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Photoregulation of Anthocyanin Synthesis[1]

VIII. EFFECT OF LIGHT PRETREATMENTS

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ALBERTO L. MANCINELLI*
Department of Biological Sciences, Columbia University, New York, New York 10027

ABSTRACT

A comparative study of the spectral sensitivity of anthocyanin production in dark-grown and light-pretreated systems was carried out in *Brassica oleracea* L., *Lycopersicon esculentum* Mill., *Secale cereale* L. and *Spirodela polyrrhiza* L. Light pretreatments bring about an enhancement of the inductive, red-far red reversible response in all systems, a decrease of the continuous irradiation response in cabbage, rye, and tomato seedlings, and an enhancement of the continuous irradiation response in cabbage leaf disks. Light pretreatments also bring about a marked change in the spectral sensitivity of the continuous irradiation response. The different effect of light pretreatments on the photosensitivity of the response to short and long wavelength irradiations suggests that two photoreceptors, phytochrome and cryptochrome, may be involved in the photoregulation of anthocyanin production.

The light-dependent production of anthocyanin is a typical HIR[2] response of plant photomorphogenesis, requiring prolonged exposures to high fluence rates of visible and near-visible radiation (17–19), and has been used quite extensively in studies of the properties of the HIR.

The spectral sensitivity of the HIR varies with species and three response groups have been identified (17). In group I, peaks of action are found in the UV-BL, R, and FR regions (1, 9, 31). In group II, the main peak of action is in the R, but the UV-BL region is also effective, whereas action in the FR is either absent or minimal (32). In group III, there is action only in the UV-BL region (4). In any given response-system combination, the spectral sensitivity of the HIR can be markedly affected by the experimental conditions (1, 3, 8–10, 16–19, 21). It has been suggested that differences in the nature and/or operational state of the photomorphogenic pigments and in the physiological state of the biological systems may be responsible for the variability in the photosensitivity of the HIR (1, 7, 9, 14, 19, 21, 22, 30), but the exact role played by these factors is still a matter of conjecture. Phytochrome is involved in the photoregulation of the HIR under R and FR (17–19, 26). Action in the UV-BL region has also been ascribed to phytochrome (1, 17, 19), but the involvement of a photoreceptor specific for the UV-BL region (*i.e.* cryptochrome) has been suggested (4, 6, 9, 17, 18, 23).

Most studies on the properties of the HIR have been carried out using young, dark-grown seedlings (17, 19); some data are available for light-grown systems (1, 3, 7, 9, 10, 16, 22, 30). Studies of photomorphogenesis in light-grown systems are complicated by the effects of Chl screening on the state of the photomorphogenic pigments (15). To reduce these effects, researchers have used light-grown seedlings treated with inhibitors (*e.g.* NF or STM) that considerably decrease the level of Chl (1, 2, 10, 13, 20).

The effect of light pretreatments on the spectral sensitivity of the HIR has been studied in detail only for the light-dependent inhibition of hypocotyl elongation in mustard seedlings (1, 9), where light pretreatments cause a considerable decrease of the response to continuous UV, BL, and FR, while having no effect on the response to continuous R. At present, it is not known if the observations in mustard can be considered a general characteristic of HIR responses, or if they are limited to this particular response-system combination. A comparison of data from different response-system combinations is necessary to clarify this point, and we studied the effects of light pretreatments on the spectral sensitivity of anthocyanin production in various species.

MATERIALS AND METHODS

Seedlings of cabbage (*Brassica oleracea* L., Burpee Red Acre), rye (*Secale cereale* L., Cougar), and tomato (*Lycopersicon esculentum* Mill., Burpee Beefsteak) were grown in Petri dishes, on filter paper moistened with the solutions indicated in the Tables and Figures. Cultures of *Spirodela polyrrhiza* were grown in Fernbach flasks, containing 900 ml of HM, with sucrose added to a final concentration of 1% (w/v); after 2 weeks growth in darkness, the material was harvested, washed with sterile water, resuspended in sterile medium, and distributed in Petri dishes containing 20 ml of incubation medium. Cabbage leaf disks, 12 mm in diameter, were cut from 10 to 12 cm long leaves of greenhouse-grown plants and distributed in Petri dishes, 10 disks per dish, on filter paper moistened with incubation medium, Operations were carried out under green safelights.

