Changes in leaf protein and pigment contents and photosynthetic activities during senescence of detached maize leaves: influence of different ultraviolet radiations

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Abstract

Senescence induced loss in pigments and proteins of detached maize (*Zea mays* L. cv. Col) leaves was significantly enhanced on the exposure of leaves to different ranges of ultraviolet (UV) radiation. Compared to UV-A (320-400 nm) and UV-B (280-320 nm), the UV-C (200-320 nm) was the most damaging for the pigments and macromolecules. A severe decline in photosystem (PS) 2 mediated photoreduction during senescence of detached leaves exposed to UV irradiation suggested a damage of the system. The PS1 mediated photoreduction of methylviologen with 2,6-dichlorophenol indophenol as electron donor was stimulated by UV-A and UV-B radiations, suggesting a reorganisation of the PS1 complex. These results were fortified by the values of fast and slow kinetics of chlorophyll (Chl) *a* fluorescence transients.

Additional key words: carotenoids; chlorophyll fluorescence; photosystems 1 and 2; UV-A, UV-B, UV-C; *Zea mays*.

Introduction

Extensive reports are available on the degradation of chloroplasts during leaf senescence (Biswal and Biswal 1988, Matile 1992, Thomas and Smart 1993). The extent of degradation of the organelle depends on the plant species (Murthy and Rajagopal 1995) and environmental conditions (Pjon 1981, Biswal and Biswal 1984). Stress-like high temperature (De Luca d'Oro and Trippi 1987), high irradiance (Pjon 1981, Biswal *et al.* 1983, Biswal and Choudhury 1986) and UV-A irradiation (Joshi *et al.* 1991, 1994) accelerate leaf senescence.

Received 30 May 1996, *accepted* 22 January 1997.
Acknowledgements: The authors thank the University Grants Commission, New Delhi for the award of a Visiting Associateship to B.B., and the Department of Science and Technology, New Delhi for financial support.

UV radiation whose fluence on the earth's surface is increasing owing to stratospheric ozone depletion (Frederick 1990) produces structural modifications of leaves in higher plants (Noorudeen and Kulandaivelu 1983) and extensive damage to thylakoid membranes (Bornmann 1989). The solar UV spectrum consists of UV-A, UV-B and UV-C; of these the UV-C causes non-specific damage to the photosynthetic organelle and inhibits photosynthesis (Bishop 1961, Noorudeen and Kulandaivelu 1982a), while the UV-B inactivates the PS2 reaction centre (Tevini and Pfister 1985, Renger *et al.* 1989, Greenberg *et al.* 1989, Friso *et al.* 1994) and impairs the electron transport path between PS2 and PS1 (Strid *et al.* 1990). On the other hand, the primary target of UV-A, whose damaging effects are rather mild, is around PS2 (Hirasawa 1984, Joshi *et al.* 1994). These UV-induced changes are expected to modify the senescence process in leaves. Further, the cellular DNA remains stable with conservation of all information to control leaf senescence (Woolhouse 1982, Biswal and Biswal 1988, Thomas and Smart 1993). Since UV radiation causes a significant damage to leaf DNA (Noorudeen and Kulandaivelu 1982a), it is important to investigate whether UV exposed leaves exhibit any differential kinetics of senescence induced degradation. Therefore, the objectives of the present investigation were to examine (*a*) the action of different regions of UV spectrum on the photosynthetic apparatus of *in vitro* senescing detached maize leaves, and (*b*) the alteration in the course of leaf senescence as modulated by these radiations.

Materials and methods

Plants and UV treatment: Maize (*Zea mays* L. cv. Col) seeds were presoaked for 24 h in continuously running water and grown in soil-filled plastic trays in the dark for 2 d. The pots were then transferred to continuous irradiation (55 μmol m^{-2} s^{-1}) of "white light" at 28±2 °C. After 7 d of growth, 15 segments, each measuring 1×3 cm^2 were excised from the second leaf and incubated on 4 layers of moist filter paper at 28±2 °C. Detached leaf segments were allowed to senesce for 3 d under similar irradiation. The samples for different measurements were taken at 24 h intervals. The leaf segments were irradiated in separate Petri dishes with either a *Philips* "black light" type *05* (UV-A, emission maximum 365 nm) or a *Philips* 20 W/12 sunlamp (UV-B, 285-320 nm) filtered with cellulose acetate 5 mil filters, or a *Philips* 15 W germicidal lamp (UV-C, emission maximum 254 nm). The duration of exposure was 60, 30, and 15 min per day at a fluence rate of 75, 64 and 50 μmol m^{-2} s^{-1} for UV-A, UV-B and UV-C radiations, respectively. Under these fluence rates, the extent of inhibition of overall photosynthesis in non-senescing organelle was identical in all UV bands.

