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# Oxidative stress injury in tomato plants induced by supplemental UV-B radiation

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## Abstract

Tomato (*Lycopersicon esculentum* Mill. cv. PKM 1) plants growing under field conditions were exposed for 15 d to solar radiation with UV-B component (280 - 320 nm) enhanced to 6.3 kJ $m^{-2}$ $d^{-1}$. This simulated a 15 % stratospheric ozone depletion over Madurai (9° 50′ N latitude). Lipid peroxidation in the leaves of UV-B treated plants was 32 % higher compared to the control. Superoxide dismutase (SOD) and catalase activities registered parallel promotion by 126 and 50 %, respectively, in the UV-B treated plants. Further, the contents of total phenols and anthocyanins in the leaves have also been enhanced by 40 and 156 %, respectively. On the contrary, polyphenol oxidase activity demonstrated a 58 % inhibition in the leaves of UV-B treated plants. While anthocyanins and phenols are proposed to act as antioxidants, the reduction in polyphenol oxidase activity may maintain the turnover of phenols in the UV-B treated plants.

*Additional key words*: lipid peroxidation, oxidative stress, phenols, superoxide dismutase.

## Introduction

The direct consequence of the stratospheric ozone depletion is increase in the input of mid band solar ultraviolet radiation (UV-B, 280 - 320 nm) reaching the earth's surface. Therefore, the stratospheric ozone depletion is gaining attention both in the

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*Abbreviations*: UV-B - ultraviolet-B radiation (280 - 320 nm); SOD - superoxide dismutase.

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scientific community and in the perspective of man. UV-B radiation inhibits a variety of growth and metabolic processes in crop plants, consequently diminishing the agricultural productivity (Caldwell *et al.* 1989, Tevini and Teramura 1989, Balakumar 1992, Stapleton 1992, Tevini 1993). Albeit a large body of literature is available on the UV-B effects, most of the investigators have addressed these effects at the whole plant level. Less information on the UV-B induced damages at the cellular level are found, *e.g.*, at the chloroplast level (Nedunchezhian and Kulandaivelu 1991, Renger *et al.* 1989) and at the enzyme level (Balakumar 1992, Dohler 1988, Jordan *et al.* 1992). UV-B radiation induces a wide range of oxidative stress injuries in plant cells (Balakumar *et al.* 1993, Larson 1988). Therefore, we decided to envisage the mechanism of tolerance of the oxidative injuries caused by UV-B radiation in vegetable crops. Tomato has been chosen as the model system. The main objective of the present study is: (*1*) to characterize the kinds of oxidative stress injuries elicited by UV-B radiation in tomato leaves; (*2*) to identify the kinds of enzymatic antioxidants which serve as defence agents against the oxidative stress injury mediated by UV-B radiation; and (*3*) to elucidate as to whether the metabolism of phenols plays any protective role against the oxidative stress injury.

## Materials and methods

**Plants:** Seeds of tomato (*Lycopersicon esculentum* Mill. cv. PKM 1) obtained from the local market, were surface-sterilized by immersing in 0.01 % $HgCl_2$ solution for 1 min, thoroughly rinsed in running water and soaked overnight. Seedlings were grown in small plastic troughs containing a mixture of garden soil and sand (1:1), or 15-d-old seedlings raised in the garden beds were transplanted to field in plots adopting a completely randomized block (CRB) design.

