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UV Actions in Plant Photomorphogenesis

—Induction and Amplification of Anthocyanin Synthesis in Broom Sorghum —

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In broom sorghum, *Sorghum bicolor* Moench, UV causes anthocyanin synthesis having action peaks in UVA and UVB regions. We previously reported that UV induces anthocyanin synthesis through UVB photoreceptor and phytochrome activated by UV. Furthermore, UVA and UVB amplify phytochrome-induced anthocyanin synthesis (PIAS). Our action-spectroscopic research indicated that a UV-receptor for amplification of PIAS is likely to be the same or same type of UVB photoreceptor for induction of anthocyanin synthesis. UVA-amplification of PIAS can be explained by the action of a cryptic red light signal (CRS), an amplification factor for PIAS produced by a distinct phytochrome-species activated by UVA. We suggest that UVA photoreceptors are not involved in anthocyanin synthesis in the broom sorghum.

Key words: action-spectrum, anthocyanin, cryptic red-light signal, UVB photoreceptor, phytochrome, *Sorghum*

Introduction

Ultraviolet light (UV) radiation not only causes damage but also induces photomorphogenic responses in plants. The biosynthesis of anthocyanin has been used as a representative indicator of light action. Anthocyanin is independently induced by red-light (R) and UV through phytochrome (phy) and UVB photoreceptor in broom sorghum (*Sorghum bicolor* Moench), respectively [1]. Recently, we indicated dual effects of R. A cryptic red-light signal (CRS) produced by a phy-species, which is different from the one for anthocyanin induction, amplifies the inductive response of phy [2, 3, 4]. Furthermore, we showed that the UV-range of the light wavelength also strongly amplifies the phy-induced anthocyanin synthesis [2], implying an unknown UV-photoreceptor(s) is involved in the amplification of phy-induced anthocyanin synthesis other than CRS produced by UV-activated phytochrome.

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Abbreviations CRS, cryptic red-light signal; FR, far-red light; Pfr, far-red light-absorbing form of phytochrome; phy, phytochrome; PIAS, phy-induced anthocyanin synthesis; Pr, red-light absorbing form of phy; R, red light; UVA, 320-400 nm UV light; UVB, 280-320 nm UV light.

The coaction between phy and specially cryptochromes, the blue and UVA photoreceptors, is well known [5]. Boccalandro et al. [6] indicated UVB photoreceptor whose nature is still obscure enhances action of phytochrome B by use of photoreceptor-deficient mutants.

In this report, we attempted to make clear the action-spectroscopic characteristics of UV photoreceptors by comparing the action spectrum for amplification of phy-induced anthocyanin synthesis (PIAS) after excluding the phy-induced amplification factor (CRS) to the ones for the induction and for the inhibition [7] of anthocyanin synthesis based on our previous data.

Materials and methods

Plant materials. Broom sorghum, *sorghum bicolor* Moench., cvs. Acme Broomcorn and Sekishokuzairai Fukuyama were used. Seeds were soaked for 24 h at 24 °C. For determining action spectra 80-120 seeds were sown in a plastic "Seedling Case" [15 (width) x 5 (depth) x 10 (height) cm^3]. Seedlings were grown to 9-10 cm in height in absolute darkness at 20 °C or 24 °C. After irradiation, seedlings were kept in the dark at 24 °C for 24 h until

harvested. All handling of seedlings except for seed sowing and harvesting were made under dim green safelight.

Light sources. Monochromatic lights were supplied from the Okazaki Large Spectrograph. Red light (R) source for R-pulses was light-emitting diode (LED) (λ_{max}, 672 nm; half-bandwidth, 32 nm) irradiator or R-fluorescent tubes (λ_{max}, 657 nm). Far-red light (FR) source for FR-pulses was LED (λ_{max}, 752 nm; half-bandwidth, 36 nm) irradiator or FR-fluorescent tubes (λ_{max}, 759 nm).

Anthocyanin determination. A segment of approximately 5 cm was excised from the pigmented or corresponding non-pigmented (non-irradiated control) part of the first internode so as to include total anthocyanin, and 20 segments per sample were extracted with 6 ml of 1% (v/v) hydrochloric acid in methanol. The differential absorbance, A_{528}-A_{650}, of the extract was measured as an index of anthocyanin synthesis [8].

Results and Discussion

Action spectra for induction of anthocyanin synthesis. Dark-grown seedlings of sorghum were exposed to various monochromatic lights (λ) of the Okazaki Large Spectrograph and the inductive effects of λ on anthocyanin synthesis were compared [1]. A curve of solid line in Fig. 1 shows the action spectrum having three peaks in red, UVA and UVB regions of wavelength. To know the involvement of phy, the action spectrum (dotted line in Fig. 1) for anthocyanin induction by the irradiation of λ-FR (monochromatic light λ immediately followed by phy-saturated FR) was also determined. The action spectrum of λ-FR had only one peak at around 290 nm. Both spectra indicated that phy and UVB photoreceptor induce independently anthocyanin synthesis in sorghum. [1].

Action spectrum for amplification of phy-induced anthocyanin synthesis.

R-effects: Although phy-induced anthocyanin synthesis is FR-reversible, a sequential pulse-irradiation of R-FR-R induced much more anthocyanin than an FR-R irradiation, leading to an assumption of a non-reversible R-action amplifies phy-induced anthocyanin synthesis (PIAS). To clarify the dual effects of R, which might be caused by a multiplicative coaction of unknown photoreceptor(s) and phy, we investigated amplification-effects of λ on PIAS by means of determination of an action spectrum for anthocyanin synthesis caused by a sequential pulse-irradiation λ-FR-R (Fig. 2) [3]. In λ-FR-R irradiation, an FR-pulse after λ reverses Pfr induced by λ and the last R-pulse fixed in fluence rate and length provides a fixed amount of Pfr or Pfr/P ratio for anthocyanin induction. The obtained action spectrum was similar to the absorption spectrum of Pr, having a main peak at 657 nm and a subpeak at 378 nm, leading to an assumption of phy-action which escapes rapidly from FR-reversion.

