

Oxidative damage and changes in activities of antioxidant enzymes in wheat seedlings exposed to ultraviolet-B radiation

Prabhat Kumar Sharma*, Preetha Anand and Sangeeta Sankhalkar

Department of Botany, Goa University, Goa 403 206, India

The effect of supplementary UV-B exposure (1 mW per cm²) on damage to photosynthetic membranes, and enzymatic and nonenzymatic processes metabolizing active oxygen species were studied in young wheat seedlings (*Triticum aestivum* L. cv DWR162). Supplementary UV-B radiation caused changes in thylakoid protein profile. Thylakoid protein of 47 and 43 kDa molecular mass decreased significantly. Pigment-protein complexes were also affected. Chlorophyll-protein complexes CP1a, CP1, CPa and light harvesting complexes LHCP2 and LHCP3 showed decreases while LHCP1 increased due to the treatment. Exposure to UV-B caused oxidation of thylakoid proteins and lipids. The activities of superoxide dismutase (EC 1.15.1.1) and peroxidase (EC 1.11.1.7) increased with increasing duration of exposure. While the activity of the peroxidase enzyme increased continuously, activity of superoxide dismutase decreased after 10 days of UV-B exposure. Catalase (EC 1.11.1.6) activity increased initially, but was followed by a decrease. The supplementary UV-B treatment also induced two isoenzymes of peroxidase, and the total amount of peroxidase was also increased. Ascorbate and dehydroascorbate pools increased initially, followed by a decrease. The study suggests that exposure to supplementary UV-B radiation results in oxidative damage of protein including light harvesting complexes and lipids in thylakoid membranes, and changes in the activities of enzymes involved in the metabolism of oxygen radicals.

UV-B (280–320 nm of light) radiation reaching the earth's surface is increasing because of depletion of its stratospheric ozone layer primarily owing to the continuing release of chlorofluorocarbons. Plants use sunlight for photosynthesis, and as a consequence are exposed to the solar radiation which also includes ultraviolet radiation. High doses of photosynthetically active radiation (400–700 nm light) cause photoinhibition which has been extensively studied. However, only few reports exist on the effect of enhanced UV-B on plant metabolism. A large number of plants exhibit UV-B response,

but the photoreceptor for UV-B radiation involved in photochemical reaction of photosynthesis is yet to be conclusively identified. Damage to physiological processes of plants such as photosynthesis and components like protein, by UV-B result in a decline in plant productivity.

UV-B exposure is believed to enhance the amount of activated oxygen species. The active oxygen species H₂O₂, O₂⁻, OH[·] and ¹O₂ are present in all plants in varying degrees as a result of normal aerobic metabolism. However, there are reports suggesting that UV-B exposure causes accumulation of these active oxygen species^{1–4}. Cellular UV-B chromophores such as aromatic amino acids, NADH and phenolic compounds could be activated by the absorption of UV-B light which could react with oxygen to form singlet molecular oxygen and super oxide radicals⁵.

Plants metabolize activated oxygen species by invoking the antioxidant defense system of low molecular weight antioxidant^{6,7}, such as ascorbate, glutathione and α -tocopherol, and carotenoids⁸ as well as enzymes such as superoxide dismutase (SOD), catalase, peroxidase and glutathione reductase⁹. Flavonoids, apart from possessing antioxidant properties, are believed to protect plants against UV-B radiation^{10,11}.

In this study we report that exposure to supplementary UV-B radiation causes oxidative damage to lipids and proteins of the thylakoid membranes. We also demonstrate the differences in the response of antioxidant enzymes to supplementary UV-B in wheat seedlings under laboratory conditions.

Materials and methods

Material wheat seeds (*Triticum aestivum* L. cv DWR162) were obtained from Agricultural Research Station, Dharwad and grown in 10 cm × 10 cm plastic pots, containing vermiculite, by routinely bottom irrigating with 1/2 strength Hoagland's solution. Plants were grown in a growth chamber illuminated with incandescent bulbs and fluorescent tubes for 12 h photoperiod, having a low photon flux of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic active radiation (PAR) (in order to avoid photo-

*For correspondence.

inhibition of photosynthesis) measured with a radiometer (model Li-189, Licor, USA). The day/night temperature was maintained at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

UV-B irradiation. Pots containing 4-day-old wheat seedlings were transferred to another growth chamber with identical PFD, photoperiod and temperature described under growth conditions, and were supplemented with an average 1 mW cm^{-2} UV-B radiation. The source of UV-B was a Vilbour-Lourmat (France) T-6 M source with a glass filter with an intensity of 6 mW cm^{-2} . The UV-B radiation was measured using a UV-B radiometer from the same manufacturer. The intensity of UV-B irradiation was measured at the base, middle and top of the seedlings by placing the sensor of the UV-B radiometer in close contact with the leaves. Care was taken to maintain the average level of UV-B irradiance at $1 \pm 0.2 \text{ mW cm}^{-2}$, in growing plants, by changing the distance between source and seedlings, and placing the bandage cloth in-between. The UV-B level observed in Goa, India, ranged between 0.5 and 0.6 mW cm^{-2} depending on the season.

