

EFFECT OF EXCLUSION OF AMBIENT SOLAR UV-A/B COMPONENTS ON GROWTH AND ANTIOXIDANT RESPONSE OF COTTON (*GOSSYPIUM HIRSUTUM* L.)

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The influence of ambient solar UV-A or UV-B radiation on growth responses was investigated in three varieties of cotton (*Gossypium hirsutum* L.) after exclusion of solar UV-A/B radiation: JK-35, IH-63 and Khandwa-2. Cotton plants were grown from seeds in UV-exclusion chambers lined with selective UV filters to exclude either UV-B (280–315 nm) or UV-A/B (280–400 nm) from the solar spectrum under field conditions. Excluding UV-B and UV-A/B significantly increased plant height, leaf area and dry weight accumulation in all three varieties of cotton. The varieties differed considerably in their sensitivity to ambient UV-A/B. Khandwa-2 was most sensitive and JK-35 least sensitive to ambient solar UV. We monitored the activity of the antioxidant enzymes superoxide dismutase (SOD), ascorbic acid peroxidase (APX), glutathione reductase (GR) and guaiacol peroxidase (GPX), as well as the level of the antioxidant ascorbic acid (ASA), in primary leaves of the most UV-sensitive variety (Khandwa-2). The level of UV-B-absorbing substances was significantly decreased by exclusion of solar UV-B and UV-A/B. Exclusion of solar UV decreased the activity of all the antioxidant enzymes monitored and the level of ascorbic acid versus control plants (+UV-A/B) grown under filters transparent to solar UV. Reduction of the antioxidant defense after UV exclusion indicates that ambient solar UV exerts significant stress and induces some reactive oxygen species to accumulate, which in turn retards the growth and development of cotton plants. Ambient solar UV stresses cotton plants, shifting their metabolism towards defense against solar UV. Exclusion of solar UV eliminates the need for that defense and leads to enhancement of primary metabolism.

Key words: Antioxidant enzymes, cotton, sensitivity index, UV exclusion.

INTRODUCTION

Anthropogenic and natural destruction of stratospheric ozone increases solar UV-B radiation at the Earth's surface (Mckenzie et al., 2007). Solar UV-B affects many aspects of plant growth and metabolism. Multiple target sites for the action of UV-B have been reported. Responses to elevated and ambient UV-B include increased DNA damage and antioxidant response (Mazza et al., 1999), alteration of plant morphology and architecture (Albert et al., 2005) and lower biomass accumulation (Kakani et al., 2003).

UV-B radiation increases the production of reactive oxygen species (ROS), which include superoxide radical ($O_2^{\cdot-}$) and hydroxyl radical ($\cdot OH$), hydrogen peroxide (H_2O_2) and singlet oxygen (1O_2), all of which can cause oxidative damage to membrane lipids, nucleic acids and proteins (Foyer et al., 1994). UV-B affects enzymatic antioxidants at both

activity level (Rao et al., 1996) and mRNA level (Willekens et al., 1994). The activities of antioxidant enzymes like superoxide dismutase, ascorbic acid peroxidase and glutathione reductase are enhanced by supplemental UV-B in *Arabidopsis* (Rao et al., 1996), cucumber (Jain et al., 2004) and wheat (Sharma et al., 1998). UV-B also increased the level of the antioxidant ascorbic acid in *Arabidopsis* (Rao et al., 1995), wheat (Sharma et al., 1998) and cucumber (Jain et al., 2003), and glutathione in cucumber (Rao and Ormrod, 1995). In response to elevated ambient levels of UV-B there is also an increase in the concentration of UV-absorbing substances, especially flavonoids and other phenolic compounds (Mazza et al., 2000).

Abbreviations: APX – ascorbic acid peroxidase; ASA – ascorbic acid; EDTA – ethylenediaminetetraacetic acid; FC – filter control; PVP – polyvinylpyrrolidone; SOD – superoxide dismutase; TCA – trichloroacetic acid; UAS – UV-B-absorbing substances; -UV-B – UV-B-excluded plant; -UV-A/B – UV-B and UV-A-excluded plant.

