Plant Physiol. (1994) 105: 1059–1066

Ultraviolet-B-Responsive Anthocyanin Production in a Rice Cultivar Is Associated with a Specific Phase of Phenylalanine Ammonia Lyase Biosynthesis[1]

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Seedlings of 17 rice (*Oryza sativa* L.) cultivars were classified on the basis of anthocyanin pigmentation into three groups: an acyanic group with 9 cultivars, a moderately cyanic group with 5 cultivars, and a cyanic group with 3 cultivars. Seedlings of the cyanic group were deep purple in color, possessing copious amounts of anthocyanin in shoots. Sunlight (SL)-mediated anthocyanin and phenylalanine ammonia lyase (PAL) induction in a cyanic cultivar, purple puttu, was compared with an acyanic cultivar, black puttu. A brief exposure of dark-grown purple puttu seedlings to SL induced anthocyanin formation during a subsequent dark period with a peak at 24 h. The magnitude of SL-mediated anthocyanin induction is age dependent, the 4-d-old seedlings being the most responsive to SL. The anthocyanin induction in purple puttu seedlings is mediated exclusively by the ultraviolet-B (UV-B) component of SL. The SL-triggered anthocyanin induction was reduced by about 30% by a terminal far-red light pulse and was restored by a red light pulse, indicating the role of phytochrome in modulation of anthocyanin level. The SL-mediated induction of PAL showed two peaks, one at 4 h and the other at 12 h. Whereas the first PAL peak (4 h) was induced by phytochrome and was seen in both cultivars, the second PAL peak (12 h) was inducible by UV-B only in the cyanic purple puttu cultivar.

The depletion of the stratospheric ozone layer by chlorofluorocarbon pollution is expected to significantly increase the amount of UV-B (280–320 nm) radiation impinging on the earth's surface in the coming years (Anderson et al., 1991). UV-B radiation is reported to be harmful to plants in many ways, e.g. by reducing plant growth (Teramura, 1983; Tevini and Teramura, 1989; Stapleton, 1992), reducing photosynthetic efficiency and biomass production, and diminishing the ability of crop plants to compete with weeds (Barnes et al., 1990). The imminent increase in the level of UV-B radiation in the biosphere might damage the performance of many crop species. Studies on the deleterious effects of UV-B radiation have been largely confined to temperate plant species and not much is known about its effect on tropical plant species.

In Asia, it has been observed that the thickness of the ozone layer has declined from 1 to 4% over the past 20 years (Watson, 1988). Moreover, in the tropics, the amount of UV-B radiation reaching the earth's surface is significantly higher than that in temperate areas, because the ozone layer above the tropics is thinner and solar angles are maximal (Caldwell et al., 1980). It has been predicted that every 1% decline in ozone layer would lead to a 1% decrease in crop yield (Coohill, 1991). Among tropical crop species, rice (*Oryza sativa* L.) is the foremost food crop of the developing world, providing food to more than three billion people (Singh, 1993). Even though rice is a staple food crop, and the reduction in its yield by UV-B can have adverse consequences in developing countries, there are only a few studies on the effects of UV-B radiation on this species.

In a recent study on 16 rice cultivars from different geographical locations, Teramura et al. (1991) showed that the increased UV-B radiation induces a significant reduction in the total biomass in a number of rice cultivars, accompanied by a reduction in tiller number and photosynthetic capacity of the plants. Prolonged exposure to UV-B light affects plant height, leaf area, dry weight, net assimilation rate, and relative growth rate in some rice cultivars (Dai et al., 1992; Ziska and Teramura, 1992).

Accumulation of the UV-B-absorbing pigments is one of the ways by which plants alleviate the harmful effect of UV-B light (Caldwell et al., 1983; Beggs et al., 1986). The UV-B light-absorbing flavonoids are implicated as protective pigments in shoots and leaves exposed to UV-B light, and their specific location in the epidermal layer protects internal cell layers by attenuating the impinging UV-B radiation at the epidermis (Tevini et al., 1991; Braun and Tevini, 1993). It has been shown that the photoinduced accumulation of these flavonoids is preceded by an induction of several enzymes of phenylpropanoid biosynthetic pathway such as PAL and chalcone synthase of the flavonoid biosynthetic pathway (Hahlbrock and Scheel, 1989; Schmelzer et al., 1989). Information on the presence of UV-absorbing pigments and their role in ameliorating the harmful effect of UV light in rice is scanty. A few investigations have reported that UV-B treatment increases the amount of UV-absorbing pigments in some rice cultivars (Dai et al., 1992; Ziska and Teramura, 1992). Since an increase in the level of UV-absorbing com-

[1] This project is funded by the Rockefeller Foundation, International Rice Biotechnology Program to A.R.R. and the University Grants Commission, New Delhi, grant (3-42/91/SR-II) to R.S. V.S.R. and K.V.G. are recipients of senior research fellowship from the Council of Scientific and Industrial Research, New Delhi.