Pigments were extracted with acidified (1% HCl, w/v) methanol, as described previously (20). The absorbance of the extracts, clarified by filtration, was measured at 530 (peak of absorption of anthocyanin) and 657 nm. The formula, $A_{530} - A_{657}$, was used to compensate for the contribution of Chl derivatives to absorption at 530 nm; the corrected A_{530} values provide an estimate of anthocyanin content and the A_{657} values provide an estimate of Chl content (20, 21). The reported values represent means of eight replicates in two independent experiments; standard deviations of the means were between 4 to 7% of the mean values. For *Spirodela*, absorbance values are expressed on a g fresh weight basis.

Exposures to light were given in growth chambers equipped with UV-A, BL, R, FR, and WL light sources described in Table

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[2] Abbreviations: HIR, high irradiance response; BL, blue; FR, far red; R, red; WL, white light; CMU, Monuron, 3-(*p*-chlorophenyl)-1,1-dimethylurea; HM, Hutner's medium; LPT, light pretreatment; LT, light treatment; D, dark; NF, Norflurazon (SAN-9789), 4-chloro-5-(methylamino)-2-(α,α,α,-trifluoro-m-tolyl)-3-(2H)-pyridazinone; Ptot, total phytochrome = Pr + Pfr; STM, streptomycin; SU, sucrose.

Table I. *Light Sources*

Plexiglass is a Rohm & Haas product. Roscolux is a Rosco product.

Light Source	Lamps	Filter	Fluence Rate	Wavelength Range	Pfr/Ptot[a]
			w/m²	*nm*	
UV-A	14 × F20T12/BLB FL[b]	None	3.0	320–400	0.60–0.70
BL	10 × F36T12/CW/HO FL	Plexiglas Blue 2045	3.8	400–500	0.30–0.40
R	8 × F36T12/CW/HO FL	Plexiglas Red 2444	3.9	600–700	0.70–0.75
FR	12 × 25 w + 12 × 40 w IL[c]	Plexiglas FRF-700	7.8	700–800	0.03–0.05
WL	8 × F36T12/CW/HO FL + 4 × 60 w IL	Plexiglas diffuser	6.8 7.9 4.8	400–500 600–700 700–800	0.65–0.70
GS[d]	F20T12/CG FL or F40T12/CG FL	Roscolux Green 90		480–540	

[a] Pfr/Ptot ratios measured after 15, 30, and 60 min irradiations in cabbage and rye seedlings.
[b] Fluorescent lamps.
[c] Incandescent lamps.
[d] Green safelight: no effect on the germination of light-requiring Grand Rapids lettuce seeds after exposure from 15 to 30 min.

Table II. *Effect of Light Pretreatment on Inductive and Continuous Irradiation Responses*

Light treatment applied immediately following the end of the pretreatment. Temperature: 22 to 23°C. Incubation media: water for the seedlings; 1% sucrose for cabbage leaf disks and *Spirodela*.

Response	Anthocyanin Production					
	Red Acre cabbage			Rye	Tomato	Spirodela
	seedling		leaf discs	seedlings		
Time of application of light treatments[a]	48	72	24	72	96	24
	A. Pretreatment: Dark					
R-FR reversible response[b]	0.034	0.070	0.024	0.037	0.007	0.272
Response to 24 or 48 h WL[c]	0.784	0.908	0.699	0.169	0.348	3.042
	B. Pretreatment: (8 h (rye) or 24 h (all others) continuous WL					
R-FR reversible response	0.126	0.150	0.254	0.078	0.090	0.693
Response to 24 or 48 h WL	0.548	0.656	0.879	0.139	0.321	2.566

[a] In hours from sowing for cabbage, rye, and tomato seedlings; in hours from cutting for cabbage leaf disks; in hours from transfer of cultures to Petri dishes for *Spirodela*.
[b] Difference between '10 min R + D' and '10 min R + 10 min FR + D'; D (dark) = 24 h for cabbage and tomato seedlings and 48 h for cabbage leaf disks, rye, and *Spirodela*.
[c] 24 h continuous WL for cabbage and tomato seedlings; 48 h continuous WL for cabbage leaf disks, rye, and *Spirodela*

Table III. *Effect of Duration and Time of Application of Continuous Irradiations on Anthocyanin Production in Cabbage Seedlings*

Time of application of light treatments is given in parentheses, in hours from sowing. Absorbance values were corrected by subtraction of the values of dark controls which were 0.050, 0.153, 0.241, and 0.337 at 48, 72, 96, and 120 h after sowing, respectively. Pigments extracted at end of indicated light treatments. Temperature: 22 to 23°C. Incubation medium: water. L, light; D, dark.

