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ISSN: 2637-7721

Journal of Plant Biology and Crop Research

Open Access | Research Article

Impact of Exclusion of Solar UV on Growth, Performance Index of Photosystem II and Leghemoglobin Content of Soybean Var. JS 335

***Juhie Joshi-Paneri*[1]*; *Sonika Sharma*[2]; *KN Guruprasad*[3]**

[1]*Plant Science Department, McGill University, Montreal, Canada*

[2]*Defence Research Laboratory (DRDO), Tezpur, Assam, India*

[3]*Shri Vaishnav Institute of Science, Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore, Madhya Pradesh, India*

***Corresponding Author(s): Juhie Joshi-Paneri**

Plant Science Department, McGill University, Montreal, Canada

Tel: +1-5192005662;

Email: juhiejoshi@yahoo.com

Received: May 25, 2020

Accepted: Jun 24, 2020

Published Online: Jun 26, 2020

Journal: Journal of Plant Biology and Crop Research

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Biomass; Glycine max; Leghemoglobin content; Nodules; PI (abs); Peroxidase activity.

Abstract

Introduction: A field study was conducted to determine impact of UV (280-400 nm) radiation on a popular Indian soybean variety JS 335. Specifically, the influence of ambient UV radiation on crop growth, PS II efficiency, performance index and leghemoglobin along with peroxidase activity was investigated by the exclusion of UV radiation from solar spectrum.

Method: The plants were grown in specially designed UV exclusion chambers, wrapped with filters that excluded both UV-A/B (<400 nm), UV-B (<315 nm), transmitted all the UV or lacked filters. Growth, biochemical and physiological analysis were carried out on 40th Day After Emergence (DAE) of soybean seedlings.

Results: Exclusion of UV significantly enhanced growth parameters of above-ground (plant height, fresh weight, dry weight, leaf area) as well as underground parts (root length, root fresh weight and dry weight) of soybean as compared to control plants. Performance index in leaves and Leghemoglobin (Lb) content in root nodules also showed a remarkable increase after UV exclusion. Reduced peroxidase activities in leaves and nodules of soybean show alleviation of oxidative stress after UV exclusion. The results are discussed in the light of advantage of UV exclusion for enhancing carbon as well as nitrogen fixation and hence yield of soybean.

Cite this article: Joshi-Paneri J, Sharma S, Guruprasad KN. Impact of Exclusion of Solar UV on Growth, Performance Index of Photosystem II and Leghemoglobin Content of Soybean Var. JS 335. J Plant Biol Crop Res. 2020; 3(1): 1023.

Introduction

Light is a well-known damaging factor of the photosynthetic apparatus both in the visible and in the ultraviolet spectral range. Nitrogen fixation and photosynthesis process posses their obvious importance by which atmospheric nitrogen comes in the plant system and light energy from the sun is trapped and converted to chemical energy in the form of ATP, NADPH and organic matter. Depletion of ozone in stratosphere causes harmful effects on human health and crop growth. Ozone layer in stratosphere protects us from harmful UV radiation of sun. The depletion of ozone by human activities has become the subject of interest from last few decades, since reduction in ozone increases UV radiation on earth.

While harvesting light, photosynthetic organisms are unavoidably exposed to the UV region of solar spectrum. Crop plants grown under tropical conditions receive approximately 50% higher dose of UV-B in the natural solar radiation compared to temperate regions due to small solar zenith angle and thin stratospheric ozone layer. The solar spectrum received at the earth's surface has a small component of UV-B (280-315 nm) and a large component of UV-A (315-400 nm) (22% and 72% of original solar radiation, respectively). These UV radiations damage lipids, nucleic acid and proteins in the leaves of higher plants and specifically target the photo system II (PS II) reaction center, Rubisco, chloroplast, ATPase and violaxanthin deepoxidase [1-3]. The enhanced exposure to UV-B is potentially detrimental to all living organisms but is especially harmful for plants due to their obligatory requirements of sunlight for survival, and its interaction with UV absorbing biological molecules such as nucleic acid, proteins, lipids and phytohormones [4]. The effects of UV-B radiation on terrestrial plants have been studied in considerable details over the last two decades, but the underlying mechanism governing these integrated responses are not fully known.

Increase in the UV-B radiation is known to reduce biomass, leaf area, yield and photosynthesis [5-10]. Compilation of the data of the last two decades suggest that nearly 50% of the crop plants are affected by elevated level of solar UV-B making it one of the most important abiotic stress factors to influence most of the plant growth parameters [11-13]. UV exposure causes loss in Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity, and a down regulation of mRNA for Rubisco transcripts [14], increased expression of stress response and ribosomal protein genes, whereas photosynthesis associated genes were down- regulated [15]. UV-B radiation damage photosynthetic apparatus, leading to decreased oxygen evolution and CO_2 fixation [16]. Chlorophyll a fluorescence transient indicates the UV-B damage on PS II by reduced electron flow from reaction center to plastoquinone [17]. also support the view that damage to the PS II quantum yield is robustly and linearly related to carbon fixation. Using chl *a* fluorescence induction curve it is possible to resolve that UV-B treatment inhibits the energy transfer within PS II reaction centre [18,19]. Vass [20] showed that UV-A irradiance causes loss in maximal fluorescence yield and rate of fluorescence rise from F_0 via level J and I to the peak P. Kautsky curve is highly affected by ambient UV-B light [21] followed by strong reduction in the variable to maximal fluorescence values (Fv/Fm) of dark adapted leaves [22] leading to decreased potential efficiency of PS II.

On the other hand, exclusion studies have indicated enhanced growth of plants like radish [23], *Cymopsis* and *Vigna* [24], cucumber [25], cucumber and cotton [26] and soybean [11,27]. Exclusion of UV radiation from the natural solar spectrum resulted in an elevated overall activity of Rubisco, related to an increase in its cellular concentration [28], CO_2 uptake and photosystem I efficiency [29] and increase in the root biomass [30]. These findings indicate alleviation of stress after UV exclusion.

Most of the deleterious effects of UV-B radiation are related to the formation of free radicals, resulting in oxidative stress. Reactive oxygen species (ROS) are routinely generated in low levels in non-stressed plant cells in chloroplast, mitochondria and also by membrane bound exocellular enzymes involved in redox reactions, its concentration increases under chemical and environmental stress conditions. Peroxidase have been used as marker of stress, as they are involved in defense against stress [31], they act to scavenge the deleterious species O_2^-, H_2O_2 and their derivatives, thus limiting: (a) the deterioration of cellular membranes [32] and (b) the functional inactivation of Lb^{2+}(reduced Lb)/ $Lb^{2+}O_2$ oxyleghemoglobin) [33,34]. Peroxidase activity increases substantially in Arabidopsis following UV exposure [35]. Increased peroxidase activity in response to supplemental UV-B radiation is observed in detached Hibiscus leaves [36] and *Beta vulgaris* [37]. Exposure of plant tissue to UV-B results in enhancement of production of oxyradicals and activates the plant antioxidant defence system against oxyradicals [38,39]. In the present study, peroxidase activity is assessed in both leaf as well as nodules. Legumes and other N_2-fixing plants face oxidative risks especially leghemoglobin protein beyond those proteins associated with photosynthesis. As is the case with leaves, nodules are rich in strongly reducing compounds, polyunsaturated fatty acids and O_2-labile proteins (most notably, nitrogenase itself), which can readily react with O_2 and generate ROS. Nodules have high rates of respiration due to the extensive energy demands of N_2 fixation, which results in a high flux of O_2 into the nodule and, hence, an elevated risk of ROS formation [40].

