Phytochemistry, 1972, Vol 11, pp 183 to 187 Pergamon Press Printed in England

EFFECT OF LIGHT INTENSITY ON THE FORMATION OF CAROTENOIDS IN NORMAL AND MUTANT MAIZE LEAVES

G. HORVÁTH, J KISSIMON and Á FALUDI-DÁNIEL

Institute of Plant Physiology, Hungarian Academy of Sciences and Institute of Evolution and Genetics, Eotvos University, Budapest, Hungary

(*Received* 24 *June* 1971)

Abstract—Carotenoid composition in leaves of normal, lycopenic and ζ-carotenic mutants of *Zea mays* were investigated In lycopenic leaves, in addition to lycopene, phytoene, phytofluene, δ- and γ-carotene, trace amounts of α- and β-carotene and antheraxanthin were identified Low light promoted accumulation of α- and β-carotene, high light brought about an increase in antheraxanthin content In the leaves of the ζ-carotenic mutant, phytoene, phytofluene and ζ-carotene were synthesized Illumination of low intensity stimulated carotenoid synthesis to a slight extent Relative amounts of carotenoid components were essentially the same as in etiolated material, except for a small increase in *cis*-ζ-carotene Under high intensity illumination, carotenoids were rapidly destroyed

INTRODUCTION

LIGHT promotes the biosynthesis of carotenoids and consequently there is a considerable difference between the carotenoid content of dark grown and that of illuminated plants This difference is rather of a qualitative[1,2] than a quantitative character [3] Xanthophylls are formed mainly in etiolated leaves, and—in contrast to leaves exposed to light—they contain only a small amount of β-carotene The point of carotenoid synthesis affected by light has not yet been identified The difference between the xanthophyll contents of etiolated and green plants results partly from increased xanthophyll synthesis[4] and partly from the interconversion between the different xanthophylls, a process stimulated by illumination.[5,6]

The present study was designed to compare carotene formation in the dark-grown and illuminated leaves of normal maize and in those of two mutants in which the pathway of biosynthesis had been blocked at different stages

RESULTS

The carotenoid content of normal maize leaves is illustrated in Table 1, which presents comparative data between etiolated leaves and leaves grown in a light of 25 lx, and shows the effect of sunlight on the formation of carotenoids Both the etiolated leaves and those grown at 25 lx contained, in addition to β-carotene, lutein-5,6-epoxide, lutein, neoxanthin, traces of violaxanthin, but no zeaxanthin The latter could be detected only after a 3-hr exposure to sunlight Etiolated leaves contained mainly xanthophylls, and only a small amount of β-carotene Light preferentially stimulated β-carotene formation while the ratio of xanthophylls between green and etiolated leaves remained about the same Synthesis

[1] T W GOODWIN and S PHAGPOLGARM, *Biochem J* **76**, 197 (1960)
[2] F T WOLF, *Plant Physiol* **38**, 649 (1963)
[3] H CLAES, *Z Naturforsch* **12b** 401 (1957)
[4] C A TAKEGUCHI and H Y YAMAMOTO, *Biochim Biophys Acta* **153**, 459 (1968)
[5] H Y YAMAMOTO, T O M NAKAYAMA and C O CHICHESTER, *Arch Biochem Biophys* **97**, 168 (1962)
[6] U BLASS, J M ANDERSON and M CALVIN, *Plant Physiol* **34**, 329 (1959)

Table 1 Carotenoid composition of normal maize leaves

Material	Etiolated	'Low light'*	Green* 'High light' after 60,000 lx	'Low light' / etiolated
β-Carotene	8 3	31 7	29 0	3 82
Lutein-5,6-epoxide + violaxanthin	25 0	17 0	13 1	0 68
Lutein	58 3	44 0	46 4	0 74
Neoxanthin	8 3	7 3	6 2	0 88
Zeaxanthin	—	—	5 3	—

Values are given as mole per cent of the total carotenoid content
* 'Low light' grown continuously at 25 lx, 'high light' the same, illuminated with 60,000 lx for 3 hr

of zeaxanthin and lutein were considerably enhanced by sunlight Carotenoids in the leaf of the lycopenic mutant are shown in Table 2 In addition to colourless carotenoids it contained small amounts of α + β-carotenes and somewhat larger quantities of δ-, γ-carotenes and lycopene Among xanthophylls the presence of antheraxanthin and traces of other xanthophylls were detected. The ratio 'low light'/etiolated was highest in respect of δ- and γ-carotenes. Three hr of sunlight increased the concentration of xanthophylls quite considerably. Carotenoids accumulating in the leaf of the ζ-carotenic mutant are shown in Table 3