Duration and Time of Application of Light Treatments	Absorbance of Extracts					Effectiveness Ratio		
	UV-A	BL	R	FR	WL	UV/FR	BL/FR	R/FR
24 h L (24–48)	0.010	0.008	0.016	0.017	0.018	0.59	0.47	0.94
24 h L (48–72)	0.404	0.499	0.417	0.664	0.792	0.61	0.75	0.63
24 h L (72–96)	0.644	0.812	0.537	0.921	0.970	0.70	0.88	0.58
24 h L (24–48) + 24 h D	0.099	0.120	0.129	0.211	0.263	0.47	0.57	0.61
24 h L (48–72) + 24 h D	0.697	0.822	0.582	0.984	0.181	0.71	0.84	0.59
48 h L (24–72)	0.232	0.413	0.297	0.663	0.664	0.37	0.65	0.47
48 h L (48–96)	0.952	1.291	0.815	1.743	1.817	0.55	0.74	0.47
48 h L (72–120)	0.804	0.950	0.525	0.993	1.431	0.81	0.96	0.53

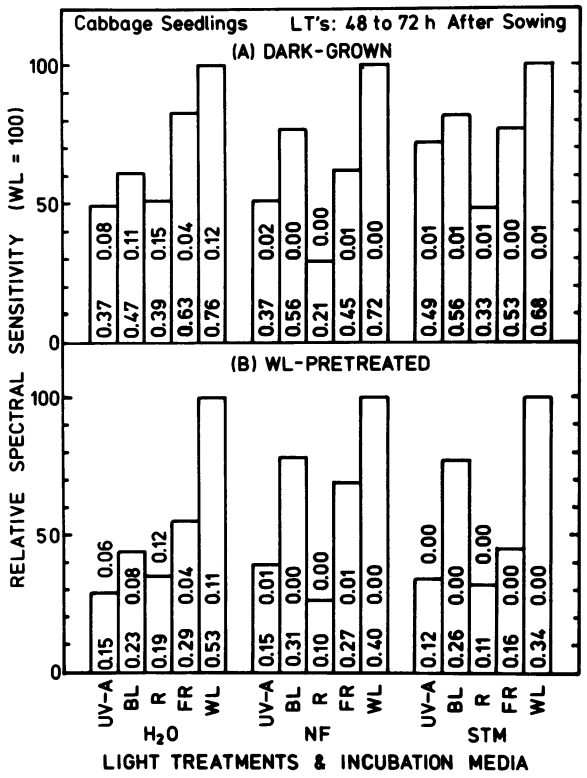

FIG. 1. Spectral sensitivity of anthocyanin production in dark-grown and WL-pretreated cabbage seedlings. Continuous UV-A, BL, R, and FR treatments applied from 48 to 72 h after sowing to dark-grown (A) and WL-pretreated (B) seedlings. Light pretreatment = 24 h WL from 24 to 48 h after sowing. Temperature: 22 to 23°C. Incubation media: water, 5 μM NF and 200 μg/ml STM. Numbers inside bars represent anthocyanin production due to the 'Light treatment' portion of the 'Pretreatment + Treatment' sequence, estimated as indicated in "Materials and Methods" (relative spectral effectiveness calculated on the basis of these numbers) and total Chl content at the end of the LT's.

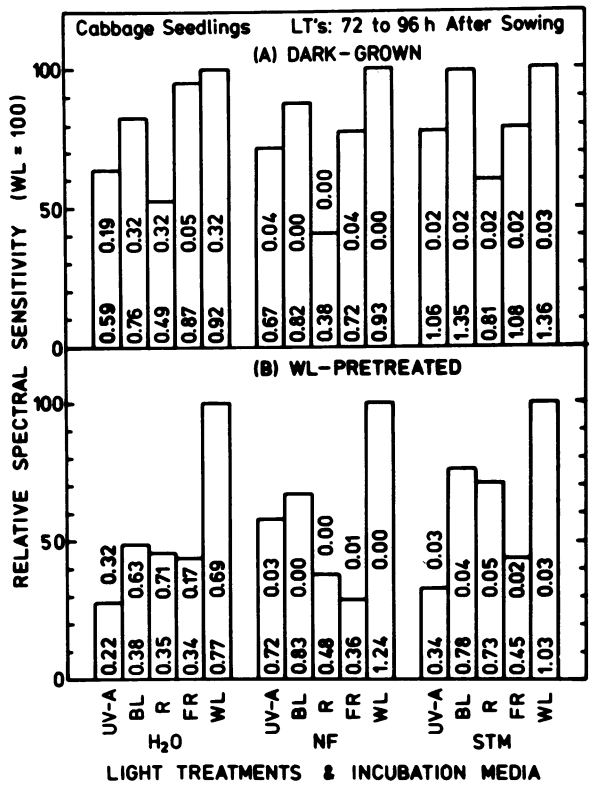

FIG. 2. Spectral sensitivity of anthocyanin production in dark-grown and WL-pretreated cabbage seedlings. Continuous UV-A, BL, R, and FR treatments applied from 72 to 96 h after sowing to dark-grown (A) and WL-pretreated (B) seedlings. Light pretreatment = 24 h WL from 48 to 72 h after sowing. Other details as in Figure 1.