The present study has been undertaken with the model plant soybean as soybean is widely known to be cheap, easily available and a good source of rich and cheap protein compared with expensive animal protein and other nutrients. India is 5th largest producer of soybean in the world after USA, Brazil, Argentina and China. In India, it is the 3rd largest oilseed crop next to groundnut and mustard. About 98% of land under cultivation is predominantly in 3 states in central India, M.P., Maharashtra, and Rajasthan, with Indore city, the epicenter of soybean renaissance, situated at 22^{0}4' N and 75^{0}50'E.

We paid particular attention in designing this experiment, to make photosynthetically active radiation (PAR) and other microclimatic factors same through out the filter excluded chambers and control chambers. In this report we are giving possible reason for the enhancement in biomass and nitrogen fixation of soybean plant grown under UV exclusion.

Material and methods

Plant material and treatments

Seeds of soybean (*Glycine max* L. merill) var. JS 335 were collected from Directorate of Soybean Research, Indore, India. Field experiments under natural sunlight were conducted in the botanical garden of School of Life Sciences, Indore, India at 22°43' north latitude and 75°49'60" east longitude. Seeds of uniform size were selected and treated with recommended fungicide viz. Bavistin and Diathane M @ 2g/kg seeds, and then inoculated with powdered *Rhizobium japonicum* (National Fertilizer

limited, New Delhi, India) before sowing. A basal dose of N:P:K @ 20:60:20 kg/ha was applied at the time of sowing. The seeds were sown in plastic bags (34 cm H x 34 cm B; filled with mixture of sand, soil and manure- 1:4:1) and kept inside iron cages (4ft.L.x 3ft.W.x 3ft.H.) with UV cut-off filters (Garware polyester ltd., Mumbai, India) that selectively cut-off UV-B (280-315 nm) and UV-A+B (280-400 nm) radiation. The plots were watered as needed and weeds were controlled manually. Two types of control were taken under study, for FC (Filter Control) plants the cages were covered with polythene film which transmits all the ambient solar radiation, OC (Open Control) plants were directly exposed to natural solar radiation (Figure 1). Metal cages received full solar radiation during the day without shading. There was no significant temperature difference between control and UV-excluded chambers as horizontal holes in chambers allowed passive air ventilation. Absolute solar irradiance, with and without UV-B or UV-A+B, was measured with a radiometer (PMA2100, Solar Light, Glenside, PA). The average ambient solar irradiance at midday was 1455 µmol m^{-2} s^{-1}, the loss in light intensity at midday was 11.34% (1290 µmol m^{-2} s^{-1}) under −UV-A+B filters and −UV-B filters was 12.37% (1275 µmol m^{-2} s^{-1}) and 4.32% (1392 µmol m^{-2} s^{-1}) under the polyethylene filter transmissible to UV (filter control). The PAR intensity for normal plant growth was optimal saturating light. Emission characters of these filters were measured by Shimadzu spectrophotometer (model UV-1601, Shimadzu, Duisburg, Germany) (Figure 2).

Sampling and statistical analysis

Samplings were done on 40th DAE before 8:00 AM for obtaining all the data. The data were replicated in triplicate (n = 3); five plants were taken from each replica for each parameter. Data were subjected to analysis of variance using Prism 4 software for windows (Graf Pad Software, Inc., LaJolla, CA) and means separated with SEM.

Growth parameters of above-ground parts

Growth parameters of above ground parts of the plants were measured on 40th DAE for all the treatments. Plant height was measured from the ground level to the raised leaf-tip with meter rule. Plant fresh weight and dry weight were determined on a top-loading balance. Plants were dried at 60 ^{0}C for 72 hours for dry weight. Leaf area was taken by pressing the blotted dry leaf on a cm graph paper, tracing the exact outline and weighing the cut paper outlines. The calibration curve was prepared by weighing 0-100 cm^2 area graph paper.

Growth parameters of below ground parts

Root length was measured in all the treatments on 40th DAE. Plant roots with nodules were taken out carefully, washed and measured against a centimeter scale. The roots were dried on filter paper and weighed for the fresh weight. For dry weight, roots were dried at 60 ^{0}C for 72 hours. The number of root nodules and the nodule fresh weight were recorded per plant. Nodules on each root were counted, removed carefully and washed. They were dried on filter paper and weighed for the fresh weight in gm/plant.

Chlorophyll a fluorescence

Chl *a* fluorescence transient exhibited by dark-adapted (30 min) leaves was measured by a *Handy PEA* fluorimeter (*Hansatech Instruments*, Pentney, King's Lynn, UK) in fully opened third trifoliate leaves of soybean plants from each treatment. The transients were induced by red light (peak at 650 nm) of 600 W m^{-2} (3,200 µE m^{-2} s^{-1}) provided by an array of six light emitting diodes, focused on the leaf surface in the clips on a spot of 4 mm diameter to provide homogenous illumination over the exposed area of the sample. Data were recorded for 1 s with 12-bit resolution; the data acquisition was every 10 µs for the first 2 ms and every 1 ms thereafter. All the measurements were recorded at 25±1 ^{0}C. The Chl *a* fluorescent transient, when plotted on a logarithmic scale clearly showed a polyphasic fluorescence rise kinetics (O–J–I–P phase). The fluorescence intensity at 20 µs was considered as the intensity F_o (O phase) when all reaction centers are open, the fluorescence intensity at 2 ms was J phase, 30 ms was I phase, and the maximum fluorescence (F_m) was the P phase. (F_p equals here to F_m since the excitation intensity is high enough to ensure the closure of all reaction centers of PS II). We calculated the quantum efficiencies, such as maximum quantum yield of PS II photochemistry (F_v/F_m), which is equal to the efficiency by which absorbed photon will be trapped by the PSII reaction center with the resultant reduction of Q_A to Q_A- (TR_o/ABS), phenomenological fluxes, such as electron transport per leaf CS (ET_o/CS_m), and the performance index based on absorption of light energy (PI_{abs}). All these parameters were measured using the software *Biolyzer HP 3* (Chl fluorescence analyzing program by *Bioenergetics Laboratory,* University of Geneva, Switzerland).

