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Impact of ambient and supplemental ultraviolet-B stress on kidney bean plants: an insight into oxidative stress management

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Abstract In the present study, the response of kidney bean (*Phaseolus vulgaris* L. cv. Pusa Komal) plants was evaluated under three different levels of ultraviolet-B (UV-B), i.e., excluded UV-B (eUV-B), ambient UV-B (aUV-B; 5.8 kJ m^{-2} day^{-1}), and supplemental UV-B (sUV-B; 280–315 nm; ambient+7.2 kJ m^{-2} day^{-1}), under near-natural conditions. eUV-B treatment clearly demonstrated that both aUV-B and sUV-B are capable of causing significant changes in the plant's growth, metabolism, economic yield, genome template stability, total protein, and antioxidative enzyme profiles. The experimental findings showed maximum plant height at eUV-B, but biomass accumulation was minimum. Significant reductions in quantum yield (Fv/Fm) were observed under both aUV-B and sUV-B, as compared to eUV-B. UV-B-absorbing flavonoids increased under higher UV-B exposures with consequent increments in phenylalanine ammonia lyase (PAL) activities. The final yield was significantly higher in plants grown under eUV-B, compared to those under aUV-B and sUV-B. Total protein profile through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and analysis of isoenzymes, like superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR), through native PAGE revealed major changes in the leaf proteome under aUV-B and sUV-B, depicting induction of some major stress-related proteins. The random amplified polymorphic DNA (RAPD) profile of genomic DNA also indicated a significant reduction of genome template stability under UV-B exposure. Thus, it can be inferred that more energy is diverted for inducing protection mechanisms rather than utilizing it for growth under high UV-B level.

Introduction

Levels of solar ultraviolet-B (UV-B) radiation (280–315 nm) reaching the Earth's surface vary depending on changes in altitude, latitude, season, and time of the day (Paul and Gwynn 2003). Several models predicted reduction in cloud cover and increased aridity for low- to midlatitudes and increased cloudiness and precipitation at high latitudes at both hemispheres (Ballaré et al. 2011). These changes in cloud patterns associated with climate change could have strong effects on the levels of UV-B, which could be increased in tropical and subtropical regions (McKenzie et al. 2011). The amount of UV-B radiation reaching tropical latitudes is higher than that in temperate latitudes because the lower solar zenith angle leads to a less atmospheric UV-B absorption in the tropics. Sahoo et al. (2005) observed a significant declining trend of total ozone column (TOC) over numerous stations lying in the northern part of India, suggesting potential vulnerability of plants to UV-B under field conditions.

Some plants are sensitive to even ambient UV-B fluences (Krizek et al. 1998), while many plants appear quite tolerant to even high fluences of UV-B (Allen et al. 1998). Previous studies have demonstrated that UV-B radiation has many direct and indirect effects on plants, ranging from phenotypic damage to genome, proteome, and metabolome alterations (Casati et al. 2005; Singh et al. 2010; Tripathi et al. 2011). Most of the available data concerning UV-B sensitivity has been focused to understand the effect of increasing UV-B

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irradiances on plants. Solar UV-B radiation may be supplemented with UV-B radiation from special filtered fluorescent lamps as an effort to simulate different scenarios of solar radiation with stratospheric O_3 depletion (Warren et al. 2003). A contrasting approach uses filters to remove much of the radiation at shorter wavelengths from the solar spectrum, thereby assessing the influence of current solar UV-B flux (Zavala and Botto 2002). There have only been a few attempts to compare the biological effects of the above two approaches in simultaneous experiments (Germ et al. 2002). Higher level of UV-B triggers non-specific pathways in plants resulting towards a general stress response. In contrast, lower UV-B rather acts as an information signal (Jansen et al 2008), stimulating the expression of major gene(s) and/or gene network involved in the UV-B protection of plants, like the UV-B-specific photomorphogenesis signaling pathway (Jenkins 2009). There have been many studies on the damage of enhanced UV-B to plants, including alterations in growth and development, and declines of plant biomass and yields (Feng et al 2007).

Kidney bean (*Phaseolus vulgaris* L.) plant species originated at higher altitude that naturally receives high UV-B irradiances. It may be possible to identify some specific characteristics or physiological traits that can be correlated well with the tolerance of plants to ambient UV-B level in natural ecosystems by comparing them with plants grown under reduced UV-B radiation. In the present study, we excluded ambient UV-B and an increased level under natural field condition to estimate the effects of UV-B radiation on kidney bean plants. We tried to find out the underlying mechanisms operating to make changes due to various levels of UV-B on morphological and physiological responses, cellular metabolism, and the proteome and genome of kidney bean plants. Furthermore, these results are an initial report on the effect of UV-B on kidney bean genome and proteome and will definitely pave the way for further characterization of specific responses under higher UV-B atmosphere by using latest "-omics" techniques.

Materials and methods

Experimental site and plant material

The field experiment was conducted at the experimental field of the Department of Botany, Banaras Hindu University, Varanasi, Uttar Pradesh (25° 81′ N, 25° 81′ E and about 76 m above mean sea level) situated in the eastern Gangetic plains of India for two consecutive experimental years. The soil of the experimental site was sandy loam in texture (sand 45 %, silt 28 %, and clay 27 %) and had a pH close to neutral (7.2–7.4). During the experiment, mean temperature ranged from 7.3 to 25.8 °C, mean relative humidity ranged from 40.4 to 62.5 %, and rainfall was 112 mm. Photosynthetic photon flux density (PPFD) averaged $1,100\pm109$ μmol m^{-2} s^{-1} at midday.

The seeds of the test plant, kidney bean (*P. vulgaris* L. cv. Pusa Komal), were collected from the Indian Agricultural Research Institute (IARI), New Delhi, India. This high-yielding variety was developed by IARI, New Delhi, India, and is widely cultivated in Northern Indian states.

