Journal of Plant Biology, March 2008, 51(2) : 132-138

Enhancement in Leghemoglobin Content of Root Nodules by Exclusion of Solar UV-A and UV-B Radiation in Soybean

Swapnil Chouhan, Kanchan Chauhan, Sunita Kataria*, and Guruprasad, KN
School of Life Sciences, Devi Ahilya University, Vigyan Bhawan, Khandwa Road, Indore-452001, India

The impact of exclusion of solar UV-B (280-320 nm) and UV-A+B (280-400 nm) radiation on the root nodules was studied in soybean (*Glycine max* var. MACS 330). Soybean plants were grown in the tropical region of Indore (Latitude-22.4°N), India under field conditions in metal cages covered with polyester exclusion filters that specifically cut off UV-B (<320 nm) and UV-A+B (<400 nm) radiation; control plants were grown under ambient solar radiation. Leghemoglobin content was analyzed in the root nodules on the 50th day after emergence of seedlings. Exclusion of UV radiations significantly enhanced the leghemoglobin content in the nodules on fresh weight basis; 25% and 45% higher amount of leghemoglobin were present in the nodules after the exclusion of UV-B and UV-A+B radiation respectively. Analysis by native and SDS-PAGE showed high intense bands of leghemoglobin after the exclusion of UV-A+B as compared to control. Exclusion of UV radiation also enhanced the growth of roots as well as aerial parts of the plants. UV Exclusion increased nodulation by increase in the number and size of nodules. The results are discussed in the light of advantage of exclusion for enhancing protein/nitrogen content in the plants.

Key words: ambient, leghemoglobin, root nodules, soybean, UV-A+UV-B exclusion

Several studies have shown that enhanced level of UV-B (280-320 nm) has detrimental effects on plant growth, development and morphology, photosynthesis and biomass production in a number of cultivated and native plant species (Caldwell, 1971; Teramura, 1983; Tevini and Teramura, 1989; Tevini et al., 1991; Teramura and Sullivan, 1994; Mazza et al., 1999). 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. The level of ambient UV-B radiation in sunlight varies with reference to latitudes. 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 in tropics (Caldwell et al., 1989; Madronich et al., 1995). Reduction in plant height, leaf area and dry weight after exposure to UV-B has been reported in sunflower, corn, rye (Tevini et al., 1991), *Rumex patentia* (Lindoo and Caldwell, 1978), *Cucurbita pepo* (Sisson, 1981), soybean (Lyndon et al., 1986) and *Gossypium hirsutum* (Kakani et al., 2003). Tezuka et al. (1993) found that the growth of plants with radiation of waveband region above 320 nm was superior to the growth of plants with that above 290 nm. Tezuka et al. (1994) have also found that UV radiation (320-400 nm) promotes physiological activities and growth of radish plants. In soybean cultivars exposure to UV-B radiation under green house or field conditions reduces vegetative growth in some cultivars but same treatment enhances growth in other cultivars (Teramura and Murali, 1986).

All the previous studies have shown suppression of photosynthesis and biomass by enhanced levels of UV-B but whether UV-B has any affect on underground organ like roots and on beneficial processes linked to nitrogen (N_2) fixation in leguminous plants is not clear yet. Singh (1997) showed that supplemental UV-B induced reduction in N_2 fixation in tropical crops like *Phaselous mungo* and *Vigna radiata*. However, Shiozaki et al. (1999) found that extra UV (300-400 nm) applied to leaves increased the nodulation and symbiotic N_2 fixation in pea plants. Ambient UV radiation enhanced the number of nodules per plant than plant deprived of UV-B in bean (*Phaseolus vulgaris*) plants (Pinto et al., 2002).

UV (280-400 nm) radiation significantly influences the growth and yield of crop plants and their influence is best studied by growing the plants after the exclusion of solar UV-A+B. Such exclusion studies have indicated enhanced growth of plants like radish (Zavala and Botto, 2002), *Cyamopsis* and *Vigna* (Amudha et al., 2005), cucumber (Krizek and Mirecki, 2004), cucumber and cotton (Solanki et al., 2006) and soybean (Varalakshmi et al., 2003; Guruprasad et al., 2007). Most of these UV exclusion studies are on the aerial parts of the plants and showed the promotion of biomass by exclusion of solar UV-B and very few on the underground parts (Zavala and Botto, 2002). In legumes, root nodules have an important role in nitrogen metabolism. Like most legumes soybean has the potential to fix atmospheric nitrogen through symbiotic relationship with soil organisms (Sanginga et al., 1996; Stefan and Christian, 2002). The present investigation on soybean (*Glycine max* cv MACS 330) was designed to study the impact of exclusion of UV-B and UV-A+B on the root nodules.