I. Light pretreatments were terminated with a 10-min exposure to FR to prevent further action by the Pfr present at the end of the pretreatments. The amount of anthocyanin produced under LT applied after the LPT was estimated as the difference between the absorbance values of the extracts for the 'LPT + 10 min FR + LT' and 'LPT + 10 min FR + D' sequences.

RESULTS

The extent of the inductive, R-FR reversible response is small, compared to the response brought about by continuous irradiation (Table II). The extent of the R-FR reversible response is larger in light-pretreated than in dark-pretreated systems. The R-FR reversibility of the response indicates involvement of phytochrome in the photoregulation of anthocyanin production under inductive (short irradiations) conditions.

Anthocyanin production in dark-grown cabbage seedlings exposed to continuous irradiation is an HIR response with a group I spectral sensitivity (Table III). The extent of the response is affected by age. Anthocyanin continues to accumulate during a 24-h-d period following exposure to light. The values of the UVA/FR and BL/FR effectiveness ratios increase with age, as observed for the action of light on hypocotyl elongation (3, 8). The addition of NF and STM results in a decrease of the Chl level and has a considerable effect on the spectral sensitivity of anthocyanin production in both dark-grown and WL-pretreated seedlings (Figs. 1, 2). Exposure to WL pretreatments causes a considerable decrease in anthocyanin production during the subsequent continuous light treatments (Table II; Figs. 1, 2), with the exception of the older seedlings incubated in NF (Fig. 2B), in which only the response to FR is decreased. Differences in the spectral sensitivity of anthocyanin production between dark-grown and WL-pretreated seedlings are smaller in water-grown than in NF- and STM-treated seedlings, and, in general, smaller in the younger (Fig. 1) than in the older (Fig. 2) seedlings. An attempt was made to determine if pretreatments with different spectral regions would show a specific effect on anthocyanin production under the subsequent light treatments. Continuous UV-A, BL, R, and FR pretreatments are about equally effective in decreasing the sensitivity of the response to BL, while continuous R is slightly more effective than the other spectral regions in decreasing the response to FR (Fig. 3). The 2 × 5R and 4 × 15R pretreatments are more effective in decreasing the sensitivity of the response to FR than to BL.

The spectral sensitivity of anthocyanin production in rye and tomato seedlings, with maximum action in the UV-BL and some action in the R-FR (Figs. 4, 5), seems to be an intermediate between groups I and III HIR. In rye, UV-A is the most effective region with the exception of the dark-grown, NF-treated seedlings where maximum action is shifted to the BL (Fig. 4). In tomato, the most effective region is the BL (Fig. 5). In both rye and tomato, anthocyanin production under continuous UV-A, BL, and R was lower, in general, in the WL-pretreated than in the dark-grown seedlings. An 8-h WL pretreatment was used in rye because preliminary experiments showed an almost total loss of the response to irradiation in all spectral regions after a 24-h WL pretreatment. Differences in the spectral sensitivity of the response between dark-grown and light-pretreated seedlings are very evident in both species. Norflurazon, in rye, and STM, in tomato, enhanced anthocyanin production.