**UV-B treatment** (*cf.* Balakumar *et al.* 1993 for details): The source of supplemental UV B radiation was *FS-40* sunlamps (*Westinghouse Co.*, Bloomfield, USA). Distance between the light source and the plants was 300 mm. The spectral characteristics of the sunlamps and the transmittance characteristics of the filters used are presented in Fig. 1. Irradiance in the UV-B waveband (mainly 290 - 320 nm) was measured using a factory calibrated double-holographic-grating spectro-radiometer (model *742*, *Optronics*, Orlando, USA). The radiation filtered through cellulose acetate filters supplied a weighted irradiance of 6.3 kJ $m^{-2}$ $d^{-1}$ of biologically effective enhanced UV-B using the generalized plant action spectrum (Caldwell 1971) normalized at 300 nm, simulating a 15 % ozone depletion over Madurai (9° 50′ N). During UV-B treatment, the control plants also were kept under the *FS-40* sunlamps wrapped with *mylar* type *D* plastic films which prevent any radiation below 320 nm to be transmitted. The supplemental UV-B was given around the noon time every day for 15 d. On the 15$^{th}$ day, measurements of growth parameters and biochemical analyses were carried out.

**Growth parameters:** Shoot and root lengths and fresh and dry masses of the plants were measured. Leaf area was measured using a *LiCOR 3100* leaf area meter.

**Biochemical estimations:** Anthocyanin in the leaf tissue was determined after extraction in the methanol, distilled water and HCl (80:19:1) mixture following the method of Mancinelli and Walsh (1978). The quantification of soluble protein was done according to Bradford (1976) after precipitating the proteins in the buffer extract (pH 7.5) with 10 % (m/v) ice-cold trichloroacetic acid (TCA). The estimation of total phenols was done according to Harborne (1984) adopting the acid hydrolysis (6 M HCl) method, after extracting phenols in ether. Catechol served as standard for phenol estimation. Lipid peroxidation was measured following Dhindsa *et al.* (1981), adopting the thiobarbituric acid (20 % TCA containing 0.5 % thiobarbituric acid) test.

The enzyme assays were carried out using the crude enzyme extract (CEE) from the leaves as the enzyme source. Leaves (200 mg) were cut into small pieces and homogenized in a mortar and pestle at 4 °C in 50 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol and 0.25 mM EDTA. The homogenate was centrifuged at 2 000 *g* for 5 min, and the clear yellowish-green supernatant was collected and used as the crude enzyme extract.

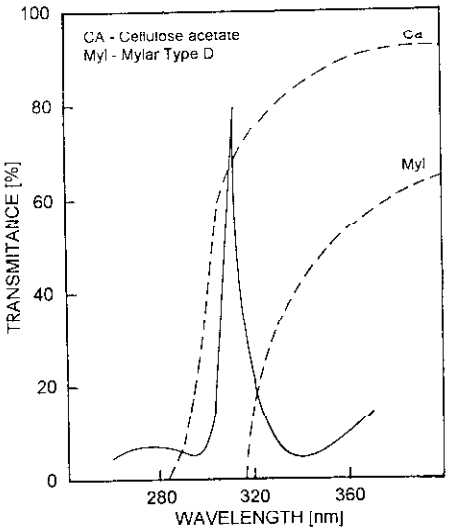


Fig. 1. Spectral characteristic (*full curve*) of FS-40 fluorescent sunlamp and transmittance properties of cellulose acetate (0.13 mm thickness) and *Mylar* type *D* plastic filters (0.13 mm). The lamps were preburnt for 100 h. The transmittance spectra of the filters were obtained using a *Hitachi 2000* double wavelength spectrophotometer. *CA* plastic films filter all radiation below 280 nm and *Mylar* films filter all radiation below 320 nm. During the experiments, *CA* filters were replaced every 70 h while *Mylar* filters were changed at 120 h intervals. *CA* and *Mylar* films are similar in the transmittance of radiation above 320 nm.

Activity of catalase was measured following the method of Kar and Misra (1976). One unit of catalase was defined as the amount of enzyme which broke down 1 nmol $H_2O_2$ per min under the assay conditions. Superoxide dismutase activity (SOD) was determined by the method of Sawada *et al.* (1972) and polyphenol oxidase activity was assayed following Mukherjee and Ghosh (1975).

**Statistical analysis:** The growth parameters were measured on 10 samples; all the other analyses were done using at least four independent replicates. The data are presented as means ± SE. The differences in the responses between the control and irradiated plants were evaluated for their significance using the Student's *t*-test and the significance was defined at the 5 % probability level.

