Differential responses of growth and photosynthesis in *Cyamopsis tetragonoloba* L. grown under ultraviolet-B and supplemental long-wavelength radiations

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Abstract

Cyamopsis tetragonoloba L. seedlings were subjected to continuous ultraviolet (UV)-B radiation for 18 h and post-irradiated with "white light" (WL) and UV-A enhanced fluorescent radiations. UV-B treatment alone reduced plant growth, pigment content, and photosynthetic activities. Supplementation of UV-A promoted the overall seedling growth and enhanced the synthesis of chlorophyll and carotenoids with a relatively high photosystem 1 activity. Post UV-B irradiation under WL failed to photoreactivate the UV-B damage whereas a positive photoregulatory effect of UV-A was noticed in electron transport rates and low temperature fluorescence emission spectra.

Additional key words: chlorophyll fluorescence induction; photoreactivation; post-irradiation; photosystems 1 and 2.

Introduction

Although UV-B radiation constitutes only 1.5 % of the total solar energy, the impacts caused by this radiation on biological systems are of great concern (Teramura *et al.* 1980, Frederick *et al.* 1989). Sensitivity of plants to UV-B depends on the relative changes in overall growth, seedling morphology, photosynthesis, and yield (Sisson

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Abbreviations: BQ - *p*-benzoquinone; Chl - chlorophyll; DCPIP - 2,6-dichlorophenol indophenol; LHCP - light-harvesting chlorophyll protein; MV - methyl viologen; PPFD - photosynthetic photon flux density; PS - photosystem; UV-A - ultraviolet-A (320-360 nm); UV-B - ultraviolet-B (280-320 nm).

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and Caldwell 1977, Teramura *et al.* 1980). Under low WL irradiance, increased UV-B sensitivity is noticed in plants grown in growth chambers and greenhouses (Teramura *et al.* 1980). Reductions in growth and chlorophyll (Chl) content were reported in plants exposed to UV-B under a background of low PPFD (Cen and Bornman 1990). High PPFD reduces the effects of UV-B on plant growth (Mirecki and Teramura 1984). High WL irradiance photoprotects UV-irradiated chloroplasts (Lingakumar and Kulandaivelu 1996). Thus critical evaluation of PPFD levels is obligatory in studies of UV-B effects in higher plants. Combination of visible radiation and UV-A at a particular ratio may be highly suitable for enhanced growth of seedlings (Middleton and Teramura 1994). Amelioration of photosystem (PS) 2 activity by the addition of UV-A to UV-B irradiated chloroplasts was shown by Panagopoulos *et al.* (1990). Till date, conflicting reports arise about the regulatory effects of UV-A. Hashimoto and Tajima (1980) and Biswal *et al.* (1997) found inhibition of total Chl and carotenoid contents induced by UV-A. Promotory effects of UV-A on the synthesis of Chl and carotenoids were reported by Senger and Schmidt (1986) and Rau and Schrott (1984). Photorepair and photoreactivation processes may be stimulated by radiations in the blue and UV-A spectral regions which activate photolyase (Sutherland 1981, Pang and Hays 1991). Since UV-B induced changes are photoreactivated by long-wavelength radiations, we selected two spectral regions (WL and UV-A) and studied their efficiency in moderation or photoregulation of UV-B effects on plant growth, photosynthetic pigment composition, and photosynthetic activities in higher plants.

Materials and methods

Plants: Seedlings of *Cyamopsis tetragonoloba* L. cv. Pusa navabhakar were grown in a growth chamber (*Hot Pack*, USA) at 25±0.2 °C with a 12/12 h light/dark cycle. Irradiance at the seedling surface was 50 W m^{-2}. Temperature was maintained at 25±0.2 °C. Seven d old seedlings at their early cotyledonary leaf stage were used.

Isolation of chloroplasts from the cotyledonary leaves was done as described by Kulandaivelu and Daniell (1980) and Lingakumar and Kulandaivelu (1996). Chloroplasts were washed with 10 mM NaCl, and the membranes were obtained after centrifugation at 3 000×*g* for 5 min.

