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EFFECT OF LIGHT AND EXOGENOUSLY APPLIED PRECURSORS ON AMARANTHIN SYNTHESIS IN *AMARANTHUS CAUDATUS*

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Key Word Index—*Amaranthus caudatus*; Amaranthaceae; love-lies-bleeding; betacyanins; phytochrome control; biosynthesis.

Abstract—Light stimulates the synthesis of amaranthin in *Amaranthus caudatus* var. *viridis*. Evidence suggests that this stimulation is markedly dependent on seedling age. Synthesis is controlled by both a "low-energy" red/far-red reversible phytochrome system and an HER at least partially under phytochrome control. In seedlings exposed to light, synthesis is promoted by exogenously applied DOPA and tyrosine. It is suggested that at least two light-promoted reactions occur in the biosynthetic pathway; one between tyrosine and DOPA and a second between DOPA and amaranthin.

INTRODUCTION

PREVIOUS studies on the biosynthesis of betacyanins in *Amaranthus* species have indicated that the system is under phytochrome control,[1,2] although *Amaranthus salicifolius* apparently provides an exception.[3] The participation of other photoreceptors, for instance the photosynthetic pigments, has been suggested in *A. tricolor*.[4] The effect of light on pigment synthesis varies considerably according to the species,[5] in some material irradiation stimulates the rate of synthesis; in others no synthesis occurs in the absence of light. It has been variously reported that the age of the seedlings at the onset of illumination affects the subsequent stimulation of pigment synthesis.[6,7]

L-Tyrosine and L-3,4-dihydroxyphenylalanine (DOPA) have been shown to be likely precursors of betacyanins,[8,9] although the fine details of the biosynthetic pathway remain obscure. The present work describes the effects of light, exogenously applied tyrosine and DOPA and suggests possible loci of light action in the biosynthetic pathway of amaranthin.

RESULTS AND DISCUSSION

The seed-coat of dry seeds of *Amaranthus caudatus* var. *viridis* was found to contain a significant amount of amaranthin ($6{\cdot}0 \times 10^{-11}$ mol/seed). Dark grown material, after 36 hr of germination, contained $7{\cdot}8 \times 10^{-11}$ mol/seedling. By contrast with the limited

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[4] GUIDICI DE NICOLA, M., PIATTELLI, M., CASTROGIOVANNI, V. and AMICO, V. (1972) *Phytochemistry* **11**, 1011.
[5] WOHLPORT, A. and MABRY, T. J. (1968) *Plant Physiol.* **43**, 457.
[6] GIMESI, N., GARAY, A., POZSAR, B. and FARKAS, G. (1951) *Agrokemia es Talajtan* **1**, 339.
[7] KOEHLER, K. (1965) *Naturwissenschaften* **51**, 561.
[8] GARAY, A. S. and TOWERS, G. H. N. (1966) *Can. J. Botany* **44**, 231.
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synthesis in darkness, seedlings which were grown for the same period but exposed to white light for 4 hr formed substantial amounts of amaranthin. Maximum sensitivity to light was evident when the exposure was given between 24 and 38 hr after sowing (Fig. 1) and the amaranthin content increased by more than 200% compared with the original content of the dry seed.

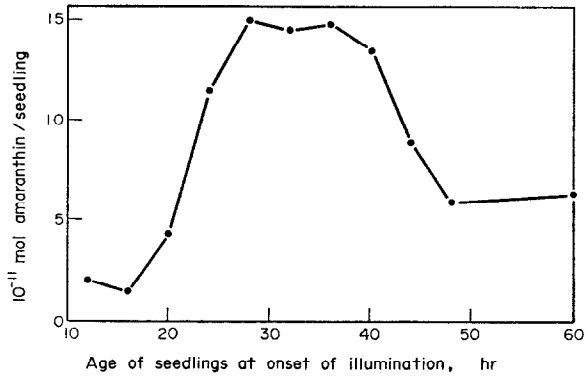

FIG. 1. EFFECT OF SEEDLING AGE ON LIGHT STIMULATED AMARANTHIN SYNTHESIS. Seedlings were germinated in the dark for the appropriate time, exposed to 4 hr white light and extracted after a further 32 hr in the dark. Values shown are light treated pigment contents minus dark controls.

Amaranthin synthesis under continuous illumination for a period of 48 hr was found to be light saturated at 250 lx. However, an intensity of only 10 lx over the same period of time stimulated synthesis to approximately 50% of the level obtained under light-saturating conditions. Thus the photoreceptor involved must be responsive to low levels of irradiation.

