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BRIEF COMMUNICATION

Photosystems activities and polypeptide composition of *Cyamopsis tetragonoloba* and *Vigna mungo* thylakoids as affected by exclusion of solar UV radiation

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Abstract

The impacts of solar UV (280 - 400 nm) radiation on photosynthetic activities and polypeptide composition of thylakoids of cluster bean (*Cyamopsis tetragonoloba* L, UV-B sensitive) and black gram (*Vigna mungo* L., UV-B resistant) plants were compared. The activity of photosystem 1 and especially photosystem 2 increased in cluster bean while decreased in black gram, when either UV-B or UV-B + UV-A radiation was removed as compared to control plants. Exclusion of UV-B radiation caused changes in the protein composition of the thylakoids particularly in the 33, 23 and 17 kDa proteins of photosystem 2.

Additional key words: black gram, cluster bean, photosystems 1 and 2.

UV-B radiation has long been known to affect the plants from molecular to ecosystem level. However, the effectiveness varies with plant species, cultivars, leaf age, prevailing growth conditions and geographical locations (Ziska 1996, Kulandaivelu *et al*. 1997). The effects of UV-B radiation have been extensively studied in plant communities, plant species, isolated chloroplasts and thylakoid membranes (Teramura and Sullivan 1994, Caldwell *et al*. 1995, Murthy and Rajagopal 1995, Jayakumar *et al.* 2004, Amudha *et al*. 2005, Mahdavian *et al*. 2008). UV-B affects photosynthesis and causes a reduction in the crop yield (Tevini and Teramura 1989). Existence of multiple target sites for UV-B action was reviewed by Bornman (1989). In thylakoid membranes, photosystem 2 (PS 2) is highly sensitive to any type of stress. UV-B radiation induced damage to membrane integrity is one of the factors contributing to photosynthetic reduction (Noorudeen and Kulandaivelu 1982). Renger *et al*. (1989) studied the possibility of an altered D1/D2 protein configuration causing functional disruption of the Mn association with the 33 kDa protein. Nedunchezhian and Kulandaivelu (1991) have shown that loss of PS 2 mediated electron transport activity and O_2 evolving capacity was primarily due to the depletion of 33, 23 and 17 kDa polypeptides during *in vitro* UV-B treatment. Analysis of the changes in the polypeptide pattern would therefore yield valuable information regarding the structural changes in the thylakoid membranes. The present study was aimed to study the changes in the polypeptide composition of thylakoids isolated from *Cyamopsis tetragonoloba* (UV-B sensitive) and *Vigna mungo* (UV-B resistant) plants at ambient and UV-B or UV-B and UV-A filtered radiation.

The certified seeds of cluster bean (*Cyamopsis tetragonoloba* L. cv. Pusa Navbagar) and black gram (*Vigna mungo* L. cv. T9) were used in all the experiments. All experiments were conducted under natural sunlight in the University Botanical garden. The average day/night temperature was 33/23 °C. The natural photoperiod varied between 10 and 12 h. Plants grown in the field were covered with polyester film mounted on metal mesh cages. The experimental treatments were obtained using a 0.12 mm thick polyester film for the UV-B exclusion and 0.30 mm for the UV-B and UV-A

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Abbreviations: BQ - *p*-benzoquinone; DAT - days after treatment; DCPIP - dichlorophenol indophenol; MV - methyl viologen; PAR - photosynthetically active radiation; PS 1 - photosystem 1; PS 2 - photosystem 2.
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exclusion. Polyester films have a sharp transmittance cut-off at around 320 nm or 380 nm as was proved in preliminary experiments. The bottom sides of all the frames were left uncovered to allow normal ventilation. The frames received full solar radiation for most of the day without any shading. The seedlings were exposed to the solar UV radiation from the time of germination.