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Abbreviations: BL, blue light; FR, far-red light; λ, wavelength; PAL, phenylalanine ammonia lyase; RL, red light; SL, sunlight; WG, sunlight obtained after filtering through a window glass.

pounds may offer protection against the potential UV-B damage, information about the presence and regulation of biosynthesis of these pigments is highly valuable in selecting rice cultivars with increased resistance to UV-B radiation.

In the present study, we provided evidence for the UV-B-mediated photoinduction of anthocyanin synthesis in seedlings of a cyanic rice cultivar, purple puttu. We show that UV-B-mediated induction of anthocyanin pigments in purple puttu is associated with a specific phase of PAL biosynthesis.

MATERIALS AND METHODS

Screening of Rice Cultivars

Rice (*Oryza sativa* L.) cultivars of both *indica* and *japonica* groups were obtained from various sources (Table I). The seedlings of these cultivars were grown in the field in nursery beds under continuous irrigation for 20 d during *kharif* (July-November) season in 1992 at Hyderabad, India (longitude 78°4′ E; latitude 17°3′ N; altitude 600 m above mean sea level) in black loam soil. The average day/night temperature was 30.1/20.7°C and RH ranged between 76 and 60%. At midday (12 noon) SL intensity was 2800 to 3300 μmol m^{-2} s^{-1}. Twenty-day-old seedlings were transferred from the nursery bed to the field and grown until maturity. The plants experienced the environmental conditions described above. Both seedlings (7 and 20 d old) and mature plants were examined visually for the presence of anthocyanin pigmentation and were classified into three groups (Table I). The first group was acyanic, with nine cultivars; the second group was moderately cyanic, with five cultivars; and the third group was cyanic, with three cultivars. The cultivars belonging to the cyanic group possessed abundant amounts of anthocyanin in shoots but not in roots. Of the three cyanic cultivars, purple puttu was chosen for further experimental studies, and an acyanic cultivar black puttu was used for comparison. Both cultivars, obtained in the homozygous state in 1989, were further selfed for six generations at Hyderabad. Mature dry seeds of the above cultivars were harvested and used for further experiments.

Table I. *Classification of rice cultivars on the basis of anthocyanin pigmentation*

Field- and laboratory-grown plants were visually screened for anthocyanin pigmentation and classified on the basis of amount of anthocyanins in leaf and shoot.

Group	Cultivar	Source[a]
Acyanic	A5	HU, Sapporo, Japan
	A58	HU, Sapporo, Japan
	A136	HU, Sapporo, Japan
	H113	HU, Sapporo, Japan
	100013	IRRI, Manila, Philippines
	Black Puttu	TNAU, Coimbatore, India
	White Puttu	TNAU, Coimbatore, India
	N22	DRR, Hyderabad, India
	Hamsa	DRR, Hyderabad, India
Moderately Cyanic	H126	HU, Sapporo, Japan
	G962	DRR, Hyderabad, India
	Crossa	DRR, Hyderabad, India
	TN1013	DRR, Hyderabad, India
	100015	IRRI, Manila, Philippines
Cyanic	Purple puttu	TNAU, Coimbatore, India
	G2237	DRR, Hyderabad, India
	R27(P)	DRR, Hyderabad, India

[a] DRR, Directorate of Rice Research; HU, Hokkaido University; IRRI, International Rice Research Institute; TNAU, Tamilnadu Agricultural University.

Growth of Seedlings

The seeds were sterilized in 5% (v/v) sodium hypochlorite for 5 min, extensively washed, and then soaked overnight in sterile, distilled water. The seeds were sown on 25 mL of solid 0.4% (w/v) agar medium in 4-inch-diameter Petri dishes and were kept in black cardboard boxes at 28 ± 1°C in complete darkness. Etiolated seedlings at indicated time points were exposed to light of various wavebands for a short period and were returned to darkness. Unless otherwise indicated, all experiments were carried out with 4-d-old etiolated seedlings exposed to 30 min of SL.