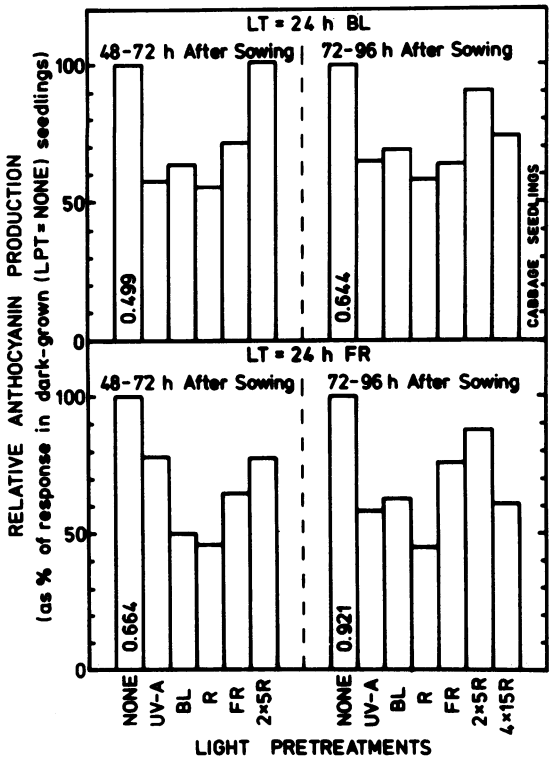

FIG. 3. The effect of different light pretreatments on the sensitivity of anthocyanin production to continuous BL and FR in cabbage seedlings. The 24-h continuous BL and FR treatments were applied immediately after the end of the light pretreatments; the numbers inside the bars represent anthocyanin production due to the continuous 24-h BL and FR light treatments applied to dark-grown (LPT = NONE) seedlings. Light pretreatments: 24 h UV-A, BL, R, and FR from 24 to 48 or 48 to 72 h after sowing; 2 × 5R: 2 × (5 min R + 55 min D) from 46 to 48 or 70 to 72 h after sowing; 4 × 15R: 4 × (15 min R + 345 min D) from 24 to 48 or 48 to 72 h after sowing. Temperature: 22 to 23°C. Incubation medium: water.

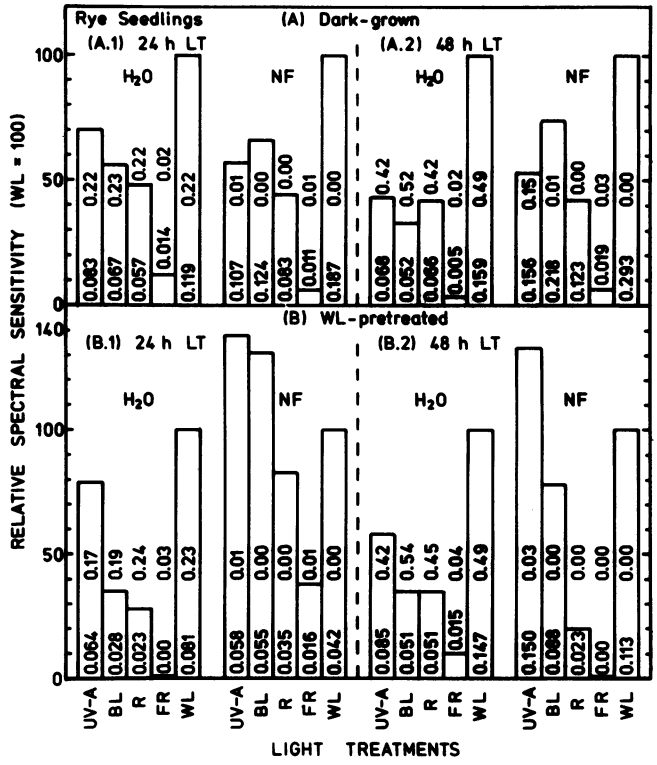

FIG. 4. The spectral sensitivity of anthocyanin production in dark-grown and WL-pretreated rye seedlings. Continuous, 24- or 48-h UV-A, BL, R, and FR treatments applied from 72 to 96 or 120 h after sowing to dark-grown (A) and WL-pretreated (B) seedlings. Light pretreatment: 8 h WL from 64 to 72 h after sowing. Temperature: 22 to 23°C. Incubation media: water and 10 μM NF. Other details as in Figure 1.

Anthocyanin production in cabbage leaf disks and *Spirodela* requires photosynthetic activity, as shown by the inhibitory action of CMU (Table IV); addition of sucrose to the CMU-containing medium restores anthocyanin production. Photosynthetic activity is required for anthocyanin production in apple skin sections (5), but not in young seedlings (18–20). The spectral sensitivity of the response in cabbage leaf disks and dark-grown *Spirodela* is that typical of group II HIR, with maximum action in the R region (Figs. 6, 7). Sucrose enhances anthocyanin production under UV-A, BL, and R and increases the values of the UVA/R and BL/R in both species. However, sucrose enhances anthocyanin production under FR only in *Spirodela* (Figs. 6, 7). In cabbage leaf disks, anthocyanin production under continuous UV-A, BL, R, and WL is higher in the WL-pretreated than in the dark-pretreated disks (Fig. 6). The differences in the spectral sensitivity of the response between dark-pretreated and WL-pretreated cabbage leaf disks and *Spirodela* are rather large in both species. In *Spirodela*, NF causes a shift in the region of maximum action from R to UV-BL (Fig. 7); WL pretreatments decrease anthocyanin production under continuous UV-A and FR in cultures incubated in HMSU and enhance anthocyanin production under continuous UV-A in cultures incubated in HMSU + NF.

