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Photocontrol of Anthocyanin Synthesis

I. ACTION OF SHORT, PROLONGED, AND INTERMITTENT IR RADIATIONS ON THE FORMATION OF ANTHOCYANINS IN CABBAGE, MUSTARD, AND TURNIP SEEDLINGS[1]

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ABSTRACT

Red far red reversibility (phytochrome control) of anthocyanin synthesis can be easily demonstrated for the response induced by short (5 minutes) and relatively short (4 hours) irradiation. Red far red reversibility of the response induced by longer irradiations can be demonstrated by the use of cyclic irradiations alternating short exposures to red and far red light.

The level of anthocyanin formed during the dark incubation period following exposure to light depends upon the duration of the irradiation and becomes proportionally smaller as the length of the irradiation increases.

Production of anthocyanins under cyclic irradiations depends upon the total energy applied and upon the length of the dark interval between successive irradiations.

The relative efficiencies of radiations in various spectral ranges change with changes in the length of the irradiations.

The synthesis of anthocyanins, as well as several other photomorphogenic responses of plants, requires prolonged periods of irradiation at relatively high light intensities (2, 8, 10, 11, 18, 20, 24, 31). The photochemical system controlling the morphogenic responses of plants to high dosages of visible radiation has been called the "high energy reaction" system of plant photomorphogenesis (2, 9–11, 18–20, 24, 25, 32). This term was introduced to distinguish this system from the phytochrome system which controls the red-far red reversible photomorphogenic responses of plants to low dosages of visible radiation, several orders of magnitude smaller than those required to elicit HER[2] responses, applied as a single exposure of short duration or as multiple short exposures separated by relatively long dark intervals (19–22, 24, 30, 31).

The nature of the photoreceptor(s) controlling HER responses is a matter of much discussion. The blue and far red regions of the spectrum which are most effective in inducing HER responses are effective also in the photoconversion of phytochrome (2, 8, 13, 18–20, 24, 30, 31). In addition, many of the physiological responses induced by prolonged periods of irradiation are also controlled by phytochrome (2, 8–12, 14, 16–19, 21–27, 31, 32). Some authors support the idea that phytochrome is the only photoreceptor involved in HER responses (1, 2, 18, 31, 32), whereas others support the idea that a second photochemical system, beside phytochrome, takes part in the expression of these responses (3, 7, 11, 14, 19, 26–29). There is some evidence suggesting that one of the photochemical systems involved in HER responses might be the photosynthetic system (4, 5, 8, 9, 19, 26).

The results discussed in this communication were obtained in a preliminary phase of our studies of anthocyanin synthesis. Our interest in HER responses was stimulated by results obtained in our work in seed germination. Using intermittent irradiations we had been able to demonstrate that the germination response (inhibition) induced by continuous far red irradiation was actually phytochrome controlled (21, 22). We decided to find out if the use of cyclic treatments, similar to those used to demonstrate phytochrome control of seed germination responses induced by prolonged periods of irradiation, could be of some help to investigate the nature of the photoreceptor(s) involved in HER responses.

MATERIALS AND METHODS

Seeds of cabbage (*Brassica oleracea* L., var. Red Acre) and of turnip (*Brassica rapa* L., var. Purple Top White Globe) were purchased from Seeds Research Specialties; seeds of white mustard (*Sinapis alba* L., var. Fine White) were purchased from Thompson and Morgan Ltd. The seeds were germinated and grown in darkness at 20 C in Petri dishes on a disk of heavy filter paper (Eaton-Dikeman grade 923) moistened with distilled water. Ages of the seedlings (from sowing) at the beginning of the light treatments were: 96 ± 2 hr for cabbage; 48 ± 1 hr for mustard; 72 ± 2 hr for turnip. Temperature throughout the experiments was 20 C.

Anthocyanins were extracted at the end of the light treatments (E.L.T.), or after a 24-hr dark incubation period following the light treatments (E.D.T.). The solution used for the extraction was 1% (w/v) HCl in methanol; the seedlings were extracted in this solution for 2 days at 4 to 6 C. The absorbance of the extracts was measured with a Model 300-N Gilford spectrophotometer (absorbancies of Fig. 1 and Tables II and III were measured with a Coleman Junior II photometer) at 525 nm for cabbage and at 530 nm for mustard and turnip. Absorbancies of the dark controls were: 0.45 to 0.55 for cabbage and 0.07 to 0.10 for mustard and turnip.