Experimental design

We adopted a randomized split plot design for the present experiment. Each experimental site was organized in three blocks, each with three experimental plots (three biological replications, $n=3$). This split plot design allowed us to test for effects of excluded UV-B (eUV-B), ambient UV-B (aUV-B; 280–315 nm; 5.8 kJ m^{-2} day^{-1}), and supplemental UV-B (sUV-B; 280–315 nm; 7.2 kJ m^{-2} day^{-1}).

The recommended dose of NPK was 20:40:80 kg ha^{-1} for kidney beans. N, P, and K were given in the form of urea, superphosphate, and muriate of potash, respectively. A half dose of N and full doses of P and K were given as basal dressing, and another half dose of N was given as top dressings after 7 days of germination (DAG).

Pure-line seeds of kidney beans were sown in rows (30 cm apart) in each plot. For each treatment, three replicate plots were maintained ($n=3$). There were nine plots of 1 m^2 each with three rows in each plot. After germination, plants were thinned to one plant every 15 cm in each row for uniformity in growth and development. Plants were watered once in every 2 days with the same amount of water through channel irrigation.

Irradiation conditions

Three contrasting spectral irradiance condition, i.e., eUV-B, aUV-B, and sUV-B, were achieved by combining filtered solar radiation and UV-B supplementation using UV-B lamps. Supplemental UV-B was artificially provided by Q-panel UV-B 313 40 W fluorescent lamps (Q Panel Inc., Cleveland, OH, USA). For each treatment, three replicate plots were used with a separate frame. Each steel frame contains three lamps (120 cm long) fitted 30 cm apart and was suspended perpendicular to the planted rows (three) of each plot. The lamps were covered by a 0.13-mm cellulose diacetate filter (transmission down to 280 nm) for supplemental UV-B radiation. UV irradiance was measured with a double-monochromator spectroradiometer (Scientech, Boulder, CO, USA). aUV-B (5.8 kJ m^{-2} day^{-1}) on the summer solstice was weighted against the generalized plant response action spectrum of Caldwell (1971). The plants beneath the cellulose diacetate film received ambient+sUV-B (ambient+7.2 kJ m^{-2} day^{-1}) at Varanasi during clear-sky condition (Green et al. 1980)

normalized at 300 nm, at 0 albedo and 1.0 scatter. Lamps in frames were adjusted weekly to a distance of 45 cm to provide a mean enhanced UV-B of 7.2 kJ m^{-2} day^{-1} (weighted) to plant apices for 3 h daily over the middle of the photoperiod (10:00 a.m. to 1:00 p.m.).

Solar UV-B radiation was filtered by using a UV-B absorbing filter. Filter treatments excluded a larger part of the UV spectrum in natural daylight; UV-B$_{ex}$ (Mylar®, type D, DuPont Teijin Films, Wilmington, DE, USA) absorbs $\lambda <$ 320 nm. These filters were mounted on aluminum frames fixed approximately 5 cm above the plant height.

Growth parameters and biomass

For growth and biomass determinations, three random samples were taken from each plot at the age of 60 DAG by carefully digging monoliths ($10 \times 10 \times 20$ cm^3) containing intact roots. These were thoroughly washed by placing on a sieve of 1-mm diameter under running tap water to remove soil particles adhering to the roots. Plant height, root lengths, leaf area, and number of leaves were quantified. Leaf area was measured using a portable leaf area meter (Model LI-3000, LI-COR, Inc., USA). For biomass determination, root and shoot portions were separated and oven-dried at 80 °C till a constant weight was achieved. Biomass allocation pattern was determined by calculating the growth indices such as root shoot ratio (RSR) and specific leaf weight (SLW) based on dry weight data using the formulae modified by Hunt (1982).

Chlorophyll fluorescence

Chlorophyll fluorescence characteristics such as initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv=Fm−Fo), and Fv/Fm ratio were measured between 8:00 and 10:00 a.m. using a portable plant efficiency analyzer (PEA; Hansatech Instruments Ltd., UK). Leaf clips for dark adaptation were placed on the adaxial side of the leaves for 20 min before measurement at a saturating flash of 3,000 μmol m^{-2} s^{-1}.

Biochemical assays

Biochemical analyses were conducted in the third fully expanded mature leaves from all three treatments. Total chlorophyll, carotenoid, and flavonoids were determined as described previously by Maclachlan and Zalik (1963), Duxbury and Yentsch (1956), and Mirecki and Teramura (1984), respectively. Ascorbic acid, phenol, and protein were determined by using the methodologies of Keller and Schwager (1977), Bray and Thorpe (1954), and Lowry et al. (1951), respectively. Malondialdehyde (MDA) content, a product of lipid peroxidation, was estimated using thiobarbituric acid (TBA) as described by Heath and Packer (1968).

Activities of peroxidase (POX), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and ascorbate peroxidase (APX) enzymes were determined as described by Britton and Mehley (1955), Fridovich (1974), Aebi (1984), Anderson (1996), and Nakano and Asada (1987), respectively. Phenylalanine ammonia lyase (PAL) activity was estimated following the methodology proposed by Rao Subba and Tower (1970).

Analysis of genome responses

Genomic DNA (gDNA) from the third fully expanded leaf of kidney bean plant was isolated according to the method described by Khanuja Suman et al. (1999). The isolated genomic DNA was used as a template for PCR amplification. Twenty-one different random amplified polymorphic DNA (RAPD) primers (synthesized from Metabion, Germany) (Table 1) were used for the PCR amplification as described by Welsh and McClelland (1990). PCR reaction of a 25-μL solution contains 2 μL gDNA (20 ng μL^{-1}), 2.5 μL 10× PCR buffer, 50 mM MgCl$_2$ (2.0 μL), 10 mM dNTPs (1 μL), 1 μL of primer, 0.2 μL Taq DNA polymerase, and 16.3 μL autoclaved Millipore water. The amplifications were performed in a DNA thermal cycler (Mastercycler pro, Eppendorf, USA), with the following temperature cycle: an initial denaturation of 60 s at 94 °C, followed by 45 cycles of 1 min at 94 °C, 1 min at 36 °C,