Thylakoid protein profile

Chloroplasts isolation. Chloroplasts were mechanically isolated according to Sharma and Singhal¹². 25 g leaves were chopped into 0.5 cm pieces and ground in 200 ml of grinding medium (10 mM $\text{Na}_4\text{P}_2\text{O}_7$, 4 mM MgCl_2 , 2 mM ascorbate, 0.33 M sorbitol (pH 6.5) and 0.25% (w/v) bovine serum albumin) for 10–15 s. The homogenate was filtered first through two layers and then eight layers of muslin. The filtrate was centrifuged at 1000 g for 5 min. The surface of the chloroplast pellet was washed twice with resuspending medium (50 mM HEPES; 0.33 M sorbitol, pH 7.6; 2 mM EDTA; 1 mM MgCl_2 ; 1 mM MnCl_2 and 0.1% BSA (w/v)) and then resuspended in the same medium. All steps were performed at $0-4^{\circ}\text{C}$.

SDS-PAGE. The polypeptides were separated on SDS-PAGE according to Sharma *et al.*¹³, using a resolving gel (10–20% T, 2.7% C linear gradient) (top to-bottom) and 5% stacking gel (Hoefer, Germany). The gel was run at 80 V, and stained using 0.125% Coomassie blue R-250 in 40% ethanol and 10% acetic acid.

Sample preparation. Thylakoid membranes were washed twice with buffer containing 0.065 M Tris-HCl (pH 7.8) and centrifuged at 9000 rpm for 5 min. The pellet was dissolved in buffer containing 0.125 M Tris-HCl (pH 6.8), 4% SDS, 20% glycerol and 10% 2-mercaptoethanol. The sample was heated at 80°C for 3 min, centrifuged at 9000 rpm for 5 min, and transferred to ice. Protein equivalent of 10 μg was loaded.

Chlorophyll-protein complex. Isolated thylakoid membranes were solubilized in octyl-B-D-glucopyranoside and sodium dodecyl sulphate in a ratio of 1:20:1 (thylakoid membrane, chlorophyll: octyl-B-D-glucopyranoside:sodium dodecyl sulphate). The chlorophyll-protein complexes were separated on a 10% non-denaturing gel. Rest of the conditions were the same as described by Sharma *et al.*¹³.

Estimation of lipid peroxides. Lipid peroxides were determined by 2-thiobarbituric acid-malonaldehyde (TBA-MDA) adduct formation as described by Halliwell and Gutteridge¹⁴ in isolated chloroplasts (thylakoid membranes) according to Sharma and Singhal¹² (described above), and in total cell membrane by grinding 0.5 g of fresh tissue in 0.1% TCA according to Somashekaraiah *et al.*¹⁵. Sample equivalent of 5 μg of protein was used for the estimation.

Assay of enzymes

Extraction. Leaves (0.5 g) were homogenized in chilled buffer containing 50 mM potassium phosphate (pH 7.5), 1 mM EDTA, 0.1% triton x-100 and 1.0% (w/v) insoluble polyvinyl pyrophosphate. The homogenate was filtered through four layers of muslin, and the filtrate was centrifuged at 12,000 g for 15 min at 4°C . Protein content of the supernatant was measured according to Lowry *et al.*¹⁶. Supernatant equivalent of 50 μg was used for the estimation of enzyme activity.

Superoxide dismutase (EC 1.15.1.1; SOD) was assayed at 25°C according to Mishra *et al.*¹⁷. The assay medium contained 50 mM potassium phosphate (pH 7.8), 0.1 mM EDTA, 18 μM cytochrome c, 0.1 mM xanthine and the leaf homogenate. The reaction was started by the addition of xanthine oxidase, and was followed spectrophotometrically as reduction in the rate of cytochrome c at 550 nm. One unit of SOD activity was defined as the amount of enzymes required to inhibit the rate of cytochrome c reduction by 50% under the specified conditions.

Catalase (EC 1.11.1.6) activity was determined spectrophotometrically by following the decomposition of H_2O_2 at 240 nm at 25°C in a reaction mixture containing 50 mM potassium phosphate, 11 mM H_2O_2 and the leaf extract, according to Mishra *et al.*¹⁷.

Peroxidase (EC 1.11.1.7) activity was monitored according to Afithile *et al.*¹⁸. Activity was assayed in a reaction mixture containing 3.4 mM guaiacol, 0.9 mM H_2O_2 and 50 mM potassium phosphate (pH 6.0) and the leaf extract. The mixture was incubated for 5 min at 25°C , and the reaction was stopped by adding 0.5 ml of 5% (v/v) sulphuric acid. Enzyme activity was calculated using an extinction coefficient of $25.5 \text{ mM}^{-1} \text{ cm}^{-1}$ at 470 nm for tetra-guaiacol.

Peroxidase isozyme. Sample equivalent to 50 µg of protein was loaded onto a nondenaturing polyacrylamide (7% T and 2.5% C, supported with 10% glycerol) separating gel. Electrophoresis was carried out at 40 V at 4°C. The gel was stained using a solution of saturated benzidine, 30% NH₄Cl (w/v) and 0.4% H₂O₂ (w/v) (50:10:2 ratio) at 40°C for 15 min and quantified, after destaining, using an LKB densitometer (model ultrascan XL).