Light treatments were given in growth chambers (Percival Model E-57) equipped with various combinations of lamps and filters as indicated in Table I. The irradiance of these

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[2] Abbreviations: HER: high energy reaction; D: dark; R: red; FR: far red; CW, CWI, B, RI: various types of wide spectrum radiation sources, their properties are specified in "Materials and Methods" and in Table I; Pt: total phytochrome; E.L.T.: end of light treatments; E.D.T.: end of a 24-hr dark incubation period following the light treatments.

Table I. *Irradiance in the Blue, Far Red, and Red Regions of the Wide Spectrum Light Sources used in Various Experiments*

Irradiances were measured at seedlings level with an IL-150 photometer (International Light Inc.). FL: fluorescent lamps; IL: incandescent tungsten lamps. Other lamp symbols are those of the General Electric large lamp catalog.

Light Sources	Irradiance		
	Blue	Far red	Red
	uw cm⁻²		
1. CWI, 8 × F36T12/CW/HO FL + 4 × 50W IL	715	430	862
2. CW, 8 × F36T12/CW/HO FL	638	55	529
3. R, 8 × F36T12/CW/HO FL + red filter 2444	ND[1]	75	380
4. FR, 12 × 25W IL + FRF-700 filter	ND	400	98
5. B, 10 × F36T12/CW/HO FL + blue filter 2045 + 5 cm of 10% $CuSO_4 \cdot 5H_2O$ solution	222	ND	ND
6. RI, 4 × 50W IL + red filter 2444	ND	345	288
7. R, 4 × F36T12/CW/HO FL + red filter 2444	ND	ND	125
8. FR, 12 × 40W IL + red filter 2444 + FRF-700 filter	ND	700	161
9. R, 4 × F20T12-R FL	ND	ND	126
10. FR, 6 × 150W/PAR38, reflector flood IL + 8 cm of water + FRF-700 filter	ND	2500	615

[1] ND: not detectable (less than 0.5 μw cm^{-2} nm^{-1}) with IL-150 photometer.

wide spectrum light sources were measured with an IL-150 photometer (International Light Inc.). This instrument measures irradiance (μw cm^{-2} nm^{-1}) in three wavelength ranges using three different detectors with peaks at 460 nm for the blue region (one-half bandwidth = 110 nm), at 640 nm for the red region (one-half bandwidth = 115 nm), and at 730 nm for the far red region (one-half bandwidth = 100 nm). The values reported in Table I (μw cm^{-2}) were obtained by multiplying the measured values (μw cm^{-2} nm^{-1}) by the one-half bandwidth of the detector used. Monochromatic radiation was obtained by filtering the radiation from 500-w reflector lamps through 9 cm of water and through second order interference filters with bandwidths from 4 to 16 nm (Corion Instrument Co.). The irradiance of the monochromatic sources, measured at seedlings level with a YSI-65 radiometer (Yellow Spring Instrument Co.), was 500 ± 50 μw cm^{-2}. Manipulations of seeds and seedlings were done under a dim green safelight (15 w cool green fluorescent lamp and one layer each of Rhom and Haas Amber 2451 and Blue 2045 Plexiglas).

The abbreviations used to indicate the various light treatments are as follows: (*a*) "X hr (m, s) light" corresponds to X hours (min or sec) of continuous irradiation; (*b*) "X hr (6s FR/54s D)" indicates an intermittent light treatment extended over an X-hour period in which successive FR irradiations—6 sec each—are separated by 54 sec of darkness; (*c*) "X hr (1m R/1m FR/8m D)" corresponds to a cyclic light treatment extended over an X-hour period in which, in each cycle of a 10 min duration, each 1 min R is followed by 1 min of FR and then by 8 min of darkness before the next R irradiation.