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Most of the above studies were conducted indoors using a growth chamber or greenhouse in which plants were exposed to high UV-B radiation and relatively lower UV-A and PAR. There are very few studies on the effects of solar UV-B radiation on ROS metabolism under natural environmental conditions (Agrawal and Rathore, 2007) and under UV-exclusion conditions (Mazza et al., 1999; Xu et al., 2008). Extensive research on the status of various antioxidants after exposure to ambient UV-B is essential to a proper assessment of their contribution to the defense system. To evaluate the possible consequences of increased UV-B radiation for tropical plants, the effects of present ambient UV-B and UV-A levels need to be known. Although UV-A is less damaging per photon than UV-B, UV-A comprises a much larger portion of the solar spectrum than UV-B, and UV-A is able to penetrate to greater depths within the leaf than UV-B (Liakoura et al., 2003). UV-A can cause oxidative damage (Yao et al., 2006) and growth inhibition (Krizek and Chalker, 2005) in higher plants. Tropical countries like India receive a large amount of solar UV-B, which influences plant growth and development. Sahoo et al. (2005) observed a significant decline in the total ozone column at numerous stations in northern India.

Cotton is one of India's major crop plants, grown in southern districts of Madhya Pradesh State. Cotton is sensitive to UV-B; supplemental UV-B radiation reduces leaf area, plant height, photosynthesis and biomass (Kakani et al., 2003). There has been no study on intraspecific variation between varieties, nor on the growth/antioxidant response of cotton to exclusion of ambient solar UV-A/B. The purpose of the present study was to evaluate the impact of ambient UV-A and UV-B on the sensitivity of three varieties of cotton plants, and on the growth and antioxidant defense system of the most UV-sensitive variety of cotton by growing the plants in the presence (+UV-A/B) or absence (-UV-A/B) of solar UV.

MATERIALS AND METHODS

All the field experiments were conducted under natural sunlight in the Botanical Garden of the School of Life Sciences, Devi Ahilya University, Indore (24°N), India. The experiments were carried out from February to March 2010, when the average daily solar UV-B dose is about 50% higher than the average daily dose received in the temperate region. Seeds of three cotton (*Gossypium hirsutum*) varieties (JK-35, IH-63, Khandwa-2) were obtained from the Cotton Improvement Project of the Agriculture College, Indore, and treated with recommended fungicides (Bavistin and Diathane M, 2 g/kg seeds). The seeds were sown inside iron wire mesh cages (120 cm × 90 cm × 150 cm) in 90 cm

rows with 30 cm between rows. The cages were wrapped with UV-B and UV-A/B cutoff filters (Garware polyester Ltd., Mumbai) which specifically eliminate UV-B (<300 nm) and UV-A/B (<400 nm) radiation. The filter control plants were grown under an ordinary polyethylene filter transparent to UV (280–400 nm) radiation. The transmission of the filters, measured with a spectrophotometer (Shimadzu UV-1601), was given earlier (Guruprasad et al., 2007).

The transmission characteristics of the 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. The plants were irrigated and fertilized with NPK at regular intervals to avoid nutrient deficiencies. The plants were grown for 30 days. Samples were taken 30 days after seedling emergence for growth measurements (plant height, leaf area, dry weight) and for biochemical analysis.

RADIATION MEASUREMENT

Absolute solar irradiance without UV-B or UV-A/B was measured using a radiometer (IL 1350, International Light, U.S.A.). Ambient solar irradiance at midday during the experimental period was 382 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Loss of light intensity at midday was 43% for -UV-B filters (219 $\mu\text{mol m}^{-2} \text{s}^{-1}$), 44% (214 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for UV-A/B filters, and 7% (356 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for polythene filters transparent to UV (filter control). Intensity of PAR was at an optimal level for normal plant growth.

