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PHOTOCONTROL OF AMARANTHIN SYNTHESIS IN *AMARANTHUS TRICOLOR**†

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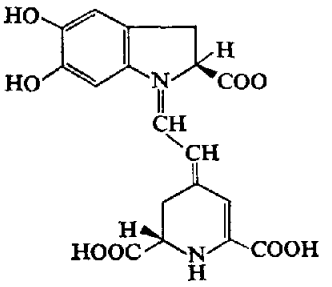
Abstract—Amaranthin formation in seedlings of *Amaranthus tricolor* is controlled by two photoreactions, one, a high-energy reaction, and the other a low-energy reaction controlled by phytochrome. Elongation of the hypocotyl in *A. tricolor* seedlings is also phytochrome controlled.

INTRODUCTION

THE RED-VIOLET pigments of the Centrospermae (betacyanins) isolated so far have all been shown to be derivatives of either betanidin (I) or its C-15 diastereoisomer, isobetanidin.[1] According to a biogenetic scheme suggested by Dreiding,[2] betanidin might be formed from two molecules of β-(3,4-dihydroxyphenyl)alanine (DOPA) and experiments on the incorporation of labelled precursors[3,4] are in agreement with this hypothesis.



(I) Betanin

Mature leaves of *Amaranthus tricolor* contain two betacyanins; amaranthin and isoamaranthin {which are betanidin- and betanidin-5-*O*-[2-*O*-(β-D-glucopyranosyluronic acid)-β-D-Glucopyranoside] respectively} in a molar ratio of about 9:1.[5,6] Synthesis of these pigments in *A. tricolor* seedlings requires illumination.[7]

In the last 10 years, studies on the induction of anthocyanin formation in many plants

* Part VIII of the series "Pigments of Centrospermae", for Part VII, see *Phytochem.* **6**, 709 (1966).

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[1] T. J. MABRY, in *Comparative Phytochemistry* (edited by T. SWAIN), p. 231, Academic Press, London (1966).
[2] H. WYLER, T. J. MABRY and A. S. DREIDING, *Helv. Chim. Acta* **46**, 1745 (1963).
[3] L. HORHAMMER, H. WAGNER and W. FRITZSCHE, *Biochem. Z.* **339**, 398 (1964).
[4] L. MINALE, M. PIATTELLI and R. A. NICOLAUS, *Phytochem.* **4**, 593 (1965).
[5] M. PIATTELLI, L. MINALE and G. PROTA, *Ann. Chim.* **54**, 963 (1964).
[6] M. PIATTELLI and L. MINALE, *Ann. Chim.* **56**, 1060 (1966).
[7] A. S. GARAY and G. H. N. TOWERS, *Can. J. Botany* **44**, 231 (1966).

by light has led to the identification of two photoreceptors. The first photoreceptor requires high light-energy levels maintained for a comparatively long period of time (HER); the second is the reversible pigment system, phytochrome.[8] Typical phytochrome-controlled reactions are stimulated by a small dose of red light (660 nm), which shifts the equilibrium $P_r \rightleftharpoons P_{fr}$ towards the physiologically active P_{fr} form, and can be neutralized by subsequent exposure to far-red light (730 nm).

It was suggested[9] that in anthocyanin synthesis, the HER controls the supply of acetyl groups to form the substrate for subsequent P_{fr}-controlled enzymatic reactions. Since the biosynthesis of betacyanins appears to follow a very different pathway from that of anthocyanins (in particular, there is no evidence that acetate is involved directly) we decided to investigate the photocontrol of amaranthin synthesis in *A. tricolor*.

RESULTS AND DISCUSSION

Nature of the Pigment

Analysis of the extract from seedlings of *Amaranthus tricolor* showed amaranthin as the only pigment. It thus appears that isoamaranthin, found in the mature plant, is perhaps not a primary product of metabolism, but is formed by isomerization of amaranthin.