MATERIALS AND METHODS

Plant Material and Treatments

All the field experiments under the natural sunlight were conducted in the Botanical garden of School of Life Science, Devi Ahilya University, Indore (Latitude-22.4°N), India. Seeds of soybean (*Glycine max* cv MACS 330) were obtained from National Research Center for Soybean, Indore and treated

*Corresponding author; fax
e-mail sunitakataria@hotmail.com

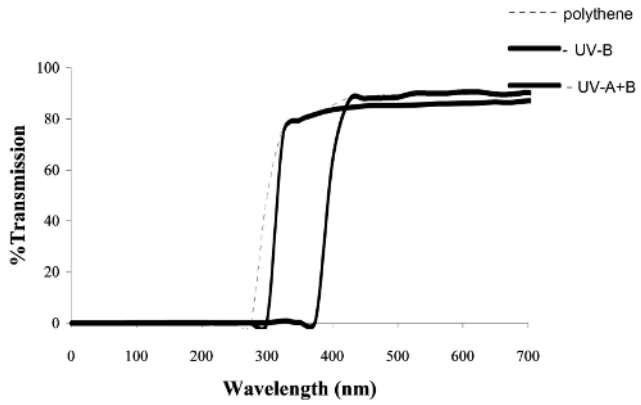

Figure 1 Transmission spectra of the UV cut off filters. The spectra between 200-700 nm by using an UV-visible spectrophotometer.

with recommended fungicides viz. Bevistin and Diathane M at 2 g/kg seeds and then inoculated with a slurry of *Rhizobium japonicum* at 3 g/kg *seeds* before sowing. The plots were watered as needed and weeds were controlled manually. The seeds were sown in plastic bags (34 cm H x 34 cm B; filled with mixture of sand, soil and manure -1:2:1) placed in metal mesh cages (4 feet L x 3 feet W x 3 feet H) covered with polyester filters (Garware polyesters Ltd., Mumbai) that cut off UV-B (<300 nm) and UV-A+B (<400 nm) radiations. Control plants were grown under a polyethylene filter, which transmits all the ambient solar radiations including the UV-A+ B components. The exclusion characters of these filters are in Figure 1. The metal cages received full solar radiation for the most of the period of the day with out any shading. The experiments were carried out in December 2004 to March 2005. Seedlings were exposed to solar radiation from the time of germination.

Plants were grown for 50 d after emergence of seedlings (DAE). Samples were taken at an interval of 10 d for collecting the growth data, number of nodules and weight per nodule.

Radiation Measurements

Absolute solar irradiance with or without UV-B or UV-A+B was measured using a radiometer (IL 1350, International light Inc.) USA. The ambient solar irradiance during experimental period at midday was 382 μ mol $m^{-2}s^{-1}$, the loss in light intensity at midday by -UV-B filter was 43% (219 μ mol $m^{-2}s^{-1}$) and 44% (214 μ mol $m^{-2}s^{-1}$) under – UV-A+B filter and 7% (356 μ mol $m^{-2}s^{-1}$) under polythene filter transmissible to UV (control filter). The PAR intensity for normal plant growth was observed to be optimal saturating light.

Growth Analysis

Plant height, leaf area, root length, root weight, number of nodules per plant and weight of nodules was measured at an interval of 10 d till the 50th (DAE) and leaf area was monitored in fully mature first, second and third trifoliate leaf. The number of pods per plant was measured at 60th DAE. Each experiment was done in triplicates of five plants each.

