

***Short Communication***

# Light, Temperature, and Anthocyanin Production<sup>1</sup>

Received for publication February 28, 1986

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## ABSTRACT

Temperature affects the total amount, the time course, and the red/far-red effectiveness ratio of light-dependent anthocyanin production in *Brassica oleracea* L. seedlings. Some of the effects of temperature on anthocyanin production in cabbage are in agreement with the predictions of a model proposed by JK Wall and CB Johnson (1983 *Planta* 159: 387-397) for the effects of temperature on the state of phytochrome and on the expression of phytochrome-mediated high irradiance responses, but others are not. The lack of a complete agreement between experimental results and model predictions might be due to factors related to the experimental system used or to limitations of the model or both.

Light-dependent anthocyanin production in young seedlings displays properties typical of HIR<sup>2</sup> plant photomorphogenic responses (8, 9). Phytochrome is involved in the photoregulation of the HIR (9).

Several theoretical models of phytochrome dynamics (2-4, 7, 15, 19) have been developed to explain the HIR in terms of the interaction between photochemical (photoconversion) and non-photochemical (synthesis, destruction, dark reversion) reactions of phytochrome. In the range of temperatures from 0 to 30°C, phytochrome photoconversion is temperature-independent and the dark reactions are temperature-dependent: for example, the  $Q_{10}$  for phytochrome destruction is about 3 between 5 and 25°C (13). Recently, Wall *et al.* (20) have proposed a model for the effect of temperature on the state of phytochrome under prolonged irradiation, and have suggested that, at low temperatures, the HIR responses might show a reduced peak of action in the FR region and that the establishment of the HIR peak of action in the FR region might be considerably delayed and therefore unlikely to be observed within the time scale of most physiological experiments.

The purpose of this study was to test if the suggestions of Wall *et al.* (20) are valid for anthocyanin production in cabbage seedlings.

## MATERIALS AND METHODS

Seeds of cabbage (*Brassica oleracea* L., Burpee Red Acre) were sown in Petri dishes on filter paper moistened with distilled H<sub>2</sub>O.

For the continuous irradiation treatments (Fig. 1), the seedlings were grown in darkness for 72 h at 14 or 25°C and then exposed

to R or FR from 12 to 72 h at 14 or 25°C. For the cyclic light treatments (Table I), seedlings were grown in darkness for 72 h at 25°C and then exposed daily for 3 d to 8 or 16 h of R or FR at constant or alternating temperatures, 14 and 25°C, during the light and dark phases of the cycle. The R and FR sources used for the light treatments have been described previously (10).

The pigments were extracted by shaking the seedlings for 48 h at 4°C in acidic (1% HCl, w/v) methanol. The absorbance of the extracts, clarified by filtration, was measured at 530 and 657 nm. The formula  $A_{530} - 0.25 A_{657}$  was used to compensate for the contribution of Chl and its degradation products to the absorption at 530 nm, as described previously (10). The values reported in Figure 1 and Table I represent the means of 8 or 16 replicates in two independent experiments; 16 replicates were used for the treatments resulting in low anthocyanin production ( $A$  in light -  $A$  in dark = 0.3 or less). The standard errors were about 3 to 5% of the values of the means.

## RESULTS AND DISCUSSION

The results (Fig. 1; Table I) show that the value of the FR/R effectiveness ratio increases with increasing duration of the light treatments. This finding is in agreement with the prediction of theoretical models for phytochrome action in the photoregulation of the HIR (2-4, 15) and confirms previous experimental results (8).

