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UV-A irradiation guards the photosynthetic apparatus against UV-B-induced damage

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Abstract

In clusterbean leaves UV-B radiation caused a reduction in contents of chlorophylls and carotenoids and in the efficiency of photosystem 2 photochemistry. The degree of damage was reduced when UV-A accompanied the UV-B radiation. This indicates the counteracting effect of UV-A radiation against UV-B-induced impairment.

Additional key words: antheraxanthin; carotenoids; chlorophylls; clusterbean; *Cyamopsis tetragonoloba*; fluorescence induction; violaxanthin; xanthophylls; zeaxanthin.

Introduction

An increase in the influx of ultraviolet-B (UV-B, 280–320 nm) radiation on the surface of the earth due to the depletion of stratospheric ozone layer has forced the photobiologists throughout the world to study the impact of the radiation on photosynthetic apparatus. The radiation is detrimental to plant growth and development (Jordan 1996, Vass 1997, Kulandaivelu and Lingakumar 2000). It inactivates the photosynthetic apparatus by inflicting damages at multiple sites (Bornman 1989, Teramura and Ziska 1996). Most of the studies pertain to the effect of UV-B radiation only without taking into consideration the role of ultraviolet-A (UV-A; 320–400 nm), another component of solar UV radiation. UV-A exhibits both positive and negative effects on plant photosynthesis (Wellmann 1983). Whereas UV-A activates gene expression for photosystem 2 (PS2) reaction centre proteins (Christopher and Mullet 1994), it inflicts damage to photosynthetic apparatus (Joshi *et al.* 1997, Turcsanyi and Vass 2000). Further, plants develop strategies to alleviate the UV-induced damage of chloroplasts. The strategies include the shielding of the organelle by inducing the accumulation of UV-absorbing phenolic compound (Jansen *et al.* 1998), repair mechanism involving both DNA repair and *de novo* synthesis of UV-sensitive proteins, especially D_1 and D_2 proteins of PS2 (Bornman 1989), and defence mechanism involving both enzymatic and non-enzymatic processes (Mohr and Schopfer 1995) or use of choline compounds (Kreslavski *et al*. 2001). Ascorbate, α-tocopherol, reduced glutathione, and carotenoids (Car) are the molecules responsible to negotiate the oxidative stress as induced by the radiation in non-enzymatic defence.

Car, the accessory pigments in photosynthetic membranes with multiple functions (Young 1991, Yamamoto and Bassi 2000), scavenge the reactive oxygen species and photoprotect the membrane from harmful quanta through xanthophyll cycle (Demmig-Adams 1990, Gilmore 1997). Their composition in seedlings exposed to UV-B radiation is different from the seedlings exposed simultaneously to UV-A and UV-B (UV-A+UV-B) radiation. So the role of the pigment in eliciting the non-enzymatic mode of defence mechanism may vary under this condition. Therefore we investigated the mode of UV-B induced changes as modulated by UV-A radiation and examined the role of Car in the change.

Materials and methods

Clusterbean (*Cyamopsis tetragonoloba* L. cv. Pusa Navbahar) seeds were surface sterilised with 30 % alcohol and then kept in running water for 3 h. The seeds were further soaked in distilled water for 12 h in

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darkness. The well germinated seeds were grown in Petri plates on cotton soaked with distilled water under continuous "white" fluorescent radiation with negligible UV component at a photosynthetic photon fluence density (PPFD) of 200 μmol m^{-2} s^{-1} at temperature of 25±2 °C without any external nutrient.

In one set of the experiments, the seedlings were irradiated with UV-B at PPFD of 60 μmol m^{-2} s^{-1} obtained from a *Philips* tube (*TL 20* type *05*, peak 315 nm) for 1 h daily from day 1. In another set, seedlings were exposed to UV-B and UV-A radiation simultaneously (UV-A+UV-B) for 1 h daily from day 1. PPFD of UV-A (obtained from *Philips TL 20* type *09*, peak 365 nm) was 75 μmol m^{-2} s^{-1}.

Pigments were extracted from primary leaves of clusterbean seedlings with pre-chilled 100 % acetone and the extract was used for spectrophotometric determination of chlorophyll (Chl) and Car as described by Wellburn and Lichtenthaler (1984).

Chloroplasts were isolated from primary leaves of clusterbean seedlings following the method of Izawa and Good (1968). Chloroplasts were suspended in a medium containing 300 mM sucrose, 50 mM NaCl, 50 mM Na/K phosphate buffer (pH 6.9) with a Chl concentration of 2 mg per cm^3.