RESULTS

Red-far red reversibility of anthocyanin formation is clearly demonstrated for the response induced after irradiations from 5 min to 4 hr (Table II). These results confirm previous findings by various workers (7, 8, 12, 23, 27). After longer periods of irradiation, anthocyanin formation apparently escapes phytochrome control and, after 24 hr of continuous irradiation, R-FR reversibility cannot be demonstrated (Table II).

The findings relative to the time course of anthocyanin formation (Fig. 1, Table III) can offer some explanation for the lack of R-FR reversibility after long periods of irradiation. The time course of anthocyanin formation is essentially similar under various light sources, CW, CWI, R, FR, RI. The level of anthocyanins formed during the dark incubation period following the irradiation becomes smaller as the length of the irradiation increases (Fig. 1, Table III). It seems possible that demonstration of R-FR reversibility of anthocyanin formation after long periods of irradiation becomes difficult because of the high level of anthocyanin already present and

Table II. *Red Far Red Reversibility of Anthocyanins Formation*

Anthocyanins were extracted at the end of a 24-hr dark incubation period following the light treatments. The figures given below were corrected by subtracting the absorbance values of the dark controls. Light sources used: for the 4- and the 24-hr irradiations: 1(CWI), 3(R) and 4(FR); for the others: 9(R) and 10(FR).

Light Treatments	Absorbance		
	Cabbage	Mustard	Turnip
10 min R	0.22	0.15	0.01
2 min FR	0.10	0.03	0.01
10 min R-2 min FR	0.08	0.05	0.00
2 min FR-10 min R	0.25	0.15	0.02
4 hr CWI-5 min R	0.76	0.62	0.10
4 hr CWI-5 min FR	0.48	0.33	0.06
4 hr CWI-5 min R-5 min FR	0.50	0.31	0.07
4 hr CWI-5 min FR-5 min R	0.76	0.58	0.11
24 hr CWI-5 min R	1.72	1.11	—
24 hr CWI-5 min FR	1.69	1.09	—
4 hr R	0.74	0.40	0.04
4 hr R-5 min FR	0.38	0.21	0.02
4 hr R-5 min FR-10 min R	0.75	0.36	0.05
4 hr FR	0.40	0.20	0.03
4 hr FR-10 min R	0.58	0.47	0.05
4 hr FR-10 min R-5 min FR	0.37	0.20	0.03

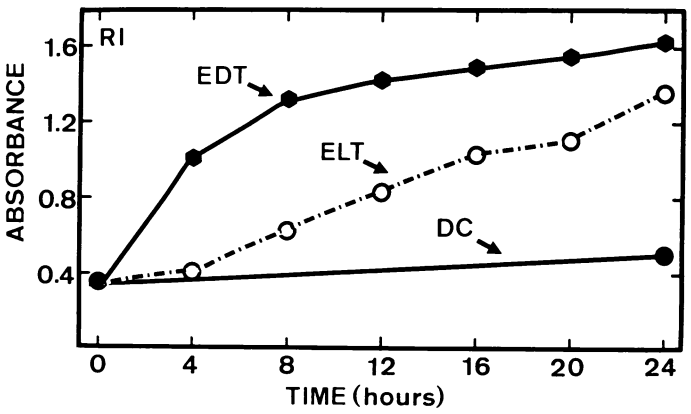

FIG. 1. Formation of anthocyanin in cabbage seedlings exposed for various times (from 4 to 24 hr) to the radiation from the RI source. DC: Dark control; ELT: anthocyanin extracted at the end of the period of irradiation; EDT: anthocyanin extracted 24 hr after the end of the irradiations. Light source used: No. 6.

Table III. *Action of Irradiations of Different Durations from Various Light Sources upon the Formation of Anthocyanins*

Anthocyanins were extracted at E.L.T. or after E.D.T. The figures given below were corrected by subtracting the absorbance values of the dark controls. Light sources used: 1, 2, 3, 4, 5, and 6.