UV-B and long-wavelength treatment: The seedlings were exposed to UV-B enhanced cool fluorescent irradiation (UV-B sun lamp, *Philips TL* 20 W/12 and *Philips* 20 W/33, India) at 25±0.2 °C. As an UV-A source, a *Philips TL* 20 W/08 was used. Radiation below 280 nm was completely removed from the UV lamps using a *Schott WG 290* filter. Irradiances at the leaf surface were 15.5, 2.5, and 2.2 μmol m^{-2} s^{-1} for UV-B, WL, and UV-A, respectively. Visible and UV irradiances were measured using an *IL-700* spectroradiometer equipped with a broad band sensor *SEE 400* (*International Lights*, USA).

Photosynthetic electron transport assays: Photosynthetic reactions mediated by PS1 and PS2 were measured using a *Hansatech* (U.K.) O_2 electrode as described by

Noorudeen and Kulandaivelu (1982). Assay media were prepared similarly as in Lingakumar and Kulandaivelu (1996).

Variable Chl *a* fluorescence was followed *in vivo* in intact leaves after excitation with broad band blue radiation (400-620 nm) filtered by the *Corning 4-96* filter. Prior to the excitation, the leaves were incubated in the dark for 10 min, and care was taken to avoid wilting of leaves in the dark. The signal was stored in a digital oscilloscope (*Iwatzu SS-5802*) and then transferred to a *Hitachi* recorder.

Low temperature fluorescence emission spectra of chloroplast samples were recorded using a *Hitachi MPF4* spectrofluorimeter. Isolated chloroplasts were resuspended in a 60 % (v/v) glycerol medium containing 20 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, and 5 mM NaCl. A specially constructed Dewar flask was used for measurements at 77 K. The emission spectra were obtained after correcting for differences in monochromator and photomultiplier responses.

Pigment analysis: Chl and carotenoids were extracted in 80 % acetone. Concentrations of Chl *a*, *b*, and carotenoids were calculated using the coefficients of Wellburn and Lichtenthaler (1984).

Results and discussion

Changes in growth characteristics: *C. tetragonoloba* seedlings were exposed for 18 h under UV-B and post-irradiated either under WL or UV-A for 72 h. Continuous UV-B treatment caused significant reductions in shoot length and leaf area by about 16 and 35 %, respectively (Table 1). The fresh mass of vegetative parts was less affected when compared to dry mass. Under UV-A supplementation, almost all these parameters increased, and maximum effect was found for leaf area. A significant

Table 1. Growth characteristics in *Cyamopsis* seedlings exposed to different long-wavelength radiations. Irradiance at the leaf surface was 15.5, 2.5, and 2.2 μmol m^{-2} s^{-1} for UV-B, WL, and UV-A, respectively. Figures in parentheses are % with reference to respective control (WL). Mean ± SE, $n = 5$.

Treatment	Shoot length [cm]	Dry matter [mg per seedling]	Leaf area [cm^2]
Control WL (18 h)	8.62 ± 0.70	20.4 ± 2.0	2.00 ± 0.12
+UV-B (18 h)	7.22 ± 0.60 (83.7)	20.0 ± 2.1 (98.0)	1.53 ± 0.15 (65.3)
Control WL (90 h)	8.22 ± 0.80	22.1 ± 2.5	2.24 ± 0.14
UV-B + WL (18+72 h)	8.24 ± 0.80 (93.4)	25.0 ± 2.0 (113.6)	1.92 ± 0.09 (96.0)
WL + UV-A (18+72 h)	9.68 ± 0.90 (109.8)	24.2 ± 1.7 (109.0)	2.67 ± 0.15 (119.0)
UV-B + UV-A (18+72 h)	6.10 ± 0.60 (69.1)	18.1 ± 1.8 (81.8)	1.47 ± 0.06 (65.6)

increase in leaf area and shoot length under UV-A irradiation is a strong evidence for its regulatory role in photomorphogenesis. Chl and carotenoid synthesis and photosynthesis are stimulated by UV-A/blue radiation (Senger and Schmidt 1986, Kawallik 1987, Rau and Schrott 1987). Middleton and Teramura (1994) report that both UV-A/blue radiation photoreceptor and phytochrome are involved in the regulation of photomorphogenetic responses. Post-irradiation with WL caused a considerable recovery of UV-B induced changes on growth parameters (Table 1). Under UV-B+UV-A such photoprotective effects were not noticed.