Ascorbate and dehydroascorbate. Quantitative estimation of ascorbate and dehydroascorbate was carried out according to Anderson *et al.*¹⁹, using an HPLC (Spectraphysics, UK) with a reversephase column (ET 250/4 nucleosil 100-5 C18 ODS) and Spectraphysics SP 8800 ternary HPLC pump with SP 4270 integrator and spectra 100 variable wavelength detector. Samples were prepared by homogenizing 0.5 g fresh leaf tissue in 6% metaphosphoric acid and 1 mM EDTA (pH 2.8), and centrifuged at 12000 g for 15 min. A portion of the supernatant was incubated with 30 mM DTT for 24 h at 25°C to reduce dehydroascorbic acid to ascorbic acid. One portion was used without DTT. Separation was carried out using a solvent system of 2% ammonium dihydrogen phosphate (pH 2.8) over 12 min with a flow rate of 1.0 ml per min and detected at 245 nm at room temperature. Dehydroascorbate was determined by comparing the difference between total reduced ascorbic acid in extracts with or without 30 mM DTT.

Results

Supplementary UV-B exposure significantly decreased thylakoid protein of 47 and 43 kDa, 32 kDa protein showed a slight decrease, however, low molecular mass proteins of 27, 24 and 17 kDa, which are associated with oxidation of water, remained more or less stable compared to control (controls are the plants grown for 19 days without UV-B treatment; Figure 1; Table 1).

LHC complexes were also affected by the exposure to supplementary UV-B radiation. A 15 days UV-B treatment caused decreases in CP1a, CP1, CPa, LHCP2 and LHCP3 due to UV-B exposure compared to control, LHCP1, however, increased due to the treatment. Free pigments also decreased due to the UV-B treatment (Figure 2, Table 2).

It was observed that thylakoids had much greater peroxidation than cell membrane (Figure 3). A 15 days exposure to supplementary UV-B radiation caused 92% increase in the peroxidation of thylakoid lipids as compared to respective control. Cell membranes showed a minimal peroxidation up to 5 days of UV-B exposure (6%). However, peroxidation of the cell membrane increased to 62% after 15 days of UV-B treatment as compared to its control (Figure 3).

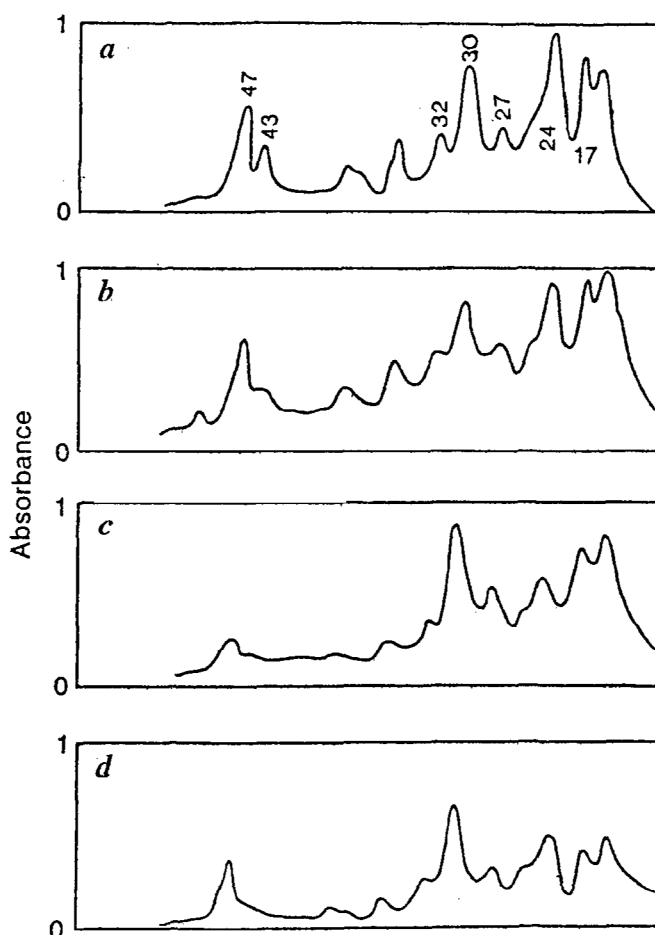


Figure 1. Densitometer scan of thylakoid protein profile of wheat seedling exposed to supplementary UV-B radiation (1 mW cm⁻²) at 25°C. *a*, Control; plants which were grown without UV-B for 19 days. *b*, 7 days UV-B treatment. *c*, 11 days UV-B treatment. *d*, 15 days UV-B treatment. Protein equivalent of 10 µg was loaded. For per cent changes in the peak area, see Table 1.