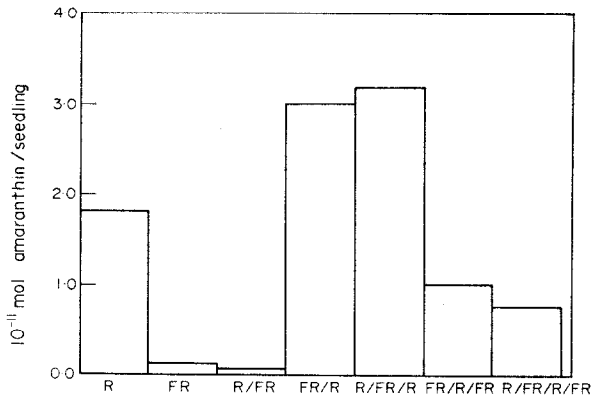

FIG. 2. EFFECT OF ALTERNATING SHORT PERIODS OF R OR FR ILLUMINATION ON AMARANTHIN SYNTHESIS. Seedlings were germinated in dark for 24 hr, exposed to ight treatment and extracted after a further 48 hr of darkness. Values shown are minus dark controls

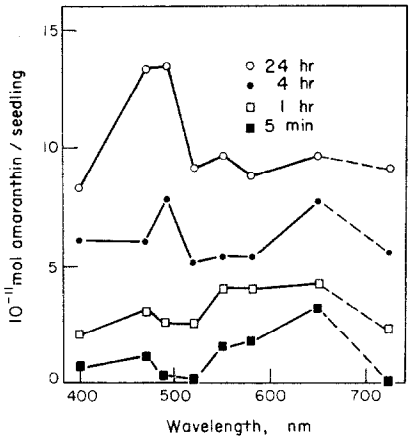

FIG. 3. EFFECT OF DIFFERENT WAVELENGTHS AND DURATION OF EXPOSURE ON AMARANTHIN SYNTHESIS. Seedlings were germinated in dark for 24 hr, exposed to light treatments and returned to darkness for a total (light plus dark) of 48 hr. Values shown are minus dark controls.

When alternating short (5 min) periods of red (R) and far-red (FR) irradiation were employed the system was found to be very light sensitive and a marked dependence on the nature of the final light exposure was evident (Fig. 2). A typical 'low-energy' phytochrome response is therefore involved. In experiments in which similar exposures to R and FR light were given immediately after a 4 hr exposure to white light, a similar dependance upon the nature of the final light exposure was observed although the pigment contents were higher than those reported in Fig. 2.

In the synthesis of anthocyanins, a 'high-energy' reaction (HER) has been proposed to account for pigment synthesis resulting from extended or continuous irradiation.[10] Amaranthin synthesis in the seedlings was promoted by increased duration of exposure to R ,FR and blue (B) wavebands (Fig. 3). The B and R effects are well shown with 4 hr irradiation; B and FR have more pronounced effects with a longer (24 hr) period of irradiation. The promotive effect of both R and FR were particularly clear when a 3 hr period of R irradiation was followed by a 3 hr FR treatment (Fig. 4). These data collectively indicate the involvement of an HER. A similar conclusion has been reached by Piattelli *et al.*[1] with *A. tricolor*. These workers have also suggested that photoreceptors other than phytochrome contribute to betalain synthesis.[4] The effect of wavebands not normally associated

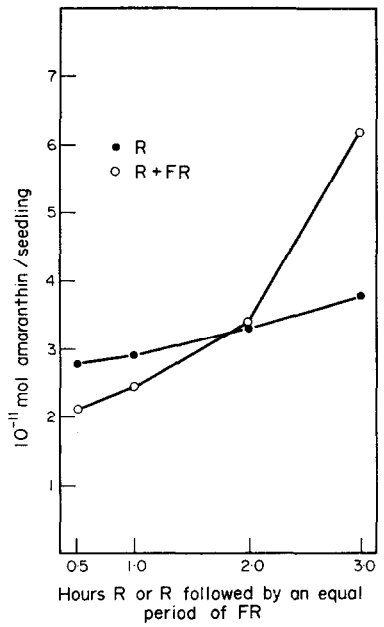

FIG. 4. EFFECT OF R, OR R FOLLOWED BY AN EQUAL PERIOD OF FR ON AMARANTHIN SYNTHESIS. Seedlings grown in dark for 24 hr, exposed to light treatments and extracted after a total of 48 hr (light plus dark). Values shown are minus dark controls.

