Assessment of impact of solar UV components on growth and antioxidant enzyme activity in cotton plant

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Published online: 16 June 2011

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Abstract A field experiment was conducted to study the impact of solar UV-B (280–315 nm) and UV-A (315–400 nm) components on the growth and antioxidant enzyme activity in cotton plant (*Gossypium hirsutum* var. Vikram). Solar UV components were excluded by filtering the sunlight through selective UV-B (<315 nm) and UV-B/A (<400 nm) absorbing polyester films. Plants grown under filters that transmitted solar UV served as controls. Exclusion of UV-B and UV-B/A enhanced plant height, leaf area and total biomass of plants. The activity of antioxidant enzymes superoxide dismutase (SOD), ascorbic acid peroxidase (APX), glutathione reductase (GR) and guaiacol peroxidase (GPx) assayed in the leaves were lesser in the UV excluded plants. The level of ascorbic acid and UV absorbing substances were also decreased by the exclusion of UV. Solar UV components exerted a limitation on the potential growth of cotton plants. Reduction in the antioxidant enzyme activity and ascorbic acid after UV exclusion indicated that ambient UV components exert a significant stress on cotton plants. Reduction in the production of UAS indicated a changed pattern of metabolism leading to improved primary metabolism. Exclusion of UV components is advantageous from the agricultural point to enhance the growth of cotton plants.

Keywords Antioxidant enzymes · Cotton · Reactive oxygen species · UV radiation · Oxidative stress

Abbreviations

APX	Ascorbic acid peroxidase
ASA	Ascorbic acid
EDTA	Ethylenediaminetetraacetic acid
FC	Filter control
OC	Open control
PVP	Polyvinylpyrrolidone
SOD	Superoxide dismutase
TCA	Trichloroacetic acid
UAS	UV absorbing substances
-UV-B	UV-B excluded plant
-UV-B/A	UV-B and UV-A excluded plant

Introduction

All plants are inevitably exposed to UV components of solar radiation during photosynthesis. The impact of the UV radiation is especially higher under tropical climate where the plants are exposed to longer duration of sunlight. One of the methods to assess the impact of ambient UV is to grow the plants in sunlight after excluding the UV components by appropriate selective filters. Such experiments have revealed the sensitivity of several plants to ambient UV. After the exclusion of UV enhancement of growth and biomass has been observed in mung bean, pea, soybean, barley and *Cyamopsis* (Mazza et al. 1999; Pal et al. 1997, 2006; Amudha et al. 2005; Varalakshmi et al. 2003; Guruprasad et al. 2007).

Biochemical changes that accompany the exclusion of solar UV components has been analysed in very few cases (Varalakshmi et al. 2003; Chouhan et al. 2008). There have been many reports however on the deleterious and inhibitory

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effects of UV-B when the natural solar light is supplemented with UV-B by artificial light sources. Supplementation experiments have often employed unrealistically high doses of UV-B (Edwards 1992; Adamse et al. 1997; Kakani et al. 2003) although the data has been useful in assessing the specific effects of UV-B on plants. Supplementation experiments have revealed enhanced production of reactive oxygen species (ROS) by UV-B (Foyer and Harbinson 1994) which can cause oxidative damage to membrane lipids, nucleic acids and proteins. Plants respond to UV-B stress by activating antioxidant enzymes like Superoxide dismutase, Ascorbic acid peroxidase, Glutathione reductase (Takeuchi et al. 1995; Rao et al. 1996; Teckchandani and Guruprasad 1998; Jain et al. 2003; Kataria et al. 2007). Plants also respond to UV-B by enhancing the endogenous level of ascorbic acid (Rao et al. 1996; Sharma et al. 1998; Jain et al. 2004a, b) and glutathione (Takeuchi et al. 1996; Kalbin et al. 1997). In addition to this synthesis of UV absorbing substances like flavonoids and other phenolic compounds are enhanced in response to supplemental UV-B (Searles et al. 2001; Day and Neale 2002).

Supplemental UV-B reduces leaf area, plant height, photosynthesis and biomass in cotton (Kakani et al. 2003; Reddy et al. 2003; Gao et al. 2003). However there has been no data on the impact of ambient UV on the cotton plants. We have investigated the response of cotton plants to solar UV components by UV exclusion experiments using selective filters under field conditions.