Effect of the Light on Amaranthin Synthesis

In darkness at 26° for 2 days *A. tricolor* synthesizes about $2{\cdot}5 \times 10^{-9}$ moles amaranthin/seedling. When the seedlings were irradiated with fluorescent light (8000 lux) for 2 hr and then returned to the dark pigment synthesis started after a lag period of about 4 hr from the beginning of illumination and attained a maximum at 24 hr. After this time, a rapid fall in amaranthin concentration was observed (Fig. 1).

When the seedlings were illuminated for different periods of time and then allowed to incubate in the dark up to 24 hr, the rate of pigment synthesis increased markedly during the first 3 hr of illumination and then slowed down approaching apparent saturation after about 20 hr.

Amaranthin synthesis stimulated by light from fluorescent lamps was appreciably inhibited by a subsequent 5 min irradiation with far-red light. The action of far-red could be reversed by subsequent exposure to red, and the response was repeatedly, although not completely, reversible (Table 1). This phenomena can be considered a typical phytochrome effect, and amaranthin formation is thus controlled by two photoreactions, a high-energy reaction and a low-energy reaction, dependent on phytochrome. In our experimental conditions, about 3 min of red or 5 min of far-red irradiation were sufficient for the maximum effect of the low-energy reaction. With longer exposure times far-red had a stimulating effect (Fig. 2). This result can be rationalized by assuming that the small but definite level of active phytochrome (P_{fr}) attained by exposure to far-red light and maintained by continued irradiation is sufficient to produce an efficient pigment synthesis, provided that a sufficient rate of supply of substrate for action of phytochrome is ensured by the high-energy reaction.

Both red (600–700 nm) and far-red (>700 nm), given alone (Fig. 3), produce pigment synthesis, showing that the spectral zone in the red and far-red regions is effective for the HER.

[8] S. B. HENDRICKS and H. A. BORTHWICK, in *Chemistry and Biochemistry of Plant Pigments* (edited by T. W. GOODWIN), p. 416, Academic Press, London (1965).
[9] H. W. SIEGELMAN and S. B. HENDRICKS, *Plant Physiol.* **33**, 409 (1958).