Extraction and estimation of Leghemoglobin (Lb) content

Leghemoglobin (Lb) was extracted from the root nodules of 40-day old soybean plants and measured by the method of Jun et al [41]. Root nodules (1.25 g) from the 40-day old plants grown under ambient UV radiation, or under exclusion of UV-B and UV-A+B were crushed in liquid nitrogen in a mortar with pestle. The resulting powder was resuspended in 25 mL of 50 mM sodium phosphate buffer (pH 7.5) containing 1 mM^{-1} EDTA, 1 mM^{-1} PMSF, betamercaptoethanol, and 10% polyvinyl pyrrolidone (PVPP). The resulting solution was filtered through cheese cloth and centrifuged at 20,000 g for 20 min. at 4 ^{0}C. The deep red supernatant was saturated to 50% with solid $(NH_4)_2$ SO_4 and then centrifuged at 15,000 g for 20 min at 4 ^{0}C. The pellet was discarded, and the red supernatant was saturated to 90% with solid $(NH_4)_2SO_4$ and then centrifuged at 15,000 g for 20 min at 4 ^{0}C. The red pellet was resuspended in 15 mL of 20 mM Tris HCl (pH 8.0) containing 1 mM $(NH_4)_2$ SO_4. The Lb-containing fractions (50 to 90% pellet) were detected at 410 nm by using UV-Visible Shimadzu Spectrophotometer (model-UV1601).

Extraction and assay of peroxidase

Extraction: 30 mg tissue was crushed in a pre-chilled mortar and pestle using chilled 80% acetone at 4^{0}C. The extract was centrifuged at 10000 x g for 10 min. The supernatant was discarded and the pellet redissolved in 10 ml of 0.02 M phosphate buffer (pH 6.4) and centrifuged for 15 min at 20000 x g. The buffered supernatant was used for the peroxidase assay.

Assay: peroxidase was assayed by the method of Maehly [42]. The reaction mixture contained 0.5 ml enzyme extract, 1 ml 20 mM guaiacol and 3 ml 0.02 M phosphate buffer. The reaction was started by addition of 0.03 ml of H_2O_2 (88.2 mM). The initial and final absorbance was noted at 470 nm for 2 min. Activity was calculated as change in OD min^{-1} mg^{-1} protein.

Results

Plant Height and Leaf Area

Plant height was significantly enhanced by solar UV exclu-

sion. Maximum enhancement of 57% and 39% in plant height was observed by UV-A+B exclusion and UV-B exclusion, respectively (Figure 3 & Figure 4).

Exclusion of solar UV increased area of leaves significantly. UV-B exclusion enhanced leaf area by 32% whereas exclusion of both UV-A+B enhanced it by 73% as compared to leaves of control plants **(**Figure 5 & Figure 6).

Fresh weight and dry weight of plant

Enhancement of 75% (-UV-B) and 97% (-UV-A+B) was recorded in fresh weight. There was also an enhancement in dry weight at this stage by 63% (-UV-B) and 111% (-UV-A+B) compared to filter control plants (Figure 7 & Figure 8).

Chlorophyll a fluorescence

Kautsky curve for chl *a* fluorescence of third trifoliate leaf showed an increase of 59% after UV-B exclusion whereas PI (abs) showed increase of 68% after UV-A+B exclusion as compared to control plants receiving ambient solar radiation (Figure 9 & Figure 10).

Root length

There was a significant increase in root length after the exclusion of UV-B and this enhancement is increased by the exclusion of UV-A/B. A 44% increase after UV-B exclusion and 94% increase after UV-A+B exclusion was recorded as compared to control plants grown under polythene filter permissible to UV-A+B (Figure 11).

Fresh weight and dry weight of root

An enhancement of 54% (-UV-B) and 80% (-UV-A+B) in root fresh weight was recorded. Root dry weight enhanced by 69% (-UV-B) and 105% (-UV-A+B) as compared to control plants (Figure 12 & Figure 13).

Number of nodules

There was a significant increase in number of nodules after exclusion of UV-B and this enhancement is increased by the exclusion of UV-A+B at all the Days After Emergence (DAE) of seedlings. An increase of 26% after UV-B exclusion and 59% after UV-A+B exclusion was recorded as compared to filter control plants (Figure 14).

Nodule fresh weight

Nodule fresh weight significantly increased in plants grown under UV exclusion filters. 70% increase after UV-B exclusion and 82% increase after UV-A+B exclusion was recorded as compared to control plants (Figure 15).

Leghemoglobin Content

Leghemoglobin was isolated from 1.25 gm of nodules on the 40th days of sampling. Crushed nodules resuspended in buffer were fractionated with $(NH_4)_2SO_4$ between 50 and 90% saturation [40]. Fractions were collected and absorbance was taken at 410 nm.

Exclusion of UV-B and UV-A+B radiation enhanced the leghemoglobin content in root nodules as compared to control plants. After the exclusion of UV-B alone, there was 64% increase in the leghemoglobin content which was further increased by 71% after the exclusion of UV-A+B both compared to control plants (Table1, Figure 16 & Figure 17).

Peroxidase activity

Solar UV exclusion had a drastic effect on peroxidase activity in leaves as well as nodules. Exclusion of UV-B reduced peroxidase activity by 30% whereas exclusion of both UV-A+B reduced peroxidase activity by 40% in leaves. In nodules, UV-B exclusion reduced peroxidase activity by 36% and UV-A+B exclusion reduced it by 45%. Also, it was found that peroxidase activity was much higher in nodules as compared to leaves (Figure 18 & Figure 19).

Figure 1: Experimental setup.

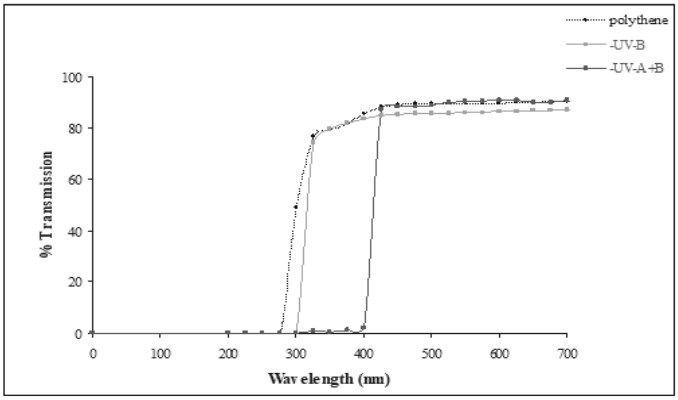

Figure 2: Transmission spectra of the UV-B and UV-B+A cutoff filters used on wire mesh cages for growing soybean plants under field conditions. The spectra between 200-700 nm by using an UV-visible spectrophotometer.

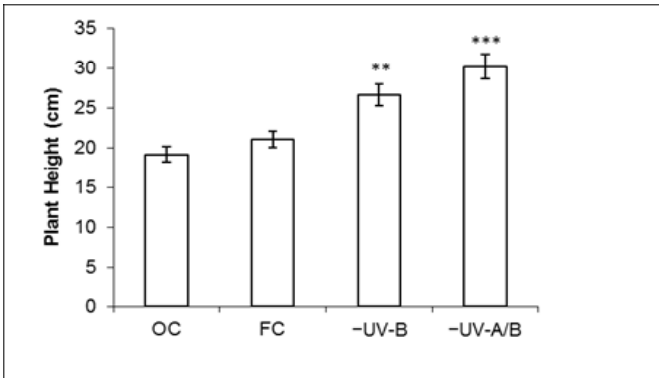

Figure 3: Effect of solar UV-B and UV-A+B exclusion on plant height of soybean var: JS 335. Vertical bars are ±SE. UV-A+B and UV-B excluded plants were significantly different at *** ($P < 0.001$) compared to controls (Newman–Keuls multiple comparison test). OC: Open control; FC: filter control; −UV-B: exclusion of solar UV-B; and −UV-A+B: exclusion of solar UV-A+B.