PS2-mediated oxygen evolution was measured with a Clark type oxygen electrode at 21 °C in rate-saturating red radiation. The basic assay buffer contained 30 mM Na/K phosphate buffer (pH 7.2), 30 mM NaCl, and 200 mM sucrose. The electron acceptor used was 0.3 mM 2,6-dichlorobenzoquinone (DCQ). Chloroplasts containing 40 μg Chl were placed in 2 cm^3 of reaction mixture. Gramicidin (2.5 μM) was used as an uncoupler.

Chl *a* fluorescence from primary leaf of clusterbean seedlings was measured with pulse amplitude modulated (PAM) fluorometer (*FMS-1*, *Hansatech*, Norfolk, UK) according to Schreiber *et al*. (1986).

Chromatography of leaf pigment extract was carried out on a 3.91×150 mm *Nova-pak* C_{18} *Waters* (USA) analytical column following the method of Rivas *et al.* (1989). 10–20 mm^3 of sample was injected with *Waters 717 plus* auto-sampler. Mobile phases were pumped by a *Waters 515* HPLC pump at a flow rate of 5 mm^3 s^{-1}. Peaks were scanned and detected by a *Waters 996* photodiode array (PDA) detector and integrated with *Waters millennium* software. The column was equilibrated prior to injection of each sample by flushing with acetonitrile : methanol (7 : 1, v/v, mobile phase A) for 10 min. The components were eluted by using a gradient of mobile phase A for 2 min and mobile phase B (acetonitrile : methanol : water : ethyl acetate, 7 : 0.96 : 0.04 : 5) for 7 min over a run time of 30 min.

Statistical analysis was carried out according to Glantz (1989).

Results

The pattern of changes in Chl *a*+*b* content of the control leaves exhibited an increasing trend till day 7 and then the pigment content remained almost constant [1.690±0.008 g kg^{-1}(F.M.)] till day 9 (Fig. 1A). UV-B irradiation decreased the content of total Chl and the decrease was furthered with the progress of UV-B dose. On day 9 the content of Chl declined by 48.4 % (p>0.005). Under UV-A+UV-B irradiation the content of total Chl was smaller than in the control, but the decline was lesser than that caused by UV-B alone. The kinetics of total Chl content in UV-A+UV-B irradiated leaves was almost parallel but at a lower level compared to that of control leaves and on day 9 the content of the pigment was only 10.3 % (p>0.005) less. The effect of UV-A radiation alone on the pigment content during the period under study was insignificant (unpublished data) and therefore its effects on other photosynthetic parameters were ignored.

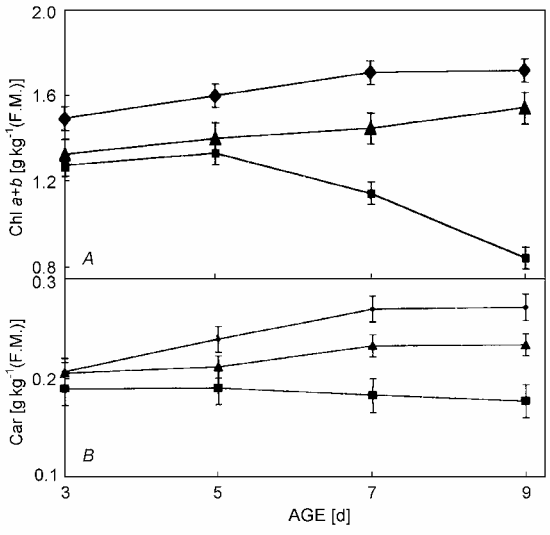

Fig. 1. Changes in the contents of total (*A*) chlorophyll (Chl) and (*B*) carotenoids in primary leaves of clusterbean seedlings under continuous irradiation (●), with UV-B (■), or UV-A+UV-B (▲) treatment of 1 h duration daily from day 1. Means of three independent estimates. n = 3. *Bar* indicates ± S.D.

The time course of changes in Car content with or without UV-B or UV-A+UV-B irradiation and the trend in loss was similar to that of Chl (Fig. 1*B*). On day 9, the loss of Car in UV-B and UV-A+UV-B irradiated primary leaves of clusterbean seedlings was 33.5 % (p>0.01) and 11.5 % (p>0.01), respectively. Chl/Car ratio calculated on day 9 (Fig. 2*A*) declined by 36 and 10 %, respectively in UV-B and UV-A+UV-B irradiated samples. The oxygen evolution characterising PS2 activity declined by 30 % in UV-B and by 20 % in UV-A+UV-B treated samples (Fig. 2*B*).

