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Changes in photosynthetic apparatus during dark incubation of detached leaves from control and ultraviolet-B treated *Vigna* seedlings

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Abstract

Changes in various components of photosynthetic apparatus during the 4 d dark incubation at 25 °C of detached control and ultraviolet-B (UV-B) treated *Vigna unguiculata* L. leaves were examined. The photosynthetic apparatus was more degraded in younger control seedlings and for a longer time UV-B treated seedlings than in the older or for a shorter time UV-B treated seedlings. This was shown by determining the losses in chlorophyll (Chl) and protein contents, variable fluorescence yield, photosystem (PS) 2, PS1 and ribulose-1,5-bisphosphate carboxylase (RuBPC) activities, and photosynthetic $^{14}CO_2$ fixation. In contrast, the Car/Chl ratio increased during the dark incubation due to less expressed degradation of Car.

Key words: carotenoids, chlorophyll, $^{14}CO_2$ fixation, *in vitro* ageing, fluorescence, photosystem 1 and 2, protein, ribulose-1,5-bisphosphate carboxylase activity

Introduction

The mechanism of chloroplast degradation in aging attached leaves is different from degradation that occurs in dark in detached leaves (Butler and Simon 1971, Choe and Thimann 1974, Biswal and Mohanty 1978, Misra and Biswal 1980). In attached leaves senescence is influenced by hormones and protein synthesizing capacity of the organelles. Pigment contents decrease faster in detached leaves than in attached leaves (Misra and Biswal 1980). The ratios of Chl *a*/*b* and Chl/Car decrease during senescence of both attached and detached leaves (Šesták 1972, Misra and Biswal 1980, 1982, Grover *et al.* 1986), probably due to the the relatively faster decrease of

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Abbreviations: BQ - *p*-benzoquinone; Chl - chlorophyll; $DCPIPH_2$ - reduced 2,6-dichlorophenol indophenol; DTT - DL-dithiothreitol; F_0 - initial fluorescence level; F_p - peak fluorescence kevel; MV - methyl viologen; PAR - photosynthetically active radiation; PS - photosystem; RuBPC - ribulose 1,5-bisphosphate carboxylase; TCA - trichloroacetic acid; UV-B - ultraviolet radiation.

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Chl *a* than Chl *b* and Chl (*a*+*b*) than Car. The decrease in non-cyclic electron transport is greater than the decline in the activities of PS1 or PS2 (Woolhouse 1984), which suggests that intersystem electron transport becomes rate-limiting (Woolhouse and Jenkins 1983).

A reduction in the ozone column continuously increases the UV-B irradiance reaching the earth and also shifts the terrestrial solar spectrum towards the more biologically damaging shorter wavelength. Plants grown under UV-B in addition to PAR show large changes in the net photosynthetic rate (*e.g.* Vu *et al.* 1981, Tevini and Teramura 1989, Teramura *et al.* 1991). The inhibition in photosynthesis is closely associated with PS2 than PS1 and coincident with ultrastructural damage of chloroplasts (Allen *et al.* 1978, Kulandaivelu *et al.* 1989). Hill activity and variable fluorescence are inhibited in chloroplasts and whole leaf after UV-B irradiation. UV-B radiation inactivates the reaction centre of PS2 (Noorudeen and Kulandaivelu 1982). UV-B irradiation deteriorates primarily water oxidation (Renger *et al.* 1989). The decrease in CO_2 uptake rates in leaves of plants exposed to UV-B radiation suggests the possible effects of this UV-B radiation on the photosynthetic enzymes (Basiouny *et al.* 1978, Kulandaivelu and Nedunchezhian 1993).

The aim of the present work was to study the changes in various photosynthetic reactions and thylakoid organization in excised leaves of *Vigna* seedlings grown under normal and UV-B enhanced radiation.

Materials and methods

Plants: Pre-soaked seeds of *Vigna unguiculata* L. were germinated in the dark for one day and then transferred to indirect daylight in the laboratory. After 2 d, when the primary leaves had fully expanded, the seedlings were put into a chamber where they were exposed for 24 or 48 h at 28 °C either to continuous irradiation from four *Philips 20 W* fluorescent tubes (type *TL/33*) plus one *Philips 20 W/12* sunlamp (*N.V. Philips Gloelampen-fabrieken*, The Netherlands) (UV-B treated), or to irradiation from four 20 W white fluorescent tubes (control).

Detached leaves incubation in the dark: Leaves from control and UV-B treated (24 and 48 h) seedlings were placed for 24 to 96 h on 200 cm^3 of distilled water in sterilized beakers in dark. Distilled water was changed every day to avoid fungal infection.

Chl and Car were estimated spectrophotometrically according to the methods of Arnon (1949) and Goodwin (1954), respectively.