Light Sources

Seedlings were exposed to midday (12–3 PM) SL (2800–3300 μmol m^{-2} s^{-1}) or colored light by opening the boxes in SL. The colored lights were obtained by filtering the SL through an RL cut-off filter (>650 nm, 1600–1900 μmol m^{-2} s^{-1}), an FR cut-off filter (>750 nm, 1300–1500 μmol m^{-2} s^{-1}), or a BL filter (λ_{max} 457 ± 40 nm, 650–750 μmol m^{-2} s^{-1}) using filters obtained from Carolina Biological Supply Co. (Burlington, NC). The SL free of UV-B (>320 nm, Klein, 1979) was obtained by filtering SL through a 4-mm-thick window glass plate (2200–2600 μmol m^{-2} s^{-1}). The seedlings were exposed to various colored lights in a black plastic box covered with a light filter. The temperature of the box was maintained at 28°C by circulating water around the box. The seedlings were also exposed to brief RL and FR pulses in a darkroom using the artificial light sources as described by Manga and Sharma (1988). RL (λ_{max} 650 ± 40 nm, 2.8 μmol m^{-2} s^{-1}) was obtained after filtering the output of two cool-white fluorescent tube lights through two layers of red plexiglass sheeting. FR (λ > 750 nm, 16 μmol m^{-2} s^{-1}) was obtained using a 150-W projector lamp whose output was filtered through an FR filter.

Estimation of Anthocyanins and UV-B-Absorbing Compounds

A pair of seedlings of uniform height were harvested and anthocyanins were extracted in 2 mL of acidified (1% [v/v] HCl) methanol for 24 h at 4°C with occasional shaking. The anthocyanins were quantitatively estimated by measuring the A_{535} (molar extinction coefficient = 31,623) (Harborne, 1967).

For qualitative analysis, aglycones were obtained by hydrolyzing 1 mL of the above extract with an equal volume of 2 N HCl at 100°C for 40 min. The hydrolyzate was extracted with isoamyl alcohol and evaporated to dryness, and the residue was dissolved in 30 μL of acidified methanol. Aliquots of 5 μL were spotted onto cellulose TLC plates and the chromatogram was run using solvent systems consisting of

n-butanol:acetic acid:water (4:1:5, v/v/v), methanol:HCl: water (190:1:10, v/v/v), and acetic acid:HCl:water (30:3:10, v/v/v). The separated compounds were identified on the basis of their R_F values, fluorescence under visible and UV light with or without ammonia, color reaction to 5% (w/v) Na_2CO_3 solution, and characteristic absorption maxima shifts with 5% ethanolic $AlCl_3$ as described by Harborne (1967).

UV-B-absorbing compounds were extracted by shaking a pair of seedlings in 2 mL of 80% (v/v) methanol at 4°C in darkness. The amount of UV-B-absorbing compounds was quantitatively determined by measuring A_{300} (Dangl et al., 1987).

PAL Assay

The PAL extraction and assay were essentially similar to those described by Goud et al. (1991). Five shoots of uniform height were homogenized at 4°C in a precooled mortar and pestle with 200 mg of sea sand and 150 mg of polyvinyl-polypyrrolidone in 3 mL of 0.1 M borate buffer (pH 8.8) containing 50 mM 2-mercaptoethanol. The homogenate was centrifuged at 18,200*g* for 30 min at 4°C and the supernatant was applied to a Sephadex G-25 column (2 cm × 10 cm) equilibrated with 0.1 M borate buffer (pH 8.8). The fractions constituting void volume were pooled together and were used for assay. The PAL assay was performed at 25°C in an assay mixture consisting of 1 mL of enzyme extract and 0.5 mL of 50 mM L-Phe (Saunders and McClure, 1975). The PAL activity was assayed by monitoring the increase in A_{290} against a control without Phe over a period of 4 h at 1-h intervals. The rate of appearance of *trans*-cinnamic acid was taken as a measure of enzyme activity using an increase of 0.01 A_{290} equal to 3.09 nmol of *trans*-cinnamic acid formed (Saunders and McClure, 1975). The PAL activity is expressed in pkat (pmol *trans*-cinnamic acid formed per second) per shoot.

Statistics

All data points in figures and tables represent the mean of values obtained from at least three independent replicates and SE values calculated therefrom.

RESULTS

Effect of SL on Anthocyanin Synthesis

The etiolated purple puttu seedlings, upon exposure to SL at different time intervals, accumulated greatly increased amounts of anthocyanin pigments (Fig. 1A), whereas the dark-grown control seedlings completely lacked anthocyanins. The induced pigments were localized primarily in the apical portions of the growing shoot, whereas the leaf sheath was almost colorless. On the other hand, roots did not show any anthocyanin pigment upon exposure to SL. The etiolated acyanic black puttu seedlings did not show any visible anthocyanin upon exposure to SL. Similarly, none of the tested acyanic lines exhibited SL-induced anthocyanin synthesis in seedlings. The induction of anthocyanin was mediated exclusively by SL, as was evident by the fact that the purple puttu

