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PHOTOCONTROL OF BETAXANTHIN SYNTHESIS IN *CELOSIA PLUMOSA* SEEDLINGS*

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Key Word Index—*Celosia plumosa*; *Amaranthus tricolor*; Amaranthaceae; betalains; betaxanthin synthesis; light; kinetin; phytochrome.

Abstract—Light stimulates the betaxanthin accumulation in *Celosia plumosa*. The induction is partially reversed by far-red and inhibited by actinomycin D, puromycin, salicylaldoxime and 2,4-dinitrophenol, while 3-(3,4-dichlorophenyl)-1,1-dimethylurea has an inhibitory effect only when photosynthesis is operative. In darkness betaxanthins synthesis is promoted by kinetin.

INTRODUCTION

RESEARCH on the light-induced production of betalains, water-soluble pigments of Centrospermae, has been focused until now on the red-violet betacyanins,[1-11] glucosides of betanidin and its diastereoisomer isobetanidin, while the effect of illumination on the closely related yellow betaxanthins has not been so far investigated.

The present study with seedlings of a betaxanthin-producing cultivar ('Golden feather') of *Celosia plumosa* (Amaranthaceae) was designed to determine whether the light induced synthesis of these pigments can be interpreted in terms of the mechanism previously proposed for the betacyanin synthesis in *Amaranthus tricolor*.[11]

RESULTS

In darkness the rate of accumulation of betaxanthin in seedlings of *C. plumosa* is slow. When 2-day-old dark-grown seedlings were irradiated (4 hr) with white light and then returned to darkness, after a lag period of approx. 4 hr the amount of pigment began to rise rapidly, attained a maximum at 24 hr and subsequently slowed down (Fig. 1). The effect of light was partially reversed by far-red (Table 1), suggesting the involvement of phytochrome. Moreover, the life-time of the photoreceptor in the active form appears to be about 8 hr, since the terminal far-red treatment had no effect if delayed by 9 hr or more from the end of the illumination.

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TABLE 1. EFFECT OF FAR-RED ON BETAXANTHIN SYNTHESIS INDUCED BY LIGHT*

Treatment	Absorbance at 485 nm
Control (24 hr darkness)	0·087
4 hr white light	0·187
plus 5 min far-red at the moment of darkening	0·157
plus 5 min far-red at the 6th hr after darkening	0·158
plus 5 min far-red at the 9th hr after darkening	0·186

* Pigment was determined 24 hr after the beginning of the light treatment.

TABLE 2. EFFECT OF ACTINOMYCIN D AND PUROMYCIN ON BETAXANTHIN SYNTHESIS STIMULATED BY A SHORT IRRADIATION TREATMENT

Treatment	Absorbance at 485 nm
Control (24 hr darkness)	0·087
plus actinomycin D (10 μg/ml)	0·086
plus puromycin (100 μg/ml)	0·085
6 hr light then 18 hr darkness	0·292
Actinomycin D added on illumination	0·174
Puromycin added on illumination	0·169

Actinomycin D or puromycin, administered at the onset of illumination, partly inhibited the light-stimulated synthesis (Table 2); it seems probable therefore that the light induction process is dependent on both *m*RNA and protein synthesis. When seedlings were briefly exposed to far-red (5 min) after 12 hr illumination with white light, the rate of betaxanthin accumulation in the subsequent dark period was significantly depressed by the addition of puromycin at the moment of darkening (Fig. 2), and this is indicative of the persistence of protein synthesis during the dark period following irradiation.

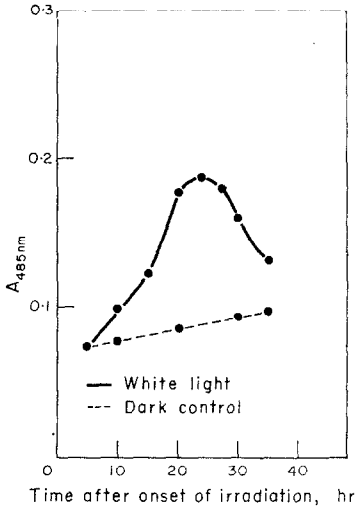

FIG. 1. VARIATION OF THE AMOUNT OF BETAXANTHIN IN RESPONSE TO VARIOUS PERIODS OF DARKNESS AFTER 4 hr EXPOSURE TO WHITE LIGHT