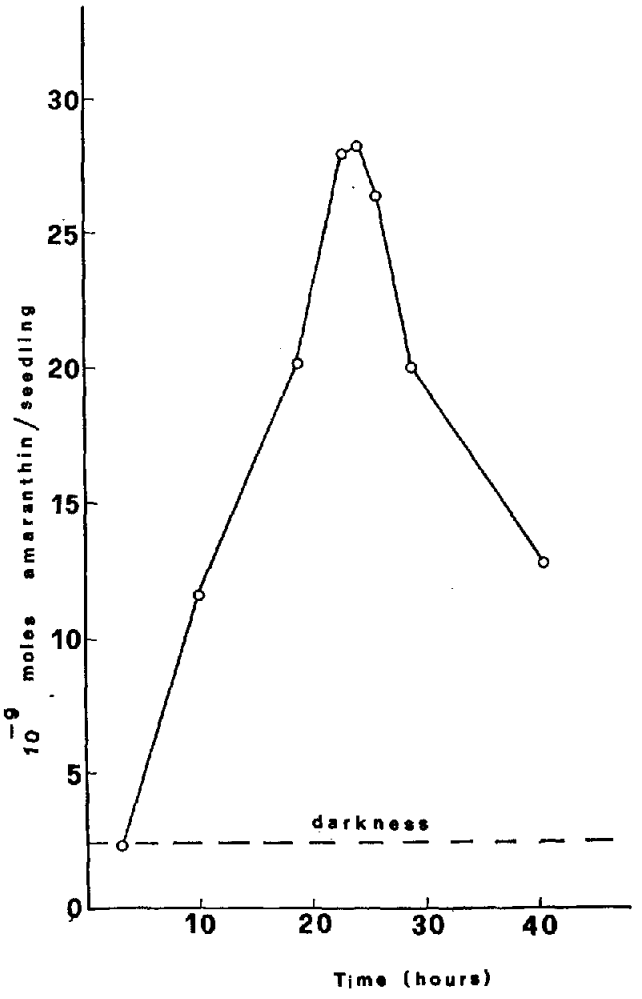

FIG. 1. VARIATION OF THE AMOUNT OF AMARANTHIN IN RESPONSE TO VARIOUS PERIODS OF DARKNESS AFTER 2 hr EXPOSURE TO LIGHT FROM FLUORESCENT LAMPS.

TABLE 1. REVERSIBILITY OF AMARANTHIN FORMATION BY RED AND FAR-RED IRRADIATION

Exposure*	Amaranthin (moles × 10^{-9})
Red	10·7
Red + far-red	7·6
Red + far-red + red	15·3
Red + far-red + red + far-red	9·3

* After 2 hr illumination with fluorescent light. Each treatment was for 5 min.