GROWTH PARAMETERS

The plants were grown for 30 days under UV-B-excluded and UV-A/B-excluded conditions and sampled 30 days after seedling emergence (DAE). Plant height was measured from the apex to the base of the stem. The mean height of 5 plants was measured.

For dry weight measurements, plants were removed, roots were washed thoroughly with water, and the plant was oven-dried at 60°C for 72 h.

Leaf area was measured from blotted-dry primary leaves at 30 DAE by tracing their outlines on mm graph paper, cutting out those outlined figures and weighing them (Shine et al., 2011). Leaf area is taken as the mean of 5 leaves.

SENSITIVITY INDEX (SI)

Differences in the UV sensitivity of the three varieties of cotton at 30 DAE were ascertained by a UV sensitivity index (UV-SI) calculated according to the following equation:

$$\text{UV-SI} = \frac{\text{Plant height} + \text{UV}}{\text{Plant height} - \text{UV}} + \frac{\text{Dry weight} + \text{UV}}{\text{Dry weight} - \text{UV}} + \frac{\text{Leaf area} + \text{UV}}{\text{Leaf area} - \text{UV}}$$

A UV-tolerant plant has an UV-SI of 3. UV-SI values below 3 indicate a UV-sensitive plant (+UV = filter control, -UV = UV-A/B-excluded or UV-B-excluded plants).

EXTRACTION AND ESTIMATION OF ANTIOXIDANT ENZYMES

All operations were performed at 4°C. The enzyme extracts for SOD, GR and GPX assays were prepared by homogenizing 100 mg primary leaves of cotton variety Khandwa-2 at 30 DAE with 5 ml phosphate buffer (50 mM, pH 7.0) containing 1 mM EDTA and 1% PVP. For estimation of APX activity, the same extraction buffer was supplemented with 1 mM ascorbic acid. The homogenates were centrifuged at 12,600 g for 30 min and the supernatant obtained was used for enzyme determination.

Superoxide dismutase (SOD) [EC 1.15.1.1] activity was assayed according to the method of Beauchamp and Fridovich (1971). The specific activity of SOD is 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). Its activity is 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. (1995) and is expressed as μmol NADPH oxidized/min/mg protein.

Guaiacol peroxidase (GPX) [EC 1.11.1.7] was assayed according to the method of Maehly (1955) and is expressed as mM guaiacol oxidized/min/mg protein.

Protein was estimated by the method of Lowry et al. (1951) using BSA as the standard.

UV-B ABSORBING SUBSTANCES (UAS)

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

ASCORBIC ACID (ASA) DETERMINATION

Ascorbate was measured based on reduction of Fe³⁺ to Fe²⁺ with ascorbic acid in acid solution followed by the formation of a red chelate between ferrous ion

and bipyridyl (Arakawa et al., 1981): 0.2 g tissue sample was homogenized in 2 ml ice cold 5% TCA containing 4% PVP-40 (w/v). The homogenate was filtered through four layers of muslin and centrifuged at 14,336 g for 10 min at 4°C. The supernatant was used for total ASA (ASA, DHA) assays. Total ASA was determined as reduction of DHA to ASA by dithiothreitol (DTT) and is expressed as μM ASA/mg leaf fresh weight.

STATISTICAL ANALYSIS

Data are expressed as means ±SE and were analyzed by ANOVA followed by the post hoc Newman-Keuls multiple comparison test (*P<0.05, **P<0.01, ***P<0.001) in Prism 4 for Windows (Graf Pad Software, LaJolla, CA, U.S.A.).

RESULTS

GROWTH PARAMETERS

As compared to plants grown under ambient UV-B/A radiation, growth parameters including plant height, leaf area and biomass accumulation increased significantly in all three cotton varieties grown under UV-B-excluded or UV-A/B-excluded solar radiation. Plant height increased significantly after exclusion of UV-B, and increased further after UV-A/B exclusion in all three varieties at 30 DAE. The maximum increase was in Khandwa-2, by 65% under UV-B exclusion and by 153% under UV-A/B exclusion (Fig. 1a).