Changes in pigment composition: On the basis of unit fresh mass, total Chl (*a*+*b*) content was slightly increased under UV-B treatment (Table 2). Similarly, the Chl *a*/*b* ratio and carotenoid content also showed an increase. UV-A supplementation caused an 11 % increase in total Chl and a 26 % increase in carotenoid content.

Table 2. The effect of UV-B and long-wavelength radiations on the amounts of chlorophyll (Chl) and carotenoids [g kg^{-1}(f.m.)] in *Cyamopsis* seedlings. Figures in parentheses are percentage with reference to respective controls. Mean ± SE, $n = 5$.

Treatment	Chl (*a*+*b*)	Chl *a*	Chl *b*	Chl *a*/*b*	Carotenoids
Control WL (18 h)	1.24 ± 0.04	0.77 ± 0.01	0.47 ± 0.02	1.66	0.30 ± 0.10
+UV-B (18 h)	1.25 ± 0.05 (100.7)	0.79 ± 0.08 (101.8)	0.46 ± 0.08 (98.9)	1.71	0.30 ± 0.04 (100.6)
Control WL (90 h)	1.42 ± 0.06	0.85 ± 0.04	0.58 ± 0.03	1.46	0.32 ± 0.03
UV-B + WL (18+72 h)	1.37 ± 0.04 (96.0)	0.80 ± 0.06 (94.6)	0.57 ± 0.03 (98.0)	1.41	0.33 ± 0.04 (105.4)
WL + UV-A (18+72 h)	1.58 ± 0.08 (110.8)	0.91 ± 0.04 (107.7)	0.67 ± 0.06 (115.4)	1.36	0.40 ± 0.06 (126.0)
UV-B + UV-A (18+72 h)	1.43 ± 0.09 (100.8)	0.84 ± 0.03 (99.8)	0.59 ± 0.04 (102.4)	1.43	0.36 ± 0.03 (114.0)

A preferential enhancement of Chl *b* concentration was observed under this treatment. Several studies have shown that Chl *b* and light-harvesting complex 2 are formed under red radiation and low WL irradiances (Terao *et al.* 1985, 1988, Droppa *et al.* 1988). Carotenoids protect against photooxidation. Post-irradiation with WL did not induce any positive response in contents of all pigments. The significant increase in carotenoid content under UV-B+UV-A treatment indicates an additional resistance mechanism to photooxidative stress effects of UV.

Changes in photosynthetic electron transport: Changes in PS2, PS1, and whole chain rates were expressed per Chl basis (Table 3). The rate of $H_2O \rightarrow MV$ electron transport was highest under WL (control). Exposure to UV-B for 18 h resulted in a 65 % inhibition. Under post-UV WL irradiation, the inhibition was further accelerated to 45 %, whereas UV-A post-irradiation greatly reversed the UV inhibition. The rate of PS2 electron transport ($H_2O \rightarrow BQ$) was more or less similar to the overall electron transport but for a small difference in the photoreversal

Table 3. The effect of UV-B and long-wavelength radiations on photochemical activities (whole chain: $H_2O \rightarrow MV$; PS2: $H_2O \rightarrow BQ$; PS1: $DCPIPH_2 \rightarrow MV$) [mmol(O_2) kg^{-1}(Chl) s^{-1}] in *Cyamopsis* chloroplasts. The seedlings at their early cotyledonary leaf stage were used for various treatments. Values are averages of five independent measurements. Mean + SE, $n = 5$. For abbreviations see the text.

Treatment	Whole chain	PS2	PS1
Control WL (18 h)	51.1 ± 4.0	78.8 ± 4.6	330.0 ± 12.8
+UV-B (18 h)	32.9 ± 7.4 (64.5)	28.5 ± 1.8 (36.2)	229.3 ± 10.7 (69.5)
Control WL (90 h)	63.7 ± 4.3	98.7 ± 5.3	349.6 ± 24.2
UV-B + WL (18+72 h)	12.2 ± 0.9 (19.1)	18.2 ± 0.8 (18.4)	178.6 ± 11.8 (51.1)
WL + UV-A (18+72 h)	62.2 ± 7.5 (97.6)	100.2 ± 6.1 (101.5)	398.3 ± 23.4 (114.0)
UV-B + UV-A (18+72 h)	54.0 ± 3.2 (84.6)	63.8 ± 5.8 (64.6)	323.8 ± 24.8 (92.7)

