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EFFECT OF CONTINUOUS FAR RED ON BETAXANTHIN AND BETACYANIN SYNTHESIS*

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Key Word Index—*Celosia plumosa*; *Amaranthus tricolor*; Amaranthaceae; betalain synthesis; phytochrome.

Abstract—Seedlings of *Celosia plumosa* under prolonged irradiation with far red light synthesize chlorophyll *a* and betaxanthin. Levulinic acid and 2,4-dinitrophenol, inhibitors of chlorophyll synthesis and cyclic photophosphorylation respectively, reduce betaxanthin synthesis. Pigment formation is also inhibited by actinomycin-*D* and puromycin, but is unaffected by 3-(3,4-dichlorophenyl)-1,1-dimethylurea, an inhibitor of noncyclic photophosphorylation. These findings are evidence of the involvement of photosynthesis, through cyclic photophosphorylation, in the far red HER associated with betaxanthin synthesis. Under continuous far red seedlings of *Amaranthus tricolor* synthesize only chlorophyll *a*. Lack of betacyanin formation is ascribed to the inactive status of the genes involved in the pigment synthesis.

INTRODUCTION

Many morphogenetic responses in plants are controlled by two photoreactions; one is the red/far red (R/FR) reversible reaction of phytochrome and the other is dependent on prolonged exposure to light of higher intensities ('high energy reaction', HER). Models have been proposed to interpret FR HER responses in terms of phytochrome as the sole photoreceptor,[1-3] but the possible contribution of photosynthesis to the red or white light HER responses was suggested for hypocotyl[4] and stem elongation,[5] for anthocyanin synthesis[6,7] and for betalain (betacyanin[8] and betaxanthin[9]) synthesis. Moreover, the involvement of photosynthesis in the FR HER associated with anthocyanin synthesis in turnip seedlings has been reported.[10]

The present study was undertaken to determine the role of photosynthesis in the FR HER responses of betaxanthin and betacyanin synthesis in seedlings of *Celosia plumosa* (cv. 'Golden feather') and *Amaranthus tricolor*, respectively.

RESULTS

In dark-grown seedlings of *C. plumosa* continuous FR promotes synthesis of chlorophyll *a* and betaxanthin, but the rate of accumulation is slower than under white light. Seedlings

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[10] Schneider, M. J. and Stimson, W. R. (1971) *Plant Physiol.* **48**, 312.

of *A. tricolor* in the same conditions of irradiation efficiently synthesise chlorophyll *a* while only a minute amount of betacyanin (amaranthin) is concurrently formed (Fig. 1).

