THE ACTIVITY OF THE PHOTOSYNTHETIC CARBON CYCLE IN GREENING MAIZE LEAVES

by

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Introduction

At the illumination of seedlings grown in dark, characteristic changes take place in the structure, nucleic-acid- and protein metabolism of the proplastids, in the activity of the plastid- and cytoplasm enzymes. Having studied the change in CO_2 fixation ensuing upon the effect of light in bacteria, algae and higher plants, researchers found that the increase in the activity of ribulose diphosphate carboxylase and the rise in photosynthetic capacity were interconnected (H u d o c k and L e v i n e 1964, CHEN et al. 1967). In the plants with low CO_2 compensation point, which function with a C_4 dicarboxylic acid cycle, phosphopyruvic acid carboxylase has the primary role in CO_2 fixation (H a t c h et al. 1969). The parallel functioning of the C_4 and C_3 cycles is concomitant with a different localization of the enzymes (S l a c k et al. 1969).

In our earlier work we found that in maize there was a close connection between the activity of both enzymes taking part in CO_2 fixation and the intensity of the illumination (N a g y et al. 1973). The aim of the examinations of the authors presented here was to try to find a connection between the changes in enzyme activity ensuing upon the effect of illumination and tissue differentiation.

Material and method

For the examinations we used the seedlings of a normal strain and of two recessive, lehtal carotenoid mutant strains of maize (*Zea mays* L.) (F a l u d i – D á n i e l et al. 1967).

Upon a preliminary swelling of two days the seeds were germinated on wet paper wadding at 25 ± 2 °C in a thermostat. The seedlings grown in dark were kept at an illumination intensity of 25 lux for 3, 6, 12, 24, and 48 hours. The moments of illumination were timed in a way that when being worked up, the seedling should be of identical age: 7 days old. Average samples of 1 g were taken from the laminae of the leaves of the

lighttreated plants. The samples were then slit into stripes of about 2 – 3 mm. along the leaf veins and cut into pieces of about 5 mm in length. The pieces were homogenized in an MSE homogenizer in 10 ml of buffer for 2 minutes at 10 000 r.p.m. The buffer was composed as follows: 4 mmol TRIS, 1 mmol $MgCl_2$, 0.02 mmol EDTA, 0.05 mmol ascorbic acid, 0.05 mmol reduced glutathione (GSH) in a volume of 100 ml; pH = 7.8. The homogenate was filtered through a nylon filter of 30 μ pore diameter. The filtrate contained the extract of the mesophyll cells. The remainder was ground in the buffer of the above composition in a braying mortar in the presence of washed sea-sand and then filtered again. The filtrate was the extract of the parenchyma sheath cells. Both extracts were centrifuged in a WAC 60 type preparative ultracentrifuge for 20 minutes, at 105 000 xg. The supernatant was used as crude enzyme extract.

Ribulose diphosphate (RuDP)-carboxylase and phosphopyruvate (PEP)-carboxylase activity were examined by the method of Björkman and Gauhl (1969), founded on $H^{14}CO_3^-$ incorporation. The quantity of ^{14}C got into organic binding was determined by means of liquid scintillation counting (Bush and Hansen 1965). Nuclear Chicago 724.

The activity of glucose-6-phosphate dehydrogenase (G6PDH) was measured spectrophotometrically with Kornberg and Horecker's (1955) method founded on NADPH formation.

The results obtained in the experiments were processed with on ODRA 1013 computer; we applied univariate linear and non-linear regression analysis (Ezekiel and Fox 1970). 13 types of curves (linear; second- and thirdorder parabola, in the normal and logarithmic versions of the variables, as well as the reciprocal function) were fitted to a series of data corresponding to each functional relationship examined by them. From among these they represented the curve fitting best to the measured data.

For characterizing the significance of the connections, the determination index and the estimated standard deviation of the estimate were calculated.