Biochemical analysis: Pigments were extracted with 80 % acetone. The content of total Chl was estimated spectrophotometrically by the method of Arnon (1949) and that of total carotenoid (Car) as described by Liaaen-Jensen and Jensen (1971). The total leaf protein in alkali (0.1 M NaOH) digests was estimated by the method of Lowry *et al.* (1951).

Isolation of chloroplasts: Chloroplasts were isolated by mechanical grinding of the leaves with ice chilled isolation medium containing 20 mM Tris-HCl buffer, pH 7.8, 5 mM $MgCl_2$, 300 mM sucrose, 10 mM NaCl and 1 mM EDTA. The homogenate was squeezed through two layers of nylon cloth and the filterate was centrifuged for 5 min at 3 000×*g*. The pellet was washed once with the isolation medium. The final pellet containing the chloroplasts was resuspended in a small volume of isolation buffer and used immediately. For thylakoid preparation, chloroplasts were lysed with 10 mM Tris-HCl buffer, pH 7.8, and collected by centrifugation at 5 000×*g* for 5 min.

Electron transport activities: The rate of PS2 electron transport was measured as 2,6-dichlorophenol indophenol (DCPIP) reduction using a *Hitachi 557* spectrophotometer as described by Noorudeen and Kulandaivelu (1982b). The PS1 electron transport from the artificial electron donor was assayed as O_2 uptake at 25 °C under saturating "white light" from a slide projector fitted with a 150 W halogen lamp (*Photophone*, India) using a *Hansatech* O_2 electrode.

Chl *a* fluorescence transient of the leaf was measured following the method of Kulandaivelu and Daniell (1980). Leaves were dark adapted for 20 min and then excited with a saturating "blue light" in the range 400-460 nm (*Corning 5113* filter) for fluorescence measurement. The photomultiplier placed 90° to the excitation beam was protected by an interference filter (*Schott*, maximum 690 nm with half-band width 12 nm). The signal was stored in a digital oscilloscope and then transferred to a recorder.

Statistical analysis: The Students' *t* test was carried out according to Glantz (1989).

Results

Effect of UV radiation on pigments and proteins of senescing leaves (Fig. 1): The loss of Chl during senescence of leaves without UV treatment was slow and gradual, UV exposure of detached leaves stimulated the loss of pigments. The UV-C induced a maximum loss of Chl (Fig. 1*A*). The decline of Chl (*a*+*b*) in untreated, UV-A, UV-B and UV-C treated samples after 3 d was 36, 50, 56 and 68 % ($p < 0.2$, 0.01, 0.001, 0.001), respectively. Compared to the Chl, the changes in Car content induced by UV-A and UV-B were not statistically significant, and only the UV-C treatment significantly ($p < 0.002$) enhanced the loss of Car in senescing leaves (Fig. 1*B*). The changes in content of total proteins during senescence of detached maize leaves (Fig. 1C) were rapid and significantly stimulated by all types of UV radiation. After 3 d of senescence, the decline in protein contents in untreated and UV-A, UV-B and UV-C treated samples was 54, 63, 65 and 82 % ($p < 0.001$, 0.001, 0.001, 0.001), respectively.

UV induced changes in primary photochemical reactions during senescence (Table 1): The PS2 mediated DCPIP reduction showed an 18 % decrease ($p < 0.01$) during the

3 d senescence period of detached maize leaves. Treatment of leaves with UV radiation accelerated this loss. The extent of loss in the photochemical reaction, however, varied with different UV treatments, the loss being maximum with UV-C (73 %, $p < 0.01$) and minimum with UV-A (33 %, $p < 0.01$) irradiation. In contrast, the photochemical reaction associated with PS1 remained stable during the study: after 3 d of senescence the PS1 activity was reduced only by 3 % (not significant). Exposure of leaves to UV-A or UV-B induced a marked enhancement in PS1 activity ($p < 0.05$ and < 0.01, respectively) while UV-C radiation caused no significant effect (not significant).