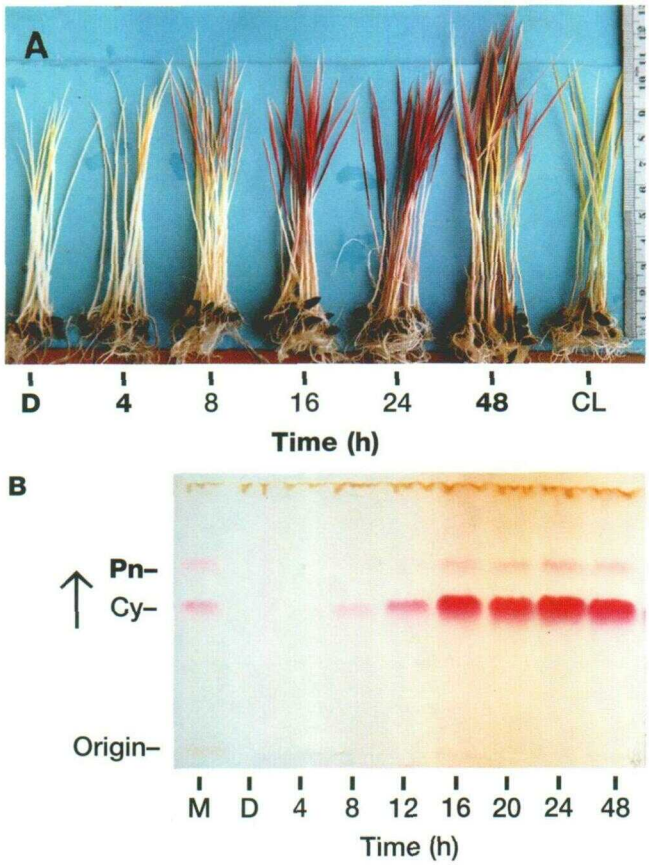

Figure 1. A, Anthocyanin accumulation in purple puttu seedlings after a saturating SL pulse. Four-day-old etiolated seedlings were exposed to 30 min of SL, transferred back to darkness, and then photographed after the indicated dark intervals. B, Separation of acid-hydrolyzed anthocyanin extracts by TLC. Four-day-old seedlings were exposed to 30 min of SL and transferred back to darkness, and anthocyanins were extracted from seedlings at the indicated time points. Anthocyanidins consisted of cyanidin (Cy) and peonidin (Pn). The arrow indicates the direction of the chromatogram. The chromatogram was run using acetic acid:HCl:water solvent. D, Dark control; M, standard markers.

seedlings grown for 5 d under cool-white fluorescent lights (26 μmol m^{-2} s^{-1}) accumulated greatly reduced amounts of anthocyanins (Fig. 1A, CL).

The extent of photoinduction of anthocyanin synthesis appears to depend on the age of seedlings, younger seedlings being the most responsive to SL. The results (Fig. 2A) reveal that photoinduction was maximum in 4-d-old etiolated seedlings and thereafter gradually declined with age.

It was observed that a 5-min exposure to SL was sufficient to induce detectable levels of anthocyanin. The magnitude of photoinduction increased with exposure to SL (Fig. 2B), and, in fact, a 30-min exposure saturated the response (Fig. 2B). The time course of photoinduction of anthocyanin pigments in purple puttu shoots shows (Fig. 3) that a 30-min SL exposure leads to a massive accumulation of anthocyanins after a lag of about 4 h, attaining a peak at 24 h, after which there is a gradual decrease in the next 24 h (40% reduction).

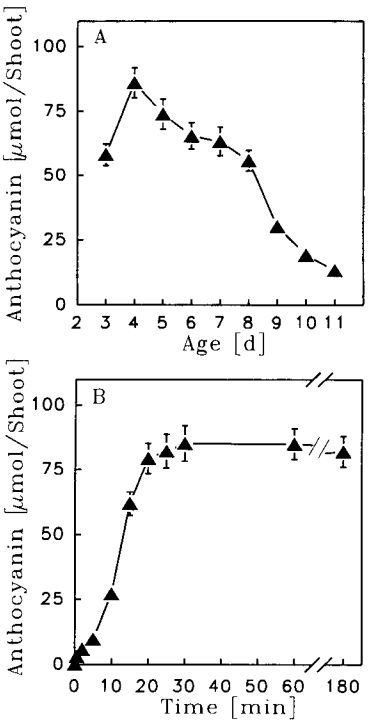

Figure 2. A, Effect of age on anthocyanin accumulation in purple puttu seedlings. Seedlings were grown in darkness from sowing. At the time points indicated on the abscissa, seedlings were removed from darkness, irradiated with 30 min of SL, and transferred back to darkness. Anthocyanin level was estimated at 24 h from the end of light treatments. B, Effect of different durations of SL on anthocyanin accumulation in purple puttu seedlings. Seedlings were grown in darkness and the 4-d-old seedlings were irradiated with SL for different durations indicated on the abscissa and transferred back to darkness. Anthocyanin level was estimated at 24 h from the end of light treatments.