Extraction and Estimation of Leghemoglobin (Lb) Content

Leghemoglobin (Lb) was extracted from the root nodules of 50 day old soybean plants and measured by the method of Jun et al. (1994). Root nodules (1.25 g) from the 50 d 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, beta-mercaptoethanol, and 10% polyvinyl pyrrolidone (PVPP). The resulting solution was filtered through cheese cloth and centrifuged at 20,000 *g* for 20 min. at 4°C. The deep red supernatant was saturated to 50% with solid $(NH_4)_2SO_4$ and then centrifuged at 15,000 *g* for 20 min at 4°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°C. The red pellet was resuspended in 15 mL of 20 mM Tris HCl (pH 8.0) containing 1 mM $(NH_4)_2SO_4$. The Lb-containing fractions (50 to 90% pellet) were detected at 410 nm by using UV-Visible Shimadzu Spectrophotometer. Total soluble protein content was measured in Lb-containing fractions by the method of Lowry et al. (1951).

Native and SDS-Polyacrylamide Gel (SDS-PAGE) electrophoresis of Lb containing crude and ammonium sulphate (50 to 90% pellet) fractions was carried out by the method of Laemmli (1970). Proteins were resolved in separating gel (12%) and a stacking gel (5%). The samples of equivalent amount of protein mixed with equal volume of sample buffer was loaded on to the gel, and was run at 60 Volts till the samples crossed the stacking layer. The voltage was then increased to 150 Volts. Electrophoresis was carried out at 20°C by using mini electrophoretic unit (Banglore Genei Pvt. Ltd. India). The gels after electrophoresis were immersed in a stain prepared by dissolving 0.25 g Coomasie brillant blue R-250 (Sigma) in 100 mL of 50% methanol containing 7% (v/v) acetic acid. The gels were stained for 20 h and destained with 50% methanol and 7% (v/v) acetic acid mixture for 12 h. The destained gels were preserved in 7% acetic acid solution and apparent molecular masses were estimated using standard marker (M_r) proteins (Banglore Genei Pvt.Ltd. India). The molecular weights of proteins were calculated by using a software-Alpha Digi Doc 1200.

Heme concentration in leghemoglobin was measured by pyridine hemechromogen assay as described by Appleby and Bergerson (1980). Fresh root nodules (500 mg) were mixed with 10 mL of 50 mM phosphate buffer (6.5 pH) and macerated. The contents were filtered through two layers of cheesecloth. Nodule debris was discarded and remaining brown filtrate was centrifuged at 20,000 *g* for 20 min. To 5 mL of extract, 5 mL alkaline pyridine reagent was added and mixed. The resulting hemechrome was equally divided into two portions. To one portion (5 mL) few crystals of sodium dithionate was added to reduce the hemechrome. To the other portion (5 mL), 5 mM of potassium hexacynoferrate was added to oxidize the hemechrome and the contents of both the test tubes were measured at 556 nm and 539 nm respectively. Leghemoglobin content was calculated using the following formula:

$$\text{Lb concentration (mM)} = \frac{A(556) - A(539) \times 2}{23.4}$$

Where, D is initial dilution.

RESULTS

Effect of Exclusion of UV-B and UV-A+B Filtered Solar Radiation on Growth and Yield Characteristics

Exclusion of UV-B and UV-A+B radiation significantly enhanced plant growth and biomass. Enhancement was more in the aerial parts such as plant height and leaf area compared to the underground parts such as root length, number of nodules and weight of nodules per plant at all the stages studied from 10th to 50th DAE. Maximum enhancement was observed at 50th DAE. Enhancement was more in the aerial parts as seen in plant height 118% (-UV-B); 136% (-UV-A+B) and second trifoliate leaf area 140% (-UV-B); 186% (-UV-A+B) (Fig. 2A, B & 3A). Enhancement in the root growth was comparatively lesser in terms of root length 122% (-UV-B); 112% (-UV-A+B) and fresh weight of roots 126% (-UV-B); 120% (-UV-A+B) (Fig. 4A, B).

There was also an increase in the number of nodules per plant 122% (-UV-B); 117% (-UV-A+B) and the weight of the nodules 134% under UV-B exclusion and further enhanced to 149% by exclusion of UV-A+B radiation as compared to the plants grown under ambient radiation at 50th DAE (Fig. 3B & 4C, D).

The number of pods was higher by 134% and 151% after the exclusion of UV-B and -UV-A+B respectively (Figure 5).