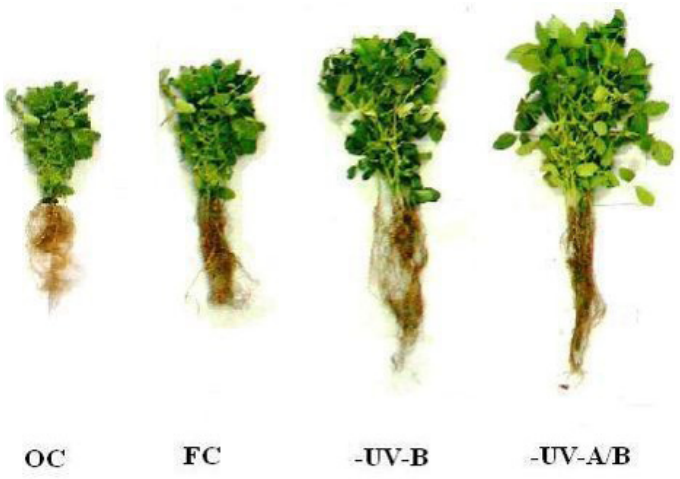

Figure 4: Effect of solar UV-B and UV-A+B exclusion on aerial and underground parts of soybean var: JS 335.

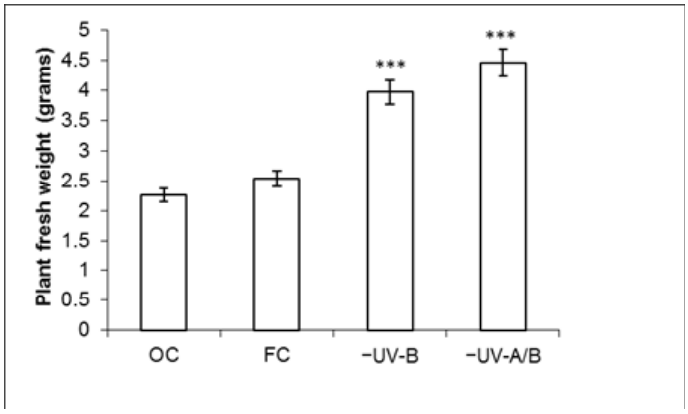

Figure 7: Effect of solar UV-B and UV-A+B exclusion on plant fresh weight of soybean var: JS 335. Vertical bars are ±SE. UV-A+B and UV-B excluded plants were significantly different at *** ($P < 0.001$) compared to controls (Newman–Keuls multiple comparison test). OC: Open control; FC: filter control; −UV-B: exclusion of solar UV-B; and −UV-A+B: exclusion of solar UV-A+B.

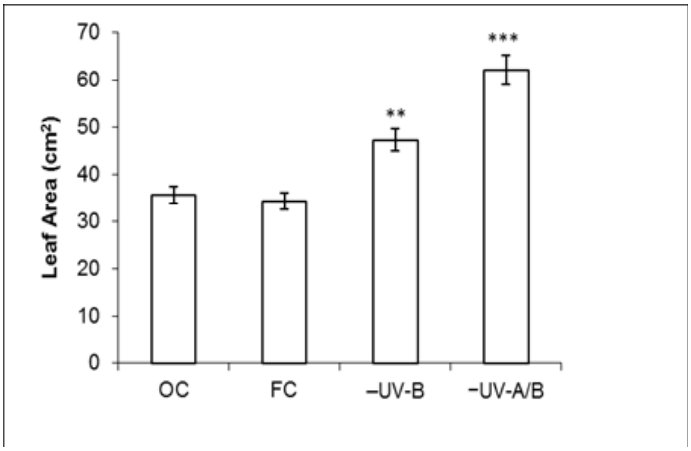

Figure 5: Effect of solar UV-B and UV-A+B exclusion on leaf area of soybean var: JS-335. Vertical bars are ±SE. UV-A+B and UV-B excluded plants were significantly different at *** ($P < 0.001$), **($P < 0.01$) compared to controls (Newman–Keuls multiple comparison test). OC: Open control; FC: filter control; −UV-B: exclusion of solar UV-B; and −UV-A+B: exclusion of solar UV-A+B.

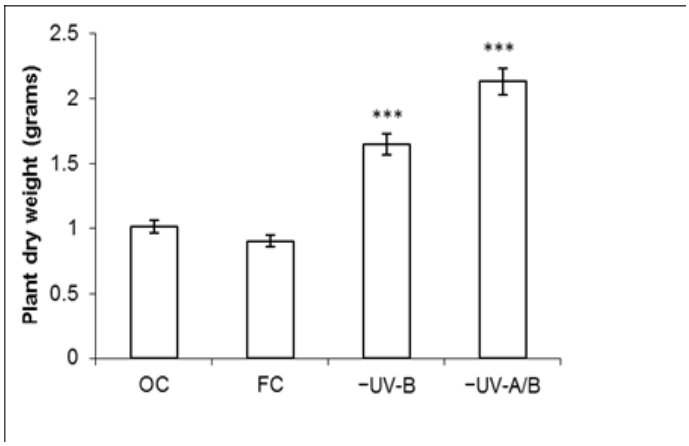

Figure 8: Effect of solar UV-B and UV-A+B exclusion on plant fresh weight of soybean var: JS 335. Vertical bars are ±SE. UV-A+B and UV-B excluded plants were significantly different at *** ($P < 0.001$) compared to controls (Newman–Keuls multiple comparison test). OC: Open control; FC: filter control; −UV-B: exclusion of solar UV-B; and −UV-A+B: exclusion of solar UV-A+B.

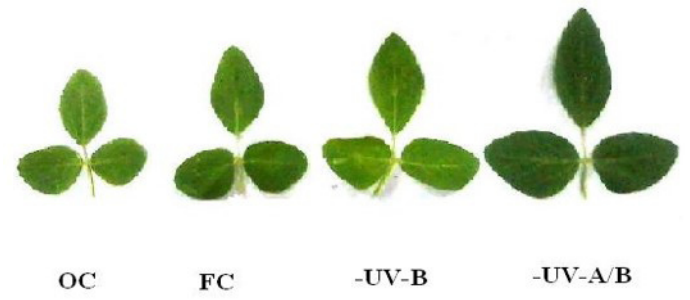

Figure 6: Effect of solar UV-B and UV-A+B exclusion on leaf area of soybean var: JS 335.