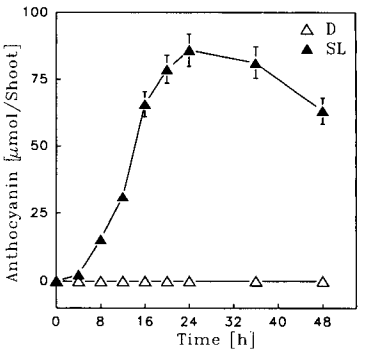

Figure 3. Time course of anthocyanin accumulation in purple puttu seedlings. Seedlings were grown in darkness and the 4-d-old seedlings were irradiated for 30 min with SL and transferred back to darkness. Anthocyanin level was estimated at the time points indicated on the abscissa.

The anthocyanin present in SL-exposed purple puttu seedlings was separated on cellulose TLC plates into two distinct bands with R_F values of 0.49 and 0.68 (acetic acid:HCl:water) at all tested time points of accumulation (Fig. 1B). The bands were identified tentatively as cyanidin and peonidin by comparing their R_F values with standards and absorbance maxima at 535 and 532 nm, respectively.

Effect of UV-B Light on Anthocyanin Accumulation

The photoinduction of anthocyanin in seedlings was characterized with respect to the effectiveness of different wave bands of light. Table II shows that in purple puttu seedlings only SL can induce anthocyanin accumulation and that this accumulation is triggered by the UV-B component of SL. This is based on the fact that the seedlings exposed to WG, which cuts off UV-B radiation (Klein, 1979), completely lack anthocyanin pigments. Also, there was no anthocyanin accumulation in seedlings exposed to RL, FR, or BL. However, the SL-mediated anthocyanin accumulation decreased by about 30% if SL exposure was followed by a 10-min FR pulse. Further, this effect of FR pulse is nullified if it is followed by a brief RL pulse. SL also induced the accumulation of UV-B-absorbing compounds in both cyanic purple puttu and acyanic black puttu seedlings (Table III). The photoinduced accumulation of UV-B-absorbing compounds was independent of UV-B, as was made evident by the fact that seedlings exposed to either SL or WG accumulated nearly the same level of UV-B-absorbing compounds. By contrast, the acyanic cultivar black puttu accumulated only a small amount of anthocyanin, which was less than one-thirtieth the amount accumulated by purple puttu seedlings.

Photostimulation of PAL Activity

The possible relationship between photoinduced anthocyanin accumulation and PAL activity was investigated by following the kinetics of PAL enzyme activity accumulation in purple puttu seedlings after a brief exposure to SL. Data

Table II. *Effect of various light treatments on anthocyanin level in purple puttu seedlings*

Seedlings were grown in darkness for 4 d and were exposed to various wavelengths of light for 30 min and transferred to darkness. In some cases, the light treatments were also followed by a 10-min FR or RL pulse before transfer to darkness. Anthocyanins were estimated after 24 h in darkness.

Irradiation	Anthocyanins	
	μmol/shoot	%
Dark	0.00	0.00
SL	85.9 ± 1.2	100.00
SL + FR[a]	58.9 ± 1.8	68.56
SL + FR[a] + RL[a]	92.4 ± 2.8	107.56
SL + FR[a] + RL[a] + FR[a]	59.8 ± 2.5	69.61
RL[a] + SL	84.4 ± 1.9	98.25
WG	0.00	0.00
RL	0.00	0.00
BL	0.00	0.00
FR	0.00	0.00

[a] Obtained using an artificial light source (Manga and Sharma, 1988).

Table III. *Effect of light treatments on the accumulation of anthocyanins and UV-B-absorbing compounds in rice shoots*

Seedlings were grown in darkness for 4 d and exposed to either SL or WG for 30 min and transferred back to darkness. Anthocyanins and UV-B-absorbing compounds were estimated after 24 h in darkness. Anth, Anthocyanin (μmol/shoot); UV-B AC, UV-B-absorbing compounds (A_{300}/shoot).