Table 1. Per cent change in relative peak area of thylakoid protein profile seen in Figure 1

Treatment	Peaks (kDa)						
	47	43	32	30	27	24	17
Control (A)	100	100	100	100	100	100	100
7 days UV-B (B)	85	61	84	88	102	81	115
11 days UV-B (C)	38	44	92	123	146	76	127
15 days UV-B (D)	85	76	91	141	138	100	98

Enzymes activity. Superoxide dismutase showed a linear increase in activity up to 10 days of UV-B treatment. Further increase in the duration of treatment caused decline in the activity (Figure 4). The UV-B treatment for 10 days resulted in an increase of 34% compared to its control, but only a 20% increase was observed in the

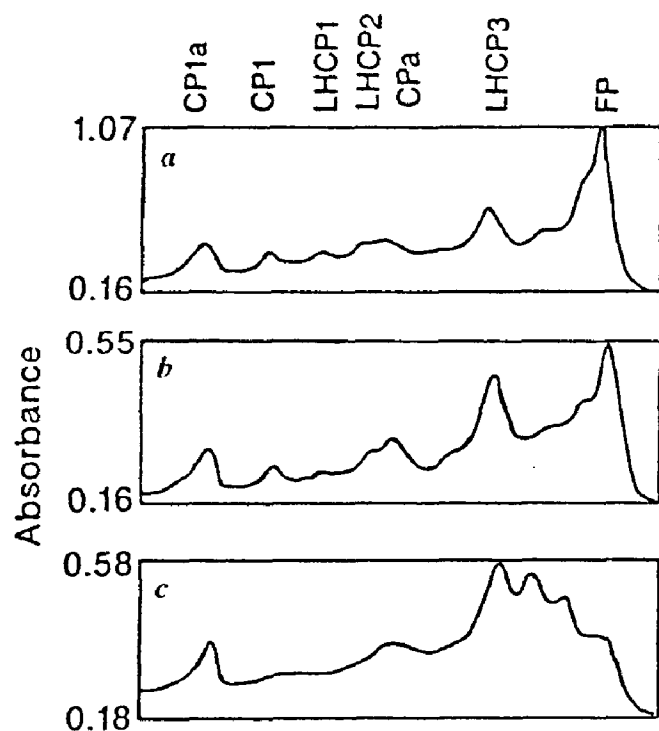


Figure 2. Densitometer scan of light harvesting complexes (LHC) of wheat seedlings exposed to supplementary UV-B radiation (1 mW cm^{-2}) at 25°C . *a*, Control; plants which were grown without UV-B for 19 days. *b*, 7-day UV-B treatment. *c*, 15-day UV-B treatment. Sample equivalent of $10 \mu\text{g}$ of protein was loaded. For per cent changes in peak area, see Table 2.

Table 2. Per cent changes in the relative peak area of light harvesting complexes, seen in Figure 2, with respect to control. Care has been taken to adjust for the difference in the 'Y' axis value

Treatment	CP1a	CP1	LHCP1	LHCP2	CPa	LHCP2	FP
Control	100	100	100	100	100	100	100
(A)							
07 days UV-B	50	27	7	106	106	57	72
(B)							
15 days UV-B	42	0	223	77	0	68	61
(C)							

plants treated for 15 days (Figure 4). Peroxidase activity also increased in response to the UV-B exposure. UV-B treatment for 15 days resulted in approximately 5 times increase in the activity of peroxidase compared to respective control (Figure 5).

The isozyme profile of the peroxidase was altered by supplementary UV-B; exposure for 5 days and further exposure resulted in an induction of two new bands (isozyme), and there was an increase in the total amount of peroxidase (Figure 6; Table 3). The observed peaks 2 and 3 represent the new isozymes.

Catalase showed an overall decrease in activity in response to the supplementary UV-B exposure. One day exposure of the seedlings resulted in a slight increase in

