Phytochemistry, 1974, Vol. 13, pp. 439 to 442. Pergamon Press. Printed in England.

EFFECT OF WHITE AND FAR-RED LIGHT ON BETALAIN FORMATION*

MARINA GIUDICI DE NICOLA, VINCENZO AMICO and MARIO PIATTELLI

Instituto di Chimica Organica dell'Università di Catania, Catania, Italia

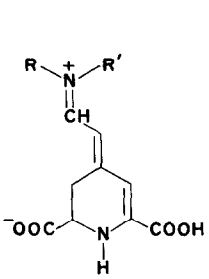
(*Received* 21 *August* 1973. *Accepted* 10 *September* 1973)

Key Word Index—*Amaranthus*; *Celosia*; Amaranthaceae; betalain biosynthesis; light; phytochrome.

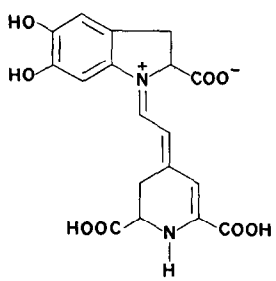
Abstract—Light-induced betalain synthesis has been studied in three species of Amaranthaceae. The results obtained suggest that the response to short-term irradiation with white light is mediated by phytochrome, while the effects of prolonged illumination are controlled by the photosynthetic system. Betalain accumulation stimulated by prolonged far-red irradiation is controlled chiefly by the photosynthetic system, the participation of phytochrome being of minor importance.

INTRODUCTION

BETALAINS, the water-soluble pigments whose occurrence is restricted to plants belonging to the order Centrospermae, possess the structure (**1**). The red–violet betalains (betacyanins) include the aglycone betanidin (**2**) and its glycosides, while the closely related betaxanthins have an amine or amino acid other than cycloDOPA attached to the dihydropyridine system.



(**1**)



(**2**)

Previous work from our laboratory has established that betacyanin synthesis in *Amatanthus tricolor* and betaxanthin synthesis in *Celosia plumosa* cv. Golden Feather are both stimulated by white (W) light.[1,2] The early responses are due to phytochrome activation while the effects of continuous irradiation are mediated mainly by the photosynthetic system. In contrast, the 2 species respond differently to prolonged far-red (FR) irradiation;[3] in *C. plumosa* betaxanthin synthesis is stimulated with the involvement of the photosynthetic system, while in *A. tricolor* formation of betacyanin does not take place at all, although chlorophyll is formed. The different behaviour of the 2 species has been ascribed to the

* This work was supported by the Consiglio Nazionale delle Richerche.

[1] GIUDICI DE NICOLA, M., PIATTELLI, M., CASTROGIOVANNI, V. and AMICO, V. (1972) *Phytochemistry* **11**, 1011.
[2] GIUDICI DE NICOLA, M., PIATTELLI, M. and AMICO, V. (1973) *Phytochemistry* **12**, 353.
[3] GIUDICI DE NICOLA, M., PIATTELLI, M. and AMICO, V. (1973) *Phytochemistry* **12**, 2163.

fact that the gene system involved in the pigment formation is activated in etiolated seedlings of *C. plumosa* while it is completely inactive in dark-grown seedlings of *A. tricolor* and requires wavelengths other than 720 nm for its photoactivation.

The object of this investigation was to determine the effects of W and FR in seedlings of other species or varieties of Amaranthaceae which in darkness produce a substantial amount of betalain and thus presumably possess an active gene system.

RESULTS

The response to light of betalain synthesis was investigated in seedlings of species or varieties of Amaranthaceae which produce either betacyanin (*A. caudatus*) or betaxanthin (*C. cristata* cv. Jewel), or a mixture of both of the pigments (*C. plumosa* cv. Fire Feather and *C. cristata* cv. Toreador).

TABLE 1. EFFECT OF VARIOUS LIGHT TREATMENT ON BETALAIN SYNTHESIS

Treatment	*A. caudatus*	*C. cristata* cv. Jewel	*C. cristata* cv. Toreador		*C. plumosa* cv. Fire Feather	
	Betacyanin*	Betaxanthin†	Betacyanin*	Betaxanthin†	Betacyanin*	Betaxanthin†
4 hr light then 18 hr dark	0·143	0·031	0·230	0·070	0·054	0·040
4 hr W light plus 5 min FR then 18 hr dark	0·111	0·022	0·161	0·050	0·038	0·028
24 hr W light then 24 hr dark	0·297	0·130	0·626	0·200	0·185	0·150
24 hr W light plus 5 min FR then 24 hr dark	0·288	0·131	0·626	0·202	0·182	0·149
24 hr W light	0·300	0·131	0·790	0·240	0·187	0·156
10^{-3} M levulinic acid applied on illumination	0·040	0·055	0·197	0·060	0·051	0·075
10^{-4} M DNP applied on illumination	0·180	0·068	0·434	0·156	0·105	0·092
10^{-5} M DCMU applied on illumination	0·198	0·070	0·553	0·161	0·133	0·107
24 hr FR	0·121	0·071	0·337	0·180	0·122	0·077
10^{-3} M levulinic acid applied on illumination	0·011	0·015	0·034	0·050	0·033	0·017
10^{-4} M DNP applied on illumination	0·051	0·023	0·135	0·060	0·056	0·032
10^{-5} M DCMU applied on illumination	0·122	0·072	0·338	0·184	0·125	0·078
6 hr R then 18 hr FR	0·095	0·055	0·253	0·126	0·100	0·056
24 hr FR then 24 hr dark	0·080	0·043	0·286	0·110	0·073	0·043
24 hr FR plus 15 min R then 24 hr dark	0·120	0·054	0·324	0·124	0·101	0·064
24 hr FR plus 15 min R plus 5 min FR light then 24 hr dark	0·108	0·048	0·314	0·119	0·087	0·058

* Absorbance at 538 nm (increase over dark control). † Absorbance at 485 nm (increase over dark control).