DISCUSSION

In seedlings of cabbage, rye, and tomato, exposures to light pretreatments bring about an enhancement of the inductive, R-FR reversible response and a decrease of the response to continuous irradiation (Table II; Figs. 1–5). A comparison with data from other response-system combinations (1–3, 6, 7, 9, 16, 22, 23, 30) suggests that the differential effect of light pretreatments on inductive and HIR responses might be a common feature of photomorphogenesis in young seedlings. It has been suggested that the decrease of the HIR response is, at least in part, a consequence of the reduction in the phytochrome level (1, 7). That suggestion is supported by our results (Table V), but it is difficult to explain the enhancement of the inductive response as a consequence of a decrease in phytochrome level. One possible explanation is that the action of Pfr under inductive conditions might be different from the action of Pfr under HIR conditions, as suggested in a model for the mechanism of action of phytochrome (26). However, some features of photomorphogenic responses predicted in this model do not agree with experimental results. For example, the response to continuous R should be smaller than that to continuous FR, a suggestion not confirmed by experimental results (1, 3, 9, 18, 19). Therefore, it is doubtful that this model can be used to explain the differential effect of light pretreatments on the inductive and HIR responses. At present, the correlation between the effect of various factors (7, 11, 12, 14, 15, 19, 25, 27–29) on the operational state ([Ptot], Pfr/Ptot, rates of photoconversion, synthesis, and degradation) of phytochrome and photomorphogenic responses is still a matter of conjecture. It is also difficult to explain the differential effect of light pretreatments on inductive and HIR responses as a consequence of changes in the state of cell functions (transduction chain, metabolic pathways) involved in the response. Perhaps, since anthocyanin production in young seedlings is a transitory process and prolonged irradiations are required for the expression of HIR responses, the light-dependent decrease of the

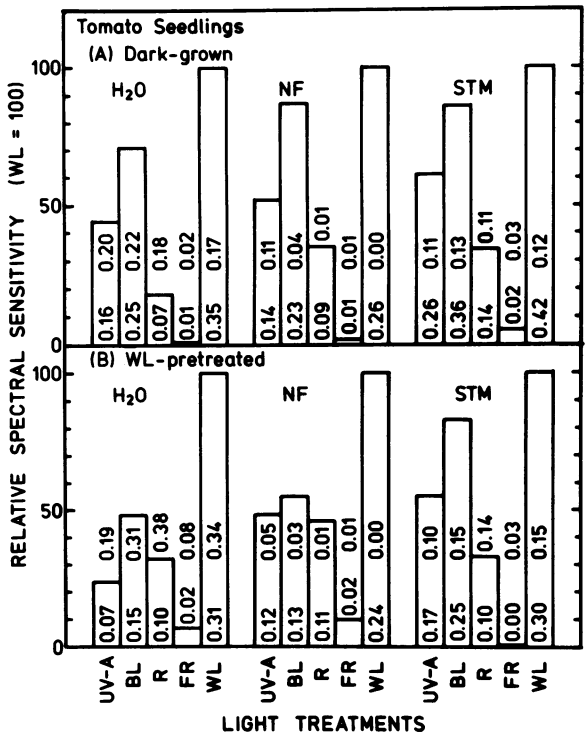

FIG. 5. The spectral sensitivity of anthocyanin production in dark-grown and WL-pretreated tomato seedlings. Continuous UV-A, BL, R, and FR treatments applied from 96 to 120 h after sowing to dark-grown (A) and WL-pretreated (B) seedlings. Light pretreatment: 24 h WL from 72 to 96 h after sowing. Other details as in Figure 1.