Light Treatments	Absorbance					
	Cabbage		Mustard		Turnip	
	E.L.T.	E.D.T.	E.L.T.	E.D.T.	E.L.T.	E.D.T.
4 hr CWI	0.14	0.87	0.01	0.47	0.00	0.09
4 hr CW	0.06	0.58	0.02	0.51	0.01	0.06
4 hr B	0.04	0.51	0.01	0.27	0.01	0.03
4 hr RI	0.05	0.58	0.01	0.50	0.02	0.07
4 hr R	0.06	0.63	0.01	0.35	0.01	0.04
4 hr FR	0.02	0.37	0.01	0.18	0.01	0.02
24 hr CWI	1.39	1.71	1.04	1.10	0.40	0.42
24 hr CW	0.93	1.14	0.85	0.99	0.30	0.30
24 hr B	0.83	1.00	0.64	0.75	0.15	0.14
24 hr RI	0.91	1.15	0.82	1.18	0.35	0.38
24 hr R	0.78	0.97	0.51	0.75	0.15	0.19
24 hr FR	0.79	1.02	0.76	0.89	0.25	0.27

because of the low level of increase during the following dark period.

The ratio between the levels of anthocyanins formed after 4- and 24-hr irradiations (Table III, E.D.T. columns) is lower in turnip than in cabbage and in mustard. The relative efficiency of various types of radiations is also different: CWI is more effective than CW in cabbage and in turnip, but not in mustard; B is slightly less efficient than RI in cabbage, but much less effective than RI in mustard and in turnip. The relative efficiencies of R and FR radiations depend upon the length of the irradiation: R is more effective than FR during the first few hours of irradiation (Tables II and III), but FR becomes as effective or more effective than R after 24-hr irradiations (Table III). These results confirm previous findings by Wagner and Mohr (32) in mustard seedlings. The differences in the levels of anthocyanins formed after 4 hr of irradiation with R or with FR are probably only a reflection of the different levels of Pfr present during and at the end of the irradiations, since these differences are reduced or eliminated when the 4-hr irradiations with R or FR are terminated with a short FR or R, respectively (Table II).

In cabbage and in mustard, at least one-half of the anthocyanins formed during a 24-hr irradiation does not depend upon this continuous irradiation, but can be formed in darkness after an irradiation of only 4 hr (Fig. 1, Table III).

Since a high level of anthocyanin formation can be induced by relatively short irradiations, the next step was to determine if cyclic irradiations could be an effective substitute for continuous ones. The effect of cyclic irradiations on anthocyanin formation has been studied before (3, 7, 14), but the results are not easy to understand because the levels of energy applied in various cyclic and continuous treatments are often quite different.

In our experiments, three different types of cyclic treatments, extending over a 24 hr period, were studied: (*a*) cycles of different lengths, but with equal total energy doses (Table IV); (*b*) cycles of equal length, 1 min, but with different total energy doses (Fig. 2); (*c*) cycles with alternating R and FR irradiations (Table VI) to investigate the presence of R-FR reversibility in a response requiring prolonged periods of irradiation.

The efficiency of cyclic irradiations depends upon the length of the cycles (Table IV) and upon the total energy dose applied (Fig. 2). Cyclic irradiations are more effective than continuous ones of short duration—144 min—applied at the beginning of the 24-hr period (Tables IV and VI); if the length of the cycles is kept short, 10 sec, cyclic irradiations are as effective as continuous ones providing the same total energy dose and extending over the same period (Table IV). When a 1-min cycle is used (Fig. 2), saturation of the response in cabbage and in mustard requires about 13 to 15% of the energy provided by continuous irradiation with the same source; this level of energy is very close to that required for saturation under continuous irradiation (data not reported

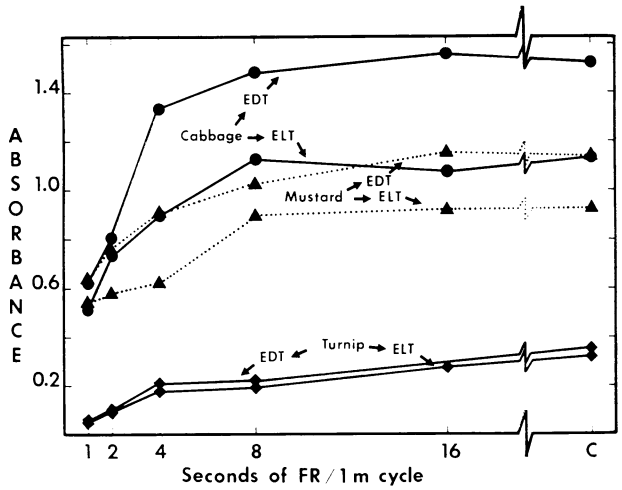

FIG. 2. Action of 24 hr of cyclic FR irradiations on the formation of anthocyanin. Values reported were corrected by subtracting the absorbance of the dark controls. ELT and EDT are as in Figure 1; light source used: No. 4; C: continuous irradiation.