Table 1 summarizes the effects of various light treatments on the pigment accumulation in the four plants used. It is clear that whilst pigment synthesis induced by 4 hr irradiation with W light is reversed by FR as a typical low-energy phytochrome-controlled response, a 24-hr irradiation elicits a larger pigment production which is not affected by terminal phytochrome manipulation. The high-energy response was reduced by the application of levulinic acid, an inhibitor of chlorophyll synthesis, and by 2,4-dinitrophenol (DNP) or 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), inhibitors of cyclic and non-cyclic photophosphorylation respectively.

Also continuous FR stimulated pigment synthesis. The enhancing effect of 24 hr FR was depressed by the administration of levulinic acid or DNP, while it was unaffected by DCMU. Moreover, when a 6-hr period in red light (R) preceded a treatment of 18 hr in FR, betalain yield was less than in 24 hr FR. On the other hand, a single 15 min exposure to R at the end of the prolonged FR treatment gave an enhancement of pigment formation.

Continuous irradiation with either white or FR light led to chlorophyll-*a* formation in the seedlings of all the species examined (Table 2).

TABLE 2. EFFECT OF CONTINUOUS W OR FR LIGHT ON CHLOROPHYLL-*a*-SYNTHESIS*

Material	Chlorophyll-*a* (μg/seedling) 24 hr W light	24 hr FR
Amaranthus caudatus	0·34	0·09
Celosia cristata cv. Jewel	0·32	0·12
Celosia cristata cv. Toreador	0·74	0·22
Celosia plumosa cv. Fire Feather	0·31	0·10

* Seedlings grown in darkness were devoid of any green colour.

DISCUSSION

In seedlings of Amaranthaceae, which in darkness produce substantial amounts of betalain, the rate of synthesis is increased by short-term irradiation with W light. This light-induced increase depends on phytochrome, as shown by R/FR reversibility. Under continuous illumination with W light the photosynthetic system seems to be the most important factor controlling pigment synthesis; the inhibition of chlorophyll formation following administration of levulinic acid is paralleled by a strong depression of pigment accumulation. A reduction of the light-stimulated pigment formation is also observed in the presence of DNP or DCMU and this is evidence for the contribution of both cyclic and non-cyclic photophosphorylation in the biosynthesis of betalains.

Continuous FR promotes betacyanin and/or betaxanthin synthesis in all the species investigated. The FR-induced pigment synthesis is strongly reduced by DNP and levulinic acid, while it is unaffected by DCMU. Therefore it seems likely that the photoreceptor for betalain synthesis under continuous FR is the photosynthetic system acting through cyclic photophosphorylation only. This could also explain the lower efficiency of FR compared with white light. However, the fact that a residual pigment synthesis also takes place when chlorophyll formation is completely suppressed by levulinic acid is evidence that under continuous FR the photosynthetic system is not the sole active photoreceptor.

Since a terminal short exposure to R has a stimulating effect which is reversed by FR it can be reasonably assumed that phytochrome is also involved in the control of the FR induced pigment synthesis. In accordance with this view, a preirradiation with R reduces the effectiveness of subsequent prolonged FR; a similar inhibitory effect was previously observed by Grill and Vince[4] in the case of anthocyanin synthesis in turnip and was ascribed to destruction of phytochrome following conversion to the labile P_{fr} form. The fact that the pretreatment with R is much less effective in depressing betalain than anthocyanin synthesis in continuous FR suggests that phytochrome is involved to a rather different extent in the control of the formation of the two types of pigment.

Our data show that the role of light in both betacyanin and betaxanthin synthesis is essentially similar and provides further evidence that the previously observed difference between *C. plumosa* and *A. tricolor* in response to FR cannot be ascribed to the different nature of the pigments elaborated by the two species. In fact, FR has a stimulating effect both in betaxanthin- and betacyanin-producing plants, which accumulate substantial amounts of pigment in darkness, but FR fails to promote synthesis in *A. tricolor*, the only species

[4] GRILL, R. and VINCE, D. (1965) *Planta* **67**, 122.

among those so far examined which produces a negligible amount of pigment in the dark. This observation favours the previous suggestion that lack of pigment synthesis in *A. tricolor* under continuous FR is caused by an inactive gene system in the etoliated seedlings of this species.

EXPERIMENTAL

Seeds were germinated in darkness and used when 2-days-old. Light sources and administration of inhibitors have been described previously.[1-3] The quantative determination of chlorophyll-*a* was carried out according to the method of Smith and Benitez.[5] Betacyanin and/or betaxanthin were extracted at the the end of each treatment in acetate buffer pH 4·5.[2] The extracts were clarified by centrifuging and the absorbance measured at 485 nm (betaxanthin) and 538 nm (betacyanin). When both pigments were present in the same sample, the absorbance at 485 nm was corrected allowing for absorption of betacyanin at this wavelength, while the absorbance at 538 nm did not require any correction since at this wavelength the absorption of betaxanthin is insignificant. Absorbancies of the dark controls were: 0·55 (538 nm) for *A. caudatus*, 0·50 (485 nm) for *C. cristata* cv. Jewel, 1·64 (538 nm) and 0·92 (485 nm) for *C. cristata* cv. Toreador, 0·96 (538 nm) and 0·45 (485 nm) for *C. plumosa* cv. Fire Feather. 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.

[5] SMITH, J. H. C. and BENITEZ, A. (1955) *Modern Methods of Plant Analysis* (PAECH, K. and TRACEY, M. V., eds.), Vol. IV, p. 142. Springer, Berlin.