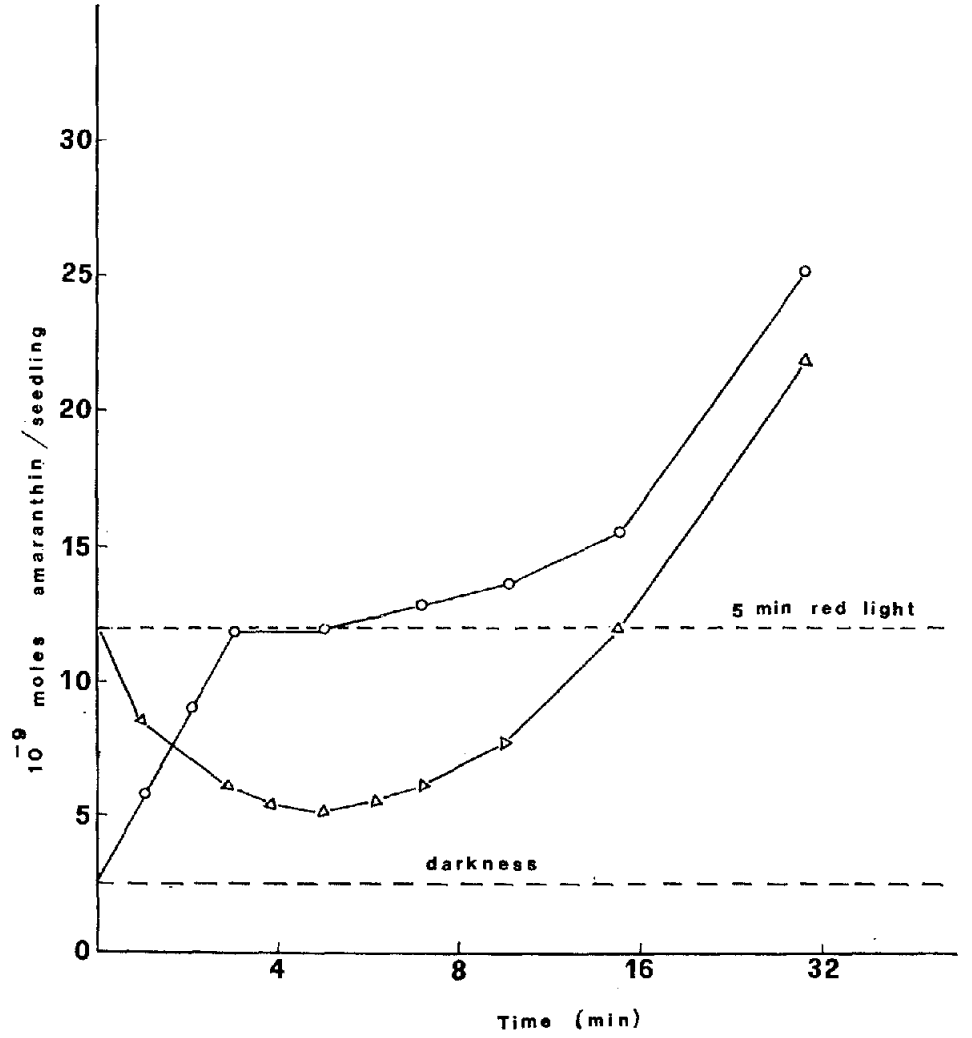

FIG. 2. EFFECT OF SHORT PERIODS OF IRRADIATION WITH RED (—○—) OR FAR-RED (—△—). Seedlings irradiated with far-red were previously exposed to red light for 5 min.

TABLE 2. EFFECT OF RED AND FAR-RED ON HYPOCOTYL ELONGATION OF *Amaranthus tricolor*

Treatment*	Length of hypocotyl (mm)
Control (darkness)	9·8
Red	7·5
Red + far-red	9·0

* 2-day-old seedlings were irradiated 5 min with red or far-red light. Length of hypocotyl was measured after 12 hr of dark incubation.

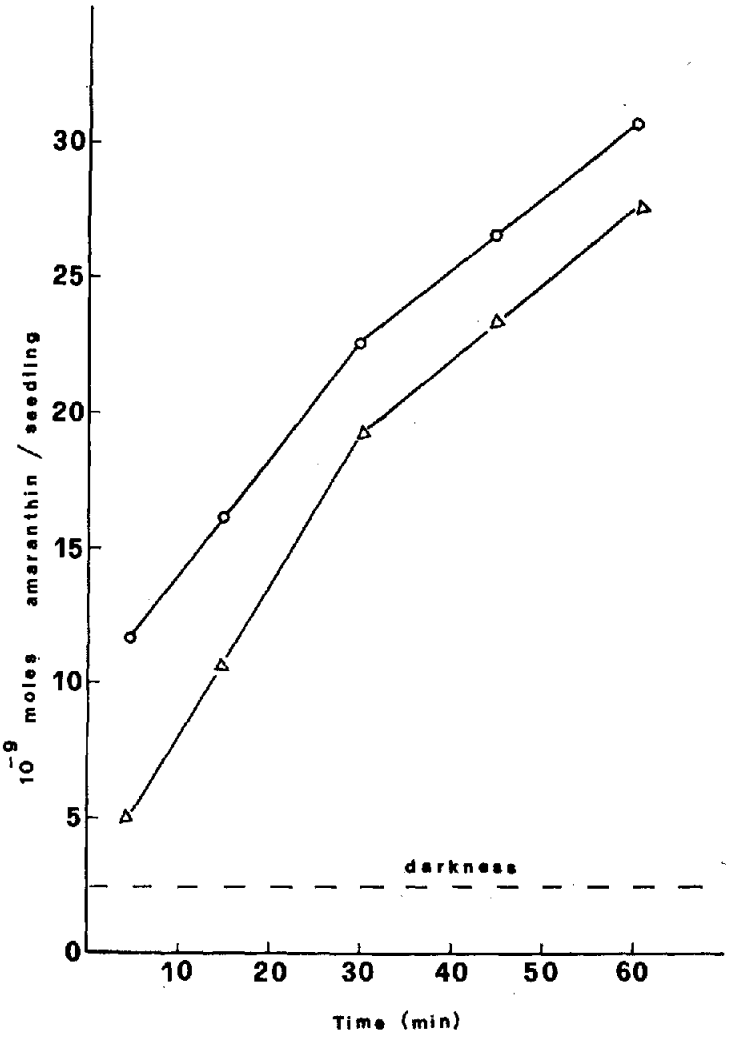

FIG. 3. AMOUNT OF AMARANTHIN FORMED IN RESPONSE TO VARIOUS PERIODS OF IRRADIATION WITH RED (—○—) OR FAR-RED (—△—).