Versus the filter control, all three varieties showed higher dry weight accumulation after exclusion of UV-B and UV-A/B radiation from the solar spectrum. Khandwa-2 again showed the maximum effect, with 53% higher dry weight under UV-B exclusion and 93% higher dry weight under UV-A/B exclusion (Fig. 1b).

The increase of primary leaf area was also highest in Khandwa-2, by 36% (-UV-B) and 69% (-UV-A/B) (Fig. 1c).

UV SENSITIVITY INDEX (UV-SI)

The UV sensitivity index (UV-SI) differed between the varieties and was less than 3 for all the cotton varieties we tested. Sensitivity to ambient UV-B was lower for Khandwa-2 (UV-SI 1.99) than for JK-35 (UV-SI 2.29). Both Khandwa-2 (UV-SI 1.50) and JK-35 (UV-SI 2.03) showed less sensitivity to ambient UV-A/B than to ambient UV-B (Tab. 1).

ANTIOXIDANT ENZYMES

We assayed the activity of the antioxidant enzymes SOD, APX, GR and GPX in primary leaves of the most UV-sensitive variety of cotton (Khandwa-2) at

TABLE 1. Sensitivity index (SI) values for three varieties of cotton under ambient UV-B and UV-A/B as found from exclusion of solar UV-B and UV-A/B radiation

Variety	UV- SI	
	UV-B	UV-A/B
JK-35	2.29	2.03
IH-63	2.20	1.94
Khandwa-2	1.99	1.50

Standard errors of five replicates are 3–5% of mean values.

30 DAE. The activity of all four antioxidant enzymes was significantly lower in plants grown under ambient UV exclusion (Fig. 2); the quantitative decreases differed between the enzymes. The difference between the control and UV-excluded plants was greatest for SOD (40%) (Fig. 2a). APX was 37% lower and GPX was 20% lower (Fig. 2b,d).

UV-B-ABSORBING COMPOUNDS

One mechanism that can protect sensitive plant tissue from solar UV is alteration of leaf transmittance properties. Most plant species synthesize epidermal UV-B-absorbing compounds in response to ambient UV, and these compounds do not absorb PAR. In exclusion experiments, methanolic extracts of leaves (especially flavonoids) had lower absorbance at 305 nm (Fig. 3a).

ANTIOXIDANT – ASCORBIC ACID (ASA)

Total ASA levels were high in plants in the filter control (+UV-A/B), and were reduced by exclusion of UV-B and UV-A/B radiation. Khandwa-2 had 17% lower ASA in the -UV-A/B treatment than in the filter control (Fig. 3b).

DISCUSSION

In this study, exclusion of UV from the solar spectrum led to significant increases in the growth parameters and biomass accumulation of all three tested cotton varieties versus the filter control (+UV-A/B). Negative effects of supplemental UV-B on the growth of field grown cotton plants have been established in the Sukan 103 variety grown in Nanjing China (Gao et al., 2003). Shorter cotton plant height due to shorter average internodal length was observed in experiments with UV-B supplementation inside sunlit plant growth chambers (Gao et al., 2003; Kakani et al., 2003). Our work supports these earlier observations by providing data on increased plant height and biomass in the absence of ambient