properties of WL. UV-B impairment of PS2 activity has been documented by several reports (see review of Bornman 1989, Melis *et al.* 1992). The UV-A post-UV irradiation brought a high UV-B inhibition as compared to WL which aggravated the UV-B effect. Generally, UV-B has less impact on PS1 (Van *et al.* 1977, Renger *et al.* 1982, Kulandaivelu and Noorudeen 1983) than on PS2. As evidenced from Table 3, UV-B inhibited the $DCPIPH_2 \rightarrow MV$ electron transport but the extent of inhibition was 50 % less than those observed for PS2 activity. WL and UV-A supplementation to UV-B-exposed seedlings brought about similar changes as observed for PS2 activity. The ineffectiveness of WL and UV-A post-irradiations for full restoration of UV-B inhibition of photochemical activities could be due to a structural reorganization under UV-B exposure. A structural reorganization under UV-B at the level of PS2 has been reported by Nedunchezhian and Kulandaivelu (1991).

Fluorescence induction kinetics: Chl fluorescence is used as a probe in tracing out the primary photosynthetic events and also to monitor the plant response to a stress affecting photosynthetic capacities (Smillie 1983). Compared to control (WL) plants, UV-A had little effect on Chl fluorescence induction parameters (Fig. 1), while UV-B inhibited the variable fluorescence (F_v). Post-UV-irradiation with WL partially restored the inhibition on F_v but with a slow O-P rise. On the contrary, UV-A supplementation to UV-B exposed seedlings severed the inhibition of F_v. The slow fluorescence changes revealed typical P, S, M, and T levels (Walker 1981). In all the controls, a rapid P-S quenching was observed. UV-B exposure brought about a slow P-S quenching and a fast attainment of the T state without an intermediate M peak. Post-UV WL and UV-A irradiations caused reappearance of the M peak in UV-B treated samples. Activation of Fd^{2+}-NADP oxidoreductase by PS1 was probably not affected by these post-irradiations (Satoh 1981). Since the M peak is attributed to the changes in CO_2 fixation efficiency, the stress imposed by UV-B on CO_2 fixation may

be relieved to a certain extent by the post-UV WL and UV-A irradiations. Furthermore, the decrease in F_v under WL+UV-B+UV-A treatments indicates that UV-A imposes an additional burden on the photosystems. Similar changes were observed in sugar beet leaves by Panagopoulos *et al.* (1990).

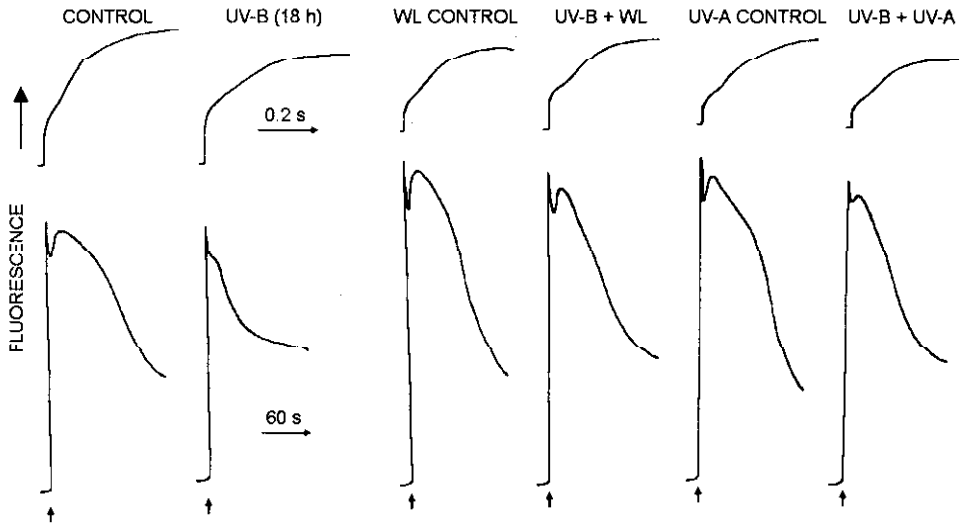

Fig. 1. Changes in fast (*top*) and slow (*bottom*) fluorescence transients obtained from *Cyamopsis* leaves exposed to UV-B and different post-UV-B treatments. The irradiances of all treatments were the same as in Table 1. The leaves were dark incubated for 10 min prior to excitation. The arrows indicate switching on the light.