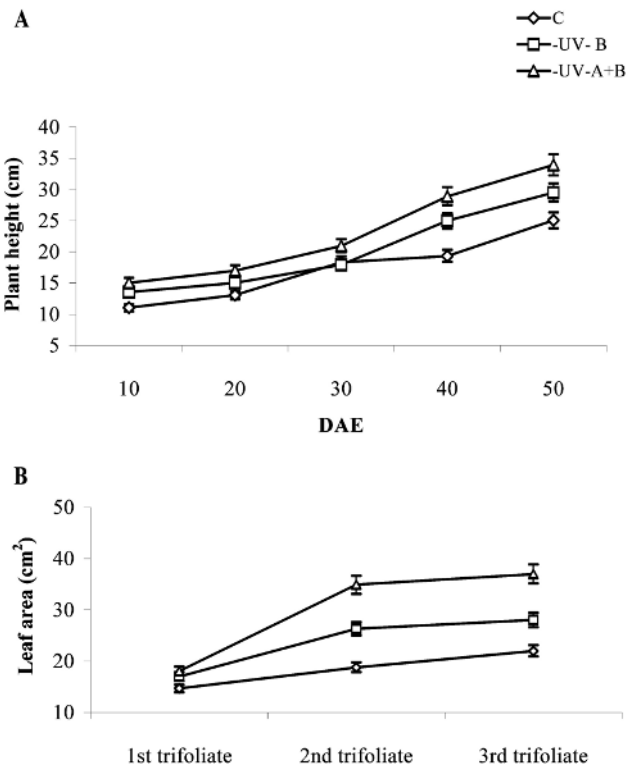

Figure 2 Effect of UV-B and UV-A+B exclusion on plant height (**A**) and Leaf area (**B**) of soybean plant (cv. MACS 330). Each bar represents the mean of three samples assayed in triplicates and the vertical lines indicates ± SE.

Leghemoglobin Content

The absorption of leghemoglobin (from 1.25 g of tissue) isolated by ammonium sulphate fractionation (50 to 90% Pellet) is shown in Figure 6. The higher amount of leghemoglobin was present in the plants grown under exclusion of UV as compared to those plants grown under ambient UV radiation. UV exclusion specifically enhanced the content of leghemoglobin in root nodules by 125% (-UV-B), which was further enhanced by 145% (-UV-A+B) on fresh weight basis (Lb/g FW) (Fig. 6, Table 1).

Along with the fresh weight of the nodules, the amount of total soluble proteins were quantified by the method of Lowry et al. (1951) in the nodules, it was also higher by 114% (-UV-B) and 124% (-UV-A+B). The percentage increase in leghemoglobin content was comparatively lesser in terms of protein (Lb/mg protein), 109% by UV-B exclusion and 117% by UV-A+B exclusion (Table 1). In view of the increase in Lb content in crude and ammonium sulphate (50 to 90% Pellet) fraction of UV excluded samples, the changes in the polypeptide patterns were analyzed by gel electrophoresis. Native and SDS gels were run to document any alteration in the protein composition of leghemoglobin containing fractions of soybean root nodules grown under ambient UV and UV filtered radiation (Fig. 7A, B). In Native

Figure 3 Photographs showing effect of UV-B and UV-A+B exclusion on plant height (**A**) and root length and number of nodules (**B**) of soybean plant (cv. MACS 330).