Under continuous light treatments (Fig. 1), the seedlings maintained at constant low temperature (Fig. 1D) produce considerably less anthocyanin than those maintained under the other temperature combinations. This is probably a consequence of the fact that the growth of the seedlings is delayed at low temperature and a longer time is required to reach system competence for anthocyanin production. The differences in total anthocyanin production among the four temperature combination treatments decrease with increasing duration of the light treatments. The values of the FR/R effectiveness ratio are the same for all temperature combinations in the 12 h light treatments. For the 24 to 72 h light treatments, the value of the FR/R effectiveness ratio is somewhat higher for the seedlings maintained at a constant low temperature (Fig. 1D) than for all other temperature combinations. For the 24 h irradiations, the lowest value of the FR/R effectiveness ratio is found for the "72 h D at 25°C + 24 h L at 14°C" treatment (Fig. 1B). The effects of temperature on the values of the FR/R effectiveness ratio are not completely consistent with the predictions of the model by Wall *et al.* (20), according to which the increase in the value of the FR/R effectiveness ratio with increasing duration of the continuous light treatments should be delayed at low temperatures.

The experimental results that are consistent with the suggestion of Wall *et al.* are those obtained with the cyclic treatments X (Table I). The FR/R effectiveness ratio for anthocyanin produc-

<sup>1</sup> Partially supported by National Science Foundation grants PCM-8008747 and DMB-8421187 to A. L. M.

<sup>2</sup> Abbreviations: HIR, high irradiance reaction; FR, far red; R, red; D, dark; L, light.

Table 1. Effect of Temperature on Anthocyanin Production under Cyclic R and FR Light Treatments  
 Schedule of light treatments (times from sowing): treatment X: 3 d D at 25°C + 3 × (8 h L at 25 or 14°C + 16 h D at 25 or 14°C); treatment Y: 3 d D at 35°C + 3 × (16 h L at 25 or 14°C + 8 h D at 25 or 14°C).

	Temperature during Light (TL) and Dark (TD) Period of Cyclic Light Treatment		Effectiveness (FR/R)	Anthocyanin		Effectiveness (FR/R)
	TL	TD		R	FR	
	°C			$A_{530}^a$		
X1.	25	25	(0.536)	0.426	0.455	1.07
X2.	25	14	(0.484)	0.709	0.696	0.98
X3.	14	25	(0.461)	0.746	0.561	0.75
X4.	14	14	(0.420)	0.628	0.508	0.81
Y1.	25	25	(0.541)	0.790	1.019	1.29
Y2.	25	14	(0.495)	0.835	1.080	1.29
Y3.	14	25	(0.498)	0.801	1.153	1.44
Y4.	14	14	(0.410)	0.605	0.862	1.42

<sup>a</sup> A for R and FR treatments corrected by subtraction of absorbance values of dark controls, given in parentheses.