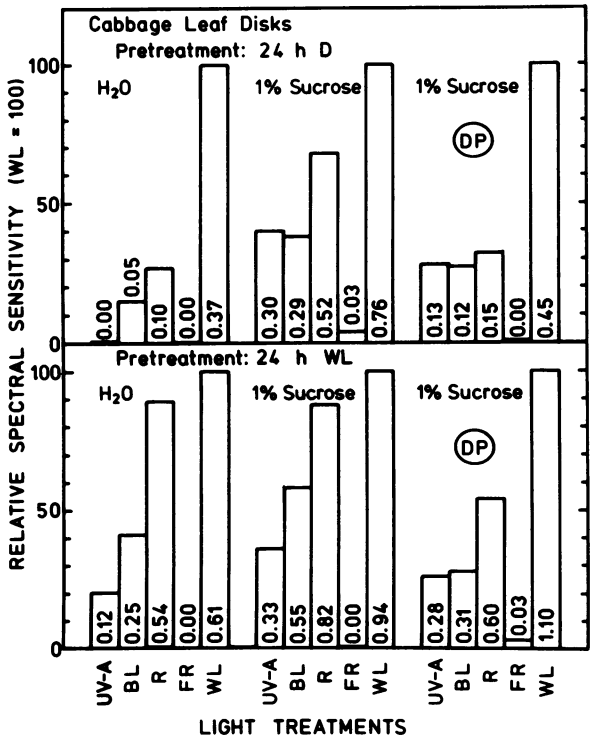

FIG. 6. The spectral sensitivity of anthocyanin production in dark-pretreated and WL-pretreated cabbage leaf disks. Continuous UV-A, BL, R, and FR treatments applied from 24 to 72 h after cutting to disks kept in darkness (A) or exposed to WL (B) during the first 24 h after cutting. Only values for anthocyanin production given inside the bars. Incubation media: water and 1% sucrose. Temperature: 22 to 23°C. DP: plants kept in darkness for 72 h before cutting the disks.

Table IV. *Effects of Monuron and Sucrose on Anthocyanin Production in Cabbage Leaf Disks and Spirodela*

Temperature: 22 to 23°C. Light treament: 48 h WL, started immediately after cutting the leaf disks and after transfer of *Spirodela* from flasks to Petri dishes. Incubation media: water for cabbage leaf disks and Hutner's medium for *Spirodela*, with or without monuron and sucrose, as indicated.

Medium Composition		Anthocyanin Production	
Sucrose	Monuron	Cabbage leaf disks	*Spirodela*
mg/ml	*μM*	A_a	
0	0	0.719	1.115
10	0	1.240	2.070
0	10	0.043	0.780
10	10	0.595	1.561
0	100	0.004	0.223
10	100	0.320	1.175

[a] Absorbance values of extracts corrected by subtraction of values of dark controls.

response to continuous irradiations might reflect a decrease in the rate of anthocyanin production limited only to the later portion of prolonged light treatments. Experiments are in progress to clarify this point.

In cabbage leaf disks, exposures to light pretreatments bring about an increase of the HIR response (Fig. 6). Anthocyanin production in the cabbage leaf disks depends upon photosynthetic activity (Table IV). Exposure to light pretreatments might result in a buildup of precursors for anthocyanin production, a suggestion supported by the observation that sucrose may substitute for the light pretreatments (Fig. 6). The spectral sensitivity of anthocyanin production in cabbage leaf disks and *Spirodela* (Figs. 6, 7) might reflect the requirement for photosynthetic activity, at least in part. Further experiments are needed to clarify this point.

Light attenuation by Chl screening may be expected to have different effects on the state of the photoreceptors under irradiation at different wavelengths for several reasons: (a) differences in Chl accumulation under irradiations at different wavelengths; (b) differences in Chl absorption at different wavelengths; (c) the rate of phytochrome photoconversion and Pfr/Ptot values under BL (which might also be affected by an interaction with flavins, 25) are more dependent on fluence rate than under R (12, 28); (d) Chl screening would affect only the operational state of phytochrome under long wavelength irradiation and the state of both cryptochrome and phytochrome under short wavelength irradiation. Possibly, the effects of NF and STM on the spectral sensitivity in both dark-grown and light-pretreated systems (Figs. 1, 2, 4, 5, 7) may be justified as a consequence of the decrease in Chl. However, the decrease in Chl may not be the only factor involved in the observed changes. It has been shown that, in darkness, NF stimulates germination of light-requiring seeds (33) and leaf expansion (24) and inhibits hypocotyl elongation (24), partly mimicking the phytochrome-mediated action of light on these responses. In studies of the effects of light pretreatments on the spectral sensitivity of the inhibition of hypocotyl elongation (1, 2), NF was used to produce Chl-poor, light-grown seedlings, but no data for the effects of NF in dark-grown seedlings were given. Perhaps the use of NF and other inhibitors to produce Chl-poor seedlings for photomorphogenic studies in light-grown systems should be subjected to critical reexamination.