Irradiation	Purple puttu		Black puttu	
	Anth	UV-B AC	Anth	UV-B AC
Dark	0.0	1.06 ± 0.05	0.0	1.02 ± 0.01
SL	85.9 ± 1.2	2.55 ± 0.02	2.5 ± 0.02	2.20 ± 0.21
WG	0.0	1.54 ± 0.01	0.0	1.38 ± 0.20

in Figure 4A show that SL irradiation led to a rapid induction of PAL activity, resulting in a more than 2-fold increase with a peak at 4 h (peak I). After a minor decline, PAL activity again showed a 4-fold increase with a peak at 12 h (peak II). Subsequently, by 20 h PAL activity declined to the level of the dark control and thereafter dipped below that of the dark-grown seedlings. However, when SL treatment was immediately followed by a 10-min FR pulse, the PAL induction was subdued during the initial 4-h period and the induction profile of PAL showed only peak II at 12 h (Fig. 4A). In contrast, either RL or BL exposure, which does not induce anthocyanin accumulation in the purple puttu seedlings, induced PAL activity, and the induction profile showed only peak I at 4 h. Thereafter, the PAL activity declined below the dark level after 8 h. The RL- as well as BL-mediated PAL induction was blocked by a 10-min FR pulse (Fig. 4, B and C), indicating that peak I is phytochrome dependent and is also RL/FR reversible.

Purple puttu seedlings irradiated with WG showed only peak I of PAL activity, similar to RL, and conspicuously lacked peak II of PAL induction (Fig. 4D). The photoinduction of PAL activity in purple puttu seedlings was also plotted by subtracting the curve obtained after RL or WG treatment from the one obtained after SL treatment. Figure 4, E and F, clearly reveal that upon such a subtraction, only peak II of PAL is discernible, substantiating the conclusion that the PAL peak II is specifically UV-B inducible in purple puttu seedlings.

The time course of photoregulation of PAL in acyanic black puttu seedlings is presented in Figure 5. It is evident that in black puttu seedlings, SL triggers an induction of PAL activity with a peak level at 4 h from the end of irradiation (Fig. 5A), which is similar to peak I observed in purple puttu seedlings with WG, BL, and RL (Fig. 4). However, black puttu seedlings do not show the second peak of PAL photoinduction, i.e. peak II at 12 h. In as much as the kinetics of photoinduction of PAL in black puttu seedlings irradiated with SL, WG, BL, and RL are essentially similar and, moreover, induction of peak I and the effect of SL, BL, or RL on PAL induction can be nullified by a terminal FR pulse, the photoinduction of peak I of PAL in black puttu seedlings is presumably mediated by phytochrome.

DISCUSSION

The results obtained in this study highlight the photoinduction of anthocyanin in shoots of purple puttu seedlings triggered by SL. Since screening of SL by window glass, which cuts off the wavelengths shorter than 320 nm (Klein, 1979), abolishes the photoinduction of anthocyanin in these seedlings, it is proposed that the SL effect is mediated primarily by the UV-B component of SL. These results are in agreement with those from maize, in which it has been shown that although SL stimulates an increase in flavonoid production, the effect of SL is significantly reduced when it is filtered through window glass (Urban, 1959).

The exclusive role of UV-B light in induction of flavonoids has been observed in cell-suspension cultures and seedlings of parsley, as well as in seedlings of sorghum. In these plants, flavonoid production was induced only by UV-B light, whereas other photoreceptors such as blue/UV-A and phy-