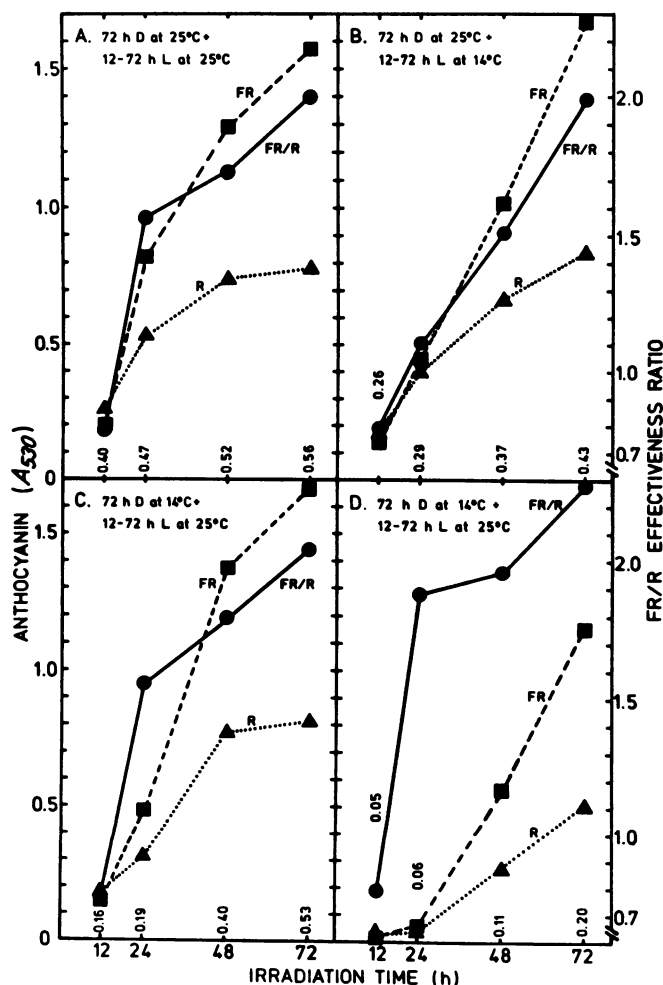


FIG. 1. Effect of temperature on anthocyanin production in cabbage seedlings exposed to continuous irradiation. The absorbance values were corrected by subtraction of the values of the dark controls, given in the figure at the corresponding irradiation times.

tion in the seedlings exposed daily to 8 h irradiations is lower when the temperature during irradiation is 14°C than when it is 25°C. However, in the seedlings exposed daily to 16 h irradiations, the value of the FR/R effectiveness ratio is higher when the temperature during irradiation is 14°C than when it is 25°C. In both cyclic light treatments, temperature differences during the daily irradiation period are more important than temperature differences during the daily dark period in determining the value of the FR/R effectiveness ratio, especially under the 16 h L + 8 h D treatments.

The results show that there is an effect of temperature on light-dependent, HIR anthocyanin production. However, the effect of temperature on the values of the FR/R effectiveness ratio are not completely consistent with the suggestions made by Wall *et al.* (20). At least two groups of factors might be responsible for the lack of a complete agreement between the predictions of the model and our experimental results.

First, the model was developed using data for the phytochrome system of mustard seedlings and our results have been obtained in cabbage seedlings. Previous studies (11) have shown considerable similarities for the effects of light on anthocyanin production in mustard and cabbage seedling. However, the differences observed in the state of the phytochrome system under continuous irradiation at low and high temperature between two batches of mustard seedlings (20) suggest that variations between biological system might be an important factor for the lack of complete agreement between model prediction and experimental results.

Second, the model takes into consideration only the effects of temperature on the state of phytochrome. The final expression of the photoresponse is the results of a sequence of events in which the state of phytochrome is only the first step. Differences in temperature sensitivity of one or more of the biochemical reactions (deamination of phenylalanine, hydroxylation of cinnamic acid, etc.) involved in anthocyanin production might modify the final expression of the response, independently of the state of phytochrome. In addition, the model for the effects of temperature on the state of the phytochrome system *in vivo* (20), as well as other models for the mechanism of action of phytochrome in the photoregulation of the HIR (2-4, 7, 15, 19) do not take into account the light-dependent variations of phyto-

chrome parameters. The rate of *de novo* phytochrome synthesis decreases after exposure to light (14). Light produces changes in the ratio between pools of phytochrome with different destruction kinetics (labile and stable phytochrome [6]), different immunochemical and spectral properties (etiolated and green phytochrome [16, 18]), and different activity (bulk and active phytochrome [5]). Perhaps, one of the reasons for the lack of a complete agreement between theoretical models of phytochrome dynamics and experimentally observed results is that the models do not take into account all the known variables. In addition, and no less important, the values of some of the phytochrome parameters (*e.g.* rates of destruction and dark-reversion) used in the development of the models are based on the results of spectrophotometric assays *in vivo*. It is known that spectrophotometric assays of phytochrome *in vivo* have low sensitivity and are adversely affected by various factors (12). Immunological techniques with a much higher sensitivity than that of the spectrophotometric assay are available to measure absolute amounts of phytochrome (12). Monoclonal antibodies with different affinities toward Pr and Pfr are being developed (1, 17). The development of satisfactory models for the action of phytochrome in the photoregulation of the HIR requires both a larger base of comparative data on the characteristics of the HIR and a better understanding of the state of phytochrome *in vivo* obtained through the use of assays more sensitive than the spectrophotometric one.

In conclusion, temperature affects light-dependent anthocyanin production in cabbage seedlings, but it is difficult to determine if the effect is mainly a consequence of the effects of temperature on the state of the phytochrome system.

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