Communication

Anthocyanin Production in Chl-Rich and Chl-Poor Seedlings

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ABSTRACT

Screening by chlorophyll (Chl) affects photoconversion rates and photoequilibrium ratios of phytochrome in vivo and may cause distortion of the action spectra of photomorphogenesis (N Kazarinova-Fukhamsky, M Seyfried, E Schäfer 1985 Photochem Photobiol 41: 689–702). Inhibitors that reduce the Chl content of seedlings are sometimes used in photomorphogenesis research to decrease the effects of Chl screening on the state of phytochrome in vivo. Streptomycin is one of the inhibitors that can be used for this purpose. The effects of streptomycin on phytochrome-mediated anthocyanin accumulation in young seedlings are significantly different in closely related systems. The use of ‘Chl-bleachers’ in photomorphogenesis studies may produce undesirable side effects. At the level of the expression of a photoregulated response, the effects of differences in the state of phytochrome between water-grown Chl-rich and inhibitor-treated Chl-poor seedlings may be difficult to evaluate because they may be masked by the effects of the inhibitor on the response.

Phytochrome is one of the photoreceptors involved in the photoregulation of HIR2 anthocyanin production in young seedlings (8). Large amounts of Chl are formed during the prolonged irradiations required to elicit maximum anthocyanin production (9, 10). As a consequence of light attenuation due to screening by Chl, the photoconversion and cycling rates and the photoequilibrium ratios of phytochrome in vivo are different from those predicted on the basis of the spectral parameters of purified phytochrome and may cause distortions in the action spectra of photomorphogenesis (6). During the course of a prolonged irradiation, as the Chl content of the tissue increases, it is reasonable to expect larger and larger deviations from the predicted values of the state of phytochrome. Chl accumulation and light absorption are higher in R than in FR, and the screening effects on the state of phytochrome in vivo are more pronounced under R than under FR (6). Thus, one may ask: how much does the presence of Chl affect the relative efficiency of R and FR in HIR responses?

The purpose of this preliminary study was to determine if a reduction of the Chl content would bring about significant changes in the relative effectiveness of R and FR on anthocyanin production in young seedlings. Inhibitors that reduce the Chl content of seedlings have been used in photomorphogenesis research to decrease the effects of Chl screening on the state of phytochrome and to allow spectrophotometric assays of phytochrome in vivo in light-grown seedlings (1, 4, 5). We also wanted to check if a specific inhibitor action on the photosresponse studied might affect the interpretation of the results in terms of the expected differences between the states of phytochrome in Chl-rich and Chl-poor seedlings (6). We measured anthocyanin production under R, FR, and simultaneous R + FR irradiations in young, water-grown and STM-treated cabbage seedlings. Streptomycin inhibits chloroplast development and Chl synthesis and enhances anthocyanin production in young cabbage seedlings (9). We used STM instead of Norflurazon, a more commonly used ‘Chl-bleacher’ (1, 4, 5), because the differences in the Chl levels of seedlings exposed to prolonged irradiations with different spectral regions are less pronounced in STM-treated than in Norflurazon-treated seedlings (7).

MATERIALS AND METHODS

Plant Material. Seeds of two lots (D and F) of cabbage (Brassica oleracea L., cv Red Acre; W. A. Burpee Co.) were sown in Petri dishes (30 seeds/dish) on filter paper (Eaton-Dikeman No. 923-70) moistened with distilled water or with a solution of STM (200 μg/ml). This concentration of STM was chosen on the basis of dose-response curves; it produces about 90% reduction in Chl content without any effect on respiration (9; and additional data not shown). The dishes were wrapped in black cloth and incubated in darkness for 72 h at 20 to 21°C.

Light Treatments. The light treatments were started 72 h after sowing and were given in two growth chambers (Percival model E-57) containing both the R and FR sources. The FR source consisted of four fluorescent lamps (F36T12/CW/HO) and a red Plexiglas filter (Red-2444, Rohm & Haas); the photon flux between 610 and 680 nm was 6.4 μE m⁻² s⁻¹. The FR source consisted of twelve 60 W incandescent lamps and black V-58015, also known as FRF-700 (Cristal-X Co., Darby, PA), and Red-2444 Plexiglas filters; the photon fluxes between 700 and 800 nm were 4.5 (1FR), 12.5 (mFR), and 24.9 (hFR) μE m⁻² s⁻¹. The measured values of the Prf/P photoequilibrium ratios in etiolated, water-grown and STM-treated seedlings of the two strains D and F were: 0.74–0.78 (R), 0.02–0.03 (FR), 0.69–0.73 (R + 1FR), 0.56–0.59 (R + mFR), and 0.44–0.48 (R + hFR). An Asco Ratiomax dual-wavelength spectrophotometer was used for the in vivo phytochrome assay.

Extraction and Measurement of Anthocyanin. Lots of 30 seedlings each were extracted with 20 ml of acidified (1% HCl, w/v) methanol for 2 d at 3 to 5°C with occasional shaking. The extracts were clarified by filtration; their absorbance was measured at 530 nm (peak of absorption of anthocyanin) and 657 nm (peak of absorption of Chl degradation products). The formula A₅₃₀ −

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2 Abbreviations: HIR, high irradiance reaction; DC, dark control; FR, far red; (l, m, h) FR, low, medium, and high FR; P, Pr + Pfr; R, red; STM, streptomycin.
0.25A_{657} was used to compensate for the absorption of Chl degradation products at 530 nm (7). The corrected A_{650} values provide an estimate of Chl content; the A_{657} values provide an estimate of Chl content. The values reported in Figure 1 are the means of 14 to 18 replicates in two independent experiments.