Material and methods

Plant material and experiment design

All the field experiments were conducted in the Botanical garden of School of Life Sciences, Devi Ahilya University, Indore, M.P. (Latitude -22.4°N) India under natural sunlight. Seeds of Cotton (*Gossypium hirsutum* var; Vikram) were obtained from Cotton Improvement Project, Agriculture College, Indore and treated with recommended fungicides viz. bavistin and diathane M at 2 g/kg seeds. Plants were irrigated and fertilized (NPK) at regular intervals to avoid nutritional deficiencies. Seeds were sown inside iron cages (120 cm L $\times$ 90 cm W $\times$ 150 cm H) in 3 ft row length and 30 cm space between the rows. The cages were wrapped with UV-B, and UV-B/A cutoff filters (Garware Polyester Ltd., Mumbai) that specifically eliminate UV-B (<300 nm) and UV-B/A (<400 nm) radiation and the control plants were grown under an ordinary polythene filter permissible to UV (280–400 nm) radiation. The transmission of the filters were measured by Shimadzu (UV-1601) as described in Guruprasad et al. 2007. The transmission characteristics of filters did not change during

the experimental period and these filters did not emit fluorescence in visible regions. The seedlings were exposed to solar UV radiation from the time of germination. Growth data; plant height, leaf area, fresh weight, dry weight has been taken after 28 days of growth of seedlings, leaves of the same seedlings have been used for biochemical analysis.

Radiation measurement

Absolute solar irradiance without UV-B or UV-B/A was measured using a radiometer (IL 1350, International light Inc) U.S.A. The ambient solar irradiance during experimental period at midday was 10 W/m²/s, the loss in light intensity at midday by -UV-B filters was 17% (8.3 W/m²/s) and 20% (8 W/m²/s) under UV-B/A filter and 5% (9.5 W/m²/s), under polythene filter transmissible to UV (filter Control). The photosynthetically active radiation intensity for normal plant growth was observed to be optimal saturating light.

Growth parameters

The plants were grown for 28 days under UV-B and UV-B/A excluded conditions. Plant height was measured from apex to the starting part of the stem and the mean height of 5 plants was taken.

Leaf area was measured in primary leaves of seedling after 28 DAE. Area of leaves was taken by pressing the blotted dry leaf on a graph paper (mm) and tracing the exact outline. The area was measured by weighing the graph cuttings of leaves (Shine et al. 2011). The mean of five leaves was taken as the average value.

Fresh weight and dry weight

Total plant weight was taken after removing the plants and washing the roots thoroughly with water. Dry weight was obtained after oven-drying of the plant at 60 °C for 72 h and weighing on an analytical balance.

UV Absorbing Substances (UAS)

UAS was measured in primary leaves by the method of Mazza et al. (1999). For spectrophotometric determination, UAS content were sampled from four leaves (each from a different plant) per plot (youngest fully expanded leaf). Each sample (one 0.50 cm diameter leaf disc) was placed in 5 ml of 99:1 (methanol: HCl) and allowed to extract for 48 h at -4°C . Absorbance of the extracts was read at 305 nm for determination of total UV-absorbing compounds. Absorbance was expressed on leaf fresh weight basis.

Ascorbic Acid (ASA)

Ascorbate level was measured based on the reduction of ferric to ferrous ion with ascorbic acid in acid solution followed by the formation of a red-chelate between ferrous ion and bipyridyl (Arakawa et al. 1981). 200 mg of tissue sample was homogenized in 2 ml of ice cold 5% trichloroacetic acid (TCA) containing 4% (w/v), polyvinylpyrrolidone (PVP-40) at 4 °C. The homogenate was filtered through four layers of miracloth and centrifuged at 13,000 g for 10 min at 4 °C. The supernatant was used for the ASA assay. The reaction mixture for ASA assay contained absolute ethanol, 5% TCA, 20% TCA, 4% H₃PO₄-ethanol 0.5% bipyridyl and 0.03% FeCl₃-ethanol. The reaction mixture was incubated at 30 °C for 90 min for the Fe²⁺ bipyridyl complex to develop the color and the A₅₃₄ was recorded. Total ASA was determined through a reduction of dehydroascorbic acid (DHA) to ASA by dithiothreitol (DTT) after which 0.24% N-ethylmaleimide (NEM) ethanol was added in addition to the reaction mixture used for estimating ASA and the color developed was recorded at A₅₃₄. A standard curve of ASA was used for calibration.

