	1.0	3.8	3.9
	30–15 min. LDC	73	21.1	3.4	6.2	8.7	1.3	3.0	2.5
	15–7.5 min LDC	53	13.8	3.8	6.0	6.9	1.8	2.7	1.7

Discussion of results

1. Is there a pigment destruction or are formed etiolated leaves in LDC of 30–15 and 15–7.5 minutes?

It was demonstrated by more than one research worker that in case of an alternating light-dark cycle, where 1 msec flashlight was followed by 15 min. darkness (Strasser and Sironval 1972, Strasser and Butler 1976) — or a light of 2 minutes by 98 minutes darkness (AKOYUNOGLOU, 1977; AKOYUNOGLOU and ARGYROUDI; AKOYUNOGLOU, 1978) — the leaves remain etiolated.

In these leaves, chloroplast is unable to develop oxygen and does not contain grana. In the so-called primary thylakoid, the synthesis of pigments, lipids and proteins, as well as their integration into functional units are inhibited. The possibility is raised that in the short rhythms, described here, leaves are etiolated, resp. some similar inhibitions of synthesis take place.

Despite the pale green colour of leaves, we do not share this opinion. According to our supposition, the decrease of the pigment content primarily originates not from the inhibition of synthesis but from the destructive effect of the 30–15 and 15–7.5 min. LDC. This is shown by the following:

— The light quantity in the 30–15 and 15–7.5 min. LDC corresponds to the considerable illumination of daily 16 hrs. The plants (beans, tomatoes) flower and may ripen fruit, in spite of their pale green colour.
— The chlorophyll a/b ratio is not high.
— We (MARÓTI and MARÁCZI, an unpublished datum) have observed that pigments also decompose if tomatoes having raised under natural conditions get a short LDC treatment.

2. In the short rhythms, the pigments of the chlorophyll a/b protein complex II are damaged

We (MARÓTI and GÁBOR, 1976; MARÓTI, 1976) demonstrated that the Lut, Neo and chlorophyll-b content of the spongy parenchyma chloroplasts, containing several grana, is more than that of the palisade chloroplasts, rich in stroma lamellae.

On the basis of literary data, we connected the high Lut, Neo and Chlorophyll-b content with the increased activity of photosystem II.

From the photochemically active photosystem II, the light-harvesting Chlorophyll a/b-protein complex (LHC) may be separated (THORNBER, 1975). The pigment composition of this complex is known from the work of Siefermann–Harms (1980) (Table 3).

Table 3. Pigment content of light-harvesting chlorophyll a/b-protein

Species	Pigment/100 Chl-a				
	Chl-b	Car	Lut	Viol	Neo
Phaesolus vulgaris	62.5	0.6	30.7	2.0	10.0
Spinacia oleracea	77.0	1.3	31.0	8.9	13.1

It is to be seen from the Table that in LHC the lutein, neoxanthin and Chl-b contents are high and carotene is in traces. It is known (SIEFERMANN–HARMS, 1980) as well, that in phtosystems I and II, 13, resp. 12.3 carotenes fall to 100 Chl-a molecules. We suppose that the quantity of the pigmenta of the Chl a/b protein complex II is proportional to the content of the complex protein. On the basis of these, the quantity of LHC can be established from the relative difference of the carotene and lutein quantity, from the neoxanthin and Chl-b contents. Among corn lines, the LHC of highest number or largest size belongs to P 165; this is followed by P 3901 and the smallest belongs to P 523. From among tomatoes, line Km has a higher LHC content than line K_3.

The fundamental effect of 30–15 and 15–7.5 min. LDC is to damage LHC. (Tables 1, 2).

As a consequence of shorter LDC the decomposition of Lut and Neo comes to a halt at the sensitive kinds, as well. In tomatoes, the different contents of Lut and Neo will be identical in 15–7.5 min. LDC (Table 2).

These results suggest that not the complete LHC is damaged by the short LDC only one of its components, on the other hand this component's different size depends on the "kind" property.

Another characteristic of the short rhythms is the increasement in the quantity of antheraxanthin. As antheraxanthin and lutein-epoxide are not separated on our thin-layer slide, probably it is not the de-epoxidation of antheraxanthin that is inhibited but just the epoxidation of lutein takes place.

From among corn lines, No. 523, with the least LHC, and from among tomatoes, Km, with the most LHC, were mostly damaged by the 15–7.5 min. LDC. Clearly the destructive effect of short rhythms is not directly connected with the quantity of LHC.

3. What causes the unfavourable effect of the short rhythms?

Since MITCHELL's chemiosmotic theory of ATP synthesis (1961, 1977), a number of researches — HIND and JAGENDORF, 1963; CROFTS *et al.*' 1972; PICK *et al.*, 1973 and others — demonstrated that in the thylakoid loculi (from one side of the

membrane to the other (light-induced proton-uptake and — as a result of darkness — from the intrathylakoid space — proton emission take place. We suppose that, in the 30–15 and 15–7.5 min. LDC, owing to the frequently repeated dark period:

— The H^+/Mg^{2+} exchange between the partition and loculus is partial.
— The Mg^{2+} bond between light-harvesting Chl-a/b-protein complexes, the close adhesion of grana thylakoids — MURAKAMI and PACKER (1970), BARBER (1976), BARBER and CROW (1979) — become loose resp. defective, owing to the repeated proton efflux.
— Owing to the defective adhesion, the surface of LHC, resp. Lut, Neo and Chl-b, on it, become and start decomposing.