Determination index: $d_y \cdot f(x) = \dfrac{s^2_{y'}}{s^2_y}$

$s^2_{y'}$ = the variance of the calculated values

s^2_y = the variance of the measured values

Estimated standard deviation of the estimate:

$$s_y \cdot f(x) = \sqrt{\frac{z^2}{} \cdot \frac{n}{n-m}}$$

z = the difference between the measured y-values and those calculated from the regression equation

n = the number of data

$$\frac{n}{n-m} = \text{correction term}$$

m = number of the coefficients of the fitted regression equation

Since the estimated standard deviation of the estimate – independently from the value of the independent variable – only characterizes the fitting of the regression curve to the measured data (in the dimension of the dependent variable), that one of the curves is called fitting best, of which the estimated standard deviation of the estimate is the lowest. In the majority of the equations fitting best the estimated standard deviation of the estimate was about 20% of the values of the dependent variable.

For the determination of each of the illumination periods, we conducted three experiments which were independent of each other. In case of PEP-carboxylase the results of a single experiment they calculated from the average of 4, in case of G6PDH from that of 2 parallels.

Experimental results

The distribution of the activity of PEP-carboxylase between the mesophyll- and parenchyma sheath cells of the normal maize leaves in the course of an illumination of 48 hours in shown in Figure 1.

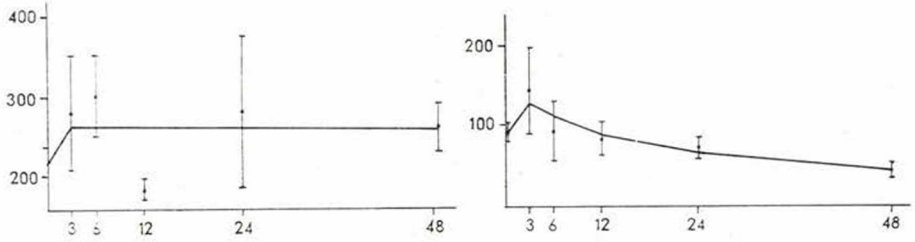

Figure 1. The change in the activity of PEP-carboxylase in the leaves of normal maize seedlings following illumination for various lengths of time

a) mesophyll (left)
b) parenchyma sheath (right)

It appears that the PEP-carboxylase activity level of the two types of tissue already differs in the etiolated leaves. The activity in the extract of mesophyll cells was nearly the double of that of the other type. An illumination of 3 hours calls forth an increase in PEP-carboxylase activity in both tissue types. Further illumination brings on a change of but slight measure, however, of contrary tendency in the mesophyll and parenchyma sheath cells.

Under similar experimental conditions the distribution of PEP-carboxylase in the mutant leaves is summarized in Figure 2.