Extraction and estimation of antioxidant enzymes

All operations were performed at 4 °C. The enzyme extracts for superoxide dismutase (SOD), glutathione reductase (GR) and guaiacol peroxidase (GPx) were prepared by homogenizing 500 mg of leaves with 5 ml of phosphate buffer (50 mM, pH 7.0) containing 1 mM EDTA and 1% PVP. For estimation of ascorbic acid peroxidase (APX) activity, the same extraction buffer was supplemented with 1 mM ascorbic acid. The homogenates were centrifuged at 12,500 g for 30 min and the supernatant obtained was used for SOD, APX, GR and GPx estimation. About 100 mg protein was used for activities of all enzymes except GR where 200 mg of protein was used for the activity assay.

Assays for antioxidant enzymes

Superoxide dismutase (SOD) [EC 1.15.1.1] activity was assayed according to the method of Beauchamp and Fridovich (1971). The reaction mixture contained 0.24 mM riboflavin, 2.1 mM methionine, 1% Triton X-100, 1.72 mM nitroblue tetrazolium chloride (NBT; in 50 mM sodium phosphate buffer, pH 7.0) and 200 µl of enzyme extract to inhibit the photochemical reduction of NBT. Glass test tube containing the reaction mixture was immersed in a thermostat bath at 25 °C and illuminated, identical tubes served as blanks. After illumination for 10 min absorbance was measured at 560 nm. SOD activity was expressed as units per mg protein. One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition in the rate of NBT photo-reduction.

The specific activity of SOD was expressed as units/mg protein.

Ascorbic acid peroxidase (APX) [EC 1.11.1.11] activity was measured by the method of Nakano and Asada (1987). The 3 ml reaction mixture contained 2.5 ml sodium phosphate buffer (pH 7.4, 50 mM), 0.3 mM ascorbate and 0.06 mM ethylenediaminetetraacetic acid (EDTA), 300 µl enzyme extract (in 50 mM sodium phosphate buffer, pH 7.4) and 200 µl of 2 mM hydrogen peroxide (H₂O₂). The decrease in absorbance at 290 nm (extinction coefficient 2.8 mM⁻¹ cm⁻¹) was recorded at 25 °C for 1 min. The activity was expressed as mM ascorbic acid oxidized/min/mg protein.

Glutathione reductase (GR) [EC 1.6.4.2] activity was determined at 25 °C as described by Rao et al. (1996). The 3 ml assay mixture contained 400 µl enzyme extract (in potassium phosphate buffer 100 mM; pH 7.0), 100 µl NADPH (1 mM), 100 µl oxidized glutathione (15 mM), and 2,400 µl potassium phosphate buffer (50 mM, pH 7.0). The decrease in absorbance was recorded at 340 nm for 10 min. The enzyme activity was calculated using the extinction coefficient (6.2 mM⁻¹ cm⁻¹), and expressed as µmole NADPH oxidized/min/mg protein.

Guaiacol peroxidase (GPx) [EC 1.11.1.7] was assayed according to the method of Maehly (1955). The reaction mixture contained 0.5 ml enzyme extract (50 mM, phosphate buffer, pH 7.0), 1 ml of 20 mM guaiacol, 3 ml of 0.02 M phosphate buffer and 0.03 ml of hydrogen peroxide (H₂O₂) (88.2 mM). The activity was calculated using the extinction coefficient (26.6 mM⁻¹ cm⁻¹), and expressed as mM guaiacol oxidized/min/mg protein.

Protein

Leaf protein was estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Total phenols

Extraction and estimation

Powdered samples (leaves) from all treatments were extracted with petroleum ether to remove fats. Fat-free materials were air dried and macerated in a water bath with aqueous methanol. Methanol (70%, v/v) was evaporated under vacuum at 40 °C and the remaining aqueous fraction was freeze dried and the residue used for determine the total phenolic contents.

The total phenolics were determined spectrometrically according to the Folin-Ciocalteau method (Hoff and Singleton 1977). Samples (0.5 ml, 0.5 mg/ml) were introduced into a test tube; 2.5 ml Folin-Ciocalteau reagent solution (10%) and 7.5 ml sodium carbonate solution

Table 1 Effect of exclusion of solar UV-B and UV-B/A on plant height, leaf area, fresh weight and dry weight of plants of cotton seedlings var. Vikram (** $P < 0.001$)

Treatments	Plant height (cm)	Leaf area (cm ²)	Fresh weight (gm/plant)	Dry weight (gm/plant)
OC	17.18±1.24	10.2±0.13	3.23±0.09	0.872±0.05
FC	18.12±0.74	11±0.12	4.1±0.045	0.916±0.004
–UV-B	28.04±0.68***	13.4±0.10***	5.8±0.08***	1.544±0.015***
–UV-B/A	45.28±2.8***	15.3±0.09***	7.28±0.01***	1.917±0.0054***

(20%) were added. The tubes were shaken and allowed to stand at room temperature in the dark for 2 h. Absorption was measured at 750 nm. Gallic acid was used as a standard. Total phenol content was expressed as gallic acid equivalents (GAE) in (mg GAE/g extract) dry material.