Effect of Red and Far-Red Light on Elongation of the Hypocotyl

Elongation of the hypocotyl in dark-grown *A. tricolor* seedling is phytochrome controlled, as shown by the results summarized in Table 2.

CONCLUSIONS

The above results are consistent with the view that two photoreceptor pigments are involved in the formation of amaranthin in *Amaranthus tricolor* seedlings. The first photoreceptor controls a high-energy reaction. The second photoreceptor (phytochrome) can be light saturated at low level of illumination, and the presence of the active P_{fr} form is a necessary condition for amaranthin production.

Apparently, therefore, the effect of light on amaranthin synthesis in *A. tricolor* seedlings is similar to that observed in other plants for anthocyanin synthesis.

Studies on apple skin[9] have shown that this plant material in darkness produces ethanol and acetaldehyde. Illumination inhibits the production of these two compounds with concomitant formation of anthocyanin (idaein). Since the A ring of anthocyanins is known to be derived from head to tail condensation of three acetyl (malonyl) units, the HER is supposed to control the supply of acetyl groups to produce the substrate for a subsequent phytochrome-controlled reaction for the synthesis of this moiety of the pigment molecule.

This mechanism of light control obviously does not apply to the formation of betacyanins. The parallel effect of light on the formation of both type of pigment might be better explained by assuming that HER controls reactions leading to the C_6-C_3 units common to betacyanins and anthocyanins.

EXPERIMENTAL

Plant Material

Seeds of *Amaranthus tricolor* were placed on two sheets of Whatman No. 1 filter paper moistened with distilled water (14 ml) on 10-cm Petri dishes and held in complete darkness at constant temperature (26°). 2-day-old seedlings were used in all experiments.

Light Source

Irradiation was effected with cool-white fluorescent source giving a maximum unfiltered illumination of about 8000 lux at the seedling level. The same source was used in conjunction with a red filter transmitting at wavelengths above 590 nm (Kodak Wratten No. 25) for irradiation with red-light. Tungsten filament lamps (4 × 40 W) were used in conjunction with a Kodak Wratten far-red filter No. 89B (transmitting at wavelengths above 690 nm) as source of far-red light.

Qualitative Analysis of Pigment

Seedlings were illuminated for 2 hr with light from fluorescent lamps and then returned to darkness. After 24 hr they were collected and homogenized in the minimum amount of distilled water. The homogenate was clarified by centrifugation, and an aliquot of the supernatant solution was used for the analysis of betacyanins by column chromatography on polyamide powder, according to a previously described procedure.[10] A single peak (amaranthin) was observed. Further confirmation of the total absence of isoamaranthin was obtained as follows: another aliquot of the supernatant solution was treated with β-glucuronidase and the reaction mixture was incubated at room temperature for 30 min. In high-voltage electrophoresis (5000 V; pH 4·5) the reaction mixture gave a single spot, identified as betanin.*

Estimation of Amaranthin

After irradiation, the seedlings were returned in darkness and generally harvested 24 hr after the beginning of the light treatment. The temperature during the incubation period was maintained at 26°.

Four replicates were used in all experiments and each experiment was repeated at least three times. The amaranthin content was determined at the conclusion of the dark period. Counted numbers (100 or 200) of seedlings were homogenized in distilled water (3 ml) with an Ultra Turrax blendor, the homogenate was centrifuged at 10,000 × *g* and the supernatant after acidification with acetic acid (0·3 ml) centrifuged at 10,000 × *g*.

The extraction procedure was carried out at 5°. The absorption of the clear solution was measured at 537 nm and the amount of amaranthin determined using a molar-extinction coefficient (ϵ) of $5{\cdot}66 \times 10^4$.

* Betanin and isobetanin are easily separated by high-voltage electrophoresis, whereas amaranthin and isoamaranthin have the same mobility.

[10] M. PIATTELLI and L. MINALE, *Phytochem.* 3, 547 (1964).