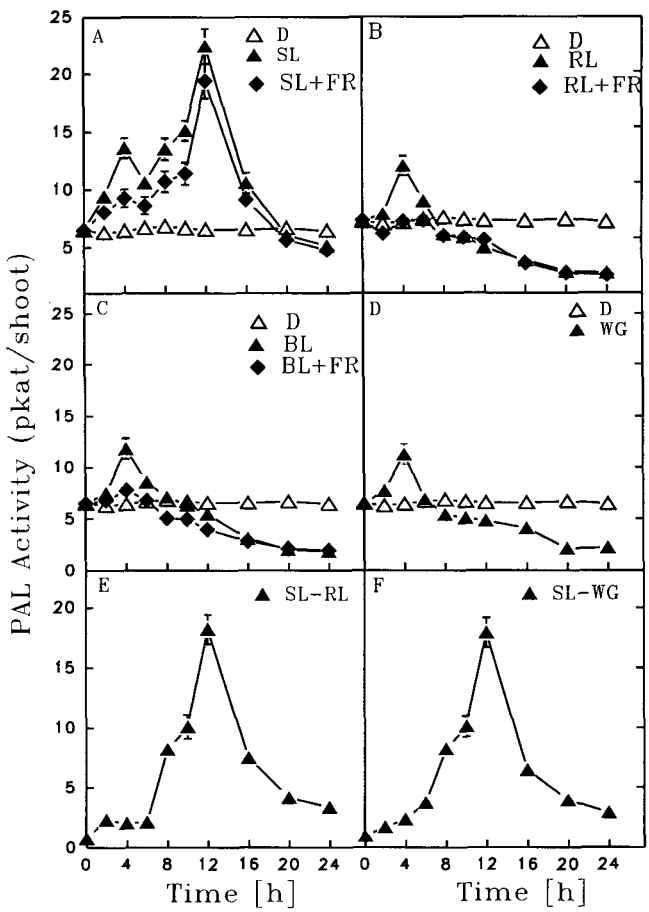

Figure 4. Time course of PAL activity in purple puttu seedlings in response to various light treatments. Seedlings grown in darkness up to 4 d from sowing were exposed to 30 min of various light treatments and transferred back to darkness. In the case of SL, BL, or RL followed by FR (A, B, and C), seedlings at the end of light treatment were irradiated with a 10-min FR light pulse using an artificial light source. The data for E and F were calculated after subtracting values obtained for the time course of PAL under SL from the values obtained with RL or WG (D), respectively.

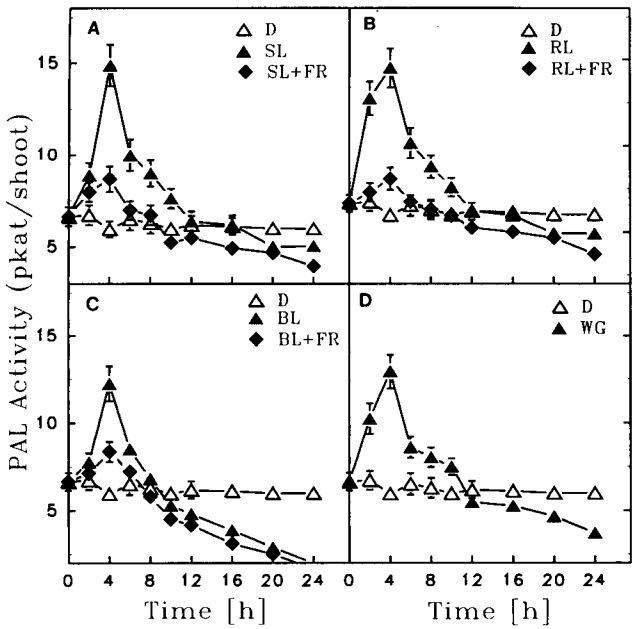

Figure 5. Time course of PAL activity in black puttu seedlings in response to various light treatments. Seedlings grown in darkness up to 4 d from sowing were exposed to 30 min of various light treatments and transferred back to darkness. In the case of SL, BL, or RL followed by FR (A, B, and C), seedlings were irradiated with a 10-min FR light pulse using an artificial light source at the end of the light treatment. D, The kinetics of PAL with WG.

tochrome could modulate only the photoresponse triggered by UV-B light (Wellmann, 1974; Yatsuhashi et al., 1982). From the present study it is clear that only UV-B induces anthocyanin induction in purple puttu seedlings, and none of the other wavelengths in the visible region of the spectrum (>320 nm) are effective in inducing any detectable levels of anthocyanins. Since anthocyanin level induced by UV-B decreased with an FR pulse, which could be nullified by an RL pulse, it is evident that phytochrome plays a secondary role in modulating the UV-B response in purple puttu seedlings, as is the case in parsley and sorghum seedlings. In comparison to the cyanic purple puttu cultivar, in the acyanic black puttu cultivar only a marginal amount of anthocyanin is induced under SL. By contrast, both cultivars induce UV-B-absorbing compounds to the same extent under SL as well as under WG. This is further evidence that the UV-B component of SL is responsible for the specific induction of anthocyanin.

It has been shown in many plant species that the photoinduced accumulation of flavonoids is preceded by an induction of several enzymes involved in phenylpropanoid metabolism (Hahlbrock and Scheel, 1989). In the present study, SL triggered photoinduction of PAL in purple puttu seedlings with two distinct peaks. The analysis of the time course of photoinduction of PAL after different light treatments revealed that the occurrence of two PAL peaks was possibly determined by an independent action of two distinct photoreceptors on PAL activity. Our observations lead to the conclusion that the PAL peak I at 4 h is induced by phytochrome, because this peak could also be seen in seedlings irradiated with RL or WG and photoinduction of this peak could be nullified by FR. The second PAL peak was induced specifically by UV-B light because it was completely missing in seedlings irradiated with WG. Moreover, FR, which abolished peak I in SL-exposed seedlings, had no effect on the appearance of peak II.