***In vivo* Chl *a* fluorescence induction** was followed in intact leaves after excitation with broad band blue radiation (100 W m^{-2}, 400 - 460 nm; filter *Corning 5113*) as described by Kulandaivelu and Daniell (1980). Leaves were kept in the dark at 28 °C for 10 min before fluorescence measurements. The signal was stored in a digital oscilloscops (*Iwatsu SS-58O2*) and then transferred to a *Hitachi* recorder.

Activities of electron transport: Chloroplasts were isolated from control and UV-B treated senescent seedlings according to the method of Reeves and Hall (1973). PS2 and PS1 activities were measured as described by Noorudeen and Kulandaivelu (1982), whole chain electron transport ($H_2O \rightarrow MV$) activity according to Armond *et al.* (1978).

$^{14}CO_2$ fixation: Leaf pieces were circulated for 5 min under irradiation (40 W "white" fluorescent tubes) in 5 cm^3 of 50 mM KH_2PO_4 - KOH buffer (pH 7.5) containing 50 mM $MgCl_2$, 35 mM NaCl, and 10 mM $NaHCO_3$. $NaH^{14}CO_3$ (1850 kBq) was injected into the reaction medium and incubated for 30 min in light. The reaction was arrested by cold acetic acid (final concentration 10 %). The leaf segments were washed and ground in small volume of inoculation medium and the volume was made up to 3 cm^3. Aliquots of 10 mm^3 of the homogenate were loaded on to the *Whatman* No. *1* filter paper discs and dried at room temperature under incandescent lamps. The radioactive carbon fixed was measured using the *Packard* model *2425* liquid scintillation counter.

Extracts and assay of RuBPC activity: Fully expanded leaves were cut into small pieces and homogenised in a grinding medium consisting of 50 mM Tris - HCl (pH 7.8), 10 mM $MgCl_2$, 5 mM DTT or 10 mM 2-mercaptoethanol, and 0.25 mM EDTA. The extract was clarified by centrifugation at 20 000 *g* for 10 min. The clear supernatant was decanted slowly and used as the crude RuBPC source. All these steps were carried out at 4 °C. RuBPC activity was determined by a modified method of Bowes and Ogren (1972). The incubation mixture contained 50 mM DTT and 10 mM $NaH^{14}CO_3$ (9.25 GBq mol^{-1}) in a total volume of 2.0 cm^3. The reaction mixture was placed in pyrex tubes. After flushing with N_2 for 3 min the tubes were sealed with serum caps and gently shaken in a water bath at 32 °C for 3 min. Aliquots of 0.2 cm^3 of the enzyme extract were then injected through the serum cap into the mixture to initiate the reaction. After 3 min at 32 °C the reaction was stopped by injecting 0.2 cm^3 of 6 M glacial acetic acid. The known aliquots were transferred to *Whatman* No. *3* filter paper discs, dried under infra-red lamp, and the radioactivity was determined using a *Packard* model *2425* liquid scintillation counter.

Extraction and determination of soluble proteins: Soluble proteins were extracted by homogenizing 0.5 g of fresh leaves in 10 cm^3 of 50 mM chilled Tris - HCl (pH 8.0). The homogenate was centrifuged at 30 000 *g* for 15 min at 4 °C, and an aliquot from the supernatant was mixed with an equal volume of cold 10 % TCA (m/v) and incubated at 0 °C for 1 h to precipitate the proteins. The protein pellet was collected by centrifugation at 5 000 *g* for 15 min at 4 °C and dissolved in 0.1 M NaOH. Soluble protein content was estimated by the procedure of Lowry *et al.* (1951).

Results and discussion

The leaves from control and UV-B treated plants (24 and 48 h) were detached and placed on distilled water for up to 4 d in dark. During this period, the Chl (*a+b*)

content decreased more rapidly in 48 h UV-B treated seedlings and 24 h control seedlings than in the 48 h control and 24 h UV-B treated seedlings (Fig. 1 *left*). The relative loss in Car content was generally less extensive throughout the dark incubation, and most expressed in the 48 h control and 24 h UV-B treated seedlings (Fig. 1, *right*). The Car/Chl ratio increased during dark incubation, more in the 48 h UV-B treated leaves than in the other ones. The comparison of observations presented in Fig. 1 shows that the degradation of Chl may induce a rise in Car content. One of the main functions ascribed to Car is a possible role in providing protection to Chl against photobleaching (Šesták 1978). The increase in Car/Chl ratio was due to the relatively faster decrease of Chl than Car during senescence *in vitro*.