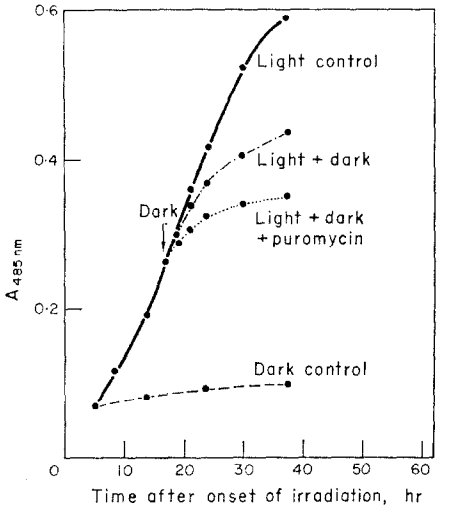

FIG. 2. EFFECT OF DARKENING AND DARKENING PLUS PUROMYCIN ON THE TIME COURSE OF BETAXANTHIN ACCUMULATION IN CONTINUOUS WHITE LIGHT.

The pigment accumulation stimulated by 6 hr illumination was depressed by the application of salicylaldoxime (SAL) or puromycin at the moment of darkening (Table 3) and this implicates dark processes related to oxidative phosphorylation and enzymatic

protein synthesis. When applied at the onset of irradiation SAL had a stronger inhibitory effect, similar to that of 2,4-dinitrophenol (DNP), whereas 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) failed to inhibit the pigment production, in accordance with its specific action on phosphorylation depending on photosynthesis.

TABLE 3. EFFECTS OF SAL, DNP, DCMU AND PUROMYCIN ON LIGHT-INDUCED BETAXANTHIN SYNTHESIS

Treatment	Absorbance at 485 nm
Control (24 hr darkness)	0·087
6 hr light then 18 hr darkness	0·292
10^{-3} M SAL added on illumination	0·151
10^{-3} M SAL added on darkening	0·230
Puromycin (100 μg/ml) added on darkening	0·210
10^{-4} M DNP added on illumination	0·147
10^{-5} M DCMU added on illumination	0·291
10^{-5} M DCMU added on darkening	0·290

TABLE 4. EFFECTS OF SAL, DNP AND DCMU ON BETAXANTHIN SYNTHESIS UNDER CONTINUOUS ILLUMINATION

Treatment	Absorbance at 485 nm
Control (24 hr darkness)	0·087
24 hr white light	0·428
10^{-3} M SAL applied on illumination	0·264
10^{-4} M DNP applied on illumination	0·213
10^{-5} M DCMU applied on illumination	0·326
10^{-3} M SAL plus 10^{-5} M DCMU applied on illumination	0·162
10^{-5} M DCMU applied at the 7th hr	0·324

Under continuous white light the betaxanthin synthesis was depressed by the application of SAL, DNP or DCMU at the onset of illumination, and the inhibition was enhanced when SAL and DCMU were given in combination. The effect of DCMU was unchanged when the inhibitor was administered 6 hr after the onset of light, that is, at the beginning of the photosynthetic activity (Table 4). These results show that betaxanthin synthesis induced by continuous illumination is dependent not only on oxidative phosphorylation but also on cyclic and non-cyclic photophosphorylation.

TABLE 5. EFFECT OF FAR-RED AND WHITE LIGHT ON BETAXANTHIN PRODUCTION INDUCED BY KINETIN*

Treatment	Absorbance at 485 nm
Control (24 hr darkness)	0·087
15 min white light	0·115
24 hr kinetin	0·146
plus 5 min far-red	0·145
plus 15 min white light	0·171
plus 15 min white light followed by 5 min far-red	0·152

* The light treatment was given at the start of kinetin application.

Seedlings treated in darkness with kinetin synthesized more betaxanthin when compared to dark controls (Table 5); the enhancement in the pigment accumulation seems not to be related to the activation of phytochrome, since far-red irradiation lacked any effect. A short irradiation with white light, sufficient for phytochrome activation, promoted a further pigment increase in the kinetin-treated seedlings; because of the poor induction by light, far-red was able to produce a low but definite reversion.

DISCUSSION

Previous research[10,11] has shown that light acts on betacyanin (amaranthin) biosynthesis in *A. tricolor* through the control of the availability of energy-rich compounds; phytochrome and photosynthetic system are involved in this regulation in conditions of short- and long-term irradiation respectively, while gene activation seems to be mediated by other photoreceptor(s) than phytochrome. In complete darkness amaranthin synthesis is stimulated by kinetin, whose action appears not to be related to the status of phytochrome.