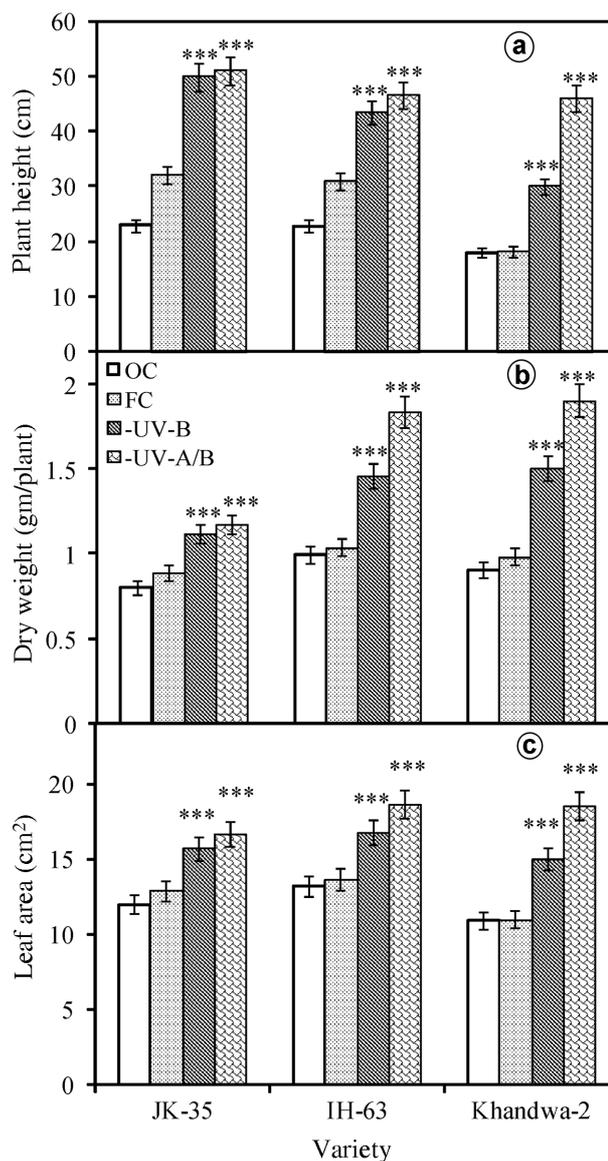


Fig. 1. Effect of exclusion of solar UV-B and UV-A/B on growth parameters of the cotton varieties Khandwa-2, JK-35 and IH-63 at 30 DAE: (a) Plant height, (b) Dry weight accumulation, (c) Leaf area. Vertical bar indicates \pm SE of mean. Values differ significantly from filter control at *** $P < 0.001$ (Newman-Keuls multiple comparison test). DAE – days after emergence.

UV. Unlike earlier experiments on cotton confined to the effect of UV-B, our present report points up the role of the UV-A component of ambient solar radiation in reducing cotton plant height and biomass. An extensive study of the effects of supplemental UV-B on the leaf growth and morphology of cotton plants showed reduced leaf area and the occurrence of chlorotic and necrotic patches on leaf surface