Table 1 List of primers used and their base sequence

Primers	Sequence
P1	5′-AAAGCTGCGG-3′
P2	5′-AACGCGTCGG-3′
P3	5′-AAGCGACCTG-3′
P4	5′-AATCGCGCTG-3′
P5	5′-AAGCGGCCTG-3′
P6	5′-ACACACGCTG-3′
P7	5′-ACATCGCCCA-3′
P8	5′-ACCACCCACC-3′
P9	5′-ACCGCCTATG-3′
P10	5′-ACGATGAGCG-3′
P11	5′-GTGACGTAGG-3′
P12	5′-CTCACCGTCC-3′
P13	5′-GGACCCAACC-3′
P14	5′-GGAAGTCGCC-3′
P15	5′-GAGTGGTGAC-3′
P16	5′-GGGTCGCATC-3′
P17	5′-ACGACCGACA-3′
P18	5′-GGATCATGTG-3′
P19	5′-GAGCGGACCA-3′
P20	5′-GACCGACCCA-3′
P21	5′-GATGACCGCC-3′

and 2 min at 72 °C. The amplification products were separated on 1.2 % agarose gels, stained with ethidium bromide, photographed using Multi Imager Gel documentation system (Bio-Rad), and quantified with Quantity One Software (Bio-Rad). The genome template stability (GTS) is one of the most reliable methods to assess toxicant-induced DNA damage in living organisms. The GTS was calculated using the formula described by Cenkci et al. (2010)

Analysis of proteome responses

In-gel assay through native PAGE was done for six specific antioxidative enzymes, i.e., SOD, POX, CAT, APX, guaiacol peroxidase (GPX), and GR. Initially, the protein for in-gel assay was isolated by crushing 1 g of the third fully expanded mature leaves of kidney bean plants in 3-mL pre-chilled 100 mM K-phosphate buffer at 4 °C as described by Sindhu et al. (1984). The homogenates were centrifuged at $18,500 \times g$ for 10 min at 4 °C to obtain the crude enzyme extract. The protein was quantified by the method given by Lowry et al. (1951). Native PAGE was performed at 4 °C and 10 mA, following Laemmli (1970), on a standard vertical gel electrophoresis unit (Bio-Rad). For native PAGE analysis, APX, SOD, and CAT enzyme extracts were subjected to native PAGE with 10 % running gel, GPX with 11 %, GR with 11.5 %, and POX with 12 % running gel. Hundred-microgram protein equivalents, from each treatment, were loaded as samples in the case of every native PAGE.

SOD activity was detected by following the method of Beauchamp and Fridovich (1971). First, the gel was equilibrated with pre-chilled 50 mM K-phosphate buffer (pH 7.8) containing 0.28 mM riboflavin and 28 mM tetramethylethylenediamine (TEMED) for 30 min at 4 °C, then washed with distilled water, and again incubated in 50 mM K-phosphate buffer (pH 7.8) containing 2.45 mM nitroblue tetrazolium (NBT) under white light at 4 °C, till the bands appeared.

CAT activity was studied as described by Woodbury et al. (1994). The gel was washed with distilled water and then submerged with 0.003 % H_2O_2 for 10 min at 4 °C. Activity was then visualized by transferring the gels to a solution of 1 % (w/v) ferric chloride and potassium ferricyanide (1:1) until the bands appeared.

POX activity was studied as described by Sindhu et al. (1984). The gel was incubated in 6 % ammonium chloride solution containing 0.15 % benzidine for 30 min in the dark at 4 °C. Later, few drops of 30 % H_2O_2 were added with constant shaking till the dark brown bands appear.

GPX activity was detected on the gel by submerging the gel in a staining solution containing 50 mM sodium citrate buffer pH 4.5, 2 mM H_2O_2, and 20 mM guaiacol. GR activity was measured as described by Neuhoff et al. (1985). The gel was soaked in the substrate solution (25-mL 50 mM Tris-HCl buffer, pH 7.9, containing 4.0 mM oxidized glutathione, 1.5 mM β-NADPH, and 2 mM DTNB) with gentle shaking for 20 min under a dark condition. After a brief rinse with 50 mM Tris-HCl buffer (pH 7.9), the GR activity was negatively stained in darkness by 50-mL 1.2 mM MTT and 1.6 mM PMS for 5–10 min at room temperature with gentle shaking. Clear zones of GR bands against the purple background were found.

APX gels were equilibrated with 50 mM potassium phosphate buffer (pH 7.0) and 2 mM ascorbate for 30 min; the equilibration buffer was renewed every 10 min. Then the gels were incubated in 50 mM potassium phosphate buffer (pH 7.0) containing 4 mM ascorbate and 2 mM H_2O_2 for 20 min. The gels were subsequently washed with 50 mM potassium phosphate buffer for 1 min and submerged and gently agitated in 50 mM potassium phosphate buffer (pH 7.8) containing 28 mM TEMED and 2.45 mM NBT. The APX activity appeared as an achromatic band on a purple-blue background. All the native gels were documented by Multi Imager Gel documentation system (Bio-Rad, Hercules, CA, USA) and analyzed and quantified with Quantity One Software (Bio-Rad).

Response to stress

Response to stress (RS) was also calculated with the reference set being taken in two ways: (i) aUV-B and (ii) eUV-B.

Statistical analyses

Results were subjected to one-way ANOVA test for assessing the significance of changes in different studied parameters due to different UV-B irradiances. Duncan's multiple range tests were performed as post hoc on parameters subjected to the ANOVA test. All the statistical tests were performed using SPSS software (SPSS Inc., version 16.0).