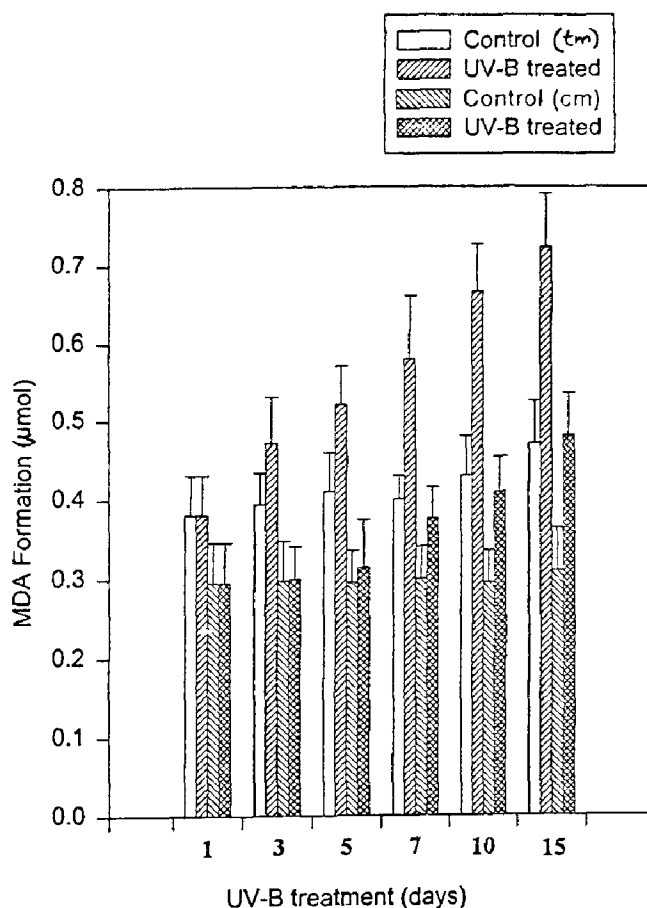


Figure 3. Effect of supplementary UV-B radiation (1 mW cm^{-2}) combined with photosynthetic active radiation (PAR) $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at 25°C on lipid peroxidation in thylakoid membrane (tn) from isolated chloroplasts and total cell membrane (cm). $n = 4$, vertical bars show standard error. Tissue equivalent of $5 \mu\text{g}$ of protein was used.

the catalase activity (7% compared to its control). However, UV-B treatment longer than 3 days caused a linear decrease in its activity resulting in 50% decrease after 15 days of supplementary UV-B exposure (Figure 7).

The ascorbate and dehydroascorbate pools also changed in response to UV-B treatment. UV-B exposure for 1 day caused many-fold increase in the content of ascorbate and dehydroascorbate compared to the control (Figure 8), after which it decreased linearly, with increasing duration of the treatment.

Discussion

The observed decrease in 47 and 43 kDa proteins (Figure 1), involved in the pigment-protein complexes (Figure 2), is probably due to the oxidation of the proteins by UV-B exposure. Proteins are the likely targets for UV-B oxidation because aromatic amino acids act as the chromophores and absorb UV-B light. Earlier

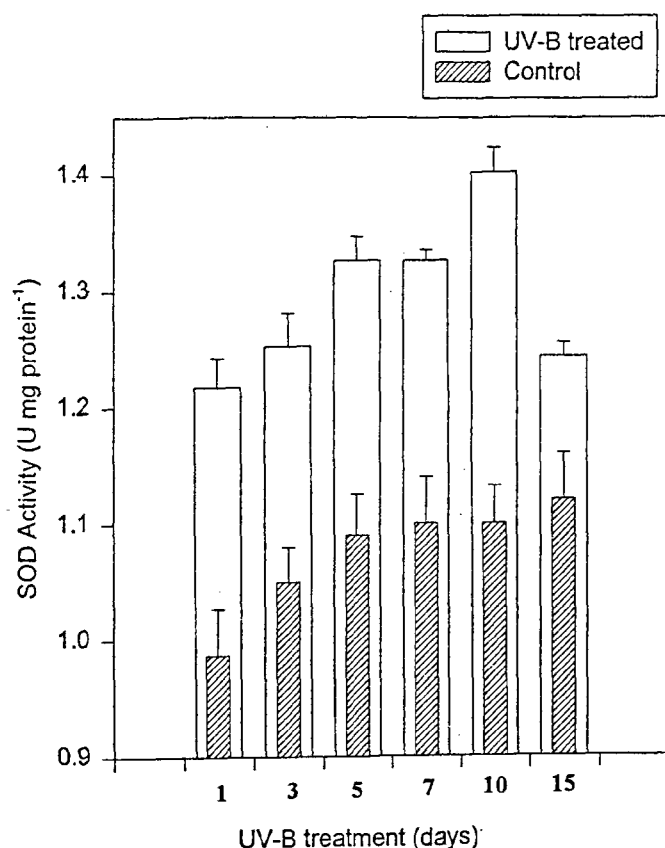


Figure 4. Effect of supplementary UV-B radiation (1 mW cm^{-2}) combined with PAR $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 25°C on activity of superoxide dismutase (SOD) in wheat leaves. $n = 3$. U = unit. Vertical bars show standard error.

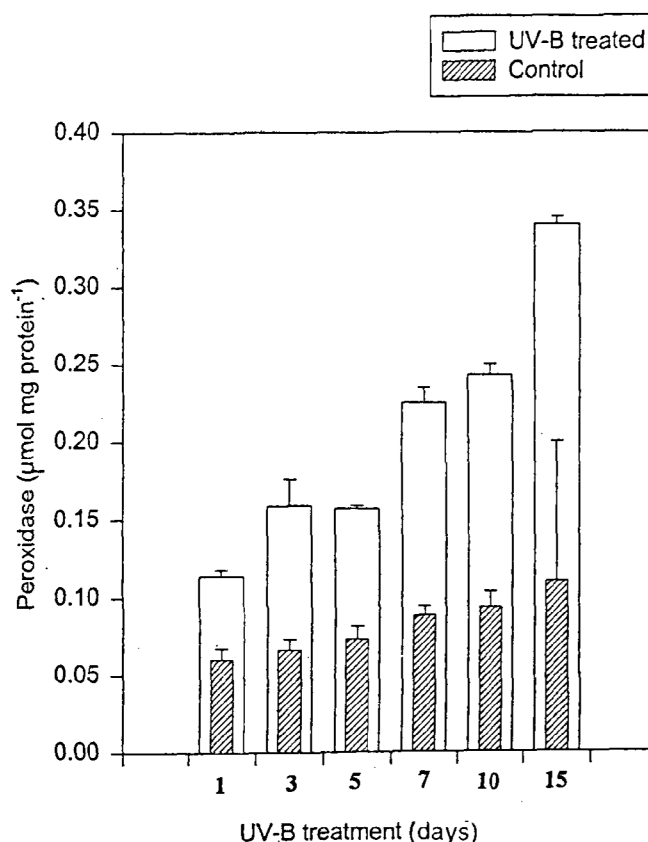


Figure 5. Effect of supplementary UV-B radiation (1 mW cm^{-2}) combined with PAR $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 25°C on peroxidase activity with guaiacol as substrate. $n = 3$. Vertical bars show standard error.