Type II broken chloroplasts were isolated from fresh leaves as described by Reeves and Hall (1973). Total chlorophyll (Chl) content was estimated according to the method of Wellburn and Lichtenthaler (1984). Activities of PS 2 [H_2O → *p*-benzoquinone (BQ)] and PS 1 [dichlorphenol indophenol ($DCPIPH_2$) → methyl viologen (MV)] were measured as described by Noorudeen and Kulandaivelu (1982) and the whole chain electron transport (H_2O → MV) as described by Armond *et al*. (1978). Plants were sampled at 10, 20, 30, 40 and 50 days after start of treatment (DAT).

To determine a profile of PS 2 particles, chloroplasts were prepared according to the method of Kuwabara and Murata (1979), using medium containing 100 mM sucrose, 100 mM NaCl and 50 mM phosphate buffer (pH 7.4). The chloroplasts were then suspended in a medium containing 300 mM sucrose and 50 mM NaCl (pH 6.9) at a Chl concentration of 2 - 3 g dm^{-3}. An aqueous solution of *Triton X-100* was added to a final Triton:Chl ratio of 25:1 (v/v). After incubation for 30 min, the suspension was centrifuged at 1 000 *g* for 2 min. The pellet was discarded and the supernatant was centrifuged at 35 000 *g* for 10 min in a *Hitachi SCP 70H* ultracentrifuge. The final pellet was used in the experiments as the PS 2 particles. The Chl *a*/*b* ratio of this fraction was less than 2.0 (Kuwabara and Murata 1982). To determine thylakoid protein composition, leaf extract was precipitated with 10 % trichloroacetic acid (TCA) and left in ice for at least 30 min before centrifugation to collect the pellet. Traces of TCA in the pellet were removed by washing thrice with ice cold acetone. The final pellet was air dried and dissolved in a small volume of 10 % sodiumdodecyl sulphate (SDS). Protein content was quantified by the method of Lowry *et al*. (1951). Plants were sampled at 20 DAT for the leaf total protein determination and profiles of PS 2 particles. The gel system described by Laemmli (1970) for polyacrylamide gradient of 8 - 18 % was used for all protein separation.

Under all treatments cluster bean showed an increase in the activities of both photosystems up to 30 DAT, thereafter a decrease was observed, while in black gram maximum activities were observed at 20 DAT. When compared to plants grown under ambient solar radiation exclusion of UV-B and UV-B + UV-A increased PS 1 and 2 activities in cluster bean, however, decreased them in black gram. As much as 32 % increase in the PS 2 activity over the respective control was observed after 30 DAT in cluster bean under exclusion of UV-B and removal of the UV-A component of solar radiation along with UV-B further increased the PS 2 activity (Table 1). In black gram, PS 2 activity decreased due to UV-B or UV-B + UV-A removal during the whole treatment, but changes observed in PS 1 activity were more or less similar at 10 and 20 DAT and only marginal at 30 to 50 DAT.

Since the changes in photosynthetic electron transport activities could be caused primarily by the changes or reorganization of thylakoid membranes, the polypeptide profiles induced by exclusion of UV-B or UV-B and UV-A from solar radiation were analyzed. As concerns the total proteins of cluster bean (Fig. 1), an increase in the content of 55, 27, 23, 17 and 14 kDa polypeptides was observed under UV-B exclusion, when compared to control plants. Comparatively the increase in contents of these polypeptides under UV-B + UV-A removal was

Table. 1. Changes in the PS 2 and PS 1 activities of *C. tetragonoloba* and *V. mungo* seedlings grown under ambient solar radiation, and under exclusion of UV-B or UV-B and UV-A. Means of three independent measurements ± SE. DAT - days after UV exclusion.