Results and discussion

Over the last several years, UV-B has been convicted as a prime stress factor for terrestrial livelihood. Several researchers had reported its deleterious effect on plants (Agrawal et al. 2009). As per the present experimental design, kidney bean plants received a gradient of UV-B exposure doses which also resulted in a proportionate damage at different levels of the plants' responses starting from phenotypic to genetic. The visible phenotypic damage on the young as well as mature leaves of the test plants was observed as cupping, curling, bronzing, marginal, and interveinal chlorosis followed by necrosis under both aUV-B and sUV-B treatments. Foliar injury percent (FIP) was 32.6 and 71.2 % in aUV-B- and sUV-B-treated plants (Fig. 1). Upward cupping of leaves
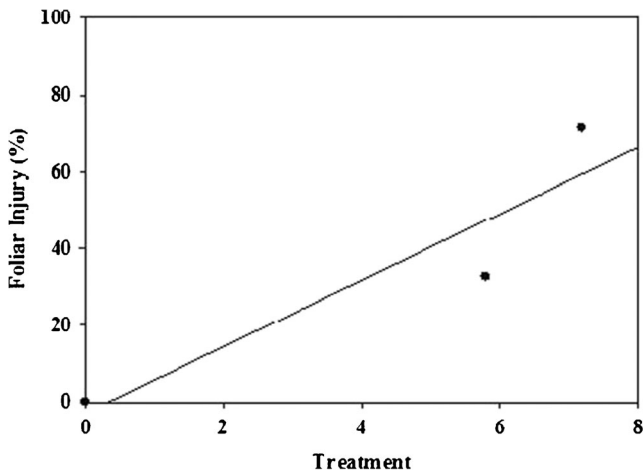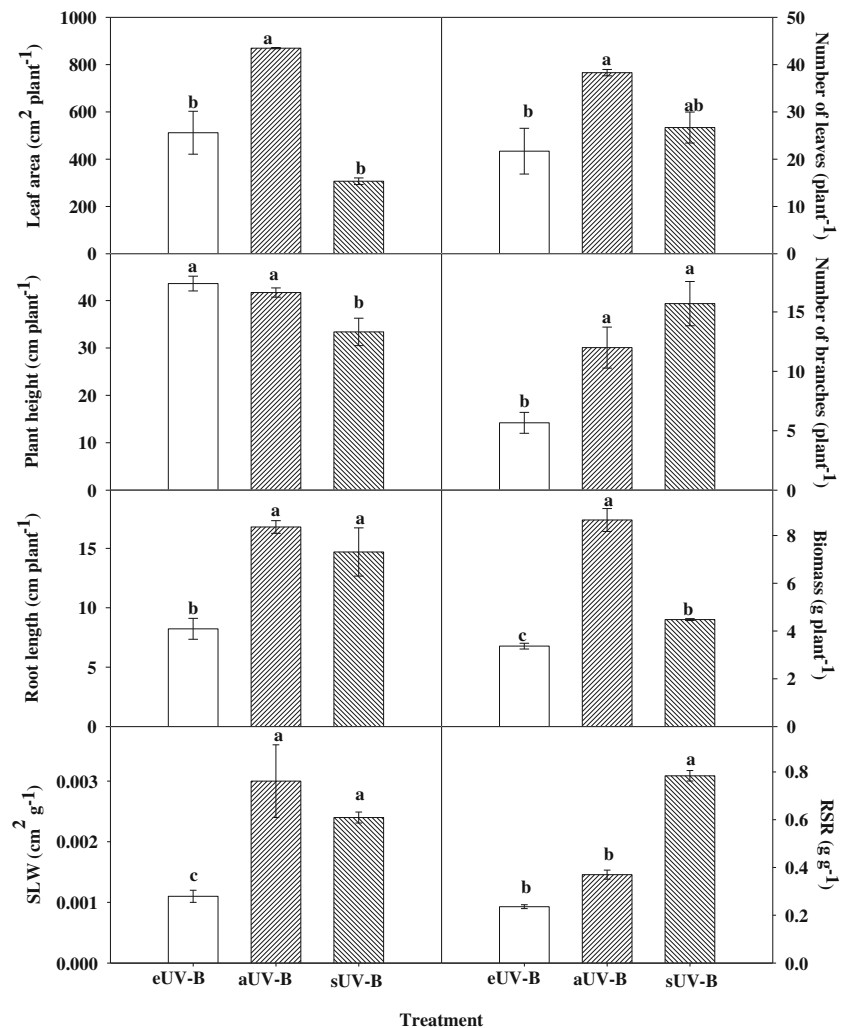
Fig. 1 Effect of ambient and sUV-B on foliar injury of kidney beans

was very pronounced under sUV-B, suggesting inhibition of cell division or expansion of the upper foliar surface under higher UV-B exposure.

Different levels of UV-B resulted in a range of photomorphogenic responses. Plants grown under eUV-B experienced a decrease in leaf area (41.1 %), number of leaves (43.3 %), number of branches (52.8 %), root length (51.0 %), and biomass accumulation (156.7 %), though plant height (4.4 %) increased compared to aUV-B (Fig. 2). However, plants grown under sUV-B displayed significant decreases in leaf area (64.7 %), number of leaves (30.3 %), root length (12.5 %), plant height (23.4 %), and total biomass accumulation (48.2 %), but an increase in the number of branches (39.8 %), as compared to aUV-B (Fig. 2). UV-B creates an imbalance in endogenous plant growth regulators by inhibiting the synthesis or enhancing the destruction of auxins and others (Lingakumar and Kulandaivelu 1993), which might directly cause the inhibition of plant height. The response of the test plant under aUV-B suggests acclimatization to aUV-B through several defense responses including various morphological changes such as increase in branches as well as other biochemical regulatory adjustments. High doses of UV-B are damaging to plants, but low doses of UV-B could act as

Fig. 2 Effects of ambient UV-B (*aUV-B*), excluded UV-B (*eUV-B*), and supplemental UV-B (*sUV-B*) on leaf area, number of leaves, plant height, number of branches, root length and biomass, SLW, and RSR of kidney beans. Values represent mean ± SE. *Bars* showing *different letters* indicate significant differences according to Duncan's test at $p \leq 0.05$

Table 2 Effects of ambient UV-B (aUV-B), excluded UV-B (eUV-B), and supplemental UV-B (sUV-B) on Fo, Fm, Fv, and Fv/Fm of kidney beans

|	Fo	Fm	Fv	Fv/Fm
Excluded UV-B	274.0±16.8 a	1,462.8±28.5 a	1,188.75±19.8 a	0.812±0.009 a
Ambient UV-B	281.3±10.7 a	995.0±17.7 b	713.8±13.1 b	0.717±0.008 b
Supplemental UV-B	323.3±24.3 a	924.7±68.3 b	601.5±83.5 b	0.642±0.046 b

Values represent mean ± SE with different letters indicating significant differences according to Duncan's test at $p \leq 0.05$

a signal factor to regulate the expression of other genes in plants. Low ambient UV-B fluence rates could stimulate protective processes that affect the plant's resistance to UV-B and thus promote growth and metabolism (Frohnmeyer and Staiger 2003).