Table 3. Per cent changes in the relative peak area of peroxidase isozymes, seen in Figure 6, with respect to control. Care has been taken to adjust for the difference in the 'Y' axis value

Treatment	Peak no.					
	1	2	3	4	5	6
Control	100	0	0	100	100	100
(A)						
1 day UV-B	106	100	0	128	85	95
(B)						
5 days UV-B	131	221	100	91	53	71
(C)						
7 days UV-B	115	311	128	113	82	90
(D)						
15 days UV-B	203	404	209	231	242	179
(E)						

studies have shown that protein oxidation can result in the derivatization of amino acid side chains to form carbonyl groups²⁰ which are indicative of direct oxidation of protein brought about by UV-B light⁴, which is more likely to occur at the site of the light absorbing pigment-protein complexes, thereby explaining the observed decrease in the 47 and 43 kDa proteins,

associated with the LHCII, compared to other proteins in the thylakoid membrane.

Our results showed increased peroxidation of thylakoid as well as cell membrane lipids (Figure 3). MDA formations caused by the oxidative degradation of polyunsaturated fatty acids, especially linolenic acid most of which is in the thylakoid glycolipids, results in greater peroxidation of thylakoid lipids as compared to total cell membrane (Figure 3). Also reactive oxygen is generated as a result of photochemistry (oxidation of water) in the thylakoid membrane. Thus, there is greater damage (peroxidation) to thylakoid lipids than to the total cell membrane (Figure 3).

Strid *et al.*²¹ have reported that UV-B exposure caused damage to the integrity of the thylakoid membrane. Studies have shown that UV-B affects both plant membrane structure and its function. Yet, it is unclear whether the main cause of membrane damage is due to direct absorption of UV-B by protein or indirect effects such as action of free radicals generated as a result of UV-B exposure. Initially there may be direct oxidative damage to protein, since aromatic amino acids can act as chromophores for UV-B radiation, which may also

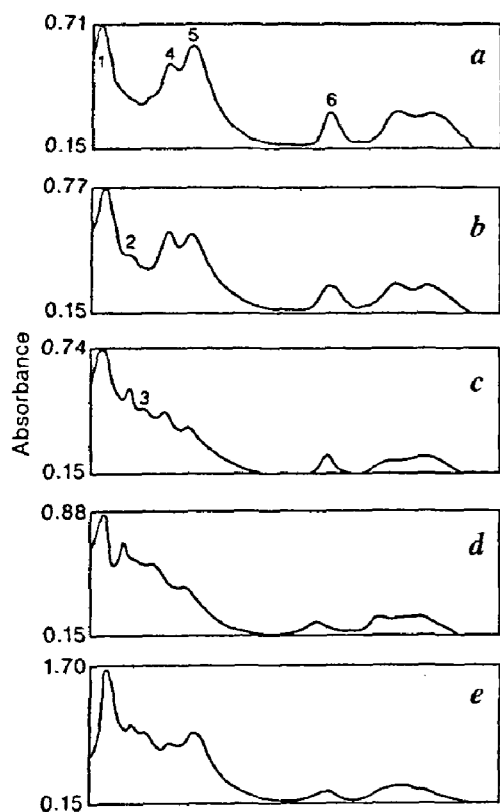


Figure 6. Separation of peroxidase isozymes from wheat seedlings exposed to supplementary UV-B radiation (1 mW cm^{-2}) combined with PAR $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at 25°C . *a*, Control; plants which were grown without UV-B for 19 days. *b*, 1 day UV-B treatment. *c*, 5 days UV-B treatment. *d*, 7 days UV-B treatment. *e*, 15 days UV-B treatment. For per cent changes in the peak area, see Table 3.

result in the formation of free radicals^{4,20} which results in further damage to lipids and proteins, and causes changes in the structure and function of membrane.

The metabolism of active oxygen species is dependent on several, functionally interrelated, antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase. High SOD activity has been linked with stress tolerance, and has been reported in plants exposed to drought²², high light²³, chilling^{17,24} and SO_2 fumigation²⁵ in various plant systems. The increase in the activity of SOD observed in our study (Figure 4) may be a consequence of the production of O_2^- during UV-B treatment of the leaves.

SOD catalyses the dismutation reaction of superoxide anions and can be placed in three classes according to their metal co-factor, Cu, Zn-Mn or FeSOD (ref. 9). Rao *et al.*⁷ reported that SOD activity induced by UV-B is due to preferential expression of the Cu and Zn SOD 3, 4 and 5 isomers. We presume that a similar kind of induction of SOD biosynthesis might be occurring in our experiments as well.