Table 2 Carotenoid composition of lycopenic maize leaves

Material	Etiolated	'Low light'*	Green* 'High light' after 60,000 lx	'Low light' / etiolated
Phytoene	25 8	13 1	16 3	0 51
Phytofluene	7 9	7 8	2 9	0 99
α- + β-Carotenes	2 2	2 1	3 3	0 99
β-Carotene	31 5	48 4	43 1	1 54
γ-Carotene	4 5	8 2	8 8	1 82
Lycopene	10 1	5 4	5 0	0 53
Antheraxanthin + other xanthophylls	18 0	13 6	20 5	0 76

Values are given as mole per cent of total carotenoid content
* As in Table 1

Table 3 Carotenoid composition of ζ-carotenic maize leaves

Material	Etiolated	'Low light'*	Green* 'High light' after 60,000 lx	'Low light' / etiolated
Phytoene	29 6	31 0	44 1	1 05
Phytofluene	21 2	18 0	23 8	0 85
ζ-Carotene	47 7	47 8	29 4	1 01
cis-ζ-Carotene	1 5	3 2	2 7	2 13

Values are given as mole per cent of total carotenoid content
* As in Table 1

This mutant contained only phytoene, phytofluene, ζ- and *cis*-ζ-carotene. The main component of both etiolated and green leaves was ζ-carotene which amounted to approximately 50% of the total carotene content. The ratio 'low light'/etiolated was the highest for *cis*-ζ-carotene and much lower in respect of other carotenes. Exposure to sunlight (for about 30 min) induced a rapid destruction of *trans*- and *cis*-ζ-carotenes. The comparatively high level of phytoene and phytofluene was a result of their slower decomposition.

TABLE 4 TOTAL CAROTENOID CONTENT IN ETIOLATED AND GREEN LEAVES OF NORMAL AND MUTANT MAIZE

Material	Etiolated	Green 'Low light'*	'Low light' / etiolated
Normal	36	218	6·1
Lycopenic	89	213	2·4
ζ-Carotenic	260	401	1·5

Values are in nmole/g fresh wt
* As in Table 1

Table 4 shows the total carotenoid contents of etiolated and green leaves and also the stimulating effect of light on carotenoid synthesis in the various strains. It is evident that the total carotenoid content of green leaves was higher in all strains studied. Light induction of carotenoid synthesis was the lowest in ζ-carotenic leaves and the highest in the normal strain.

Changes in chlorophyll concentration induced by high light intensity are shown in Table 5. Sunlight decreased the amount of chlorophyll in all three strains. Three hr of sunlight caused a slight decrease in the normal and the lycopenic leaves, while a 30-min exposure reduced the amount of chlorophyll to a third of the original value in the ζ-carotenic leaves.

TABLE 5 CHLOROPHYLL CONTENT OF NORMAL AND MUTANT MAIZE LEAVES UNDER DIFFERENT CONDITIONS OF ILLUMINATION

Material	Duration of 'High light' (hr)	Chlorophyll a+b 'Low light'	Chlorophyll a+b 'High light'	'High light' / 'Low light'
Normal	3	668	584	0·87
Lycopenic	3	270	238	0·88
ζ-Carotenic	0·5	178	67	0·38

Values are nmole/g fresh wt

DISCUSSION

Our experiments have shown that light increases carotenoid contents in both the normal and the mutant leaves.[7–10] Comparison between green and etiolated leaves of the normal,

[7] R. Z. COHEN and T. W. GOODWIN, *Phytochem.* **1**, 67 (1962).
[8] J. D. HENSHALL and T. W. GOODWIN, *Phytochem. Photobiol.* **3**, 243 (1964).
[9] K. I. TREHARNE, E. L. MERCER and T. W. GOODWIN, *Phytochem.* **5**, 581 (1966).
[10] Á. FALUDI-DÁNIEL, Á. NAGY and A. GYESKO, *Ann. Univ. Sci. Biol.* **9–10**, 143 (1968).