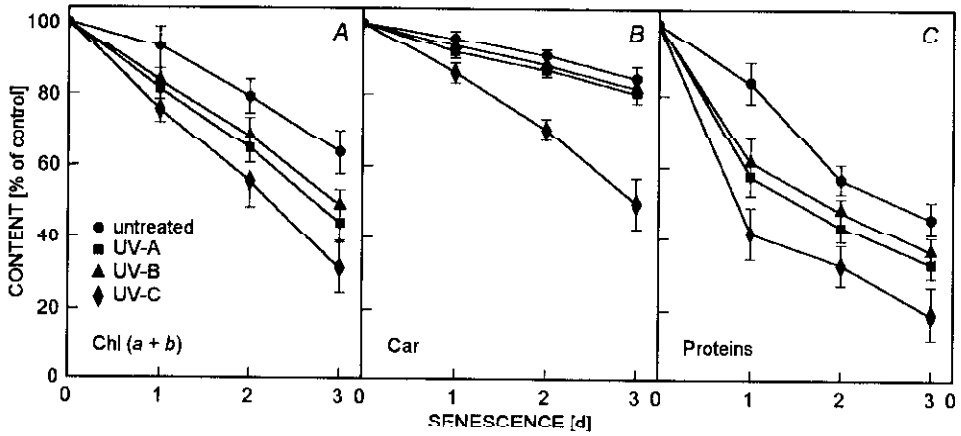

Fig. 1. Changes in contents of chlorophyll (Chl) ($a+b$) (*A*), carotenoids (Car) (*B*) and total proteins (*C*) in senescing maize leaves induced by UV-A, UV-B and UV-C. Error bars indicate SE ($n = 3$). The initial values were [g kg^{-1}(FM)]: Chl 2.09 ± 0.3, Car 0.35 ± 0.05, proteins 23.6 ± 4.1.

Table 1. Changes in the rate of photosystem 2, PS2 ($H_2O \rightarrow$ DCPIP) and PS 1 ($DCPIPH_2 \rightarrow$ MV) activity (figures in parentheses represent % activity with reference to 0 d control) and in different parameters associated with fast and slow kinetics of chlorophyll *a* flurescence induction (values in 0 d control sample taken as 100 %) in control and UV-treated maize leaves after 3 d of aging. 100 % values of F_v/F_m and $(F_p - F_s)/F_s$ were 0.8 and 2.6, respectively. Means ± SE ($n = 4$ for PS2 and PS1, 3 for fluorescence).

Treatment	$H_2O \rightarrow$ DCPIP [mmol(DCPIP) kg^{-1}(Chl) s^{-1}]	$DCPIPH_2 \rightarrow$ MV [mmol(O_2) kg^{-1}(Chl) s^{-1}]	F_v/F_m [%]	$(F_p - F_s)/F_s$ [%]
0 d control	21.2 ± 1.9 (100.0)	48.1 ± 5.8 (100.0)	100.0 ± 1.5	100.0 ± 2.1
+3d	17.4 ± 1.8 (82.3)	47.4 ± 5.1 (98.6)	66.0 ± 5.2	70.0 ± 4.9
+3d + UV-A	14.3 ± 2.0 (67.4)	62.1 ± 8.7 (129.3)	56.0 ± 3.4	60.0 ± 4.1
+3d + UV-B	11.4 ± 1.6 (53.6)	60.6 ± 6.8 (126.0)	42.0 ± 4.1	45.0 ± 2.9
+3d + UV-C	5.8 ± 0.9 (27.3)	46.6 ± 3.5 (97.0)	22.0 ± 2.6	12.0 ± 3.2

Changes in Chl *a* fluorescence transient parameters (Table 1): The F_v/F_m (where F_v is the variable fluorescence and F_m is the maximum fluorescence) decreased by 34 % at

the end of 3 d of senescence study. The % decline in the values of the parameter with UV-A, UV-B and UV-C was 44, 58 and 78, respectively. Similarly, the ratio $(F_p - F_s)/F_s$ (where F_p is peak fluorescence and F_s is steady state fluorescence of slow kinetics) decreased by 30 % during 3 d of senescence and was diminished by 40, 55 and 88 % with UV-A, UV-B and UV-C, respectively.