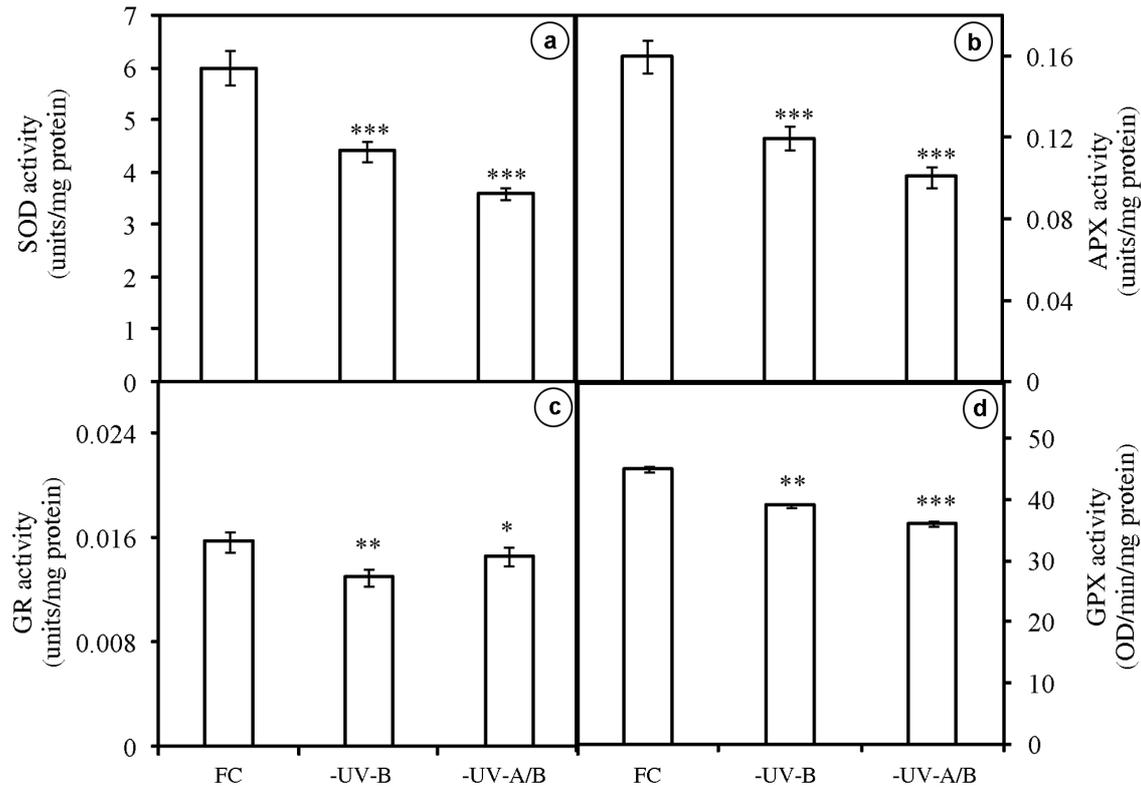


Fig. 2. Effect of exclusion of solar UV-B and UV-A/B on (a) SOD, (b) APX, (c) GR, (d) GPX activity in primary leaves of cotton var. Khandwa-2 at 30 DAE. Vertical bar indicates \pm SE of mean. Values differ significantly from filter control at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ (Newman-Keuls multiple comparison test).

(Kakani et al., 2003). In our study ambient UV reduced the leaf area, and excluding it enlarged the primary leaves. Thus a major effect of the absence of ambient UV seems to be a higher leaf growth rate. Coleman and Day (2004) reported that individual leaves as well as total plant leaf area become smaller in cotton plants as the UV-B dose approaches ambient levels, as compared to subambient levels of UV-B. Greater plant height, leaf area and biomass due to exclusion of ambient UV have been observed in several plants such as wheat, pea, soybean, barley, beans and *Cyamopsis* (Mazza et al., 1999; Amudha et al., 2005; Pal et al., 2006; Guruprasad et al., 2007). Higher growth and biomass accumulation have been linked to increased photosynthesis and carbon fixation rates in soybean (Guruprasad et al., 2007). In cotton as well, increased leaf area may boost photosynthesis.

Sensitivity indices are useful indicators of plant sensitivity to UV-B radiation (enhanced or supplemental) (Saile Mark et al., 1997). In our study we calculated sensitivity indices from plant height, total dry weight accumulation and leaf area, reflecting the overall sensitivity of the three cotton varieties to cur-

rent levels of UV radiation. The UV sensitivity index values for all the varieties were significantly less than 3, meaning that all of them are UV-sensitive to some extent. This is the first report of intraspecific responses of Indian varieties of cotton plants to ambient solar UV. Our results reveal that Khandwa-2 is most sensitive and JK-35 is least sensitive to current levels of UV (280-400 nm) radiation.

We used Khandwa-2 to assay antioxidants and measure antioxidant enzyme activity because we found it to be the most UV-sensitive variety of cotton tested. Enhanced synthesis of UV-B absorbing compounds, mainly flavonoids, has been recognized as a general response to UV-B stress (Mazza et al., 2000). Besides filtering UV-B, flavonoid compounds are also capable of scavenging free radicals, contributing to photoprotection against UV-B since UV-B radiation results in excessive production of free radicals (Mazza et al., 2000). Exclusion of ambient UV reduced the amount of UAS in cotton leaves, demonstrating the sensitivity of cotton to ambient UV. Excluding ambient UV also reduced the activity of antioxidant enzymes SOD, APX, GR and GPX (Fig. 2) and the levels of ascorbic acid (Fig. 3b) in cotton