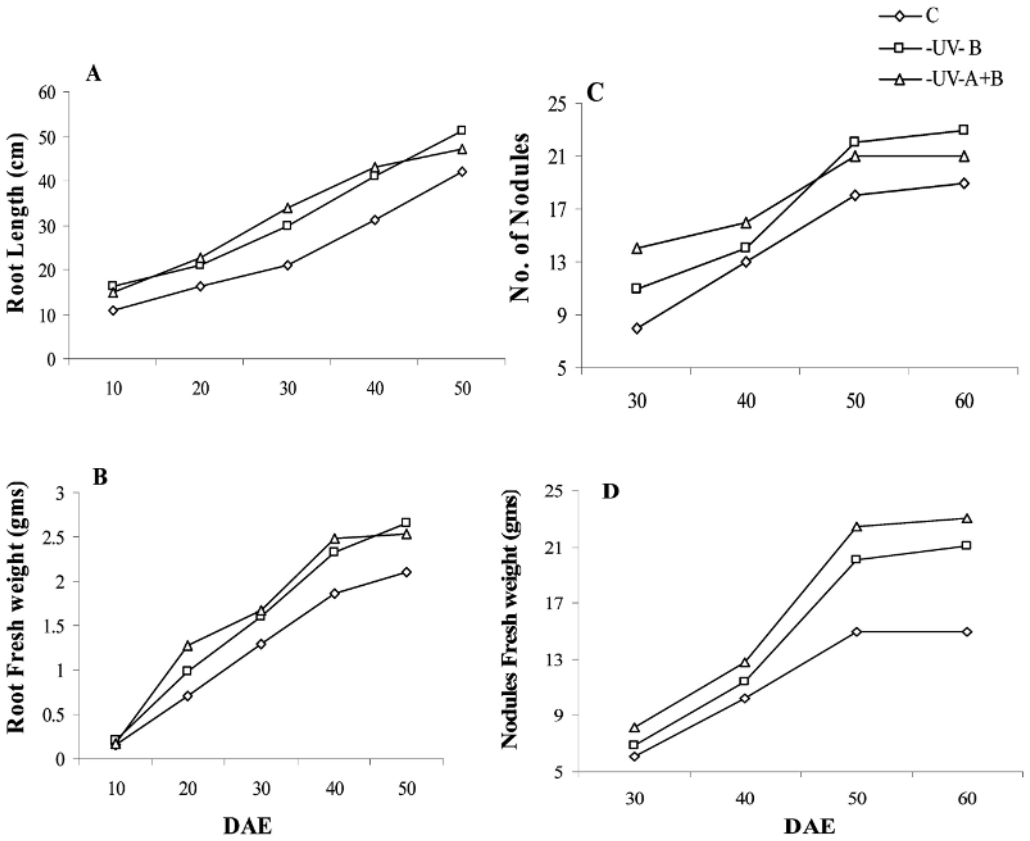

Figure 4 Effect of UV-B and UV-A+B exclusion on root length (**A**), fresh weight of roots (**B**), number of nodules (**C**) and fresh weight of root nodules (**D**) of soybean plant (cv. MACS 330). Each line represents the mean of three samples assayed in triplicates and ± SE varies from 0.02 to 5.0.

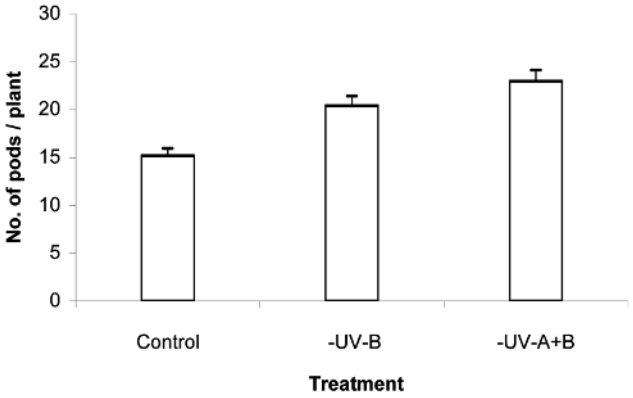

Figure 5 Effect of UV-B and UV-A+B exclusion on number of pods per plant of soybean (cv. MACS 330). Each bar represents the mean of three samples assayed in triplicates and the vertical lines indicates ± SE.

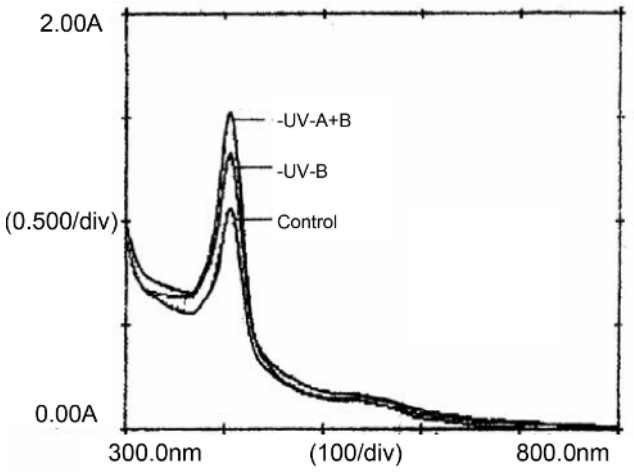

Figure 6 Spectral analysis of leghemoglobin isolated from root nodules of soybean plant (cv. MACS 330) in terms of fresh weight by ammonium sulphate fractionation (50 to 90% Pellet) at 50th DAE.