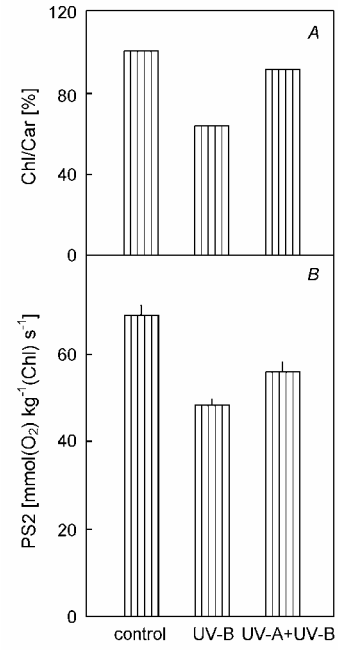

Fig. 2. Relative changes in (*A*) Chl/Car ratio and (*B*) photosystem 2 (PS2) mediated oxygen evolution in the chloroplasts isolated from 9 d old primary leaves with or without UV-B or UV-A+UV-B treatment of 1 h duration daily from day 1. Means of three independent estimates. $n = 3$.

The initial fluorescence intensity (F_0) and non-photochemical quenching (NPQ) increased by 12.0 and 58.9 % in UV-B irradiated and by 22.7 and 12.3 % in UV-A+UV-B irradiated leaves, respectively (Fig. 3). On the other hand, the measure of maximum yield of primary photochemistry (F_v/F_m) and photochemical quenching coefficient (q_p) decreased under these radiations. The decrease in F_v/F_m was 34.0 and 10.9 % in UV-B and UV-A+UV-B irradiated samples, respectively, while that in q_p was 4.0 and 10.1 % in these samples, respectively.

While the content of β-carotene declined by 40.6 % owing to UV-B treatment, it increased by 25.0 % in UV-A+UV-B treated sample (Table 1). UV-B radiation enhanced the content of violaxanthin (V), possibly at the expense of zeaxanthin (Z) component of xanthophyll cycle such that V/Z ratio increased by 18.5 %. UV-A+UV-B radiation partially restored Z and thereby V/Z. The content of antheraxanthin (A), which was almost absent in control and in UV-B irradiated samples, was enhanced considerably in response to UV-A+UV-B irradiation.

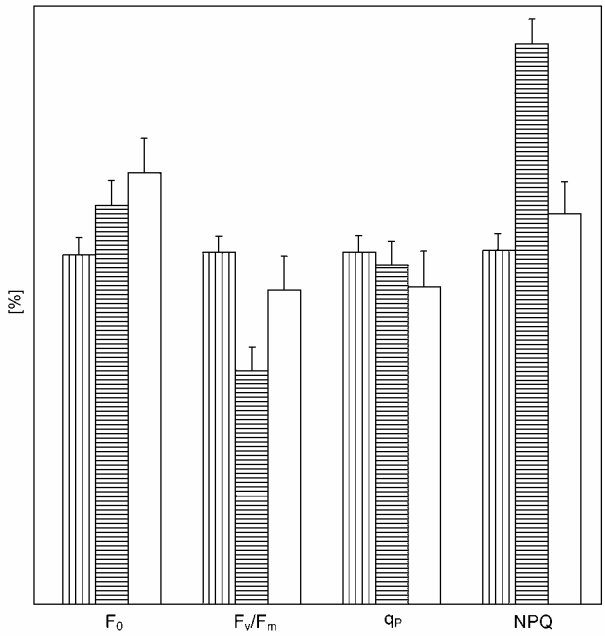

Fig. 3. Relative changes in different parameters associated with chlorophyll *a* fluorescence measured in 9-d-old primary leaves of clusterbean seedlings. *Vertical bars* = control, *horizontal bars* = UV-B, *black and white dots* = UV-A+UV-B irradiation of 1 h duration daily from day 1. Means of three independent estimates. *Bar* indicates ±S.D.

Table 1. Effect of UV-B or UV-A+UV-B irradiation on the contents of β-carotene and different component of xanthophyll cycle in primary leaves of clusterbean seedlings on day 9. The content of β-carotene after normalisation with chlorophyll (Chl) content of control sample is taken as 100 %. The values of V (violaxanthin), A (antheraxanthin), and Z (zeaxanthin) were calculated as the percentage of total xanthophyll pool after normalising to Chl content.