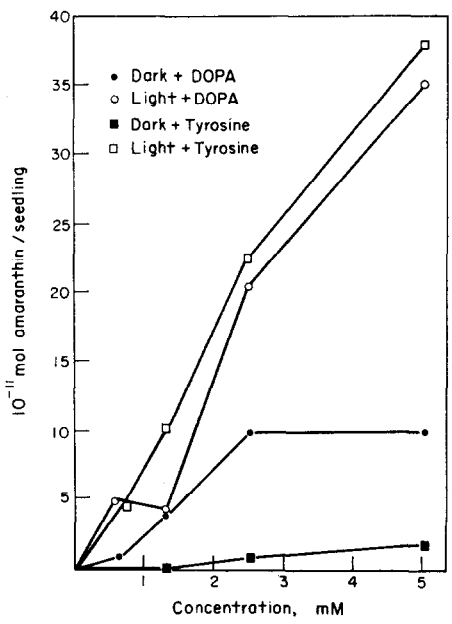

FIG. 5. EFFECT OF EXOGENOUS L-DOPA AND L-TYROSINE ON AMARANTHIN SYNTHESIS. Seedlings grown in dark for 24 hr, transferred to 2 ml of solution of DOPA or tyrosine and extracted for a further 48 hr growth in continuous light (open symbols) or continuous dark (closed symbols). Values shown are minus those of untreated controls.

[10] MOHR, H. (1966) *Photochem. Photobiol.* **5**, 469.

with phytochrome (e.g. 500–600 nm) becomes increasingly prominent, relative to the effects of B and R, with increasing duration of irradiation (Fig. 3). It may be concluded that amaranthin synthesis is controlled by; (a) a "low-energy' R/FR reversible system, and (b) an HER at least partially under phytochrome control. The addition of exogenous DOPA led to a considerable stimulation of amaranthin synthesis in illuminated seedlings and a smaller stimulation in dark grown material (Fig. 5). When tyrosine was applied instead of DOPA, a considerable stimulation of amaranthin synthesis was found in illuminated seedlings but there was only a small promotion of dark synthesis (Fig. 5). In order to determine whether the greater stimulation of amaranthin synthesis in illuminated material than in dark-grown seedlings might be a reflection of light-promoted uptake of these substrates, ^{14}C-labelled compounds were employed. No evidence was obtained which suggested that light promoted the uptake of these compounds during the first 24 hr after feeding. It therefore seems likely that the stimulatory effect of light on amaranthin biosynthesis in the presence of exogenously supplied DOPA or tyrosine is due to a specific promotion of synthesis rather than via an effect on uptake.

When labelled DOPA and tyrosine were supplied together at optimal concentrations, a small additive effect on amaranthin synthesis was observed compared with the effects of either compound on its own (Table 1). From this observation there does not appear to be substantial evidence to suggest that the two compounds are incorporated by different pathways. Assuming that tyrosine and DOPA are sequential on the biosynthetic pathway, the data suggest that the conversion of tyrosine to DOPA is light-stimulated. It is also evident that the incorporation of DOPA into amaranthin is promoted by light.

TABLE 1. EFFECT OF AMARANTHIN SYNTHESIS OF SUPPLYING EXOGENOUS TYROSINE AND DOPA IN COMBINATION

Treatment	10^{-11} mol amaranthin/seedling	Treatment	10^{-11} mol amaranthin/seedling
Dark	5·4	Light	42·0
Dark + Tyrosine	6·1	Light + Tyrosine	52·0
Dark + DOPA	12·3	Light + DOPA	70·0
Dark + DOPA + Tyr	17·5	Light + DOPA + Tyr	80·0

Seedlings were germinated in the dark for 24 hr, transferred to 2 ml of the solution of tyrosine or DOPA and extracted after a further 48 hr.

These results presented above indicate that there are likely to be at least two points on the biosynthetic pathway at which light promotes chemical conversions. This knowledge may enable subsequent studies to be focused on the mechanism of photocontrol: on the assumption that this is comparable to that operative in anthocyanin biosynthesis[11] it would be reasonable to seek evidence for photostimulation of enzyme activity.