gel profile, two protein bands were observed in crude and ammonium sulphate (50 to 90% pellet) fractions of soybean root nodules grown under ambient UV radiation (Fig. 7A; Control) and these protein bands became intense by the exclusion of solar UV-B or UV-A+B radiations (Fig. 7A; -UV-B, -UV-A+B). Similarly, the SDS-PAGE also showed the changes and increase in protein band intensities by exclusion of solar UV-B and UV-A+B (Fig. 7B). These changes indicated possible over expression of proteins of 66.0 and 18.5 kDa molecular weights due to UV omission.

Heme concentration in leghemoglobin was measured by the pyridine hemochromogen assay. Much higher enhancement was observed in the hemechrome content by 204% (-UV-B) and 269 % by exclusion of both UV-A+B (Table 1).

Table 1. Total protein, leghemoglobin and hemechrome contents in the root nodules of soybean (cv MACS 330) on 50th DAE of seedlings by the exclusion of solar UV-B and UV-A+B. The values are mean ± SE, n=6. [Values in parenthesis show percent increase].

S.N.	Parameter	Control	(-UV-B)	(-UV-A+B)
1.	Protein (mg/mL)	0.207 ± 0.09	0.237 ± 0.01(114.0)	0.257 ± 0.02(124.0)
2.	Leg hemoglobin content/gm FW	1.034 ± 0.05	1.293 ± 0.09(125.0)	1.500 ± 0.05(145.0)
3.	Leg hemoglobin content/mg protein	4.99 ± 0.04	5.46 ± 0.05(109.4)	5.84 ± 0.06(117.0)
4.	Heme chrome content/mg protein	0.227± 0.002	0.464 ± 0.005(204.4)	0.610 ± 0.007(269.0)

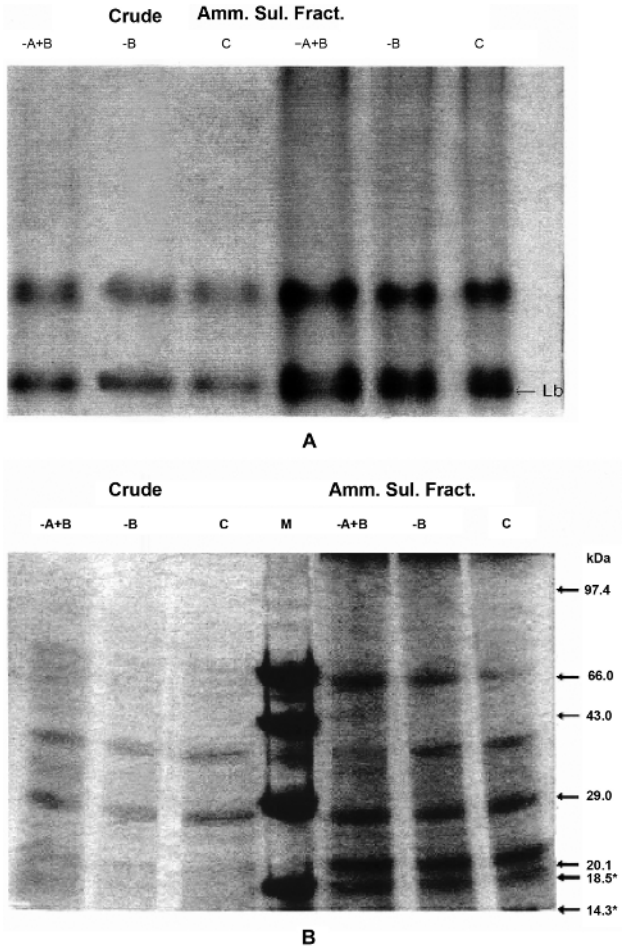

Figure 7. A Native PAGE analysis of leghemoglobin isolated from root nodules of soybean plant (cv. MACS 330) in terms of protein in crude and ammonium sulphate fractions (50 to 90% pellet). Equal amount of protein was loaded for each sample. **B.** SDS PAGE analysis of leghemoglobin isolated from root nodules of soybean plant (cv MACS 330) in terms of protein (equal amount of protein was loaded for each sample) in crude and ammonium sulphate fractions (50 to 90% Pellet) on a 12% gel (see Material and Methods for details). Mr is shown in kDa and the band of about 18.5* is of Lb.