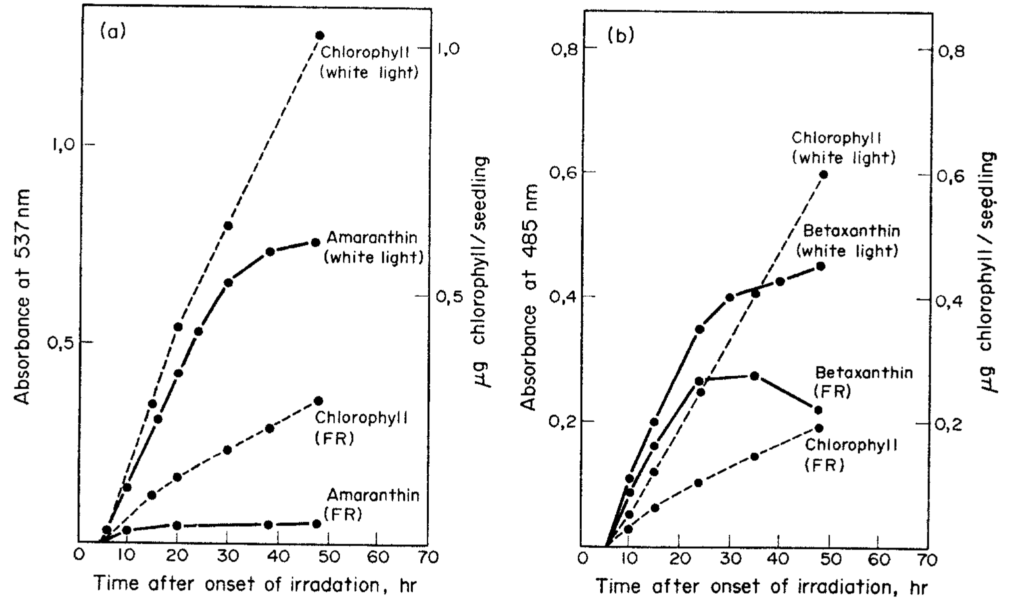

Fig. 1. Time course of the accumulation under white or far red light of (a) chlorophyll and betacyanin in *Amaranthus tricolor* and (b) chlorophyll and betaxanthin in *Celosia plumosa*.

An augmentation of the intensity of FR light (from 1·9 to 9·0 mW/cm^2) produces an increase in the accumulation of chlorophyll *a* without greatly affecting either betaxanthin or betacyanin synthesis (Table 1).

Table 1. Effect of different intensities of continuous far red on the synthesis of chlorophyll, betaxanthin and betacyanin in *Celosia plumosa* and *Amaranthus tricolor*

	Celosia plumosa		*Amaranthus tricolor*	
Intensity of far red*	Chlorophyll *a* (μg/seedling)	Absorbance at 485 nm (increase over dark control)	Chlorophyll *a* (μg/seedling)	Absorbance at 537 nm (increase over dark control)
1·9 mW/cm^2	0·034	0·100	0·069	0·025
9·0 mW/cm^2	0·057	0·114	0·146	0·029

* In each case 10 hr of FR was followed by 14 hr dark.

Betaxanthin formation in *C. plumosa* is strongly reduced by levulinic acid, an inhibitor of chlorophyll synthesis,[11] and by actinomycin or puromycin. Also 2,4-dinitrophenol (DNP), an inhibitor of cyclic photophosphorylation, depresses the FR induced betaxanthin synthesis, while 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of non-cyclic photophosphorylation, is completely ineffective (Table 2).

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[11] Beale, S. I. (1970) *Plant Physiol.* **45**, 504.

Betaxanthin synthesis is slightly reduced when the first 6 hr of prolonged irradiation with FR are replaced with R. By contrast, a brief exposure (15 min) to R at the end of the FR treatment produces an extra pigment (over the FR irradiated control) which is in part reversed by FR (Table 3).

TABLE 2. EFFECT OF DNP, DCMU, LEVULINIC ACID, ACTINOMYCIN *D* AND PUROMYCIN ON BETAXANTHIN SYNTHESIS IN *Celosia plumosa* INDUCED BY CONTINUOUS FAR RED

Treatment (24 hr far red)	Betaxanthin (absorbance at 485 nm) increase over dark control	Treatment (24 hr far red)	Betaxanthin (absorbance at 485 nm) increase over dark control
No addition	0·220	Levulinic acid (10^{-3} M)	0·084
DNP (10^{-4} M)	0·114	Actinomycin-*D* (10 μg/ml)	0·088
DCMU (10^{-5} M)	0·221	Puromycin-*D* (10 μg/ml)	0·082

DISCUSSION

Synthesis of chlorophyll *a* is stimulated in both species investigated under continuous FR irradiation, while betalain formation is significantly promoted only in *C. plumosa*. Since in dark-grown seedlings of this species the genes involved in pigment formation seem to be largely 'open', while in *A. tricolor* they are probably inactive, this result suggests that gene activation is not the primary action of phytochrome. Furthermore, the different behaviour of *A. tricolor* in white and FR light suggests that photoactivation of the genes involved in betacyanin formation requires wavelengths other than 720 nm.