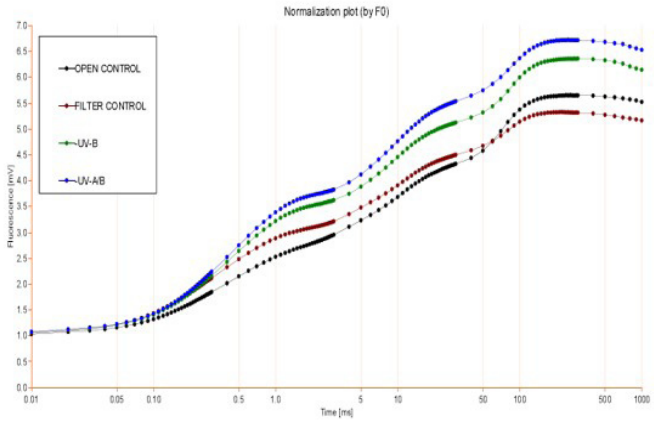

Figure 9: Effect of UV-B and UV-A+B exclusion on fluorescence emission transient of PS II in third trifoliate leaf of soybean var: JS 335.

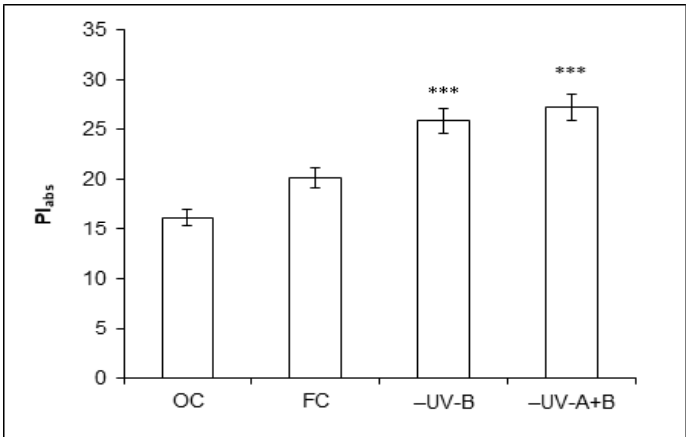

Figure 10: Effect of solar UV-B and UV-A+B exclusion on Performance index of photosystem II (PI_{abs}) of soybean var: JS 335. Vertical bars are ±SE. UV-A+B and UV-B excluded plants were significantly different at *** (P < 0.001) compared to controls (Newman–Keuls multiple comparison test). OC: Open control; FC: filter control; −UV-B: exclusion of solar UV-B; and −UV-A+B: exclusion of solar UV-A+B.

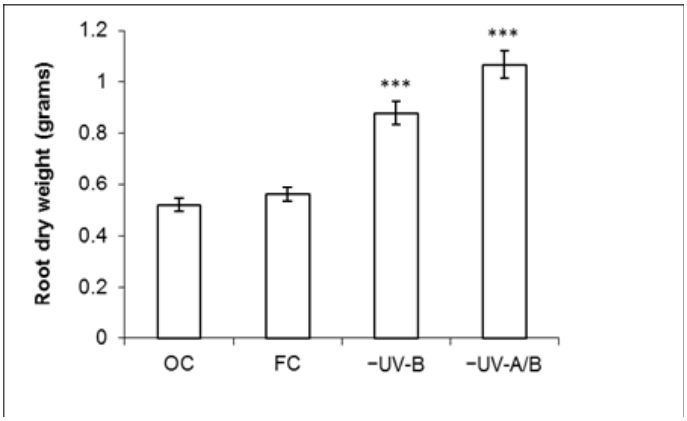

Figure 13: Effect of solar UV-B and UV-A+B exclusion on root dry weight of soybean var: JS 335. Vertical bars are ±SE. UV-A+B and UV-B excluded plants were significantly different at *** (P < 0.001) compared to controls (Newman–Keuls multiple comparison test). OC: Open control; FC: filter control; −UV-B: exclusion of solar UV-B; and −UV-A+B: exclusion of solar UV-A+B.

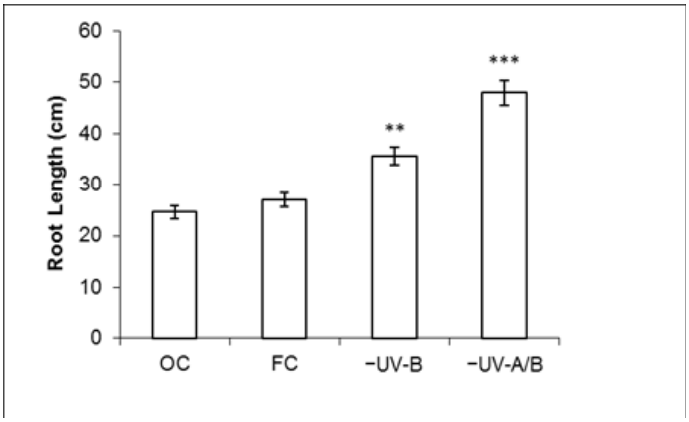

Figure 11: Effect of solar UV-B and UV-A+B exclusion on root length of soybean var: JS-335. Vertical bars are ±SE. UV-A+B and UV-B excluded plants were significantly different at *** (P < 0.001) compared to controls (Newman–Keuls multiple comparison test). OC: Open control; FC: filter control; −UV-B: exclusion of solar UV-B; and −UV-A+B: exclusion of solar UV-A+B.

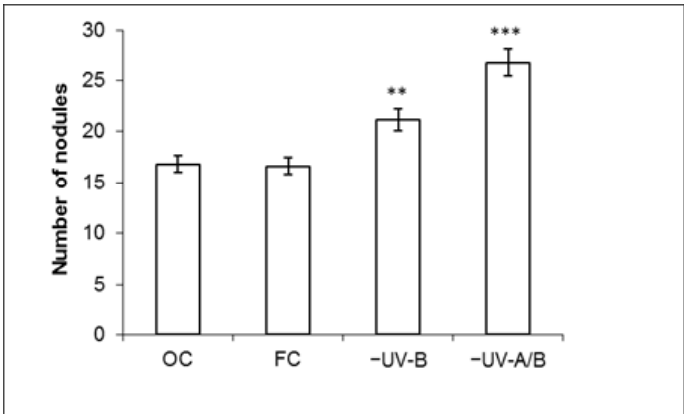

Figure 14: Effect of solar UV-B and UV-A+B exclusion on number of nodules of soybean var: JS 335. Vertical bars are ±SE. UV-A+B and UV-B excluded plants were significantly different at *** (P < 0.001), ** (P < 0.01) compared to controls (Newman–Keuls multiple comparison test). OC: Open control; FC: filter control; −UV-B: exclusion of solar UV-B; and −UV-A+B: exclusion of solar UV-A+B.