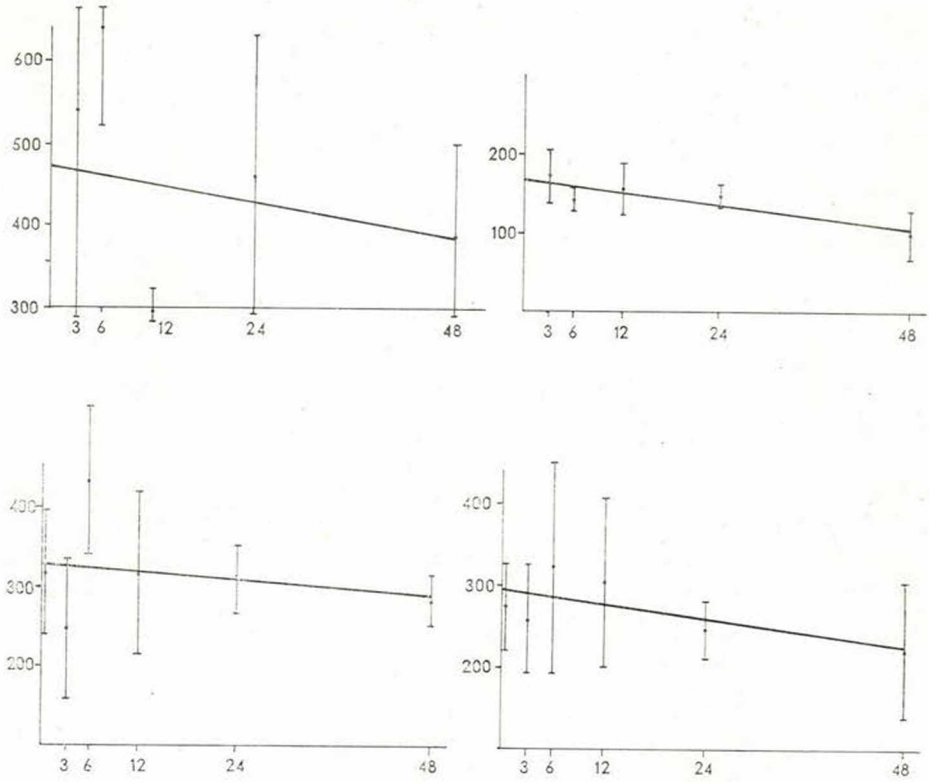

Figure 2. The change in the activity of PEP-carboxylase in the leaves of mutant maize seedlings following illumination for various lengths of time

1. lycopenic (up)
2. ζ –carotenic (down)

a) mesophyll (left)
b) parenchyma sheath (right)

In both mutants we measured a higher PEP-carboxylase activity than in the normal leaves. In the two issue types of the lycopenic leaves enzyme capacity is nearly identical. In the ζ-carotenic mutant, it was in the mesophyll extract where a higher PEP-carboxylase activity was measured. A change of decreasing tendency could be observed in the tissues of the mutant leaves upon the effect of illumination. The high values of standard deviation indicate a considerable heterogeneity of the chloroplasts and tissues of the mutant leaves.

Figure 3 shows the distribution of RuDP-carboxylase activity in the leaves of normal maize seedlings illuminated with 25 lux for various lengths of time.

RuDP-carboxylase capacity formed in 48 hours is distributed between the two tissue types in a nearly equal proportion. However, there is a difference to be observed in the tendency of the increase in activity during illumination. Illumination lasting over 3 hours does not cause a

notable increase in the mesophyll cells any more. Upon extending the illumination period we could find a steady rise in enzyme activity in the parenchyma-sheath.

RuDP-carboxylase activity measured in the mutant leaves under similar experimental conditions is shown in Figure 4.

In the lycopenic leaves grown in dark a rather low RuDP-carboxylase activity was measured, in the ζ-carotenic ones this activity resembled the normal one. In the enzyme capacity of the etiolated leaves no notable change ensues upon the effect of illumination.

The change of G6PDH activity ensuing in the mesophyll and parenchyma-sheath cells of normal leaves upon the effect of illimunation for various is presented in Figure 5.

In the extract of the mesophyll cells of the etiolated normal leaves we found a higher level of G6PDH activity. In both types of tissue, illumination has for result a decrease in enzyme capacity.

The change in activity of G6PDH in the mesophyll and sheath parenchyma-sheath tissues of the two mutants is shown in Figure 6.

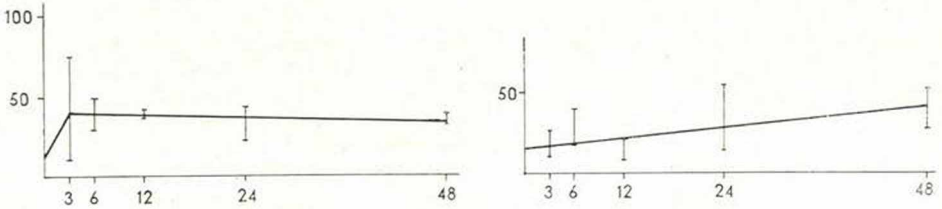

Figure 3. The change in the activity of RuDP-carboxylase in the leaves of normal maize seedlings following illumination for various lengths of time

a) mesophyll (left)
b) parenchyma sheath (right)

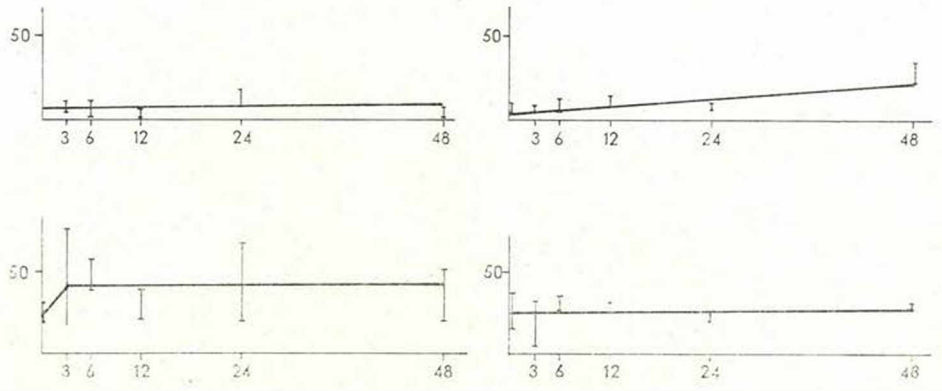

Figure 4. The change in the activity of RuDP-carboxylase in the leaves of mutant maize seedlings following illumination for various lengths of time