DAT	Treatment	PS 2 activity [μmol (O_2) mg^{-1}(Chl) min^{-1}]		PS 1 activity [μmol (O_2) mg^{-1}(Chl) min^{-1}]	
		C. tetragonoloba	*V. mungo*	*C. tetragonoloba*	*V. mungo*
10	control	3.11 ± 0.017	2.87 ± 0.016	5.43 ± 0.022	6.23 ± 0.020
	UV-B	3.35 ± 0.020	2.63 ± 0.012	5.80 ± 0.020	5.22 ± 0.018
	UV-B + UV-A	4.02 ± 0.021	2.20 ± 0.012	6.58 ± 0.022	4.98 ± 0.022
20	control	4.72 ± 0.020	4.78 ± 0.019	7.85 ± 0.023	8.82 ± 0.022
	UV-B	5.40 ± 0.023	4.13 ± 0.017	8.45 ± 0.030	8.62 ± 0.020
	UV-B + UV-A	6.27 ± 0.022	3.68 ± 0.017	9.50 ± 0.024	8.37 ± 0.022
30	control	4.97 ± 0.020	2.87 ± 0.012	8.38 ± 0.030	5.95 ± 0.020
	UV-B	6.55 ± 0.022	2.43 ± 0.012	9.62 ± 0.029	5.66 ± 0.020
	UV-B + UV-A	7.87 ± 0.020	2.02 ± 0.009	10.50 ± 0.020	5.60 ± 0.019
40	control	4.52 ± 0.018	2.32 ± 0.009	6.78 ± 0.020	5.10 ± 0.019
	UV-B	5.77 ± 0.019	1.95 ± 0.009	7.38 ± 0.020	4.77 ± 0.019
	UV-B + UV-A	6.48 ± 0.022	1.60 ± 0.007	8.77 ± 0.022	4.68 ± 0.022
50	control	1.37 ± 0.010	1.58 ± 0.009	3.12 ± 0.013	4.67 ± 0.022
	UV-B	1.40 ± 0.010	1.32 ± 0.007	3.37 ± 0.015	4.35 ± 0.017
	UV-B + UV-A	1.75 ± 0.020	1.08 ± 0.007	3.57 ± 0.014	4.18 ± 0.017

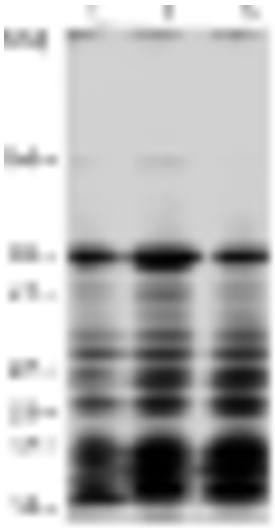

Fig. 1. SDS-PAGE analysis of total proteins isolated from *C. tetragonoloba* leaves grown under ambient solar radiation (1), and exclusion of UV-B (2) or UV-B + UV-A (3) for 20 d. The numbers of the left side refer to the molecular mass of polypeptides showing prominent changes.

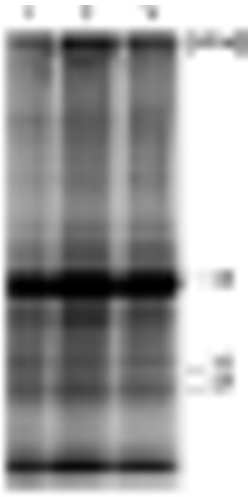

Fig. 2. SDS-PAGE analysis of total proteins isolated from *V. mungo* leaves grown under ambient solar radiation (1), and exclusion of UV-B (2) or UV-B + UV-A (3) for 20 d. The numbers of the right side refer to the molecular mass of polypeptides showing prominent changes.

less than only UV-B removal, but they were still higher than in the control plants. Total protein profile of black gram mostly did not show any significant changes under UV-B or UV-B + UV-A exclusion except for a decrease of 33 and 27 kDa polypeptides (Fig. 2). The PS 2 particles of cluster bean showed an increase in the content of 33, 23 and 17 kDa polypeptides and complete loss of 45 kDa polypeptide in plants grown under UV exclusion (Fig. 3). Exclusion of UV has brought about an increase in the content of low molecular mass polypeptides in the range of 17 - 33 kDa in black gram and decrease in the content of 43 kDa polypeptide (Fig. 4).