Low temperature (77 K) fluorescence emission spectra: At 77 K, characteristic emissions by Chl-protein complexes such as PS1, PS2, and LHCP can be distinguished (Fig. 2) after various irradiations. The fluorescence emission spectrum of control chloroplasts upon excitation with 430 nm radiation exhibited peaks at 686 nm (F_{686}) and 730 nm (F_{730}), and a shoulder at 695 nm (F_{695}). F_{686}, F_{730}, and F_{695} represent emissions from PS2, PS1, and LHCP, respectively (Bose 1982). The fluorescence emission properties of UV-B and UV-A treated chloroplasts varied from that of WL control samples. The F_{730}/F_{686} values were higher under all the UV treatments. UV-B treatment alone increased the F_{686} and F_{730} levels, but on further exposure to WL a pronounced decrease in F_{686} was noticed which correlated well with the changes in photosynthetic electron transport rates. Similarly UV-A alone affected both PS2 and PS1 emission. When UV-A was used as a post-irradiation source, a preferential increase in the F_{730} level was noticed with a low inhibition of F_{686} in UV-B irradiated chloroplasts. This indicates that UV-A has a selective regulatory effect towards PS2 rather than towards PS1. Such a similarity in the regulation of photosystems is possible since both UV-A and UV-B share a common target site, namely PS2. Thus, our results confirm the damaging effects of UV-B in higher plants. The extent of photoprotection at the organelle level was not the same

for both long-wavelength radiations. Even though WL and UV-A post-irradiations accelerated the PS2 inhibition, a significant protection occurred at PS1.

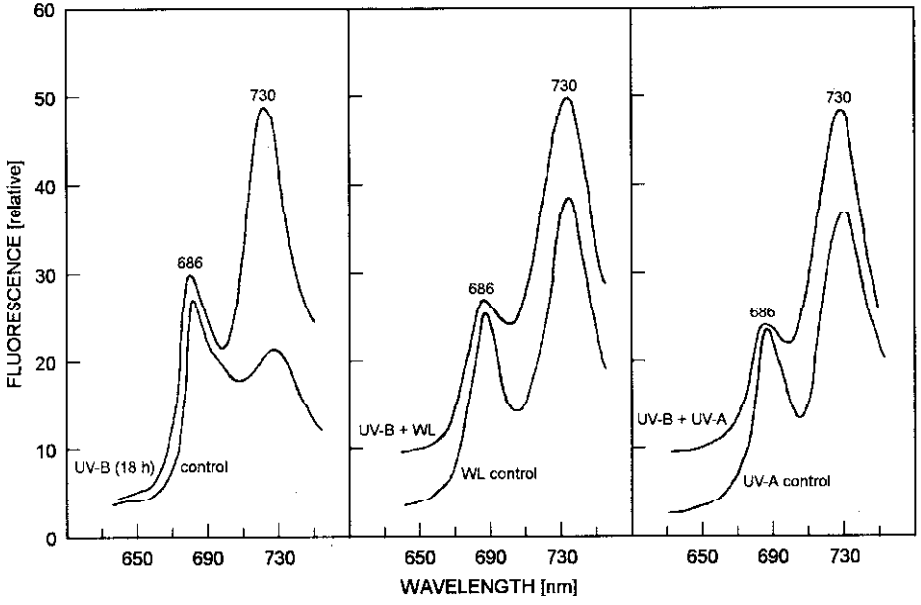

Fig. 2. Low temperature (77 K) fluorescence emission spectra of chloroplasts isolated from WL control, and UV-B and UV-A treated *Cyamopsis* seedlings. The irradiances of all treatments were the same as in Table 1. Isolated chloroplasts were resuspended at a final Chl concentration of 2 g m^{-3} in the reaction buffer containing 60 % (v/v) glycerol.

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