Statistical analysis

Data are expressed as means±S.E.M. and were analyzed by the analysis of variance (ANOVA) followed by post hoc Newman-Keuls Multiple Comparison Test (** $P < 0.01$, *** $P < 0.001$) by using Prism 4 software for window, Graf Pad Software, Inc, LaJolla, CA, USA.

Results

Growth

Exclusion of UV enhanced plant height, leaf area, fresh and dry weight (Table 1). Exclusion of UV-B/A enhanced the growth parameters to a greater extent compared to exclusion of UV-B. Plant height was enhanced by 55% and 150%; area of primary leaf was enhanced by 22% and 39% respectively by excluding UV-B and UV-B/A (Table 1). Fresh weight of plants was enhanced by 43% (–UVB) and 75% (–UV-B/A) and dry weight by 68% (–UVB) and 109% (–UV-B/A) (Table 1). All these growth parameters were also enhanced significantly, after normalizing the intensities of light in all the treatment as compared to ambient conditions (Table 2).

UV absorbing substances and total phenolic content

After the exclusion of UV leaves synthesized significantly lesser amounts of UAS (Table 3). Reduction was more

in –UV-B/A compared to –UV-B with an overall reduction of 50–60%. Total phenolics content were also significantly decreased by the exclusion of solar UV radiation as compared to filter control.

Ascorbic acid

There was also a reduction in the level of ascorbic in the leaves after the exclusion of UV (Table 3). The reduction was to the extent of 16% (–UV-B) and 32% (–UV-B/A).

Antioxidant enzymes

The activity of SOD, APX and GR were lowered by exclusion of UV. The difference between the control and UVexcluded plants was maximum with respect to SOD (68%) (Table 3).

A reduction of 27% in GR activity, 31% in APX and 20% in GPx was also recorded (Table 3).

Protein

Total soluble protein content was enormously increased by exclusion of solar UV-B (60%) as compared to exclusion of solar UV-B/A (37%) (Table 3).

Constellation of data

A RADAR plot showed all data together, in which each spine is a parameter and each relative value of each parameter is relative to the FC conditions. It showed that all growth parameters and protein content were up regulated and biochemical parameters like activity of SOD, APX, GR, GPX, ASA and UAS/total phenolics content were down regulated by exclusion of solar UV-B and UV-B/A. (Fig. 1).

Table 2 Growth parameters after normalizing the intensity of all treatments to ambient condition

Treatments	Plant height (cm)	Leaf area (cm ²)	Fresh weight (gm/plant)	Dry weight (gm/plant)
OC	17.18	10.20	3.23	0.872
FC	19.07	11.57	4.31	0.964
–UV-B	33.78	16.14	6.98	1.860
–UV-B/A	56.60	19.13	9.10	2.396

Table 3 Effect of exclusion of solar UV-B and UV-B/A on SOD, APX, GR, GPx activity and protein, UV absorbing substances and total phenolic compounds in primary leaves of cotton seedlings var. Vikram

Treatments	SOD (Units/mg protein)	GPx (mM guaiacol oxidized/min/mg protein)	GR (μM NADPH oxidized/min/mg protein)	APX (mM ASA oxidized/min/mg protein)	ASA (μM/mg FW protein)	Protein (mg/gmFW)	UAS (Units/mg FW)	Total phenolics (mg GAE/g extract)
OC	11.78±0.130	1.71±0.045	0.016±0.00034	0.175±0.002	13.9±1.3	0.505±0.0020	0.316±0.371	1.8±0.05
FC	11.48±0.139	1.69±0.047	0.015±0.0005	0.163±0.0018	13.72±1.33	0.536±0.0018	0.301±0.0036	1.793±0.045
-UV-B	04.74±0.19***	1.46±0.06***	0.011±0.0004***	0.131±0.06***	11.4±1.56***	0.91±0.02***	0.182±0.004***	0.50±0.06***
-UV-B/A	03.6±0.2***	1.35±0.07***	0.012±0.0002***	0.112±0.07***	9.22±1.65***	0.721±0.03***	0.135±0.02***	0.836±0.07***

Values are significantly different at (***) $P<0.001$) from filter control.