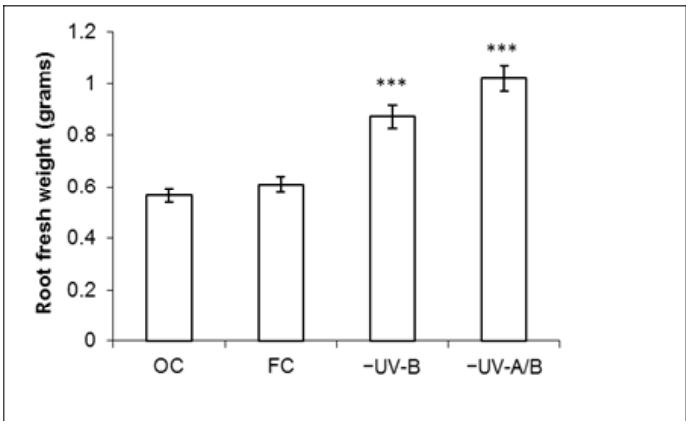

Figure 12: Effect of solar UV-B and UV-A+B exclusion on root fresh weight of soybean var: JS 335. Vertical bars are ±SE. UV-A+B and UV-B excluded plants were significantly different at *** (P < 0.001) compared to controls (Newman–Keuls multiple comparison test). OC: Open control; FC: filter control; −UV-B: exclusion of solar UV-B; and −UV-A+B: exclusion of solar UV-A+B.

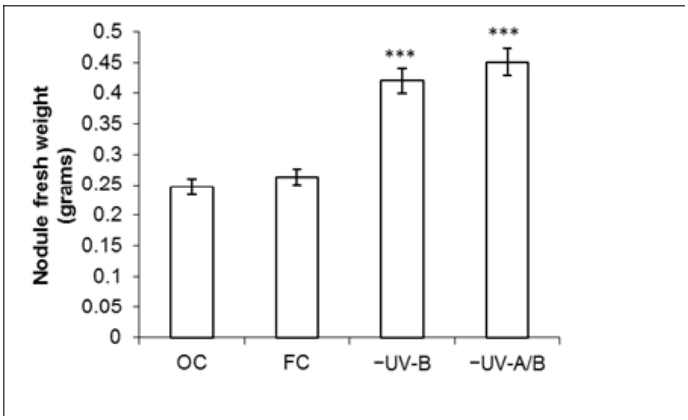

Figure 15: Effect of solar UV-B and UV-A+B exclusion on nodules fresh weight of soybean var: JS 335. Vertical bars are ±SE. UV-A+B and UV-B excluded plants were significantly different at *** (P < 0.001) compared to controls (Newman–Keuls multiple comparison test). OC: Open control; FC: filter control; −UV-B: exclusion of solar UV-B; and −UV-A+B: exclusion of solar UV-A+B.

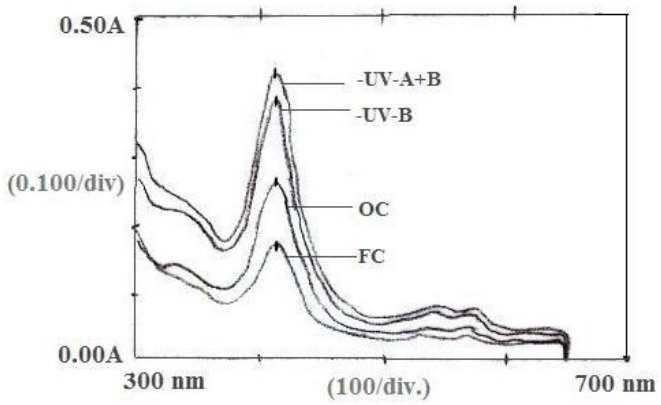

Figure 16: Spectral analysis of leghemoglobin isolated from root nodules of soybean var: JS-335 in terms of fresh weight by ammonium sulphate fractionation.

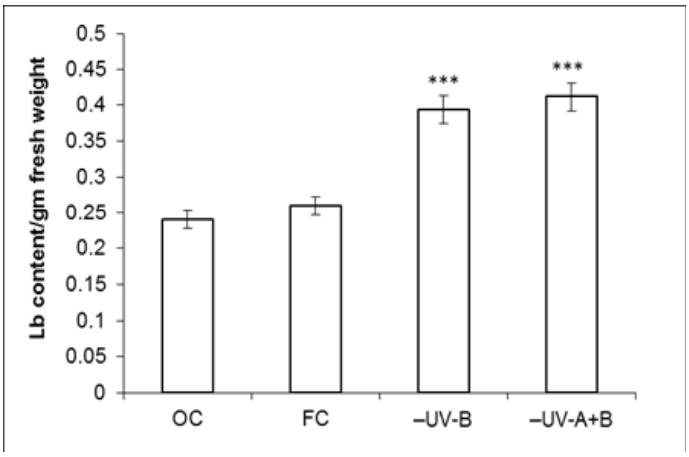

Figure 17: Effect of solar UV-B and UV-A+B exclusion on Lb content of soybean var: JS 335. Vertical bars are ±SE. UV-A+B and UV-B excluded plants were significantly different at *** ($P < 0.001$) compared to controls (Newman–Keuls multiple comparison test). OC: Open control; FC: filter control; −UV-B: exclusion of solar UV-B; and −UV-A+B: exclusion of solar UV-A+B.

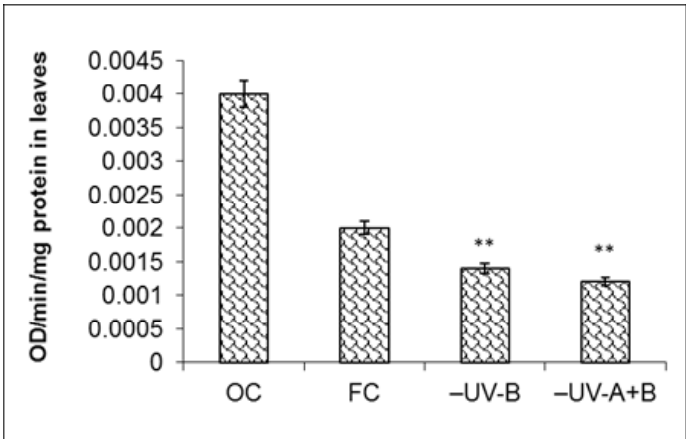

Figure 18: Effect of solar UV-B and UV-A+B exclusion on peroxidase activity in leaves of soybean var: JS-335. Vertical bars are ±SE. UV-A+B and UV-B excluded plants were significantly different at ** ($P < 0.01$) compared to controls (Newman–Keuls multiple comparison test). OC: Open control; FC: filter control; −UV-B: exclusion of solar UV-B; and −UV-A+B: exclusion of solar UV-A+B.

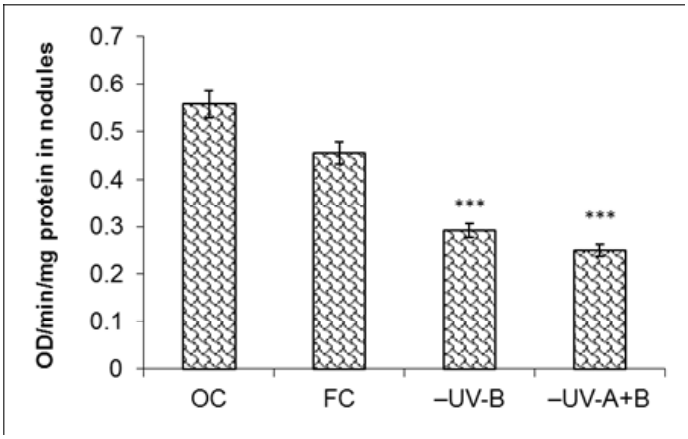

Figure 19: Effect of solar UV-B and UV-A+B exclusion on peroxidase activity in nodules of soybean var: JS 335. Vertical bars are ±SE. UV-A+B and UV-B excluded plants were significantly different at *** ($P < 0.001$) compared to controls (Newman–Keuls multiple comparison test). OC: Open control; FC: filter control; −UV-B: exclusion of solar UV-B; and −UV-A+B: exclusion of solar UV-A+B.