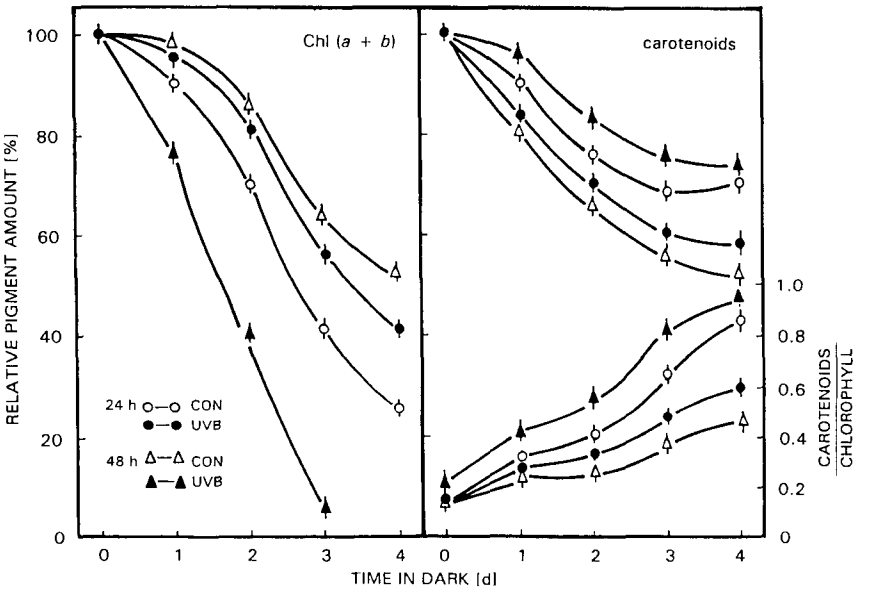

Fig. 1. Changes in chlorophyll (Chl) (*a*+*b*) and carotenoid (Car) contents and their ratio in detached control and UV-B treated *Vigna* leaves incubated at 25 °C as a function of time of dark incubation. The initial (0 h) values for Chl and Car were: control 1.80, 0.22 (24 h) and 2.60, 0.33 (48 h); UV-B treated 1.82, 0.24 (24 h) and 1.39, 0.30 (48 h) g^{-1} kg^{-1}(f.m.), respectively. Each value represents mean of three independent determinations ± SE.

Leaves collected from the 24 h control and 48 h UV-B treated seedlings showed during dark incubation a larger reduction in variable (F_0-F_p) fluorescence yield than leaves from the 48 h control and 24 h UV-B treated seedlings (Fig. 2). This indicated larger stability of PS2 reaction centre during dark incubation of both older control samples and less UV irradiated samples. The PS1 activity increased during dark incubation in both types of seedlings during the first day, while within the same period the PS2 activity gradually decreased (Fig. 3). The maximum reduction was noticed in the PS2 activity in 48 h UV-B treated and 24 h control seedlings. The rate of photochemical reactions associated with both PS1 and PS2 usually declines during dark incubation (Woolhouse 1984, Biswal and Biswal 1988), PS1 being relatively

more stable than PS2 (Biswal *et al.* 1983). This is probably connected with the disorganization of membrane structure that is also reflected in the absorption and emission characteristics of chloroplasts (Šesták 1972, Biswal and Mohanty 1976, Kulandaivelu and Senger 1976).

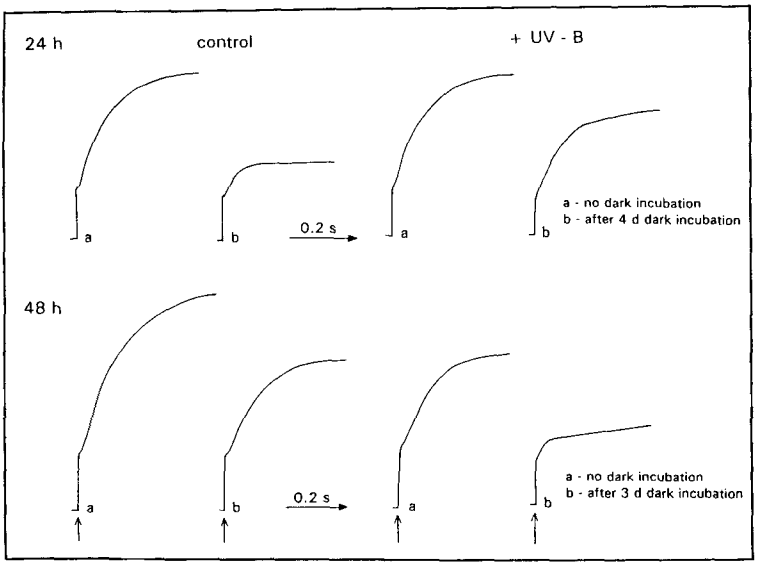

Fig.2. Changes in the typical fluorescence transients of 24 and 48 h control and UV-B treated *Vigna* leaves incubated in the dark for 3 and 4 d.