Discussion

Results presented on cotton var. Vikram emphasize the inhibitory effect of solar UV components on the growth of cotton plants especially in tropical conditions. A reduction in plant height and leaf area under ambient UV results in reduced photosynthesis and growth of plants. In earlier experiments with supplemental UV-B used inside sunlit plant growth chambers a shorter plant height of cotton was observed due to shorter average internodal length (Reddy et al. 2003, 2004; Kakani et al. 2003). Earlier studies had used high intensity of UV-B (Kakani et al. 2003, 2004) and there was no report earlier on the inhibitory effect of ambient UV-B on the cotton plants as reported here. In addition to UV-B, UV-A also exerts an inhibitory effect on the growth of cotton plants. The inhibitory effect of UV-A is more on the plant height rather than on leaf area (Table 1). The differences in the growth parameters after exclusion of solar UV components are calculated for uniform intensity in all treatments to ambient conditions. Differences in intensities of light are not responsible for the differences in physiological parameters (Table 2).

Coleman and Day (2004) have reported that individual leaves as well as total plant leaf area become smaller in cotton plants as the UV-B dose approaches ambient levels compared to sub-optimal levels. Response of cotton plants to UV exclusion is similar to that of mungbean, pea, soybean, barley and *Cyamopsis* (Pal et al. 1997, 2006; Mazza et al. 1999; Varalakshmi et al. 2003; Amudha et al. 2005; Guruprasad et al. 2007) in terms of enhanced plant height, leaf area and biomass.

Fig. 1 Radar plot showing differences regulation of growth parameters (plant height, leaf area, fresh weight and dry weight) and biochemical parameters (SOD, GR, APX, GPx, UAS, total phenolics and protein) in cotton plants var. Vikram after exclusion of solar UV-B and UV-B/A

Exclusion of solar UV reduced the synthesis of UV absorbing substances in the leaves (Table 3). Flavonoids and other phenolic compounds which are the products of secondary metabolism act as UAS and filter the ambient solar UV (Kolb et al. 2001). When the plants are grown after excluding UV, there is a signal transduction that alters the metabolism and reduces the synthesis of UAS and total phenolics content (Table 3). Besides filtering UV, flavonoid/phenolic compounds are also capable of scavenging free radicals and contribute to photoprotection against UV-B, since irradiation with UV-B lamps results in excessive production of the free radicals of O₂ in the leaves (Jain et al. 2004a, b). Since secondary and primary metabolic pathways compete for the common pool of carbon, reduction in secondary metabolism can channelize the carbon to primary metabolism. This type of enhanced channelization has been reported with respect to reduced betacyanin synthesis in *Amaranthus* plants grown under UV excluded sunlight (Sharma and Guruprasad 2009).

In cotton plants, exclusion of UV also lowered the activity of antioxidant enzymes and the level of ascorbic acid in leaves. Enhancement in ascorbic acid level under ambient UV-B and its reduction by the exclusion of UV-B has been demonstrated earlier in soybean leaves (Xu et al. 2008). The response of cotton leaves to ambient UV is similar to soybean in this respect. The reduction in the activity of SOD, APX, GR and GPx by the exclusion of UV in cotton leaves as observed here is the first report for cotton. Activation of antioxidant enzymes in response to supplemental UV-B has been recorded in several plants like *Arabidopsis thaliana* (Rao et al. 1996), wheat (Sharma et al. 1998) and cucumber (Takeuchi et al. 1996; Teckchandani and Guruprasad 1998; Jain et al. 2003, 2004a, b; Kataria et al. 2007).

The higher activity of antioxidant enzymes in cotton leaves under ambient UV suggests that the ambient UV keeps the cotton plants under some amount of stress by the production of higher amounts of ROS compared to UV excluded sunlight.

In conclusion, ambient UV exerts a stress on cotton plants inducing the plants to respond for defense in terms of enhanced activity of antioxidant enzymes, synthesis of UAS/total phenolics and synthesis of ascorbic acid (Fig. 1). Exclusion of UV removes this stress and alters the metabolism of cotton plants to favour primary metabolism resulting in enhanced plant height, leaf area and biomass (Fig. 1). Cotton plants thus have a potential for producing higher biomass which gets limited under ambient UV. Growing cotton plants under UV excluded condition may prove to be beneficial in enhancing the yield of cotton.

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