It is apparent that the control mechanism in *A. caudatus* var. *viridis* involves phytochrome but it shows no dependence on applied kinetin as reported by Koehler.[12] It seems possible that small changes in pigment content in the latter work were overlooked. Since the photosensitivity reported in the present material fell off markedly towards the end of the second day of germination it is worth noting that the observations on *A. salicifolius*, which revealed

[11] SMITH, H. and ATTRIDGE, T. H. (1970) *Phytochemistry* **9**, 487.
[12] KOEHLER, K. (1972) *Phytochemistry* **11**, 127.

little effect of R light on betacyanin synthesis, were made on 4-day-old dark grown seedlings. It would be interesting to know how the latter material behaved when irradiated at an earlier stage of development.

From the evidence on the influence of the waveband 500–600 nm (Fig. 3) the possibility that a photoreceptor(s) other than phytochrome may be involved in the HER cannot be ignored. It has been suggested in respect of betalain synthesis[13] and anthocyanin synthesis[14,15] that a component of the HER may involve the photosynthetic pigments. It seems likely that some indirect photosynthetic contribution to betacyanin synthesis, possibly via the provision of substrates or energy-rich compounds, could be involved in *A. caudatus* var. *viridis*.

EXPERIMENTAL

Plant material. Amaranthus caudatus var. *viridis* seeds were supplied by Thompson & Morgan Ltd., Ipswich. Seeds were sown in 9 cm Petri dishes on filter paper (404) supplied by Greens Ltd., Maidstone, moistened with 4 ml H_2O. Germination was in the dark at 27 ± 1°.

Light conditions. (Figs. 1, 2, 4 and 5). Seedlings exposed to white light treatments were illuminated by a bank of 80 W Atlas Super 5 Daylight fluorescent tubes which gave a light intensity of 350 lx at seedling level. R light was provided by 4 × 13 W Warmwhite fluorescent tubes in combination with Cinemoid filters which comprised, (a) 2 × layers of red filter No. 14, (b) 1 × layer of yellow No. 1. A uniform transmission spectrum was observed from 650 nm to FR. In order to remove the FR element from the light a $CuCl_2$ screen was used (2%; 1 cm deep). Energy produced at seedling level was equal to 0·10 mW/cm². FR light was provided by a bank of 22 Chryselco 60 W tungsten tubes in combination with Cinemoid filters, (a) 2 × layers deep orange No. 5A, and (b) 2 × layers of blue No. 20. This combination gave a transmission from 710 nm to FR with no definite maximum. Energy output at seedling level was equal to 0·42 mW/cm².

Light source (Fig. 3). Ilford gelatin filters were used as follows: filter number 600, 380–450 nm transmission, λ_{max} 400 nm; No. 602, 440–490, 470; No. 603, 470–520, 490; No. 604, 500–540, 520; No. 605, 530–570, 550; No. 606, 560–610, 580; No. 608, 620–FR. The energies at seedling level were measured with a thermopile and galvanometer (Kipp and Zonen) and were equal to 1·6 × mW/cm². Incident light on the filters was provided by a bank of 30 W double life Warmwhite fluorescent tubes in a Fisons Weyco refrigerated climate. cabinet. The FR source was as previously described.

Dark manipulation of material. Illumination was provided by a 15 W Chryselco tungsten bulb in conjunction with an Ilford orange safe light No. 902S and 3 × layers of green Cinemoid No. 39.

Extraction and assay of amaranthin. A method similar to that used by Piattelli *et al.*[1] was employed to extract and assay amaranthin. 100 seedlings were homogenized in 3 ml H_2O, the homogenate was centrifuged at 10 000 *g* and the supernatant after acidification with HoAc (0·3 ml) centrifuged at 10 000 *g*. The extractor procedure was carried out at 0–5°. The absorption of the solution after filtering with 4 × layers of Whatman GF/A to remove a slight precipitate was measured at 537 nm and the amount of amaranthin determined using the molar extinction coefficient (ϵ) of 5·66 × 10^4. Two replicates were used for every treatment and all experiments were repeated at least once. Variability was less than 10% between all samples.

Transfer of material into solutions of DOPA or tyrosine. In these experiments the filter disc was transferred with the seedlings into a 2 ml solution of DOPA or tyrosine. This resulted in a dilution of *ca.* 50%. The values on the figures refer to the concentration of DOPA or tyrosine before transfer.

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[14] Schneider, M. J. and Stimson, W. R. (1971) *Plant Physiol.* **48**, 312.
[15] Schneider, M. J. and Stimson, W. R. (1972) *Proc. Nat. Acad. Sci. U.S.* **69**, 2150.