DISCUSSION

The results on enhancement of vegetative growth by exclusion of UV radiation from sunlight in the present variety of soybean (cv. MACS 330) is similar to the observation made earlier on the soybean variety cv. JS 7105 (Varalakshmi et al., 2003; Guruprasad et al., 2007) and Cymopsis (Amudha et al., 2005). In these varieties, enhanced vegetative growth is further complemented by enhanced number of pods formed per plant. This suggests that the level of UV-B present in solar radiation produced a strong inhibitory effect on vegetative growth and morphological developments of soybean plants. Reduction in plant height in response to supplementary UV-B radiation has been reported for soybean (Biggs et al., 1981), cucumber (Murali and Teramura, 1986), and cotton (Ambler et al., 1975; Kakani et al., 2003). UV-B stress has also been observed to reduce root biomass in soybean (Feng et al., 2003) and agricultural weeds (Furness and Upadhyaya, 2002).

Since the level of ambient UV-B radiation in sunlight varies with reference to latitude and is relatively higher in tropical regions, a true assessment of UV radiation effect is possible by the UV exclusion studies. The impact of UV exclusion is also seen on the underground parts in the present study, there was enhancement in the fresh weight of roots and nodules and a significant increase in the number of nodules. Many nodules were noticed by UV-B exclusion and much higher number by UV-A+B exclusion in *Cymopsis* but no increase in *Vigna mungo* and *V. radiata* (Amudha et al., 2005). The soil water content was unaffected by the exclusion filters (Rinnan et al., 2005).

The present study provides the positive effect of UV exclusion on aerial parts as well as on underground parts of soybean crop grown in tropical region and such responses varies with the plant species and cultivars. According to Teramura and Murali (1986) in some cultivars of soybean plants exposed to UV-B, vegetative growth was reduced but in some other cultivars growth was enhanced. Conflicting data have been published regarding the effects of UV-B on underground part or non UV exposed part – root, number of root nodules, weight of nodules and nitrogen fixation. Shiozaki et al. (1999) found that the growth of pea plant was enhanced by near UV (300-400 nm) radiation and the nodulation and symbiotic nitrogen fixation are also enhanced about two and eight times respectively. Similarly, Tezuka et al. (1998) found that UV-A (320-400 nm) from UV lamps promotes nodulation on roots and symbiotic N_2 fixation in soybean plants. Ambient UV-B affected the biomass partitioned to tubers; and increased the tuber diameter and tuber fresh weight of radish (*Raphanus sativus* L.) (Zavalla and Botto, 2002). Pinto et al. (2002) have found that exposure of bean plants grown in green house, where UV-B is low to ambient level of UV-B light stimulated nodulation more than 2.5 fold and they have also found that the plants grown under ambient UV radiation had almost 60% more nodules per plant than plant deprived of UV-B. However, moderate and elevated UV-B exposure had no effect on number of nodules, nodule mass and nodule size but the nitrogen concentration was markedly reduced in roots of *Glycine max* and *Phaseolus vulgaris* but these parameters were not altered by below ambient UV-B exposures (Chimphango et al., 2003). The response in root biomass is consis-

tent with increased root length production of Carex spp. under reduced UV radiation reported by Zaller et al. (2002). Rinnan et al. (2005) also found 30% increase in the root biomass under reduction of both UV-A and UV-B radiation in *Vaccinium uliginosum* in an arctic heath in northern eastern Greenland.

Biochemical analysis of nodules has revealed enhancement in total soluble proteins and a specific enhancement in the level of leghemoglobin; a protein, which plays an important part in the fixation of nitrogen in the nodules. The native and SDS- PAGE analysis also showed changes and increase in protein band intensities by exclusion of solar UV-B and UV-A+B in the root nodules, suggesting that exclusion of UV components from solar spectrum altered both quality and quantity of proteins in root nodules of soybean. SDS PAGE analysis indicated that the calculated approximate molecular weight of Lb is 18.5* kDa in the root nodules of soybean plant cv MACS 330 (Fig. 7B), which is slightly higher than 15.9 kDa known molecular weight of soybean leghemoglobin (Morikis and Wright, 1996).