The observation that in *C. plumosa* betaxanthin synthesis stimulated by continuous FR is strongly affected by levulinic acid, an inhibitor of chlorophyll synthesis, points to the involvement of photosynthesis in the FR HER response. Moreover, the fact that DNP reduces betaxanthin synthesis (however to a lesser extent than levulinic acid, in accordance with its partial inhibitory effect, at 10^{-4} M, on cyclic photophosphorylation,[12]) while DCMU lacks any effect is evidence that photosynthesis contributes essentially via the cyclic pathway. Also the reduction of pigment formation observed in the presence of actinomycin-*D* or puromicin can be ascribed to the inhibition of chlorophyll synthesis[13] since these antibiotics suppress chlorophyll formation and reduce pigment synthesis to the same extent than levulinic acid.

Experiments performed with different intensities of FR irradiation show that an increase in chlorophyll formation is not paralleled by an enhancement of betaxanthin synthesis. From this it can be deduced that the larger betaxanthin accumulation observed under white light depends on a greater activity of the photosynthetic system rather than on the increase in chlorophyll formation. In contrast, in condition of HER, FR is more effective than white light on anthocyanin synthesis[13] and this can be explained assuming that in this case activation of phytochrome is the determining factor while photosynthesis plays only a minor role. In fact, a pre-irradiation with R, causing the decay of a large amount of phytochrome, results in a strong inhibitory effect on the FR stimulated anthocyanin synthesis[14] while it is much less efficient in reducing betaxanthin synthesis.

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We interpret the results above to indicate the photosynthetic system to be the main photoreceptor not only for the previously reported[9] white light HER associated with betaxanthin synthesis in *C. plumosa* seedlings, but also for the FR HER associated with the same response. The differences in the time course of pigment accumulation in the two conditions of irradiation are probably due to the fact that in analogy to what has been observed in bean leaves,[15] under FR light the capacity for photosynthesis develops more slowly than in white light, and respiration is depressed after 24 hr irradiation with FR. The decrease in betaxanthin content after 24 hr of FR irradiation is possibly due to the prevalence of catabolic over less active synthetic processes. The marked decrease in pigment which is observed in 24 hr FR irradiated seedlings after a 24 hr period of darkness (Table 3) seems to indicate that betaxanthin is in a dynamic state and does not merely represent a metabolic end-product. Furthermore, we believe that the promotion of betaxanthin synthesis which is observed when a 24 hr FR irradiation is terminated with 15 min R cannot be assumed as evidence of a direct control of the processes by phytochrome. This effect may well be related to a non-specific action of this photoreceptor on the availability of energy-rich compounds.[8]

TABLE 3. EFFECT OF VARIOUS LIGHT TREATMENTS ON BETAXANTHIN SYNTHESIS IN *Celosia plumosa*

Treatment	Betaxanthin (absorbance at 485 nm) increase over dark control
24 hr FR	0·220
6 hr R ,18 hr FR	0·172
24 hr FR, 24 hr dark	0·100
24 hr FR, 15 min R, 24 hr dark	0·130
24 hr FR, 15 min R then 5 min FR and 24 hr dark	0·119

EXPERIMENTAL

Plant material. Seedlings of *C. plumosa* and *A. tricolor* were germinated in darkness at 28° in Petri dishes on two layers of filter paper moistened with tap water and used when 2-day-old.

The FR irradiation was obtained by filtering the radiation from tungsten filament lamps through a 9-cm thick flowing water screen and through Kodak filter No. 89B. The irradiance, measured at the seedlings level with a YSI-65 radiometer (Yellow Spring Instrument Co.), was 1·9 mW/cm^{-2} unless otherwise stated. A cool-white fluorescent source giving 5000 lx at the seedling level was used. The same source was used in conjunction with a red filter (Kodak Wratten No. 25) for irradiation with R.

The quantitative determination of chlorophyll *a* was carried out according to the method of Smith and Benitez.[16] Betaxanthin and amaranthin were determined as previously reported.[9, 17]

Six replicates were used in all experiments and each experiment was repeated at least 6 times. The experimental error was normally 4% or less in any given experiment.

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