Table IV. *Action of Cyclic Far Red Light upon the Formation of Anthocyanins*

Anthocyanins were extracted at the end of the indicated treatments. The figures given below were corrected by subtracting the absorbance values of the dark controls. Light sources used: No. 4 for the 24 hr-continuous and cyclic irradiations; No. 8 for the 144-min irradiation.

Light Treatments	Total FR Energy Dose	Absorbance		
		Cabbage	Mustard	Turnip
	joules cm^{-2}			
144 min FR-1296 min D	6.0	0.19	0.13	0.01
24 hr FR	34.6	1.13	0.93	0.33
24 hr (1 sec FR/9 sec D)	3.5	0.91	0.75	0.19
24 hr (6 sec FR/54 sec D)	3.5	0.77	0.83	0.13
24 hr (36 sec FR/324 sec D)	3.5	0.73	0.66	0.12
24 hr (72 sec FR/648 sec D)	3.5	0.73	0.70	0.11
24 hr (3 min FR/27 min D)	3.5	0.49	0.42	0.04
24 hr (6 min FR/54 min D)	3.5	0.50	0.39	0.05
24 hr (15 min FR/135 min D)	3.6	0.41	0.28	0.02
24 hr (30 min FR/270 min D)	3.6	0.35	0.19	0.01
24 hr FR[1]	3.5	0.92	0.87	0.23

[1] Irradiance from this source was 40 μw cm^{-2}.

Table V. *Action of Various Combinations of Continuous Red or Far Red and Cyclic Far Red Irradiations upon the Formation of Anthocyanins*

Anthocyanins were extracted after a 24-hr dark incubation period following the end of the light treatments. The figures given below were corrected by subtracting the absorbance values of the dark controls. Light sources used: 3 and 4.

Light Treatments	Absorbance		
	Cabbage	Mustard	Turnip
24 hr FR	1.52	1.13	0.33
4 hr FR	0.40	0.20	0.04
4 hr R	0.74	0.37	0.04
8 hr FR	0.58	—	—
8 hr R	1.09	—	—
24 hr (6 sec FR/54 sec D)	1.12	0.95	0.19
4 hr FR-20 hr (6 sec FR/54 sec D)	1.39	1.08	0.31
4 hr R-20 hr (6 sec FR/54 sec D)	0.87	0.76	0.13
8 hr FR-16 hr (6 sec FR/54 sec D)	1.38	1.02	0.25
8 hr R-16 hr (6 sec FR/54 sec D)	0.82	0.85	0.13
24 hr (6 min FR/54 min D)	0.59	0.51	0.05
4 hr FR-20 hr (6 min FR/54 min D)	0.68	0.61	0.10
4 hr R-20 hr (6 min FR/54 min D)	0.48	0.36	0.03
8 hr FR-16 hr (6 min FR/54 min D)	0.86	0.78	0.16
8 hr R-16 hr (6 min FR/54 min D)	0.61	0.45	0.06

in this paper). Saturation of the response under cyclic irradiation might require different energy doses for cycles of different lengths (Table IV).

The low efficiency of intermittent irradiations separated by long dark intervals (Table IV) could have been due to a difficulty in overcoming the lag phase (15). To test this hypothesis we studied several combinations of continuous and cyclic irradiations extending over a 24-hr period. When the first 4 or 8 hr of a cyclic FR treatment were substituted by continuous R or FR, the level of anthocyanin produced was different from that formed under 24 hr of cyclic FR irradiation, but the differences in the efficiency of cycles of different lengths were not eliminated (Table V). Continuous FR, applied before cyclic FR, resulted in a slight increase of the production of anthocyanins, while continuous R, similarly applied, decreased production. This last result is very similar to those obtained by Grill and Vince (13) in turnip seedlings. Actually, anthocyanin production under the sequence 8 hr R-24 hr D is higher than under 8 hr R-16 hr cyclic FR-24 hr D.