From the results described in the present paper it is apparent that there are marked qualitative similarities between the light responses of betaxanthin and betacyanin synthesis. Indeed, betaxanthin formation is controlled by phytochrome, probably through its action on oxidative energy metabolism, as can be deduced from the effects of inhibitors of the electron transport on the pigment formation. SAL and puromycin inhibit betaxanthin synthesis also when applied at the beginning of the dark period following irradiation, and this is indicative of dark processes depending on oxidative phosphorylation and enzymatic protein synthesis. Moreover, as in the case of betacyanin formation in *A. tricolor*,[11] under continuous illumination with white light, not only is oxidative phosphorylation involved, but also cyclic and non-cyclic photophosphorylation, as shown by the inhibitory action of DNP and DCMU. Therefore, the photosynthetic apparatus is involved in the high energy reaction (HER) associated with betaxanthin synthesis in *C. plumosa*.

TABLE 6. INDUCTION BY LIGHT AND KINETIN OF THE PIGMENT SYNTHESIS IN *Celosia Plumosa* AND *Amaranthus Tricolor*

Treatment	Amaranthin/seedling (10^{-2} nmol) in *Amaranthus tricolor** Total	Increase over dark control	Betaxanthin/seedling (10^{-2} nmol) in *Celosia plumosa*† Total	Increase over dark control
Darkness (24 hr)	6·8		19·8	
15 min white light	12·1	5·3	26·2	6·4
6 hr white light	54·4	47·6	66·4	46·6
24 hr white light	93·5	86·7	97·4	77·6
Kinetin in darkness	68·7	61·9	33·2	13·4
Kinetin plus 15 min white light	104·6	97·8	38·9	18·7

* Amaranthin values are from M. GIUDICI DE NICOLA *et al.* (see Ref. 10).

† Calculated on the assumption that ϵ of the unidentified betaxanthin(s) is 43 800, that is the same as that of indicaxanthin.[12]

In opposition to these similarities between betaxanthin and betacyanin synthesis, it must be remarked that actinomycin D and puromycin, which completely block the light-induced formation of amaranthin in *A. tricolor*,[4] only partially inhibit betaxanthin accumulation in *C. plumosa* seedlings. This difference is presumably to be ascribed to a different activation level of the gene system involved in the pigment synthesis in the two species. The gene system seems to be completely inactive in dark-grown seedlings of *A. tricolor* and largely active in *C. plumosa*, and lack of complete inhibition in the latter species by the two antibiotics points to the presence in dark-grown seedlings of the enzymes controlling the biosynthesis of betaxanthin. In agreement with this interpretation, *C. plumosa* synthesizes more pigment than *A. tricolor* in darkness.

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The fact that in darkness kinetin stimulates betaxanthin synthesis much less in *C. plumosa* than amaranthin synthesis in *A. tricolor*, is probably also to be ascribed to the different status of the genes in the two species.

From Table 6, which comparatively summarizes the effect of light and kinetin in *C. plumosa* and *A. tricolor*, it is apparent that the main quantitative differences between the two species chiefly concern the dark production of the pigments and the action of kinetin.

The light responses of betaxanthin synthesis in *C. plumosa* seedlings are on the whole similar to those previously reported for betacyanin formation in *A. tricolor*, notwithstanding minor differences attributable to a different condition of the genes in the two species. As both types of pigments possess an identical dihydropyridine moiety biogenetically derived from DOPA, the resemblances in the light reactions suggest that the control of their synthesis takes place at the level of the formation of a common intermediate.

EXPERIMENTAL

Plant material. Seedlings of *C. plumosa* were germinated in darkness at 28° in Petri dishes on two layers of filter paper moistened with water and used when 2-days-old. A cool-white fluorescent source giving 5000 lx at seedling level was used. The far-red source,[3] and the administration of inhibitors[11] and kinetin[13] has been described previously.

Determination of betaxanthin. At the end of each treatment 100 seedlings were homogenized in acetate buffer pH 4·5 (3 ml) and the homogenate centrifuged at 10 000 *g*. The clear supernatant was diluted to a standard vol. and the absorbance was then measured at 485 nm. The extraction procedure was carried out at 5°. Six replicates were used in all experiments and each experiment was repeated at least 6×. The experimental error was normally 4% or less in any given experiment.

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