The results presented here indicate that exposure of cluster bean and black gram plants to two different UV excluded conditions, produces different trend in photosynthetic activities, probably because the former was UV sensitive and the later UV resistant species. Solar UV-A and UV-B radiation were found to cause reductions in photosynthetic rate. Ambient UV may induce changes in membrane integrity due to lipid peroxidation caused by the generation of free radicals (Kramer *et al*. 1991). Evidences have been already presented on the changes induced by UV-B radiation on the photosynthetic electron transport chain (Noorudeen and Kulandaivelu 1982). Among the possible target sites,

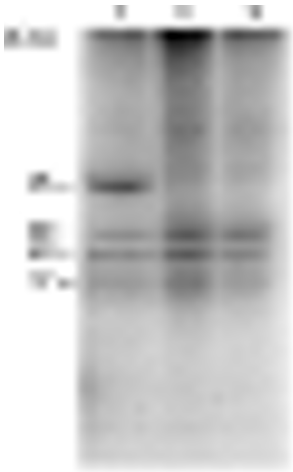

Fig. 3. SDS-PAGE analysis of proteins of purified PS 2 particles isolated from *C. tetragonoloba* leaves grown under ambient solar radiation (1), and exclusion of UV-B (2) or UV-B + UV-A (3) for 20 d. The numbers on the left side indicate the molecular mass of polypeptides showing changes.

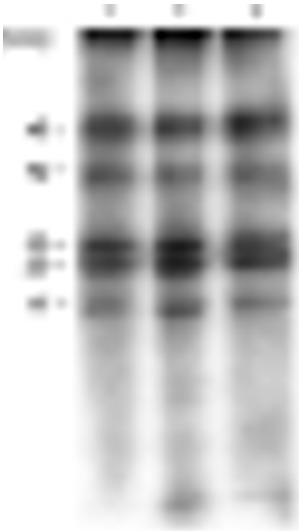

Fig. 4. SDS-PAGE analysis of proteins of purified PS 2 particles isolated from *V. mungo* leaves grown under ambient solar radiation (1), and exclusion of UV-B (2) or UV-B + UV-A (3) for 20 d. The numbers on the left side indicate the molecular mass of polypeptides showing changes.

the PS 2 has been considered as the most susceptible site to UV-B radiation (Renger *et al*. 1989). It has been established that UV-B primarily affects the reaction center components of PS 2 and H_2O oxidizing complex (Renger and Eckert 1991). Studies with artificial UV-B sources have shown that UV-B radiation may strongly affect the PS 2, whereas the PS 1 appears to be rather insensitive (Teramura and Ziska 1996). It is known that the PS 1 electron transport activity is more resistant than PS 2 to the various stresses (Baker 1991).

A remarkable increase in the rate of O_2 evolution in cluster bean seedlings grown under UV exclusion suggests that the PS 2 reactions remain unaffected. This could be due to either an increase in the number of active units in the thylakoid membranes or the efficient functioning of the primary photosynthetic light absorbing processes. On the other hand, a significant decrease in PS 2 activity was observed in black gram plants grown under UV excluded conditions. This indicates that the exclusion of solar UV-B and UV-B + UV-A created a condition similar to partial shading.

PS 2 core protein analysis of cluster bean resulted in complete loss of 45 kDa polypeptide under UV excluded conditions. As the black gram is a resistant species, no such change in the PS 2 core protein was observed. Association of the three polypeptides with the inner luminal surface of the thylakoid membranes (Enami *et al*. 1991) is needed for the complex to maintain its conformation and optimal O_2 evolution (Murata and Miyao 1987, Rutherford 1989). The 23 and 17 kDa polypeptides are considered to have regulatory roles in O_2 evolution. Decrease in these polypeptides, indicates their poor stability under solar UV-B supplementation (Nedunchezhian and Kulandaivelu 1993) and therefore it results in a loss of PS 2 activity (Nedunchezhian *et al*. 1995).

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