Red light, when applied before FR in treatments like those reported in Table V, has an inhibitory action on anthocyanin formation, but, in other types of prolonged cyclic treatments, R applied before FR has an enhancing action. In cyclic treatments in which each cycle contains both R and FR, the sequence R/FR/D is more effective than the sequence FR/D (Table VI). The various results of Table VI offer a further comparison of the relative efficiencies and of the relationships between R and FR in various types of treatments. The difference between the R/FR/D and the FR/R/D cyclic treatments seem to provide some indication of R-FR reversibility, therefore phytochrome control, of anthocyanin production under prolonged periods of irradiation.

Since our results had shown that the relative efficiencies of R and FR changed with the duration of the irradiations and that these changes were about the same for the three species, we decided to study the wavelength dependence of anthocyanin synthesis in the 650 to 740 nm region to find out if the differences in the peaks of action reported for cabbage, mustard, and turnip (17, 23, 27) could be attributed to the fact that various authors had used irradiations of different lengths, from 4 hr for cabbage (27) to 48 hr for turnip (17).

The results of our experiments are reported in Figure 3. In most of these experiments, temperature during irradiation was 22 to 24 C. But, in mustard, due to a failure of the air conditioning system, only about half of the 24-hr irradiations were run at this temperature (LT, Fig. 3); the other half was run at 27 to 30 C (HT, Fig. 3), and the line A in the same figure is an average of the results at the two different temperatures. The action of temperature is very pronounced in the red region, but almost nonexistent in the far red region.

After a 4-hr irradiation, the efficiency for the production of anthocyanins is higher in the 650 to 710 nm region than in the 710 to 740 nm region. Far red light applied at the end of the 4-hr irradiations decreases production in the 650 to 710 nm region, while R, similarly applied, increases production in the 710 to 740 nm region. After 24 hr of irradiation, production of anthocyanins in the 650 to 700 nm region is only slightly higher than that obtained after 4 hr of irradiation followed by 20 hr in darkness, but there is a large increase in the 700 to 740 nm region, and the peaks of action are at about the same wavelength, 710–720 nm, for cabbage and mustard.

After exposure to monochromatic radiations for 24 hr, the difference between the levels of anthocyanin produced in the R and FR regions (Fig. 3) is more pronounced than that obtained after 24 hr of irradiation with the wide spectrum R and FR sources (Table III). A possible explanation for these differences could be that there is some FR in a wide spectrum R source and some R in a wide spectrum FR source (Table I).

Table VI. *Action of Red and Far Red Radiation upon the Formation of Anthocyanins*

Anthocyanins were extracted at the end of the treatments indicated in the table. The figures given below were corrected by subtracting the absorbance values of the dark controls. Light sources used: 7 and 8.

Light Treatments	Absorbance		
	Cabbage	Mustard	Turnip
144 min R-1296 min D	0.27	0.27	0.04
288 min R-1152 min D	0.39	0.34	0.05
144 min FR-1296 min D	0.19	0.13	0.01
288 min FR-1152 min D	0.23	0.22	0.02
144 min R-144 min FR-1152 min D	0.18	0.18	0.02
144 min FR-144 min R-1152 min D	0.43	0.34	0.08
144 min (R + FR)-1296 min D	0.38	0.38	0.03
24 hr (1 min R/9 min D)	0.52	0.43	0.09
24 hr (2 min R/8 min D)	0.48	0.39	0.10
24 hr (1 min FR/9 min D)	0.56	0.53	0.11
24 hr (2 min FR/8 min D)	0.60	0.55	0.12
24 hr (1 min (R + FR)/9 min D)	0.55	0.54	0.11
24 hr (1 min R/1 min FR/8 min D)	0.81	0.76	0.24
24 hr (1 min FR/1 min R/8 min D)	0.42	0.46	0.08