Root length defines the extent to which a plant explores soil for water and mineral nutrients. In the present study, root length increased in aUV-B but further decreased in sUV-B, as compared to eUV-B (Fig. 2). Leasure et al. (2009) reported increased root length under UV-B in *Arabidopsis thaliana*. The growth of root and leaves were well coordinated, and their relative sizes varied with the three UV-B levels. Plants having

the most expanded leaves under aUV-B maintained the longest roots to optimize the uptake of resources. UV-B significantly induced the biomass accumulation in test plants (Fig. 2). Searles et al. (1995) observed the absence of inhibitory effects of solar ambient UV-B on total biomass production; however, several other workers reported contrasting trends (Day and Neale 2002; Zavala and Ravetta 2002). Biomass accumulation during exposure to UV-B is likely to increase the sensitivity against UV-B (Smith et al. 2000). It has been suggested that plant growth strategies are a trade-off between productivity and tolerance to stress.

Fig. 3 Effects of ambient UV-B (*aUV-B*), excluded UV-B (*eUV-B*), and supplemental UV-B (*sUV-B*) on MDA, flavonoid, total chlorophyll, carotenoid, PAL, protein, phenol, and ascorbic acid contents of kidney beans. Values represent mean ± SE. *Bars* showing *different letters* indicate significant differences according to Duncan's test at $p \leq 0.05$
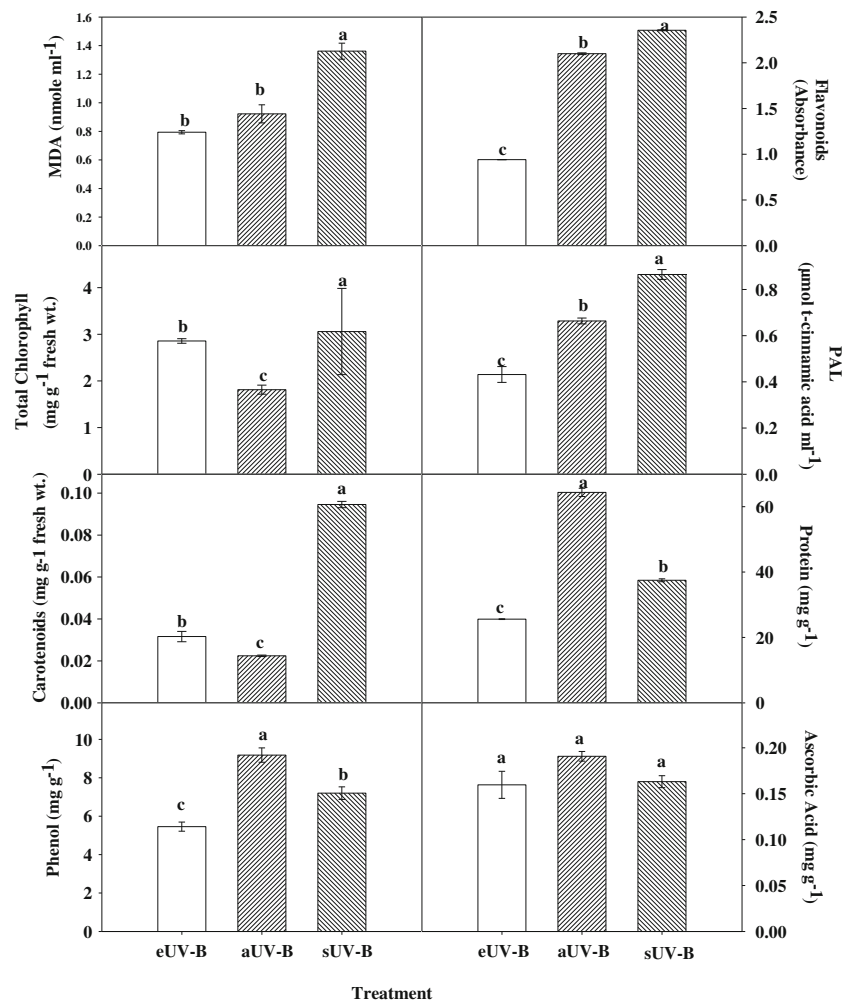
SLW was reduced by 68.2 and 31.2 % in eUV-B and sUV-B, respectively, compared to aUV-B (Fig. 2). Antonelli et al. (1998) reported that leaves generally increase their thickness to avoid penetration of UV-B to the underlying photosynthetic machinery. Changes in biomass under different UV-B levels were accompanied by substantial alteration in the partitioning of biomass to above- and belowground parts. The observed RSR of test plants was reduced by 36.4 % under eUV-B and increased by 111.6 % under sUV-B, as compared to aUV-B (Fig. 2). Reduced RSR in eUV-B suggests favored shoot growth under a comparative stress-free condition. Kriedemann and Sands (2010) depicted that under a stress-free condition, carbon assimilation is maintained by modest investment in shoots than in roots. In general, legumes maintained higher root biomass for the growth and development process of nodules and symbiotic nitrogen fixation (Voisin et al. 2003), which probably led to higher RSR under UV-B treatment.