RESULTS AND DISCUSSION

Anthocyanin production in water-grown seedlings (Fig. 1) is much higher in cabbage D than in cabbage F under all light treatments used. There are also differences in the relative efficiency of the light treatments: taking anthocyanin production under hFR as 100, the relative efficiencies of the other light treatments are significantly higher in cabbage D than in cabbage F. Chl formation is about 35% higher in cabbage F (under R, the extracts' A_{657} values are 0.24 in strain D and 0.32 in strain F) and is about the same under R and R+FR simultaneous irradiation treatments.

In STM-treated seedlings (Fig. 1), there is a reduction in Chl formation of about 85 to 90% under R, and a considerable enhancement of anthocyanin production, confirming previous reports (9). The cause of the enhancing effects of STM and other antibiotics (9, 13) on anthocyanin production has not been fully established. At the signal level, the inhibition of chloroplast development by STM might simulate a condition calling for increased production of pigments with a photoprotective function. This suggestion is based on the hypothesis that light-dependent anthocyanin production in young seedlings might represent an adaptive photoprotective mechanism, involved in the protection of the photosynthetic apparatus during its early stages of development until a certain amount of Chl has been accumulated (2).

At the metabolic level, the inhibition of chloroplast development might result in an increase in the level of precursors (e.g., phenylalanine) available for anthocyanin production (9, 13). This suggestion is consistent with the observation that the addition of phenylalanine enhances anthocyanin production in young seedlings (3).

In STM-treated seedlings, the differences in anthocyanin production between the two strains are reduced and, for the mFR and hFR treatments, eliminated (Fig. 1; Table 1, D/F ratios). Apparently, in STM-treated seedlings, the potential for anthocyanin production under medium and high FR irradiances is the same in the two strains. For the other light treatments, R, IFR, R+FR, the differences between the two strains, although reduced, are still significant (Table 1, D/F columns). The enhancing effect of STM on anthocyanin production is more pronounced in strain F than in strain D (Table 1, STM/Water columns).

In cabbage D, the differences in the relative efficiency of the light treatments between water-grown and STM-treated seedlings (Fig. 1) are not statistically significant. The differences in the values of the anthocyanin production ratios between STM-treated and water-grown seedlings (Table 1, cabbage D, STM/Water column) are also not statistically significant. The STM-induced enhancement of anthocyanin production in cabbage D is apparently independent of light quality and quantity. This suggests that the differences, if any, in the state of phytochrome between water-grown and STM-treated seedlings do not play a major role, or the role played is masked by the action of STM on the response.

In cabbage F, the differences in the relative efficiency of the light treatments between water-grown and STM-treated seedlings are rather large (Fig. 1). The increase in the relative efficiency of R in STM-treated seedlings might reflect a difference in the state of phytochrome between Chl-poor and Chl-rich seedlings. However, the situation is rather complex, as shown by the data for the values of the anthocyanin production ratios between STM-treated and water-grown seedlings (Table 1, Cabbage F, STM/Water column). The STM/Water anthocyanin production ratios vary from a minimum of 1.87 (R+hFR) to a maximum of 3.81 (R+IFR), a 104% difference. The STM/Water ratios for R, IFR, and R + IFR (12% difference between minimum and maximum values) are not significantly different. The STM/Water ratios for mFR and R+mFR (14% difference) are not significantly different and are significantly lower than the previous ones. The STM/Water ratios for hFR and R+FR are the same and significantly lower than those for the other treatments. The data suggest that the STM-induced enhancement of anthocyanin production in cabbage F is independent of light quality and inversely dependent on light quantity.

CONCLUSIONS

Streptomycin enhances anthocyanin production and inhibits Chl formation. The differences in anthocyanin production be-

**Fig. 1.** Anthocyanin production in water-grown and STM-treated seedlings of cabbage strains D and F, exposed to light from 72 to 120 h after sowing. Anthocyanin, open bars; Chl, thin, black bars. Absorbance values corrected by subtraction of DC values (anthocyanin: 0.271 (D, H2O), 0.384 (D, STM), 0.042 (F, H2O), 0.048 (F, STM); Chl: 0.022 (D, H2O), 0.022 (D, STM), 0.020 (F, H2O), 0.019 (F, STM). Numbers in open bars are relative (hFR = 100) anthocyanin production; l, m, and h refer to the FR photon fluxes used in the FR and R+FR simultaneous irradiation treatments. Photon fluxes (μE m⁻² s⁻¹): R, 6.4; IFR, 2.4; mFR, 12.5; hFR, 24.9.
between water-grown Chl-rich and STM-treated Chl-poor seedlings might be due to differences in the state of phytochrome in vivo, in the availability of precursors for anthocyanin production, and in the system's response to differences in the state of development of the photosynthetic apparatus. With the data from this study it is not possible to determine the relative contributions of these factors. The shifts in the wavelength of the action peak in the R region, observed in action spectroscopy studies (1), are probably the only cases in which differences between the responses of Chl-rich and Chl-poor seedlings can be attributed, with some confidence, to differences in the state of phytochrome caused by differences in the degree of screening by Chl.

Inhibitors that reduce the Chl content of seedlings are sometimes used in photomorphogenesis research to decrease the effects of Chl screening on the state of phytochrome in vivo (1, 4, 5). However, these inhibitors should not be used in physiological experiments without previous studies of their effects on the response/system combination: differences in the effects of the inhibitors can be quite large even for the same response in closely related systems, as shown in this report. Even Norflurazon, a more commonly used Chl-bleacher, may create problems because it decreases phytochrome accumulation (12) and has a direct effect (11, 14, 15) on several photomorphogenic responses.

**LITERATURE CITED**