The rapid removal of H_2O_2 produced by SOD is important if the generation of highly destructive OH^\cdot is to

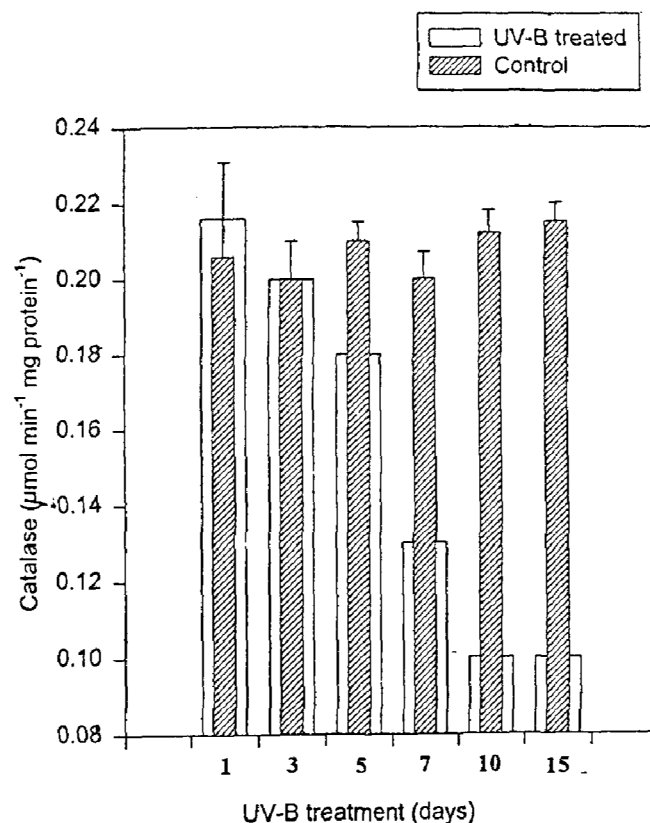


Figure 7. Effect of supplementary UV-B radiation (1 mW cm^{-2}) combined with PAR $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at 25°C on catalase activity in wheat seedlings. $n = 3$. Vertical bars show standard error.

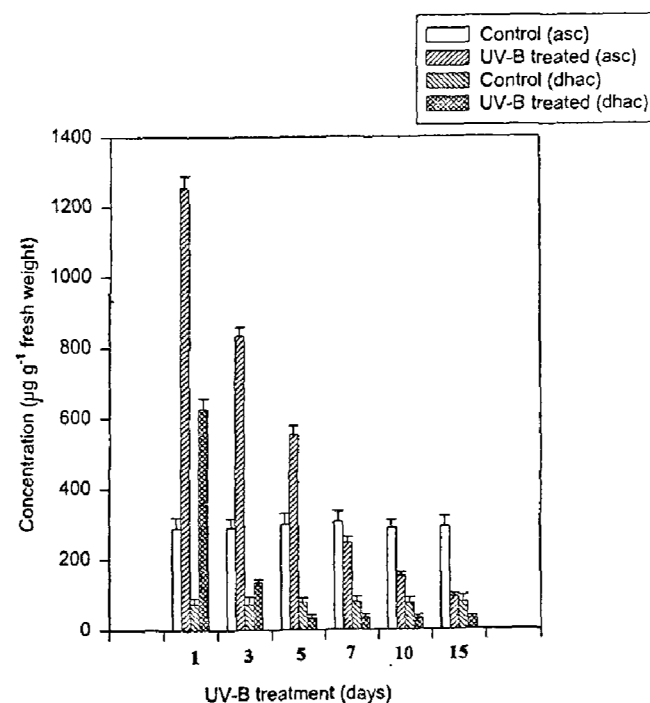


Figure 8. Changes in ascorbic acid (asc) and dehydroascorbic acid (dhac) pool in wheat seedlings exposed to supplementary UV-B radiation (1 mW cm^{-2}) combined with PAR $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at 25°C . $n = 3$. Vertical bars show standard error.

be avoided. Peroxidase also scavenges the H_2O_2 produced through the dismutation of O_2^- catalysed by SOD (ref. 26). The observed increase in peroxidase activity (Figure 5) might be due to an increase in H_2O_2 production probably as a result of induced SOD activity during the treatment. Peroxidase usually occurs as multiple molecular forms (isoenzymes)⁷. Examination of the peroxidase isozyme profile seen in Figure 6 revealed changes that occurred as a response to supplementary UV-B radiation. The increase in the peroxidase activity observed in this study may well be a result of accumulation of new isozymes (peaks 2 and 3; Figure 6) as well as overall increase in the various isozymes of peroxidase (Figure 6). Peroxidases are believed to utilize phenolic compounds as co-substrate^{27,28}. Continual enhanced peroxidase activity was observed in this study (Figure 5), despite the decrease in ascorbate level, during longer UV-B exposure which may indicate use of co-substrates such as phenolic compounds by the peroxidase. Sharma *et al.*¹¹ have reported significant increase in phenolic compounds as a result of supplementary UV-B radiation, which are suggested to be involved in protection of sensitive sites against the UV-B radiation¹⁰.