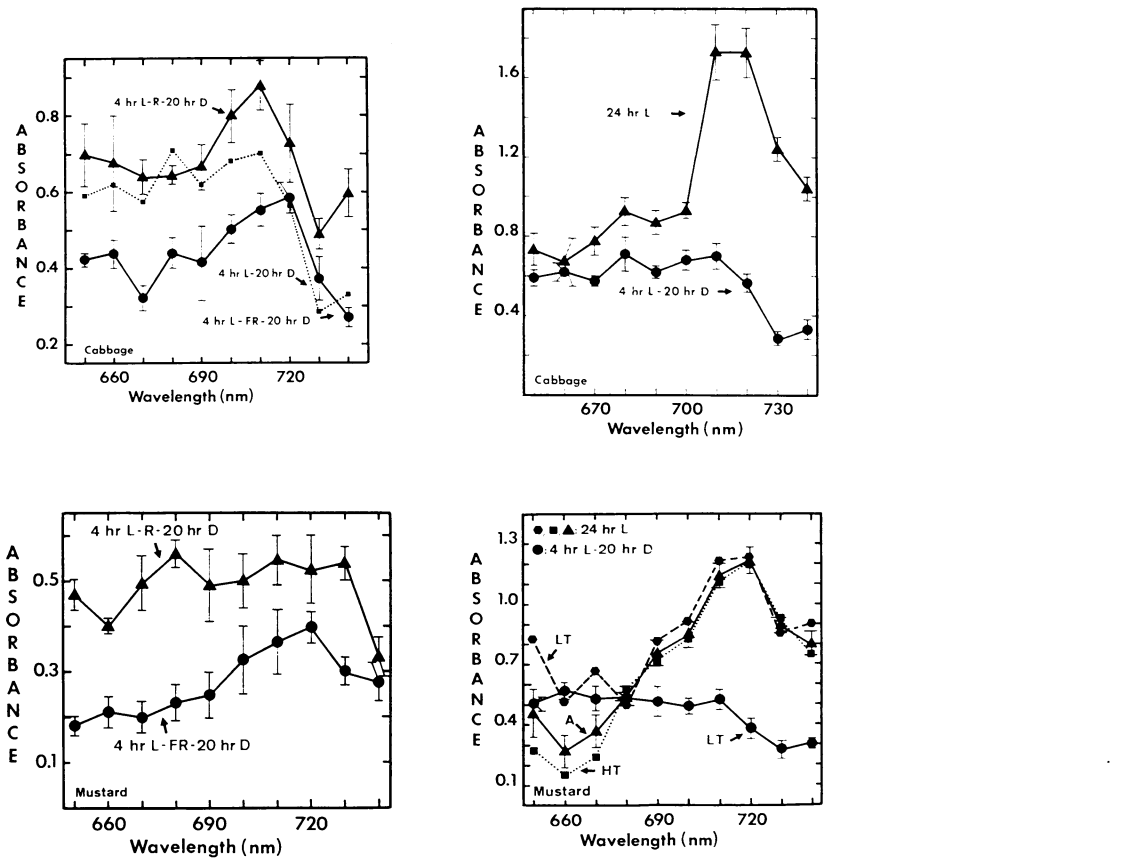

FIG. 3. Wavelength dependence of anthocyanin formation in seedlings exposed to monochromatic radiation in the 650 to 740 nm region for 4 and 24 hr. Values reported were corrected by subtracting the absorbance of the dark controls. L: Monochromatic radiation; D: dark; R: 5 min of R (source No. 9); FR: 5 min of FR (source No. 10). A, HT, LT are explained in the text.

DISCUSSION

The behavior of the three species studied is essentially similar, even though there are some quantitative (anthocyanin produced/seedling·unit of light) and qualitative differences, as we have pointed out in the previous section.

The length of the irradiations is a very important factor in the comparison of the action of radiations of different spectral regions on anthocyanin synthesis, since the relative efficiencies of the various spectral regions might change quite considerably depending upon the length of the irradiations (Table III, Fig. 3). These changes of efficiency, depending upon the duration of the irradiations, might be a direct effect on anthocyanin synthesis, but could also be an indirect one. It seems quite possible that, a few hours after the beginning of the irradiations, the physiological states of seedlings exposed to radiations in different spectral regions might be completely different, and that a difference in the rate of anthocyanin synthesis might only reflect a different balance among the rates of various metabolic reactions competing for a common substrate.