Discussion

Senescence induced loss of Chl was significantly accelerated when the detached maize leaves were exposed to different regions of UV spectrum for a short period. Among the three bands examined, UV-C induced breakdown of Chl was more rapid than that induced by UV-A and UV-B. Disorganisation of the thylakoid membrane may activate membrane bound chlorophyllase for degradation of Chl (Lambers *et al.* 1984). Since UV causes membrane disorganisation (Bornmann 1989), the rapid Chl loss may be attributed to the stimulation of chlorophyllase activity induced by the radiation. Compared to Chl, Car are stable and their content declines slowly during senescence (Biswal and Biswal 1988). Although in our experiments both UV-A and UV-B stimulated the Chl loss, they did not significantly affect the loss of Car. This is in contrast to the finding of Hsiao and Björn (1982) who demonstrated UV-A and UV-B induced carotenogenesis in the fungus *Verticillium agaricinum*. On the other hand, UV-C accelerates Car degradation during senescence.

UV irradiation of leaves also accelerated the loss of total leaf proteins. The loss of proteins was more rapid than that of the pigments during the first 24 h of senescence. The impact of different bands of UV radiation on the loss of protein was comparable to the changes observed for Chl content: UV-C caused the maximum damage followed by UV-B and UV-A. Protein loss during senescence is generally attributed to the increase in the activity of proteases (Wettern and Galling 1985, Thayer *et al.* 1987). A possible stimulation of the activity of senescence related proteases by UV radiation cannot be ruled out. Our results of pigment and protein loss owing to these irradiations are at par with the findings of other workers (Noorudeen and Kulandaivelu 1982a, Tevini and Pfister 1985, Renger *et al.* 1989, Strid *et al.* 1990). The results on the loss in electron transport efficiency of thylakoid membranes during leaf senescence of maize corroborate with the findings of Choudhury and Biswal (1980) who have demonstrated a decline in DCPIP photoreduction during dark induced senescence of detached maize leaves. The photochemical reactions associated with PS1 and PS2 of thylakoid membranes are differentially susceptible to senescence in leaves. Within the time period of our study, senescence induced decline in PS1 activity was negligible compared to the decline in PS2 activity. The relative susceptibility of PS2 mediated reaction to senescence could be attributed to a damage of the oxygen evolving complex (Biswal and Biswal 1988) and/or the content of plastoquinone (Kulandaivelu and Senger 1976). Acceleration of loss of PS2 activity during senescence of detached leaves by UV readiation suggested further inactivation of the oxygen evolving complex and/or decline in the plastoquinone level.

On the other hand, the PS1 mediated reaction was markedly stimulated by UV-A and UV-B, while UV-C had a damaging effect. It may be argued that UV radiation reorganises the PS1 complexes in the thylakoid membranes (Nedunchezhian *et al.* 1995) contributing to their stability during senescence. Further, the possibility of UV induced uncoupling of the LHC from the complex of PS2 and its subsequent migration to the PS1 complex with consequent enhancement of its photochemical reaction cannot be ruled out. The results confirmed the finding of Nedunchezhian and Kulandaivelu (1991).

In order to ascertain the differential action of the tested radiations, the Chl *a* fluorescence transients (an *in vivo* test of thylakoid functions) in detached leaves were followed. The parameter F_v/F_m (associated with fast kinetics) is a measure of PS2 activity (Krause and Weis 1991), while the parameter $(F_p - F_s)/F_s$ (associated with slow kinetics) is an indicator for complete photosynthesis (Lichtenthaler 1986, Lichtenthaler and Buschmann 1987). Relatively higher decline in the former parameter compared to the later in UV-A and UV-B irradiated samples (Table 1) suggested that the deleterious actions of these radiations were mainly around PS2. The decline in PS2 activity probably acts as a bottleneck resulting in a decline in the overall photosynthetic capacity [cf. the $(F_p-F_s)/F_s$ values of Table 1]. However, relatively low decline in these values compared to F_v/F_m in UV-A and UV-B treated samples could be due to reorganisation of PS1 owing to these radiations as proposed earlier. On the other hand, both these parameters declined drastically upon UV-C irradiation suggesting a non-specific damage of the photosynthetic apparatus.

Our results suggest the following: (*1*) UV radiation of different wavelengths causes variable response in the senescence induced loss of Chl, Car, proteins and PS2 activity; the damaging effect increases from higher to lower wavelengths of the UV bands. (*2*) Long wavelength UV (UV-B and UV-A) by reorganizing the thylakoid components provide some stability to the PS1 complex during senescence.

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