Catalase which is localized in peroxisome in higher plants functions in the decomposition of H_2O_2 . However, it is susceptible to photoinactivation and degradation. It is also limited in its effectiveness by its relatively poor affinity for H_2O_2 (ref. 2). Foyer *et al.*² also suggested that endogenous catalase may not be very effective in decomposition of H_2O_2 . This may explain low activity of catalase observed in this study (Figure 7). Anderson *et al.*²⁴ have reported that catalase may be of importance in protecting mitochondrial components from oxidative damage.

Dehydroascorbate is produced nonenzymatically in plants by disproportionation of monodehydroascorbate radicals²⁹. The initial increase in the ascorbate pool in our results (Figure 8) seems to be related to the increase in the concentration of dehydroascorbate which could be a result of increased dehydroascorbate reductase activity in detoxification of H_2O_2 in chloroplasts³⁰. Ascorbate is the substrate for ascorbate peroxidase. The higher levels of ascorbate in 1–5 days of UV-B treatment compared to their respective controls may explain the higher activity of peroxidase (we have assayed total peroxidase activity using guaiacol as a substrate; Figure 5). However, while ascorbate level declines after 5 days of UV-B treatment, activity of peroxidase continually increases and this may suggest that beside ascorbate, other co-substrates for the peroxidase, such as phenolic compounds, which increase due to UV-B treatment¹¹, may be used as substrate^{31,32} in plants exposed for longer durations of UV-B. Sarma *et al.*³³ have reported that anthocyanin not only chelates metal ions but also forms ascorbic acid-metal anthocyanin complexes which could also scavenge hydro-peroxy radicals.

In summary, this study has shown that supplementary UV-B exposure resulted in an oxidative stress in wheat plants, indicated by the peroxidation of thylakoid lipids as well as oxidation of thylakoid protein and light harvesting complexes. Also, wheat seedlings responded by changes in the activities of antioxidant enzymes responsible for metabolizing active oxygen species.

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RESEARCH ARTICLE

Late Quaternary morphotectonic evolution of Upper Indus valley profile: A cosmogenic radionuclide study of river polished surfaces

Kewal K. Sharma[§], Z. Y. Gu*, D. Lal**, M. W. Caffee[†] and J. Southon[†]

Wadia Institute of Himalayan Geology, Dehra Dun 248 001, India

*Institute of Geology, Chinese Academy of Sciences, Beijing, China

**Scripps Institution of Oceanography, Geosciences Research Division, La Jolla, CA 92093 0220, USA

[†]Lawrence Livermore National Laboratory, Center for Accelerator Mass Spectrometry, Livermore, CA 94551, USA

We have studied cosmogenic exposure ages in a set of five samples from the river polished surfaces (1–46 m above present river level) of the Indus river (Ladakh), at maximum discharge yield during August. These ages not only provide an opportunity to unravel the complex morphotectonic history of the Upper Indus valley, but also yield estimates of river incision rates during down-cutting of bedrocks. The three lower surfaces, 21 m, 6 m and 1 m above the river level have monotonically decreasing cosmic ray exposure ages of 22, 15 and 3 ka BP, suggesting that the river has been cutting the bedrocks initially (22–15 ka) at high rate of 2 mm yr⁻¹ which reduced to 0.3–0.4 mm yr⁻¹ since 15 ka BP. The latter is at par with the reported values of exhumation rate for the Ladakh region. The present data together with similar data from the Indus valley west of Skardu, reported earlier, give a better understanding of the so far poorly understood interplay of regional exhumation, incision rate and the role of climate in the morphotectonic evolution of the Upper Indus valley.

THERE exists sufficient geological evidence to suggest that Tibet and the adjoining regions of Ladakh

witnessed appreciable changes in local and regional climates during the Late Pleistocene–Holocene. These are related to the advances and retreats of the mountain glaciers, and the high altitude (> 5 km) and the middle latitude (33°–38° N) 'continental glaciers' in Tibet. Moraines, which mark the former limits of alpine glaciers present in Tibet region, are present in Gilgit–Ladakh region as well^{1–4}. Recent studies on oxygen isotopic data from the core samples of Dunde ice cap from Tibet⁵ and the magnetic susceptibility of loess and interbedded soils in central China⁶ serve as a proxy measure of palaeoclimate of the region.

All the Himalayan rivers are in their 'early' to 'immature' stages of development, and show erosional as well as depositional features characteristic of river rejuvenation with every pulse of uplift in the Himalaya⁷. Wadia⁸ suggested that the major rivers like Indus, Satluj, Ganga and Brahmaputra remained nearly confined to their channels, but worked certainly at an accelerated rate of erosion in response to uplift of the region near their source. The down-cutting (incision) of the rivers kept pace with the uplift of the mountain chains, resulting in deep valleys. He reported existence of about 5,000 m deep valley profile of Indus in Gilgit carved by the river, as evident from erosional features and fluvial

[§]For correspondence.