Treatment	β-car	V/(V+A+Z)	A/(V+A+Z)	Z/(V+A+Z)	V/Z
Control	100.00	75.3	0	24.70	3.05
UV-B	59.35	78.7	0.37	20.93	3.76
UV-A+UV-B	125.00	72.1	4.14	23.76	3.04

Discussion

UV-B-induced damage of the photosynthetic apparatus of primary leaves of clusterbean seedlings was assessed on the basis of pigment loss. The radiation induced a loss in Chl content and with the progress of doses the loss became cumulatively severe even during the steady phase of leaf development (Fig. 1*A*). Similarly, the content of total Car was reduced due to the UV-B irradiation. These results are in conformity with findings of Vu *et al.* (1982) and Strid and Porra (1992). However, Car reduction was relatively less than that of Chl as evident from the

Chl/Car ratio (Fig. 2*A*). UV-A+UV-B irradiation also induced a loss in Chl and Car contents but the losses were lesser than the losses caused by UV-B radiation alone. Hence the loss in pigments owing to UV-B was retarded significantly when UV-B was accompanied by UV-A radiation. We also examined the effects of UV-B radiation in combination with or without UV-A radiation during steady state of leaf development. The study indicated a lesser decline in the contents of photosynthetic pigments (unpublished data).

To examine such effects of the radiation on the PS2 photochemistry, the photosynthetic oxygen evolution of isolated chloroplasts from primary leaves of clusterbean seedlings with UV-B or UV-A+UV-B irradiation was measured. UV-B radiation induced a significant 30 % loss in PS2-mediated oxygen evolution with DCQ as electron acceptor and this inhibitory effect of UV-B radiation was weakened when UV-A was supplemented (Fig. 2*B*). This healing effect of UV-A radiation against the damaging effect of UV-B was further examined by measuring Chl *a* fluorescence, an *in vivo* indicator of PS2 efficiency (Krause and Weis 1991).

UV-B induces a significant loss in photochemical activity as evidenced by a decline in F_v/F_m ratio (Fig. 3). The decline in both F_v/F_m and q_p and an enhancement in F_0 in spite of loss in total Chl suggest that UV-B radiation inactivates PS2 reaction centre. The increase in NPQ by 58.9 % indicated the severity of the stress perceived by chloroplasts. On the other hand, the UV-B-induced severity seemed to be diminishing in the presence of UV-A, as NPQ value declined in UV-A+UV-B irradiated sample (Fig. 3). Although the UV-B-induced increase in F_0 was found in UV-A+UV-B irradiated sample, the values of q_p and F_v/F_m were restored. We, therefore, consider that UV-A radiation considerably restores the injuries of PS2 caused by UV-B. The likely involvement of Car in reversing the damaging effect was also investigated.

β-carotene, a major Car component in PS2 having close association with reaction centre 2 of higher plants protects the photosynthetic apparatus by quenching singlet oxygen (Mohr and Schopfer 1995). -carotene may also improve the stability of D_1 protein (Barber 1995, Deo and Biswal 2001). The mechanism of protection of PS2 by β-carotene has recently been proposed (Nayak *et al.* 2002). UV-B irradiation reduces the content of β-carotene (Table 1) indicating the vulnerability of D_1 protein to the radiation resulting in a loss in PS2 activity. On the other hand, a 25 % increase in the pigment owing to UV-A+UV-B exposure may enhance its shielding capacity to stabilise D_1 protein. Z is also important for structural integrity of light-harvesting Chl-protein complex (Ruban *et al.* 1997) and it may be the blue/UV-A light photoreceptor (Quinones and Zeiger 1994). UV-B radiation caused a decline in both V and Z components of the xanthophyll cycle (Table 1). This suggests an impairment of the cycle ensuing loss in the pigment content. But the restoration of the content of Z and a considerable rise of A in response to UV-A+UV-B irradiation may facilitate energy flow to Z through the molecular gear shift mechanism proposed by Frank *et al.* (1994) for energy dissipation, and the photosynthetic apparatus is rescued. The involvement of these pigments in dissipating harmful quanta *via* a quenching complex proposed by Nayak *et al.* (2002) could also be a plausible mode of protecting the apparatus.

Therefore we surmise that UV-A radiation accompanying UV-B guards the photosynthetic apparatus against the harmful effect of the later moderately and we believe that Car protect it at least partially.

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