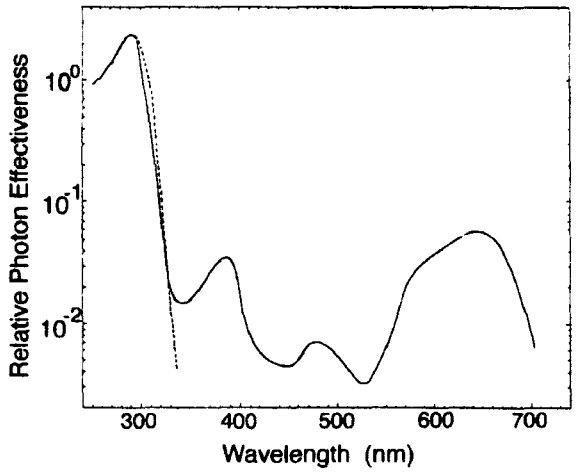

Figure 1. Action spectra for induction of anthocyanin synthesis in broom sorghum. Relative photon effectiveness was obtained from fluence-response curves of anthocyanin synthesis induced by λ (solid line) or by λ-FR (dotted line). (Yatsuhashi et al. 1982 [1])

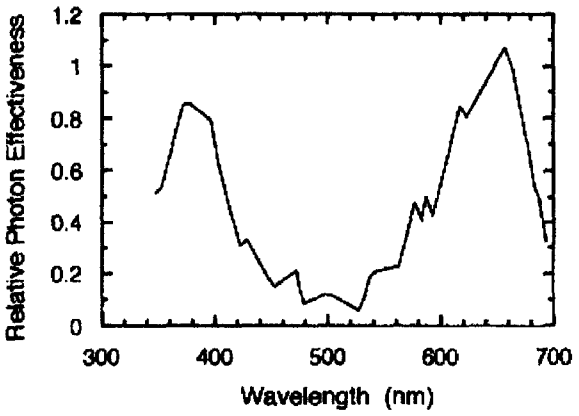

Figure 2. Action spectrum for amplification of phy-induced anthocyanin synthesis in broom sorghum. Relative photon effectiveness was obtained from fluence-response curves of anthocyanin synthesis induced by λ-FR-R. (Shichijo et al. 1999 [3])

Rapid phy-action of amplification of PIAS was also tested. In R-FR-R irradiation, a 0.2 s-pulse of the first R did not amplify the anthocyanin synthesis induced by the last R, indicating that a factor induced by a rapid response of Pfr of a distinct phy-species amplifies PIAS. We referred to this amplification factor as cryptic red-light signal (CRS).

Effects of UV: Since almost all wavelengths convert Pr into Pfr, the amplification effects of UV on PIAS cannot be assessed without excluding phy-amplification effect. Next, we determined a UV-action spectrum for amplification of PIAS by means of the simultaneous irradiation with λ and phy-saturating R (denoted by R/λ). That is R/λ-FR-R irradiation. The anthocyanin synthesis after subtracting UV-induced anthocyanin synthesis by λ-FR (see the first column of this section) from that by R/λ-FR-R was compared to the control of FR-R irradiation. Our data (detail of the results will appear in now-preparing paper) showed that an action spectrum peaked at 285 nm and sharply declined towards 390 nm, having no sub-peak in the UVA region at all. It agreed well with the action spectrum for the induction of anthocyanin (dotted line in Fig. 1) not only in peak wavelength but also in relative photon fluence effectiveness, which varied over a wide range as great as 10^4 order of magnitude and extended up to 390 nm.

These results indicated that UV-photoreceptors for anthocyanin induction and for the amplification of PIAS in broom sorghum are likely to be the same or same type of UVB photoreceptor. UVA-amplification of PIAS (Fig. 2) can be explained by CRS produced by phytochrome activated by UVA. We suggest that UVA photoreceptors are not involved in anthocyanin synthesis in broom sorghum.

Action spectrum for inhibition of anthocyanin synthesis. For an action spectrum for inhibition (Fig. 3), anthocynain synthesis was induced by irradiation of sub-optimal dose with combined UV and R, followed by another pulse irradiation with λ from 257 to 307 nm at various fluence rate [7]. The highest inhibition was shown at 257 nm and declined towards 297 nm. No inhibition was observed at longer wavelengths. It was concluded that UVB photoreceptor proposed for induction of anthocyanin synthesis and amplification of PIAS was different from that for inhibition of anthocyanin synthesis.

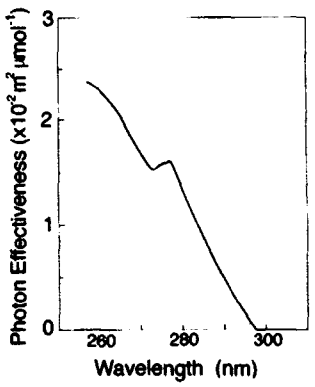

Figure 3. Action spectrum for inhibition of anthocyanin synthesis in broom sorghum. Photon effectiveness was obtained from fluence-response curves of anthocyanin synthesis induced by the irradiation of anthocyanin inductive light followed by λ. (Hashimoto et al. 1991 [7])

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