Chlorophyll fluorescence is an effective indicator of plant response to stress. A decrease in Fv/Fm ratio under sUV-B and aUV-B, compared to eUV-B, reflects photoinhibition (Table 2). Light-dependent inactivation of the photosystem II (PSII) reaction center is associated with a decline in Fm and Fv/Fm and with an increase in initial fluorescence as is observed in sUV-B-exposed plants, compared to eUV-B (Table 2) (Long and Humphries 1994). A diminished electron flow from the water-splitting complex could cause impairment on the donor side, probably lowering Fm in sUV-B-exposed plants (Demmig-Adams et al. 1989). Fv, which characterizes the photosynthetic competence of a leaf or chloroplast, reduced under ambient and sUV-B levels to a similar extent. The impact of UV-B radiation on PSII activity is commonly measured as a decrease in oxygen evolution or variable fluorescence (Vass et al. 1996). The decrease in Fv/Fm ratio was reported to be accompanied by an enhanced production of superoxide radicals (Jin and Tao 2000) which corresponded directly with higher MDA content under UV-B stress during the present study. UV-B caused more membrane damage measured in terms of MDA content with a higher magnitude of damage under sUV-B than aUV-B, compared to sUV-B (Fig. 3).

UV-B also induced flavonoids with the concomitant increase in PAL activity (30.3 %) (Fig. 3). The UV-B screening ability of flavonoids has been reported to be associated with increased tolerance of plants to UV-B, by reducing the transmittance of UV-B photons through the leaf tissue (Jansen et al. 1996). PAL is a key enzyme of the phenylpropanoid pathway and is generally induced by UV-B (Pontin et al. 2010). It was observed that the amount of extractable flavonoids in sUV-B-exposed plants did not correspond with the UV-B screening of the leaf epidermis as is evident by maximum reduction in Fv/Fm, suggesting that full UV-B screening may not be achieved with the acquired flavonoid level during the present study, but this may serve as the measure of the capacity of UV-B protection.

Interestingly, both the photosynthetic pigments, chlorophyll and carotenoid significantly increased by 57.8 and 40.9 % under eUV-B and by 167.7 and 321.5 % under sUV-B, respectively, compared to aUV-B (Fig. 3). In general, photosynthetic pigments are major stress markers in plants, but an increase in both the pigments under sUV-B might be an adaptive feature as accumulation of chlorophyll can also play a

Fig. 4 a RAPD profile of genomic DNA isolated from leaves of ambient UV-B excluded (C), ambient UV-B (A), and supplemental UV-B (S)-exposed kidney beans. b Genome template stability (GTS) of ambient UV-B (aUV-B), excluded UV-B (eUV-B), and supplemental UV-B (sUV-B). c Cluster analysis
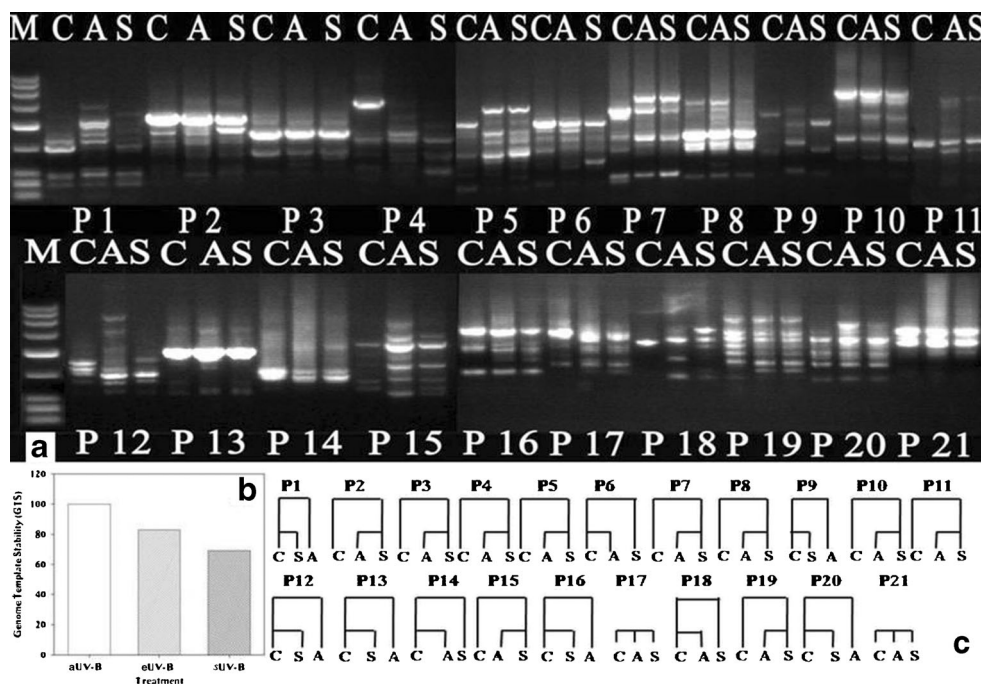
role in the antioxidant defense in addition to the normal process of photosynthesis (Roshchina and Roshchina 2003).

Plants grown under sUV-B and eUV-B radiations showed reductions in protein (41.8 and 60.2 %) and phenol (21.6 and 40.6 %) contents as compared to aUV-B (Fig. 3). Previous reports have already stated that UV-B causes severe oxidative stress which generally resulted in accumulation of enzymatic and non-enzymatic antioxidants in plants, but up to a certain limit; higher than that might cause destruction of such defense molecules (Blokhina et al. 2003; Singh et al. 2010).

In the present study, 21 RAPD primers were used to generate a total of 278 amplified DNA products from isolated genomic DNAs, of which 84 in eUV-B, 104 in aUV-B, and 90 in sUV-B (Fig. 4). In a total of 278 amplified DNA bands, only 99 were polymorphic (41.5 %). Plants grown under aUV-B yielded a large number of new amplified DNA fragments (Tripathi et al. 2011) when compared to sUV-B exposure (Fig. 4). Similarly, the total number of disappeared amplified DNA fragments was 14 under aUV-B and 13 under sUV-B as compared to eUV-B. From the obtained RAPD profile of isolated genomic DNA of kidney bean from all the experimental sets, it can be clearly depicted that UV-B can directly/indirectly damage the structure of genomic DNA, hence affects the genetic stability too. In the present study, the genome template stability (GTS) was reduced to 83.0 and 69.2 % under aUV-B and sUV-B, respectively, as compared to eUV-B (Fig. 4). Tripathi et al. (2011) also reported significant damage in genetic stability in linseed plants exposed to sUV-B. The sizes of the amplified DNA bands and the total number of the bands produced showed variation among the three doses of UV-B. Some amplified DNA fragments appeared exclusively in the plants grown under aUV-B and sUV-B, which indicate development of a specific marker depending on the magnitude of the UV-B stress. Among the 21 primers, P12, P15, P5, and P8 showed the highest degree of polymorphism in terms of unique bands. Clustering revealed that primers 1, 9, 12, 16, and 20 can be used as markers for ambient UV-B and primers 14 and 18 as markers for sUV-B (Fig. 4).