## Results

The results obtained in the pot experiments clearly indicated that tomato plants were sensitive to UV-B radiation. The UV-B treated field-grown plants also showed distinct reductions in plant height (42 %), leaf area and biomass production as compared to the control (Table 1). While the UV-B supplemental irradiation resulted in a 47 % reduction in shoot length, the inhibition in root growth was only 29 % (Table 1). Likewise, fresh and dry masses of the plants were also significantly inhibited by 76 % and 62 % respectively, under UV-B treatment (Table 1). While

Table 1. Changes in growth parameters and leaf characteristics of tomato seedlings exposed to enhanced solar UV-B radiation for 15 d. The data are means ± SE ($n$ = 10) and the differences between control and UV-B treatment are significant ($P$ = 0.05).

| Parameter | Control | UV-B treated |
|---|---|---|
| Plant height [mm] | 268 ± 19 | 141 ± 24 |
| Shoot length [mm] | 184 ± 16 | 114 ± 26 |
| Root length [mm] | 78 ± 19 | 48 ± 15 |
| Plant fresh mass [g] | 1.4 ± 0.20 | 0.4 ± 0.02 |
| Plant dry mass [g] | 0.1 ± 0.02 | 0.05 ± 0.025 |
| Number of leaves | 18.4 ± 1.60 | 15.3 ± 2.6 |
| Leaf area [cm$^2$] | 1460 ± 19 | 426 ± 3 |
| Leaf fresh mass [g] | 0.4 ± 0.02 | 0.10 ± 0.016 |
| Leaf dry mass [g] | 0.1 ± 0.02 | 0.03 ± 0.015 |

UV-B did not bring about a significant reduction in the number of leaves produced, there was an 81 % decrease in leaf area in the UV-B irradiated plants, and also the leaf fresh and dry masses showed 71 % and 40 % reductions, respectively (Table 1). Lipid peroxidation in the leaves of UV-B treated plants was 32 % higher than in control (Table 2). The superoxide dismutase (SOD) and catalase activities were also promoted by 126 % and 50 %, respectively, due to UV-B treatment (Table 2). There was a strong and positive correlation ($r$ = 0.92) between the activities of SOD and catalase under UV-B irradiation. The UV-B treated tomato plants have showed 40 % higher phenol content in their leaves (Table 2). A similar trend was noticed in the level of anthocyanins (Table 2). The phenol and anthocyanin contents in the leaves of UV-B treated plants expressed a strong and positive correlation ($r$ = 0.93). On the

contrary, the polyphenol oxidase activity was inhibited by 58 % due to UV-B treatment (Table 2).

Table 2. Changes in the lipid peroxidation, enzyme activities and other related biochemical aspects in the leaf tissue of tomato plants exposed to UV-B radiation. The data are mean ± SE ($n$ = 4) and the differences between control and UV-B treatment are significant ($P$ = 0.05).

| Parameter | Control | UV-B treated |
|---|---|---|
| Lipid peroxidation [μmol(MDA) $kg^{-1}$(f.m.)] | 78.0 ± 1.9 | 103.2 ± 0.111 |
| Superoxide dismutase activity [U $mg^{-1}$(protein)] | 30.3 ± 1.5 | 68.0 ± 1.9 |
| Catalase activity [U $mg^{-1}$(protein)] | 20.8 ± 0.148 | 31.2 ± 0.15 |
| Total phenols [mmol $kg^{-1}$(f.m.)] | 3.2 ± 0.27 | 4.3 ± 0.11 |
| Anthocyanin [$A_{530}$ $kg^{-1}$(f.m.)] | 900.0 ± 0.192 | 2200.0 ± 0.16 |
| Polyphenol oxidase activity[mmol(catechol) $kg^{-1}$(protein) $s^{-1}$] | 3.0 ± 0.03 | 1.2 ± 0.002 |

**Discussion**

The growth parameters of tomato plants exposed to enhanced solar UV-B radiation were significantly reduced (Table 1). This is in agreement with the literature reports. Reduction in the leaf area by UV-B radiation serves as the marker of the radiation effect in plants and confirms their sensitivity (Biggs *et al.* 1981, Tevini *et al.* 1989). Results obtained in the present study show that though the oxidative stress injury is more prevalent under UV-B radiation, the plants in turn adopt certain strategies to minimize the injurious effect of the oxidative stress.