1. lycopenic (up)
2. ζ – carotenic (down)

a) mesophyll (left)
b) parenchyma sheath (right)

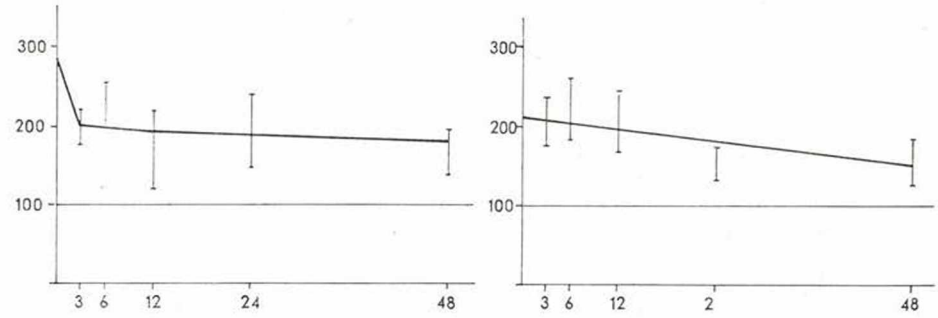

Figure 5. The change in the activity of G6PDH in the leaves of normal maize seedlings following illumination for various lengths of time

a) mesophyll (left)
b) parenchyma sheath (right)

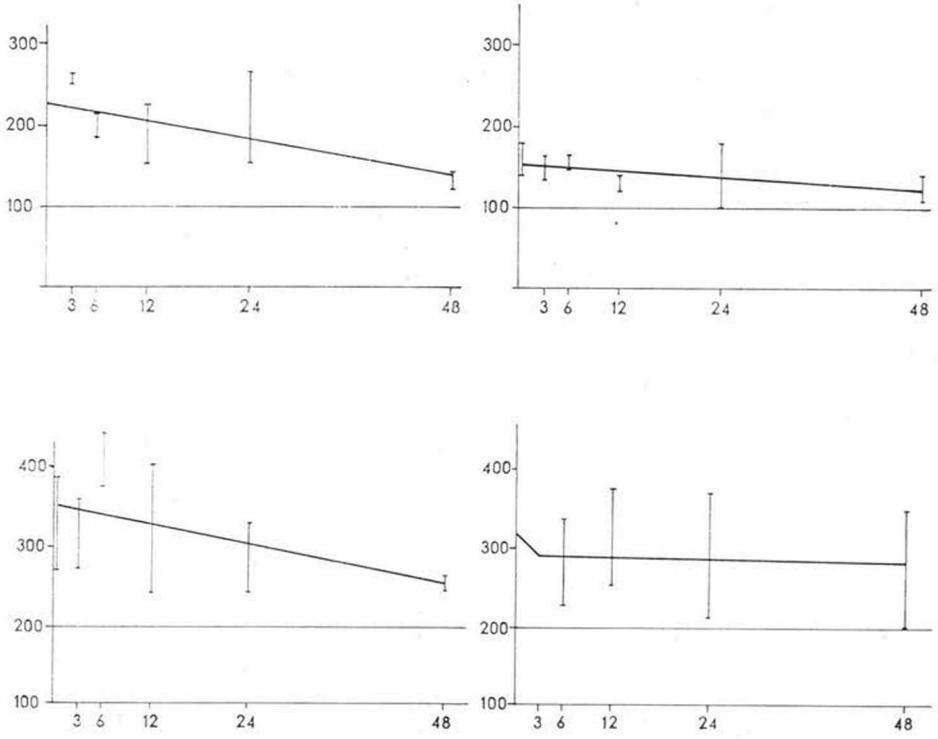

Figure 6. The change in the activity of G6PDH in the leaves of mutant maize seedlings following illumination for various lengths of time

1. lycopenic (up)
2. ζ –carotenic (down)

a) mesohyll (left)
b) parenchyma sheath (right)

Founded on the data it can be ascertained that in the tissues of the lycopenic leaves the activity of G6PDH surpasses the level measured in the normal leaves to a higher and that in the ζ-carotenic ones to a lower degree. The difference of the two types is not significant in either of the mutants, moreover, during illumination also the somewhat higher level of activity found in the mesophyll cells is compensated.