Table 1: Leghemoglobin content in the root nodules of soybean var. JS 335 after UV exclusion. UV-A+B and UV-B excluded plants were significantly different at *** ($P < 0.001$) compared to controls (Newman–Keuls multiple comparison test). OC: Open control; FC: filter control; −UV-B: exclusion of solar UV-B; and −UV-A+B: exclusion of solar UV-A+B.

Treatment	OD_{410nm}	Lb content/gm fresh weight	% Control
OC	0.150	0.240	
FC	0.163	0.260	100
-UV-B	0.246	0.394	164***
-UV-A+B	0.258	0.412	171***

Discussion

The present study revealed that exclusion of UV-B and UV-A from the solar radiation causes significant physiological and biochemical changes in soybean var. JS 335. There is a significant visible difference in the plants grown under the exclusion of UV-B and UV-A compared to those grown under the ambient solar radiation. Omission of UV-B radiation enhanced the plant height, leaf area, fresh and dry weight of plant and root, number of root nodules and their fresh weight. All these parameters are further promoted by excluding UV-A along with UV-B as compared to the plants grown under the ambient solar radiation. The results of the present study indicate that UV-A has more impact on the growth of the plants than UV-B. Exclusion of UV components leads to overall increase in biomass in terms of fresh and dry weight. These results were supported by chlorophyll *a* fluorescence data showing significant enhancement in performance index (PI_{abs}) after UV exclusion. Also, the improved efficiency of PSII has been observed after UV exclusion which in turn contributes in enhancing photosynthesis and carbon fixation as well. These results are also in accordance with recent study on soybean showing increased F_v/F_m and F_v/F_o after UV exclusion [43]. Biochemical analysis of nodules has revealed enhancement in total soluble proteins, specific enhancement in the level of leghemoglobin content a protein which plays an important part in fixation of nitrogen in the nodules, an indirect evidence for increased nitrogen fixation [44,45].

UV exclusion leads to considerable decrease in the activity of peroxidase enzyme which protects leaves and the nodular tissues against oxidative damage. Thus, reduction in the peroxidase activities after UV exclusion indicate decrease in oxidative stress of UV excluded plants [43].

Conclusion

In conclusion, these results indicate that the presence of UV-B and UV-A components in sunlight was the cause of inhibition of some morphogenetic expression and loss in carbon and nitrogen assimilation. Industrialization, transportation, population growth and energy consumption have all contributed to a sharp rise in the concentration of CO_2 from a near stationary level of 270 ppm two centuries back, to the present level of 350 ppm, with a tendency to rise further in the next few decades. Since soybean is rich in protein and exclusion of UV enhanced vegetative biomass in a fixed land area infers that soybean plant have the potential for fixing more carbon and nitrogen, this potential is suppressed by the UV component of solar spectrum. Enhancement of photosynthesis by exclusion of solar UV components is one of the very new method for carbon sequestration. The present data on exclusion of solar UV radiation indicates an effect on the activity of nitrogen fixation by enhancement in the synthesis of leghemoglobin. Increased growth of aerial parts seems to be deriving more nitrogen to support growth and enhanced protein synthesis. More carbon sequestration, nitrogen fixation and thus overall productivity after UV exclusion open more lines of investigations and avenues for research in this field.

References

1. Jordan BR. The effects of ultraviolet-B radiation on plants: A molecular perspective. Adv Bot Res. 1996; 22: 97–162.
2. Strid A, Chow WS, Anderson JM. Effect of supplementary ultraviolet-B radiation on photosynthesis in *Pisum sativum*. Biochim. Biophys. Acta. 1990; 1020: 260-268.
3. Nogues S, Baker NR. Evaluation of the role of damage to photosystem II in the inhibition of CO2 assimilation in pea leaves on exposure to UV-B radiation. Plant Cell Environ. 1995; 18, 781-787.
4. Berkelaar EJ, Ormrod DP, Hale BA. The influence of photosynthetically active radiation on the effects of UV-B radiation on *Arabidopsis thaliana*. Photochem Photobiol. 1996; 64: 110-116.
5. Jayakumar M, Amudha P, Kulandaivelu G. Effect of low doses of UV-A and UV-B radiation on photosynthetic activities in *Phaseolus mungo L*. J Plant Biol. 2004; 47: 105-110.
6. Ballaré CL, Rousseaux MC, Searles PS, Zaller JG, Giordano CV, Robson TM, Caldwell M M, Sala OE and Scopel AL. Impacts of solar ultraviolet-B radiation on terrestrial ecosystems of Tierra del Fuego (southern Argentina)-an overview of recent progress. J Photochem Photobiol B. 2001; 62: 67 –77.
7. Searles PS, Flint SD, Caldwell MM. A meta-analysis of plant field studies simulating stratospheric ozone depletion. Oecollogia. 2001; 127: 1-10.
8. Mazza CA, Battista D, Zima AM, Szwarcberg-Bracchitta M, Giordano CV, Scopel AL, Ballare CL, Acevedo A. The effects of solar UV-B radiation on growth and yield of barley are accompanied by increased DNA damage and antioxidant responses. Plant Cell Environ. 1999; 22: 61-70.
9. Golaszewska-Zuk K, Upadhyaya MK, Golaszewski J. The effect of UV-B radiation on plant growth and development. Plant Soil Environ. 2003; 49: 135-140.
10. Pal M, Zaidi PH, Voleti SR, Raj A. Solar UV-B exclusion effects on growth and photosynthetic characteristics of wheat and pea. J New seeds. 2006; 8:
11. Guruprasad KN, Bhattacharjee S, Kataria S, Yadav S, Tiwari A, Baroniya S, Rajiv A, Mohanty P. Growth enhancement of soybean (*Glycine max*) upon exclusion of UV-B and UV-A components of solar radiation characterization of photosynthetic parameters in leaves. Photosynthetic Research. 2007; 94: 299-306.
12. Eichholz I, Rohn S, Gamm A, Beesk N, Herppich WB, Kroh LW, Ulrichs C, Huyskens-Keil S. UV-B-mediated flavonoid synthesis in white asparagus (*Asparagus officinalis* L.). Food Res Intl. 2012; 48: 196–201.
13. Kataria S, Guruprasad KN, Ahuja S, Singh B. Enhancement of growth, photosynthetic performance and yield by exclusion of ambient UV components in C3 and C4 plants. J Photochem Photobiol B. 2013; 127: 140–152.
14. Jordan BR, He J, Chow WS, Anderson JM. Changes in mRNA levels and polypeptide subunits of ribulose 1,5-bisphosphate carboxylase in response to supplementary UV-B radiation. Plant cell physiol. 1992; 42: 141-145.
15. Paula C, Virginia W. Gene expression profiling in response to UV radiation in maize genotypes with varying flavonoids content. Plant Physiol. 2003; 132: 1739-1754.
16. Vass I, Sass L, Spatea C, Bakou A, Ghanotaki D, Petrouleas V. UV-B induced inhibition of PS II electron transport studied by EPR and chlorophyll fluorescence, impairment of donor and acceptor side components. Biochem. 1996; 35: 8964-8973.
17. Grzymski J, Orrico C, Schofield OM. Monochromatic ultraviolet light induced damage to photosystem II efficiency and carbon fixation in the marine diatom *Thalassiosira pseudonana* (3H). Photosynthetic Res. 2001; 68: 181-192.
18. Noorudeen AM, Kulandaivelu G. On the possible site of inhibition of photosynthetic electron transport by UV-B radiation. Physiol. Plant. 1982; 55: 161-166.
19. Iwanzik W, Tevini M, Dhont G, Voss M, Weiss W, Graber P, Renger G. Action of UV-B on photosynthtic primary reactions in spinach chloroplasts. Physiol Plant. 1983; 58: 401-407.
20. Vass I, Turcsanyl E. Inhibition of photosynthetic electron transport by UV-A radiation targets the photosystem II complex. Photochem Photobiol. 2000; 72: 513-520.
21. Krause GH, Schmude C, Hermann G, Koroleva O, Winter K. Effects of solar ultraviolet radiation on the potential efficiency of photosystem II in leaves of tropical plants. Plant Physiol. 1999; 121: 1349-1358.
22. Krause G.H., Virgo A., Winter K. High susceptibility to photoinhibition of young leaves of tropical forest trees. Planta. 1995; 197: 583-591.
23. Zavala JA, Botto JF. Impact of solar UV-B radiation on seedling emergence, chlorophyll fluorescence, growth and yield of radish (*Raphanus sativus*). Funct Plant Biol. 2002; 29: 797-804.
24. Amudha P, Jayakumar M, Kulandaivelu G. Impact of ambient solar UV (280-400 nm) radiation on three tropical legumes. J Plant Biol. 2005; 48: 284-291.
25. Krizek DT, Merecki RM. Evidence of phytotoxic effects of cellulose acetate in UV exclusion studies. Environ Exp Bot. 2004; 51: 33-43.
26. Solanki R, Lakshmi N, Athale R, Baroniya S, Guruprasad KN.