Both the manifestation and magnitude of major antioxidative enzymes increased significantly under UV-B exposure. In SOD activity, staining resulted in four bands in eUV-B, which increased up to five under aUV-B and sUV-B and with higher intensity (Fig. 5). Spectrophotometric analysis of SOD also supported the in-gel assay by showing a significant increment at aUV-B and sUV-B, compared to aUV-B (Fig. 5). In the entire aerobic system, SOD acts as the first line of action against oxidative stress. It catalyzes the dismutation of O_2^- into O_2 and H_2O_2. To cope with the elevated H_2O_2 level, plants need a higher amount and activity of the H_2O_2-scavenging system. Singh et al. (2010) found increased amounts in the transcript levels of CAT and POX in radish plants, under supplemental UV-B exposure. In the present study, only one

isoform of APX and CAT was observed in the in-gel assay, but the magnitude of amount and activity of both the enzymes were significantly varied in proportion to the UV-B doses (Fig. 5). The spectrophotometric analysis also showed a significant increase in CAT (48.5 and 100 %) and APX (8.1 and 147.7 %) activities under aUV-B and sUV-B, respectively (Fig. 5). The other H_2O_2 scavengers, POX and GPX, also showed similar increments in both amount and activity as observed in CAT and APX (Fig. 5). In GR, the in-gel assay revealed a total of five isoforms under aUV-B and sUV-B but four in eUV-B (Fig. 5). The percent decrease in GR activity was 28.2 % at eUV-B, and the increase was 30.2 % at the sUV-B level compared to aUV-B (Fig. 5). GR and APX act in conjunction through the ascorbate-glutathione cycle for the utilization and regeneration of ascorbic acid; their increased amount and activity might suggest a better UV-B management in kidney bean plants.
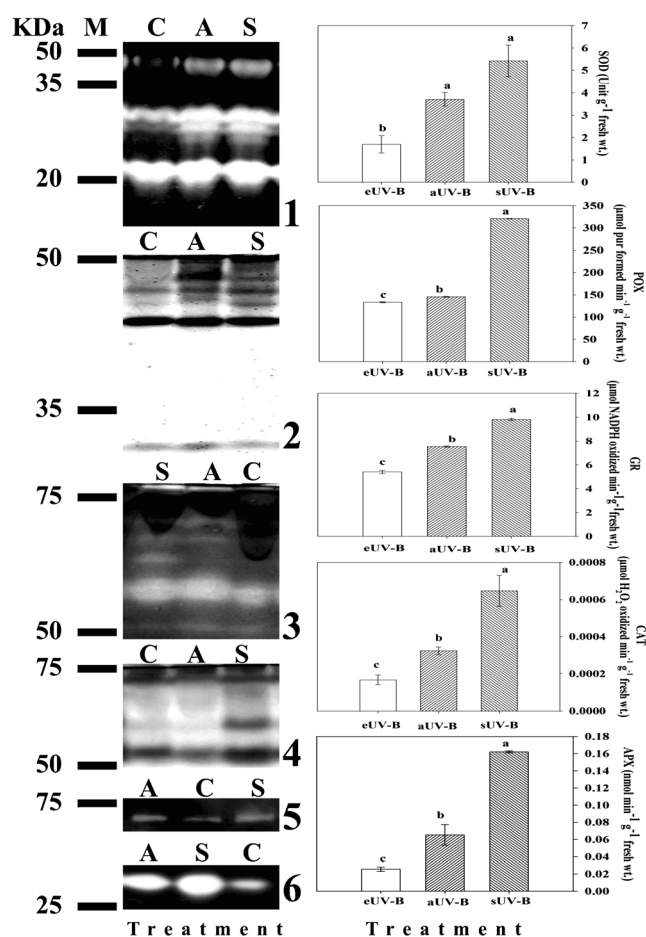

Fig. 5 Native gels for the activities of (*1*) SOD, (*2*) POX, (*3*) GR, (*4*) GPX, (*5*) CAT, and (*6*) APX and spectrophotometric analysis of SOD, POX, GR, CAT, and APX of kidney beans exposed to supplemental UV-B (*sUV-B*), ambient UV-B (*aUV-B*), and excluded UV-B (*eUV-B*). Equal amounts of protein (200 μg) from plants exposed to supplemental, ambient, and excluded UV-B. *A* aUV-B, *S* sUV-B, *C* eUV-B pur purpurogallin. Bars showing different letters indicate significant differences according to Duncan's test at *p*<0.05
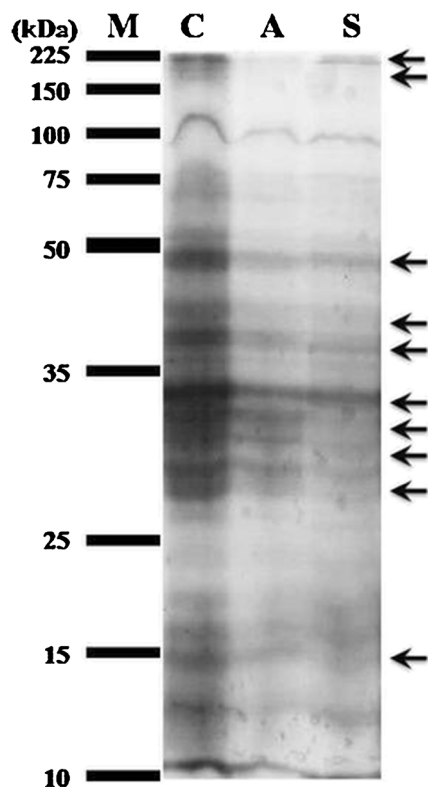
Fig. 6 Comparative SDS-PAGE profile of isolated protein from ambient excluded, ambient, and supplemental UV-B-treated leaves of kidney beans at 60 DAG (*A* protein from ambient, *S* protein from supplemental UV-B-treated leaves, *C* protein from ambient excluded). Arrows indicate the point of the difference in the protein profile