Discussion

Founded on a survey of the experimental results it can be stated that both in the etiolated and in the continuously illuminated normal seedlings the activity of PEP-carboxylase many times surpasses the one of RuDP-carboxylase, the other important enzyme of CO_2 fixation. No similarly considerable difference was found in the C_3-type bean leaves under similar experimental conditions (B r a d b e e r 1970). On the other hand, the characteristic two tissue types of the plants with the C_4 pathway also differed as to enzyme capacity even in the maize leaves grown in the dark. The mesophyll cells showed nearly twice as high PEP-carboxylase activity than the cells of the parenchyma-sheath. Also continuous illumination affects the two types of tissue in a different manner. Enzyme capacity developed in the mesophyll cells in 3 hours does not differ essentially from the 48 hours' value, which refers to the possibility that the low intensity of illumination might be a limiting factor in the development of the activity level. The decreasing PEP-carboxylase activity found in the parenchyma-sheath cells is indicative of changes resembling those of the C_3-type bean leaves during illumination (B r a d b e e r et al. 1970).

In the leaf tissues of the mutants the activity of PEP-carboxylase in markedly higher than the normal one. This activity level refers to regulation troubles of the enzyme synthesis in the mutants. The two types of tissue show a difference only in the etiolated ζ-carotenic mutant. According to our earlier electron microscopic examinations, similarly to the situation in normal plants, in this mutant etioplasts show a different structure in the mesophyll- and parenchyma-sheath cells (N a g y et al. 1973). In both mutants it can be observed that illumination does not affect PEP-carboxylase activity.

The activity of RuDP-carboxylase in normal leaves shows a characteristic change in the course of illumination. In the mesophyll cells an equilibrium is developing within a short time (3 hours) while in the parenchyma-sheath we measured a gradually increasing activity. This latter change resembles the observations made with the bean leaves of the C_3 type, although the level of activity is markedly higher here (B r a d b e e r 1969).

From among the mutants it is the ζ-carotenic one which shows a RuDP-carboxylase activity level resembling the normal value; the values to be measured in the lycopenic leaves were much lower. Also regarding this enzyme light regulation is ineffective in both tissue types.

The low level of activity of RuDP-carboxylase occurs at a high G6PDH activity in the lycopenic mutant. This experimental finding refers to the conditions of regulation prevailing between the two cycles. Feierabend (1966) experimentally proved the significance of the activity level of G6PDH in the swith-over of the oxydative and reductive pentose phosphate cycles. A high activity level of G6PDH may hinder the formation and activation of RuDP-carboxylase. Conditions similar to these can be observed in the leaves of the lycopenic mutant. Another important step of regulation is manifested through the formation of $NADPH_2$ in a way that through inhibition of the product it suppresses the activity of G6PDH and by this the reductive pentose phosphate cycle comes into function. Also phosphorylation of an adequate measure is required for the process (Lendzian and Ziegler 1970). In consequence of inadequate functioning of the photosystems of the mutants, of the insufficiency of phosphorylation and $NADPH_2$ the action of the oxidative pentose phosphate cycle is not suppressed and the conditions of the action of the reductive pentose phosphate cycle are not given (Faludi–Dániel et al. 1970, Nagy et al. 1971).

Summary

The authors measured the activity of RuDP- and PEP-carboxylase as well as of G6PDH in normal and mutant maize leaves following continuous illumination for various time. They found that PEP-carboxylase showed a considerably higher activity than RuDP-carboxylase. Both in the etiolated and in the illuminated leaves they could measure different levels of activity in the mesophyll- and parenchyma-sheath cells. Tissue differentiation and light regulation in the mutants, as well as the enzyme level measured in the etiolated leaves equally differed from those observed in the normal plants.

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