ζ-carotenic and lycopenic strains makes it clear that light stimulates the terminal stages of carotene synthesis with greater intensity Acceleration of carotene synthesis begins with the δ- and γ-carotenes, light affects cyclization to a considerable degree, and promotes the synthesis of δ- and γ-carotenes in a similar manner [11] Difference between the respective concentrations of δ- and γ-carotenes points to the fact that the intensity of biosynthesis is not the same on the two pathways The relatively high percentage of *cis*-ζ-carotene in ζ-carotenic leaves is in agreement with Goodwin's theory that carotene synthesis advances via *cis*-isomers [12] With a low light intensity, zeaxanthin (present in the conventional xanthophyll cycle) could not be identified, although its synthesis in trace amount cannot be excluded The fact that the formation of zeaxanthin becomes marked when the intensity of light is raised points to the possibility that the interconversion between violaxanthin, antheraxanthin and zeaxanthin is not operative at low intensity of illumination [5] The rapid rate at which the amount of zeaxanthin increases in strong light shows that the plant responds rapidly to changes in light intensity The amount of carotene synthesized at low light intensity is not sufficient to prevent photodestruction under conditions of high light intensity, the result being a decrease in the amount of chlorophyll [13,14] Photodestruction of chlorophyll is moderate in normal and lycopenic leaves but is considerable in the ζ-carotenic mutant While under prolonged illumination the pigment content in normal leaves will be restored in the leaves of lycopenic and ζ-carotenic mutants the pigment-synthesizing apparatus is irreversibly damaged [15] However, lycopenic leaf, which needs about three days for bleaching, still behaves normally after a 3-hr exposure to sunlight in respect of both carotene and chlorophyll synthesis

EXPERIMENTAL

The leaves of 7- to 9-day-old seedlings of normal maize as also of the ζ-carotenic and lycopenic mutants were used [16] On account of the mutant's photosensitivity they were raised in a light of 25 lx (low light), and the etiolated plants were grown in the dark To study the effect of high light intensity, plants grown in a light of 25 lx were exposed for a predetermined period to sunlight of 60,000–70,000 lx (high light)

In order to extract the pigments, the leaves were ground in a mixture of acetone and light petroleum Acetone was removed from the crude extracts by washing (H_2O), and the extracts were then dried over an NaCl [17] Separation of the carotenoids was carried out by TLC according to Hager and Meyer-Bertenrath,[18] and their method was also used for the quantitive elution of pigments

Identification of the pigments was based on the following parameters shape of the spectra in hexane,[19] the absorption maxima of carotenoids in hexane, light petroleum, *iso* octane, $CHCl_3$ and CS_2,[20] iodine-catalysed *cis–trans*-isomerization,[21] distribution ratio between hexane and 95% MeOH,[22] colour reactions of the carotenoids in conc H_2SO_4, conc HCl and in $SbCl_3$ dissolved in $CHCl_3$,[23] isomerization of epoxides with conc HCl [20]

[11] T W GOODWIN, in *Progress in Photosynthesis Research* (edited by H METZNER), Vol 2, p 669, Tubingen (1969)
[12] T W GOODWIN, *J Sci Ind Res* **27**, 103 (1968)
[13] I C ANDERSON and D S ROBERTSON, *Plant Physiol* **35**, 531 (1960)
[14] Á FALUDI-DÁNIEL, B FALUDI, I GYURJAN and I SZARAZ, *Ann Univ Sci Bp* **5**, 69 (1962)
[15] Á FALUDI-DANIEL, A H NAGY, Á NAGY, in *Progress in Photosynthesis Research* (edited by H METZNER), Vol 2, p 592, Tubingen (1969)
[16] Á FALUDI-DÁNIEL, J AMESZ and A NAGY, *Biochim Biophys Acta* **197**, 60 (1969)
[17] S W JEFFREY, *Biochim Biophys Acta* **162**, 271 (1968)
[18] A HAGER and T MEYER-BERTENRATH, *Planta* **69**, 198 (1966)
[19] A HAGER and T MEYER-BERTENRATH, *Ber Dtsch Bot Ges* **80**, 426 (1967)
[20] A HAGER and T MEYER-BERTENRATH, *Planta* **76**, 149 (1967)
[21] L ZECHMEISTER, in *Fortschritte der Chemie Organischer Naturstoffe* (edited by L ZECHMEISTER), Vol 18, p 224, Wien (1959)
[22] F J PETRACEK and L ZECHMEISTER, *Analyt Chem* **28**, 1484 (1965)
[23] T W GOODWIN, in *Moderne Methoden der Pflanzenanalyse* (edited by K PEACH and M V TRACEY), Vol 3, p. 272, Springer-Verlag, Berlin (1955)

Carotenoids eluted from TLC plates were brought to the desired volume and their absorbances were measured at the characteristic λ_{max} values Molar extinctions were calculated from Goodwin's $E^{1\%}_{1\,cm}$ values[23] and mol wts Degradation occurring in the course of the chromatography was determined separately for each carotenoid [24]

Chlorophylls were measured in the ether extract of the leaves, and the amount of pigments was determined according to Smith and Benitez [25]

[24] G Horváth, J Kissimon and Á Faludi-Dániel, *Acta Biol Acad Sci Hung* (in press).

[25] J H C Smith and A Benitez, in *Modern Methods of Plant Analysis* (edited by K Peach and M. V Tracey), Vol 4, p 143, Springer-Verlag, Berlin (1955)

Key Word Index—Zea mays, Gramineae, carotenoids, light intensity, mutants