The molecular events leading to the induction of biphasic PAL profile under SL can only be speculated upon at the moment. It is plausible that these two peaks of PAL activity arise by a stimulation of PAL activity by phytochrome and UV-B photoreceptor on different temporal scales. Since PAL is encoded by a small multigene family in rice (Minami et al., 1989), it is also possible that individual members of the PAL gene family may respond differentially to photoinduction, leading to the biphasic appearance of PAL. In *Arabidopsis*, transcripts encoding PAL and other enzymes of the flavonoid biosynthetic pathway are induced independently by three photoreceptors, viz. phytochrome, blue/UV-A photoreceptor, and UV-B photoreceptor, in a temporally determined fashion (Kubasek et al., 1992). By contrast, in rice, BL induced only peak I of PAL activity, which was qualitatively similar to the RL effect; moreover, the BL effect was nullified by a terminal FR pulse. So, in rice seedlings, the effect of a short exposure to BL (30 min) on PAL activity is evidently mediated by a phytochrome rather than a specific BL/UV-A light photoreceptor.

Since the photoinduction of these two PAL activity peaks precedes the anthocyanin accumulation, it is conceivable that the photoinduction of PAL is a part of the overall induction of the anthocyanin biosynthetic pathway in purple puttu seedlings. The above possibility arises from the observation that the photoinduction of both peak II of PAL and anthocyanin depends exclusively on UV-B light. A correlation between the induction of peak II of PAL activity and anthocyanin formation is also supported by the observation that peak II is restricted to the cyanic purple puttu line. Although the anthocyanin synthesis is induced by UV-B and this induction is possibly dependent on peak II of PAL activity, it is apparent that in purple puttu seedlings peak I of PAL activity also contributes to the anthocyanin production. This becomes evident from the observation that a terminal FR exposure, which blocks the SL-mediated induction of peak I, also reduces the anthocyanin level by about 30% in purple puttu seedlings.

In summary, our results demonstrate that there is an apparent correlation between UV-B induction of anthocyanin and induction of peak II of PAL activity in seedlings of the cyanic cultivar purple puttu. At the moment, the genetic determinants in rice associated with anthocyanin synthesis, both UV-B dependent and independent, are absolutely unknown. Therefore, a comparison of rice with the genetically well-defined maize system becomes necessary. In maize, at least 14 nonallelic genes are involved in the synthesis and distribution of anthocyanin pigments in various plant parts, and of these at least 4 are found to be regulatory (Coe et al., 1988; Dooner et al., 1991). Of these 4, the *R* locus has been shown to regulate the tissue-specific expression of several genes associated with the anthocyanin pathway. The product of the *R* locus was found to be a transcription activator

protein of the helix-loop-helix class that interacts with the regulatory elements of a number of structural genes of the pathway. Most importantly, the *R* locus was found to be largely responsible for the photoinduction of anthocyanin synthesis in seedlings (Taylor and Briggs, 1990). It is reasonable to suggest, therefore, that the UV-B-induced anthocyanin synthesis in rice may be mediated by the UV-B-dependent expression of a regulatory gene analogous to the *R* locus of maize. In fact, recent experiments elegantly demonstrate that this maize gene exerts a similar regulatory influence on anthocyanin pathway in transgenic plants of diverse origin, including dicots, such as *Arabidopsis* and *Antirrhinum* (Lloyd et al., 1992). We speculate that the purple puttu seedlings are homozygous for a gene analogous to the *R* gene of maize mediating the photoinduction of anthocyanins, whereas the recessive acyanic black puttu seedlings are defective in this function. We are currently working on the molecular isolation of both structural and regulatory genes of the anthocyanin pathway in rice using the corresponding maize genes as probes.

Although, at the moment, no conclusive data are available on the possible protection of rice seedlings from UV-B radiation by anthocyanin and other UV-B-absorbing compounds, the available information implies such a role (Robberecht and Caldwell, 1986; Tevini et al., 1991). For instance, seedlings of certain *Arabidopsis* mutants deficient in flavonoids including anthocyanins are hypersensitive to UV-B radiation (Li et al., 1993) and exhibit a lethal response, suggesting that the UV-B-absorbing compounds play a protective role. Similarly, the *Rumex patientia* plants are more sensitive to UV-B damage than *Rumex obtusifolius*, which have a higher level of UV-B-absorbing compounds in the epidermal layers (Robberecht and Caldwell, 1986). It can be argued that the UV-B-induced anthocyanin pigmentation in rice seedlings may have a role in minimizing the UV-B damage. More direct physiological and molecular genetic data on anthocyanin synthesis and regulation in rice are essential to evolve a strategy to protect this important crop plant from an impending threat of enhanced UV-B radiation in the biosphere.

ACKNOWLEDGMENTS

We are thankful to Dr. E.A. Siddiq and his colleagues at the Directorate of Rice Research, Hyderabad, Prof. S.R. Sree Rangasamy of Tamilnadu Agricultural University, Prof. T. Kinoshita of Hokkaido University, and the International Rice Research Institute, Manila, for providing us with rice lines.

Received November 29, 1993; accepted April 7, 1994.

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