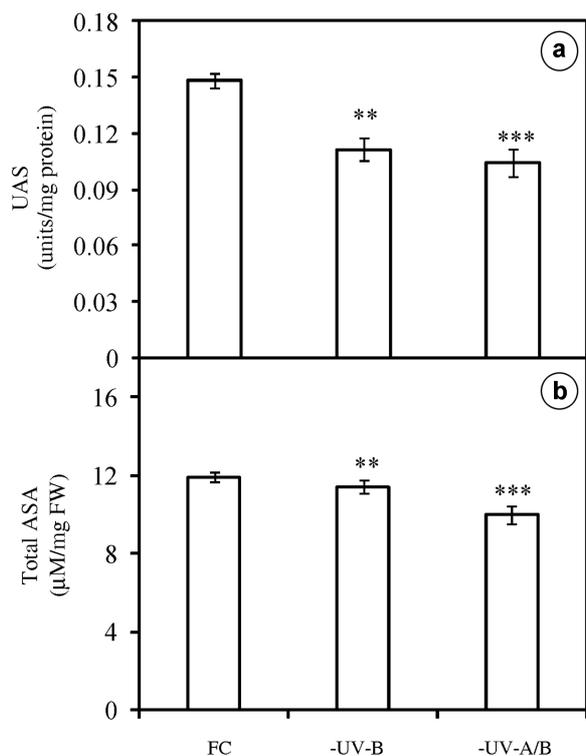


Fig. 3. Effect of exclusion of solar UV-B and UV-A/B on (a) UAS, (b) Total ASA content in primary leaves of cotton var. Khandwa-2 at 30 DAE. Vertical bar indicates \pm SE of mean. Values differ significantly from filter control at ** $P < 0.01$ and *** $P < 0.001$ (Newman-Keuls multiple comparison test).

leaves. These results indicate that the presence of ambient UV (i) activates the antioxidant enzymes, (ii) enhances UAS synthesis and (iii) increases ascorbic acid synthesis. Common signal transduction by ambient UV seems to augment the defense mechanism in cotton plants. Our data also indicate that both UV-A and UV-B in the solar spectrum are involved in this activation, since there were significant quantitative differences in all the tested antioxidant biochemical parameters between the UV-B-exclusion and the UV-A/B-exclusion treatments.

Activation of antioxidant enzymes in response to supplemental UV-B has been recorded in several plants including *Arabidopsis thaliana* (Rao et al., 1996), wheat (Sharma et al., 1998) and cucumber (Jain et al., 2004; Kataria et al., 2007). Elevation of ascorbic acid levels in response to UV-B has been observed in soybean leaves (Galatro et al., 1991), *Arabidopsis* (Rao and Ormrod 1996) and cucumber (Jain et al., 2004). In soybean leaves, Xu et al. (2008) found higher ascorbic acid levels under ambient UV-B and lower levels under UV-B exclusion.

The response of the antioxidant enzymes in cotton to ambient UV has not been tested previously.

Most studies have emphasized the role of UV-B in oxidative stress but our data indicate that UV-A stress is quite significant in cotton. Wilson (2001) found that UV-A can be even more detrimental to a plant than UV-B, depending on the parameters tested.

In this work we demonstrated that ambient UV-B and UV-A reduced the growth and biomass of all three varieties of cotton, and that excluding ambient UV removed the stress. Khandwa-2 is the most sensitive and JK-35 the least sensitive to current levels of solar UV. In the absence of ambient UV, the activity of antioxidant enzymes, antioxidant level and UAS were reduced in the most sensitive variety, Khandwa-2. Exclusion of solar UV eliminated the defense against UV-B stress and led to enhanced growth and biomass accumulation in the cotton plants. This indicates that the current ambient levels of UV-A and UV-B are high enough to induce accumulation of reactive oxygen species, which trigger antioxidant defense systems. In turn, the growth and development of cotton plants are retarded.

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