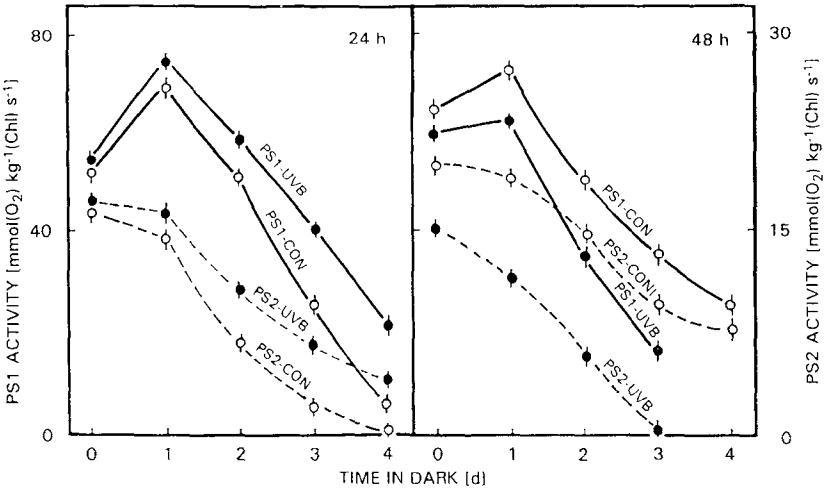

Fig. 3. Changes in the photosystem 2, PS2 ($H_2O \rightarrow BQ$) and photosystem 1, PS1 ($DCPIPH_2 \rightarrow MV$) electron transport activities of chloroplasts isolated from detached 24 and 48 h control and UV-B treated leaves. Each value represents mean of 3 independent determinations ± SE.

A general decline in the total leaf protein content, RuBPC activity and $^{14}CO_2$ fixation during the entire incubation period was observed in both control and UV-B treated seedlings (Fig. 4): the largest reduction was found in the 24 h control and 48 h UV-B treated seedlings. UV-B enhanced radiation drastically reduces the RuBPC activity, probably due to protein destruction or enzyme inactivation (McLaren and Luse 1961, Piras and Valle 1966, Peterson and Huffaker 1975, Giese 1976, Nedunchezhian and Kulandaivelu 1991). The decline in the activity is observed usually earlier than the loss of either Chl or primary photochemical reactions activity of chloroplasts in many plant systems (Biswal and Biswal 1988). A decline in the rate of CO_2 fixation and photophosphorylation is observed during leaf senescence of *Populas deltoides* (Hernandez-Gill and Schaedle 1973). Our results suggest that prolongation of UV-B treatment supports the dark induced degradation of photosynthetic apparatus.

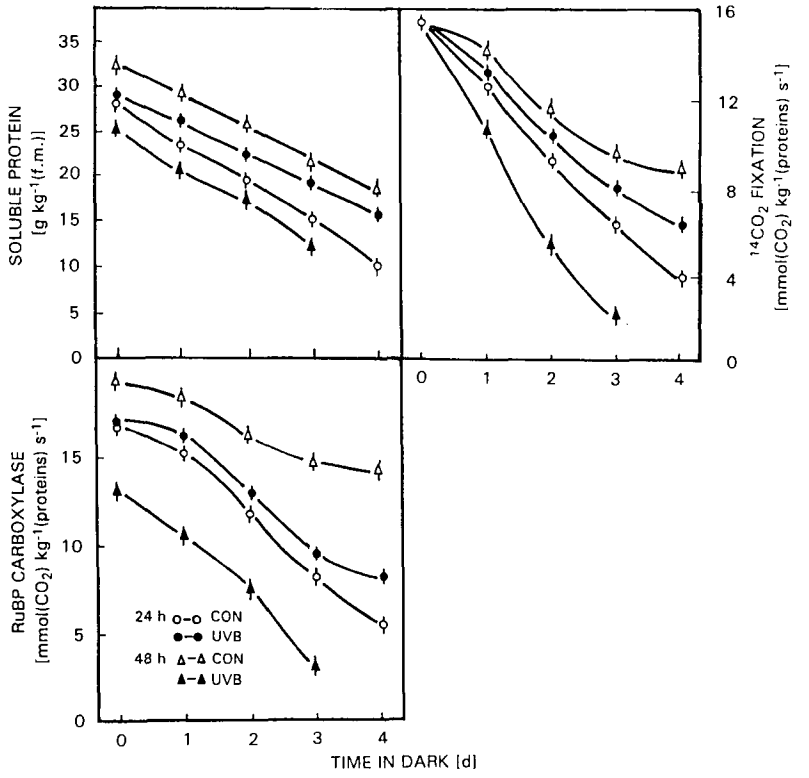

Fig. 4. Changes in the total soluble protein content, RuBPC activity and $^{14}CO_2$ fixation of control and UV-B treated *Vigna* leaves incubated in dark at 25 °C. Each point represents mean of 4 independent determinations ± SE.

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