Our suppositions are supported by the electron-microscopical photographs, as well, being in preparation, but we need a further many-sided investigation in order to decide the question.

4. The violaxanthin cycle is the indirect indicator of proton-transport

According to HAGER (1969), the de-epoxidation (transforming into zeaxanthin through anteraxanthin) of violaxanthin in the isolated chloroplast can be elicited by the low pH both of the light and medium. HAGER supposed that the de-epoxidase enzyme is to be found in the loculus and is activated by the low pH.

It was demonstrated by MARÓTI and SZAJKÓ (1972) that at the light-induced transformation of violaxanthin in leaf-disks:

— Violaxanthin occurs in chloroplasts in strongly and weakly bonded forms.
— In de-epoxidation, primarily the weakly bonded violaxanthin takes part.

Later on, SIEFERMANN and YAMAMOTO (1974, 1975) observed more exactly on isolated lettuce chloroplasts that:

— Maximum two-thirds (67 p.c.) of the entire violaxanthin quantity can be transformed and only this participates in de-epoxidation.
— The violaxanthin quantity, able to be de-epoxidized, changes depending on light intensity.
— They have supposed that the violaxanthin which is able to be de-epoxidized is to be found in the loculus (mainly as a "free pigment"), and the one-third of violaxanthin that is unable to be transformed is to be found at the outer surface of the membrane (mainly as protein-bound) (SIEFERMANN–HARMS, 1980).

It follows from the above mentioned that — at the given light intensity — the activity of de-epoxidase (the quantity of violaxanthin transformed within the time unit (may be the endogenous indicator of the light-induced acidification of the loculus

In our experiments it has been observed that:

— As a result of high light-intensity (900 W/m^2), the light-induced decrease of. violaxanthin (the percentage of dark control), in corn line 523, is three times faster in the first two minutes than in 165 (Fig. 1).
— As a result of the 15–7.5 min. LDC, in corn line 523, violaxanthin considerably decreases, and in corn line 165, it increases (Table 1).
— The mesophyll chloroplasts of line 523 shrink in LDC and those of 165 swell. At the same time, line 523 produces in this rhythm the most, and line 165 the least dry material (Maróti and Pataky, 1981).

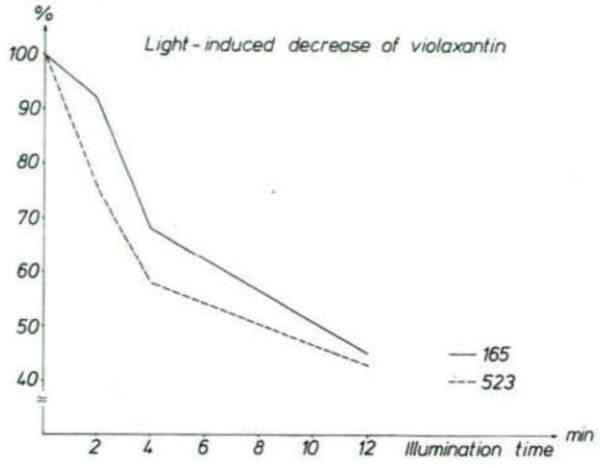

Fig. 1. Light-induced decrease of violaxanthin (percentage of dark control) in corn lines 165 and 523. Light intensity is 900 W/m². The experiment was performed with disks of 6 mm diameter taken from the third and fourth leaves of 5-week maize, on wet filter-paper.

Consequently in the chloroplasts of line 523, the proton gradient, necessary for ATP, is earlier formed (even at light intensity 32 W m²) than in corn line 165. The 15 min. illumination of chloroplasts 523 is satisfactory for the de-epoxidation of a violaxanthin; the same illumination of corn line 165, however, is insufficient. This may be one of the causes of the opposed change of the violaxanthin content.

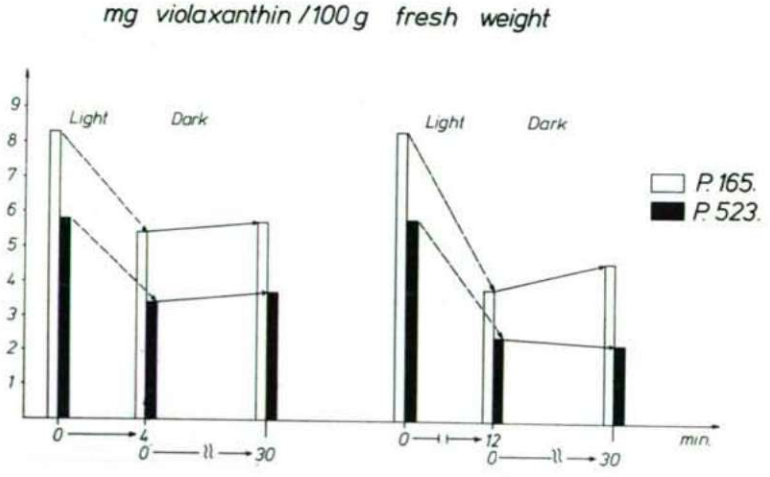

Fig. 2. De-epoxidation of violaxanthin in light and epoxidation of zeaxanthin in the dark. The time of illumination was 4, resp. 12 minutes, the dark period 30 minutes. The experimental conditions are identical with those of Figure 1.

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