Growth and chlorophyll contents as affected by UV-A and UV-B components in cucumber and cotton. Physiol and Mol Biol of Plants. 2006; 12: 321-323.

27. Varalakshmi D, Lakshmi N, Guruprasad KN. Physiological changes in soybean CV. JS 71-05 after the exclusion of UV-A and UV-B from the solar radiation. Indian Journal of plant physiol. 2003; (special issue): 602-606.

28. Bischof K, Krabs G, Wiencke C, Hanelt D. Solar UV radiation effects the activity of ribulose -1,5-bisphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L. Planta 2002; 215: 502-509.

29. Krause HG, Grube E, Aurelio V, Winter K. Sudden Exposure to Solar UV-B Radiation Reduces Net CO2 Uptake and Photosystem I Efficiency in Shade-Acclimated Tropical Tree Seedlings. Plant Physiol. 2003; 131: 745–752.

30. Rinnan R, Keinanen MM, Kasurinen A, Asikainen J, Kekki TK, Holopainen T, Poulsen-Ro H, Mikkelsens TN, Michelsen A. Ambient UV radiation in the Arctics reduces root biomass and alters microbial community composition but has no effects on microbial biomass. Global Change Biol. 2005; 11: 564-574.

31. Hammerschmidt R, Nuckles E, and Kuc J. Association of enhanced peroxidase activity in induced systemic resistance of cucumber to Colettotrichume lagenarium. Physiol. Plant Pathol. 1982; 20: 73-82.

32. Dhindsa RS, Plumb-Dhindsa P, Thorpe TA. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot. 1981; 32: 93-101.

33. Puppo A, Rigaud J, Job D. Role of superoxide anion in leghemoglobin autoxidation. Plant Sci Lett. 1981; 22: 353-360.

34. Puppo A, Dimitrijevic L, Rigaud J. Possible involvement of nodule superoxide dismutase and catalase in leghemoglobin protection. Planta. 1982; 156: 374-379.

35. Rao MV, Paliyath G, Ormrod DP. Ultraviolet-B and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. Plant Physiol. 1996; 110: 125–136.

36. Panagopoulos I, Bornman JF, Bjorn LO. The effect of UV-B and UV-C radiation on Hibiscus leaves determined by ultraweak luminescence and fluorescence induction. Physiol. Plant. 1989; 76:461-465.

37. Panagopoulos I, Bornman JF, and Bjorn LO. Effects of ultraviolet radiation and visible light on growth, fluorescence induction, ultraweak luminescence and peroxidase activity in sugar beet plants. J Photochem Photobiol. 1990; 8:73-87.

38. Jain K, Kataria S, Guruprasad KN. Oxyradicals under UV-B stress and their quenching by antioxidants. Indian J Exp Biol. 2004; 42: 884.

39. Kataria S, Jain K, Guruprasad KN. UV-B induced changes in antioxidant enzymes and their isoforms in cucumber (*Cucumis sativus* L.) cotyledons. Indian J Biochem Biophys. 2007; 44: 31-37.

40. Dalton DA. Antioxidant defenses of plants and fungi. In: Ahmad S (ed.) Oxidative Stress and Antioxidant Defenses in Biology. Chapman and Hall, New York, NY, USA. 1995; pp 298–355.

41. Jun HK, Sarath G, Wagner FW. Detection and purification of modified leghemoglobins from soybean root nodules. Plant Sci. 1994; 100: 31-40.

42. Maehly AC. Plant peroxidase. In: Methods in Enzymology (Eds. Colowick PS and Kaplan, NO). Academic Press, New York. 1955; vol. II: 271-285.

43. Kataria S, Baghel L, Guruprasad KN. Alleviation of Adverse Effects of Ambient UV Stress on Growth and Some Potential Physiological Attributes in Soybean (*Glycine max*) by Seed Pre-treatment with Static Magnetic Field. J Plant Growth Regul. 2017; 36: 550–565.

44. Chouhan S, Chauhan K, Kataria S, Guruprasad KN. Enhancement in leghemoglobin content of root nodules by exclusion of solar UV-A and UV-B radiation on soybean. J plant biol. 2008; 51: 132-138.

45. Joshi J, Sharma S, Guruprasad KN. Foliar application of pyraclostrobin fungicide enhances the growth, rhizobial-nodule formation and nitrogenase activity in soybean (var. JS 335). Pest Biochem Physiol. 2014; 114: 61–66.