Singlet oxygen ($^1O_2$), superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide ($H_2O_2$) and hydroperoxide (HO•) are the major reactive forms of oxygen with greater toxicity potentials (Larson 1988). The peroxides damage the cell and organelle membranes and lead to their disfunction (Knox and Dodge 1985). A 32 % enhancement in lipid peroxidation in the UV-B treated tomato plants (Table 2) confirms the induction of oxidative stress injury by UV-B radiation. Panagopoulos *et al.* (1990) and Kramer *et al.* (1991) have also observed higher level of lipid peroxidation due to UV-B treatment in sugar beet and cucumber leaves, respectively. UV-B treated tomato plants registered nearly 2.3-fold higher SOD activity. These results concord with the findings of Balakumar *et al.* (1993). Though the superoxides are not directly reactive with biomolecules like $H_2O_2$, they give rise to more reactive species of oxygen with higher toxicity potential. Further, even if the nonenzymatic conversion of superoxide to hydrogen peroxide is possible at physiological pH's, SOD is a highly powerful catalyst increasing the rate of the reaction by several orders of magnitude. Therefore, we propose that the increase in the SOD activity could be a powerful antioxidant mechanism to scavenge the superoxides generated due to UV-B irradiation. Hydrogen peroxide is generated as a result of direct UV-B photochemical reactions in plants (Larson 1988) and also due to the promotion in SOD activity under UV-B irradiation (Table 2). Albeit $H_2O_2$ is not specifically reactive with most

biomolecules, it acts as the intracellular precursor for highly reactive oxidants like hydroperoxide (HO·). Catalase, which cleaves the toxic precursor $H_2O_2$, registers a 50 % promotion in tomato leaves on exposure to UV-B radiation. By bringing out a concomitant increase on par with SOD activity and mediating the sequential catalysis of hydrogen peroxide, catalase complements SOD activity (Table 2). Similar parallelism between the activities of SOD and catalase have been reported in our previous work (Balakumar *et al.* 1993). Increased antioxidant capacity has been suggested as a stress tolerance mechanism in plants (Burke *et al.* 1985, Monk *et al.* 1989).

In the UV-B treated tomato plant leaves, considerable increase in total phenol and anthocyanin content has been recorded. Anthocyanin accumulation is common under UV-B treatment (Robberecht *et al.* 1986). The accumulated isoflavonoids and anthocyanins are primarily explained as solar screens against UV-B radiation. Anthocyanins also may have an antioxidant activity as a secondary function in plants experiencing oxidative stress injuries. However, the precise role of the anthocyanins as antioxidants remains to be elucidated. Further, triggering of anthocyanin synthesis, which is a rapid response of plants to UV-B radiation, can be also correlated with the synthesis of phenols (Zaprometov and Zagoskina 1987). While the phenol level was increased, on the contrary, the polyphenol oxidase activity was reduced to 42 % in the UV-B treated plants. By reducing the polyphenol oxidase activity, plants can maintain the required turnover rate of phenols in the cells and they do so only as an adaptive strategy to cope with oxidative stress injury. This observation substantiates the hypothesis that apart from the enzymatic antioxidants such as SOD and catalase, also the phenols may act as antioxidants. Our results suggest that to endure the oxidative injuries caused by UV-B radiation in plants, both enzymatic (SOD and catalase) and non-enzymatic (phenolic compounds) antioxidants come into play. Nevertheless, further work to gain more information about the role of phenols as antioxidants under UV-B radiation stress would be rewarding.

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