Determination of the energy requirement for anthocyanin synthesis should take into account the importance of the length of the irradiations and the fact that considerable amounts of this pigment can be formed in darkness after relatively short periods of irradiation (Tables II and III, Figs. 1 and 3).

Cyclic irradiations can be an effective substitute for continuous ones if some conditions (Table IV) are observed: (*a*) cycles of short duration, (*b*) cyclic treatments extending over the same period and furnishing the same total energy dose as the continuous ones. One should also make sure that the relative efficiencies of continuous and cyclic irradiations in different spectral regions remain the same. With R and FR the relative efficiencies of 24 hr of continuous or cyclic irradiations remain about the same: continuous FR is slightly more effective than continuous R (Table III), and cyclic FR is slightly more effective than cycle R (Table VI). Since cyclic treatments seem to be, qualitatively and quantitatively, an effective substitute for continuous ones, and since cyclic treatments allow alternate short irradiations in different spectral regions, they could be of some help in investigating the nature of the photoreceptors involved in responses induced by prolonged irradiations.

After short or relatively short irradiations (Table II), phytochrome seems to be the most important or, at least, the most evident factor controlling light-induced anthocyanin formation, and a high value of the Pfr/Pt ratio seems necessary for a high level of anthocyanin production.

After long periods of continuous irradiation, phytochrome control is much less evident (Table II), and there is very little correlation between anthocyanin production and the Pfr/Pt ratio maintained during continuous irradiations in various spectral regions (Table III). The R/FR cyclic treatments (Table VI) provide some evidence of a R-FR reversibility extending over prolonged periods of irradiation, but also provide evidence that the value of the Pfr/Pt ratio is not the only factor controlling anthocyanin synthesis under these conditions; otherwise the FR/D and the R/FR/D sequences should have about the same efficiency. Several different factors might contribute to the results obtained after various types of prolonged treatments: rates of irreversible destruction and of *de novo* synthesis of phytochrome (16, 20, 30, 32), relative

(percentage of Pt) and absolute levels of Pfr, and rate of dark conversion from Pfr to Pr. Some preliminary measurements of the levels of Pfr and Pt under various R and/or FR cyclic treatments seem to show a close correlation between total levels of Pfr and the efficiency of the various cyclic treatments of Table VI, but these results are not yet sufficient for a complete analysis.

Anthocyanin synthesis after prolonged periods of irradiation is energy dependent, as it has been shown by various authors (7, 8, 11) and as shown by the results of Figure 2. Since the length of the cycle used in these experiments—1 min—is such that no appreciable reduction of the Pfr should take place during the dark intervals between successive irradiations, one would not expect such a large difference in the production of anthocyanins under the various cycles if the level of Pfr was the only factor controlling the response. Our results do not exclude the possibility of the participation of a second photorection, beside phytochrome, in the processes leading to the formation of anthocyanins. It has been suggested that photosynthesis might be one of the photoreactions involved in anthocyanin synthesis (4, 5, 8, 9, 26), and there is some evidence for the formation of chlorophyll and the development of the photosynthetic apparatus under prolonged FR irradiations (6, 26). Assuming that photosynthetic system I is involved in anthocyanin formation (26) in the three species studied, the fact that a high efficiency for the production of anthocyanins under FR is developed only after prolonged periods of irradiation (Table III, Fig. 3) would be in agreement with the fact that the development of the photosynthetic system is slower under FR than under R or white light (6).

In conclusion, our results have shown: (*a*) that while the production of very high levels of anthocyanins might require prolonged periods of irradiation, it is possible to obtain quite high levels with irradiations of few hours followed by a long dark incubation period; (*b*) that cyclic irradiations are an effective substitute for continuous ones; (*c*) that phytochrome control, as indicated by R-FR reversibility, is an important feature of anthocyanin synthesis either after short irradiations or during prolonged ones, but one cannot exclude the possibility of the participation of a second photoreaction; (*d*) that the relative efficiencies of radiations of different spectral regions depend upon the length of the irradiation; (*e*) that the relative efficiencies and the relationships between R and FR applied alone or in various types of combinations and sequences depend upon the type of treatment—short, long, or cyclic.

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