Exposure to light pretreatments results in considerable changes of the spectral sensitivity of anthocyanin production under con-

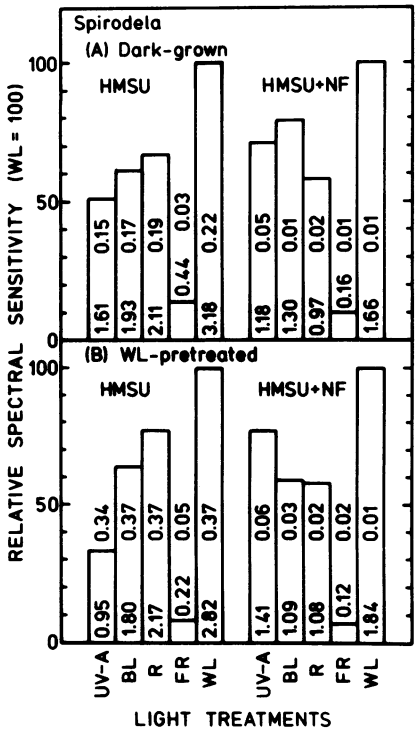

FIG. 7. The spectral sensitivity of anthocyanin production in dark-grown and WL-pretreated *Spirodela*. Continuous UV-A, BL, R, and FR treatments applied from 24 to 72 h after transfer into Petri dishes to *Spirodela* kept in darkness (A) or exposed to WL (B) during the first 24 h after transfer. Temperature: 22 to 23°C. Incubation media: 1% sucrose in Hutner's medium (HMSU) and 10 μM NF in HMSU (HMSU + NF). Anthocyanin production in dark-grown *Spirodela*, incubated in Hutner's medium without sucrose and exposed to light from 24 to 72 h after transfer, was 0.21 (UV-A), 0.51 (BL), 1.08 (R), and 0.00 (FR).

Table V. *Effect of Light on the Phytochrome Content of Cabbage, Rye, Tomato, and Spirodela*

A Ratiospect with measuring beams at 728 and 801 nm was used for the spectrophotometric measurements of phytochrome, made at the end of the light treatments. One phytochrome unit = 0.001 Δ (ΔA). ND, not detectable.

Light Treatments	Phytochrome Content at Following Times (h)[a]				
	Cabbage		Rye	Tomato	*Spirodela*
	48	72	72	96	24
			units		
Dark[b]	14.7	16.3	35.1	2.4	3.6
24 h UV-A, BL, R	1.0	1.0			
24 h FR	5.9	5.7			
8 h WL[b]			ND		
24 h WL[b]	1.0	1.0	ND	ND	ND
2 × 5R[c]	5.8	5.7			
4 × 15R[c]	2.9	3.1			

[a] In hours from sowing for cabbage, rye, and tomato; in hours after transfer to Petri dishes for *Spirodela*.

[b] The results of spectrophotometric measurements were the same for materials grown with or without NF and STM.

[c] 2 × 5 R = 2 × (5 min R + 55 min D) from 46 to 48 or 70 to 72 h after sowing. 4 × 15R = 4 × (15 min R + 345 min D) from 24 to 48 or 48 to 72 h after sowing.

tinuous irradiation (Figs. 1–7), generally confirming observations in other response-system combinations (1, 3, 9, 16), but also showing large qualitative and quantitative differences in the extent of the changes. For example, the spectral sensitivities of anthocyanin production in dark-grown cabbage seedlings (Table II; Figs. 1, 2) and inhibition of hypocotyl elongation in dark-grown mustard seedlings (1, 9) are very similar. The spectral sensitivity of the two responses in light-pretreated seedlings is quite different. In mustard, there is a strong, parallel reduction of the response to BL and FR and almost no effect on the response to R (1, 9). In cabbage, the response to R can be reduced as much or more than the response to BL and FR (Figs. 1, 2). Research is in progress to determine if these differences are response related or species specific.

One common feature of the effects of light pretreatments on the spectral sensitivity of anthocyanin production (Fig. 1–7) is the difference in the extent of the changes in the photosensitivity of the response to long (R and FR) and short (UV and BL) wavelength irradiations. Possibly, as a consequence of the decrease of phytochrome in light-pretreated systems (Table V), the optimal conditions (insofar as the operational state of phytochrome is concerned) for anthocyanin production may be shifted from one region to another. A second interpretation is based on the assumption that two photoreceptors might be involved in the photoregulation of anthocyanin production (phytochrome in the R-FR region; phytochrome and/or cryptochrome in the UV-BL region). The different effects on the sensitivity of the response to short and long wavelength irradiation might reflect different effects of the light pretreatments on the operational state of the two photoreceptors. In view of recent observations (6, 23), the second interpretation may be more plausible than the first one, but further research is needed to clarify this point.

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