To obtain an overall idea on the effect of UV-B exposure on the leaf proteome of kidney bean plants, we also examined the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles of the total soluble protein extracted from aUV-B and sUV-B, over eUV-B. The CBB-stained protein pattern (Fig. 6) showed differences among the treatments (lane A for aUV-B and lane S for sUV-B) over the control from eUV-B (lane C). A major visible change was the reduced amounts of the large subunits (LSU; 49 kDa) and small subunits (SSU; 15 kDa) of RuBisCo under UV-B treatments in the test plant. Interestingly, the magnitude of reduction in the amount of LSU and SSU of RuBisCo was proportional to the amount of UV-B doses (as the highest reduction was observed at sUV-B). Some other proteins,

Table 3 Effects of ambient UV-B (aUV-B), excluded UV-B (eUV-B), and supplemental UV-B (sUV-B) on pod number and pod weight of kidney beans

	Pod number (plant^{-1})	Pod weight (g plant^{-1})
Excluded UV-B	9.33±2.03 a	29.3±3.59 a
Ambient UV-B	7.67±0.88 ab	14.73±1.87 b
Supplemental UV-B	4.00±1.00 b	11.6±2.04 b

Values represent mean ± SE with different letters indicating significant differences according to Duncan's test at $p \leq 0.05$

present in between 50 and 150 kDa, also degraded under high UV-B doses in lanes A and S, as compared to lane C, respectively. Although both the UV-B doses caused prominent damage on the kidney bean leaf proteome, the magnitude of damage varied between them.

The number of pods as well as pod weight per plant increased in eUV-B (21.6 and 98.9 %) and decreased in sUV-B (47.8 and 21.2 %) plants, respectively, when compared to aUV-B (Table 3). In the present experiment, a yield reduction occurred in aUV-B and sUV-B instead of activated photoprotection responses in these plants through reduced epidermal screening and increased activities of the antioxidant defense system.

The whole discussion mainly centered on considering aUV-B as a reference, but the manifestation of the present result could also be explained if we take exclusion as a reference. So, responses to stress (RS) were calculated for both the conditions, considering ambient as a reference in the first (Fig. 7) and exclusion as a reference in the second (Fig. 7). eUV-B plants maintained the membrane integrity, chlorophyll and carotenoid contents, and photosynthetic machinery which enabled them to have a higher yield. aUV-B plants capitalize the resources by
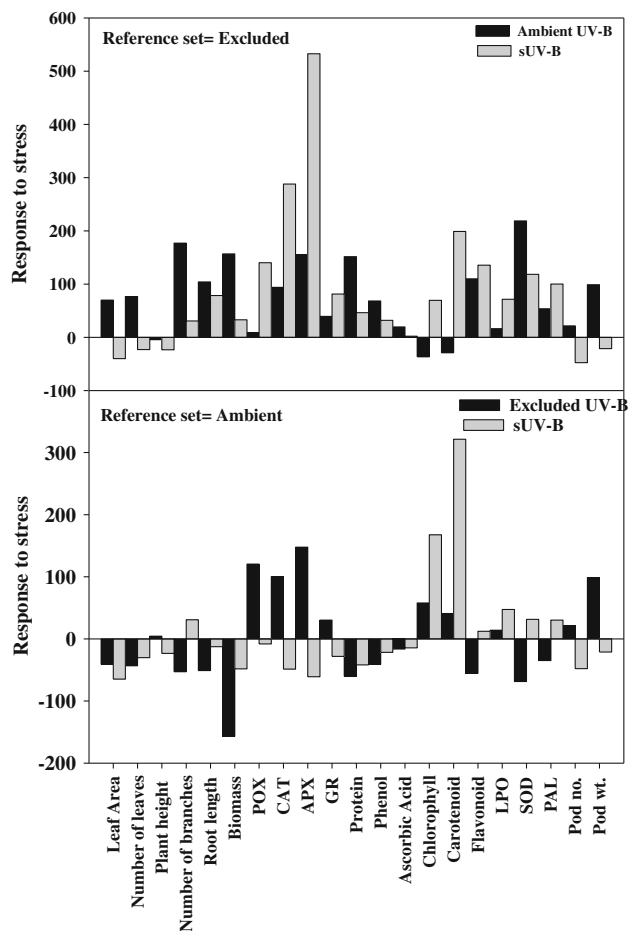

Fig. 7 Response to stress of kidney beans from excluded UV-B (*eUV-B*) and supplemental UV-B (*sUV-B*) treatments where ambient was taken as a reference and where excluded was taken as a reference

maintaining better morphogenic response with a moderate investment in antioxidants and flavonoids, while sUV-B plants solely focused on the need to activate the defense response and thus had the lowest yield.

Conclusions

Evidences obtained in this field study indicate that the present level of solar UV-B represents an environment stress for plants, which caused significant reduction in the yield. The reduction in yield under ambient and supplemental UV-B is correlated with the direct damage to the photosynthetic machinery and DNA or as a consequence of the diversion of most of the plant resources to repair the damage and afford the protection. Genome template stability reduced under ambient as well as supplemental UV-B. Upon excluding the ambient UV-B, the plants maintained higher photochemical efficiency. Under sUV-B and aUV-B, either more intense bands and/or extra isoforms of various studied antioxidant enzymes were observed, while the metabolic enzymes like LSU and SSU of RuBisCo showed a decline. Acclimation responses like accumulation of UV-B screening compounds and increased antioxidant activity at a higher UV-B level imply an energy cost with consequent redistribution of resources for production of protective compounds or structures.

Conflict of interest The authors declare that they have no conflict of interest.

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