These changes in the gel profiles did suggest that the changes in the relative abundance of total soluble proteins occurred in the root nodules of soybean cv. MACS 330, due to omission of UV components from ambient solar spectrum.

Higher the content of leghemoglobin more will be the efficiency of the plant in terms of its capacity to fix atmospheric nitrogen (Gurumoorthi et al., 2003). 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. Tezuka et al. (1994) have found that promotion of the plant growth by UV radiation involves the promotion of carbon and nitrogen metabolism in radish plants. High protein content of UV treated plants correlates with higher number of nodules found in plants (Pinto et al., 1999, 2002; Shiozaki et al., 1999). It is evident that absence of solar UV alters the metabolism in soybean in favor of primary metabolism especially in enhanced synthesis of proteins. Guruprasad et al. (2007) found that solar UV radiation primarily affect the photomorphogenic regulatory system that leads to an enhanced growth of leaf, enhanced rate of net photosynthesis as well as protein content in soybean cv-JS 7105. Investigations are continuing to identify the limiting factors for leghemoglobin synthesis in the presence of solar UV radiation.

ACKNOWLEDGMENTS

The work received financial support from CSIR research associatship- 9/301(102)/2K3-EMR-I.

Received February 3, 2007; accepted January 18, 2008.

REFERENCES

Ambler JE, Krizek DT, Semeniuk P (1975) Influence of UV-B radiation on early seedling growth and translocation of Zn from cotyledons in cotton. Physiol Plant 34: 177-181

Amudha P, Jayakumar M, Kulandaivalu G (2005) Impacts of ambient solar UV (280-400 nm) radiation on three tropical legumes. Journal of Plant Biol 48: 284-291

Appleby CA, Bergersen FJ (1980) Preparation and experimental use of leghemoglobin. *In* FJ Bergersen, ed, Methods for Evaluating Biological Nitrogen Fixation. Wiley- Chichester, pp 315-335

Biggs RH, Kossuth SV, Teramura AH (1981) Response of 19 cultivars of soybeans to Ultraviolet-B irradiance. Physiol Plant 34: 19-26

Caldwell MM (1971) Solar UV-irradiation and the growth and development of higher plants. *In* AC Geise, ed, Photophysiology. Academic Press, New York, pp 131-177

Caldwell MM, Teramura AH, Tevini M (1989) The changing solar ultraviolet climate and the ecological consequences for higher plants. Trends Ecol Evol 4: 363-367

Chimphango SB, Musil CF, Dakora FD (2003) Effects of UV-B radiation on plant growth, symbiotic function and concentration of metabolites in three tropical grain legumes. Funct Plant Biol 30 (3): 309-318

Feng H, An L, Chen T, Qiang W, Xu S, Zhang M, Wang X, Cheng G (2003) The effect of enhanced ultraviolet-B radiation on growth, photosynthesis and stable carbon isotope composition ($d^{13}C$) of two soybean cultivars (*Glycine max*) under field conditions. Environmental and Experimental Botany 49: 1-8

Furness NH, Upadhyaya MK (2002) Differential susceptibility of agricultural weeds to ultraviolet-B radiation. Canadian Journal of Plant Science 82: 789-796

Gurumoorthi P, Senthil Kumar S, Vadivel V, Janardhnan K (2003) Studies on agro botanical characters of different accessions of velvet bean collected from Western Ghats, South India. Tropical and Subtropical Agroeco 2: 105-115

Guruprasad K., Bhattacharjee S, Kataria S, Yadav S, Tiwari A, Baroniya S, Rajiv A, Mohanty P (2007) 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 94: 299-306

Jun H, Sarath G, Wagner RW (1994) Detection and purification of modifies leghemoglobins from soybean root nodules. Plant Sci 100: 31-40

Kakani VG, Reddy KR, Zhao D, Mohammed AR (2003) Effects of ultraviolet-B radiation on cotton (*Gossypium hirusutum* L.) morphology and anatomy. Ann of Bot 91: 817-826

Krizek DT, Mirecki RM (2004) Evidence for pytotoxic effects of cellulose acetate in UV exclusion studies. Environ and Exp Bot 51: 33-43

Laemmli UK (1970) Cleavage of structural proteins during the assemble of the head of bacteriophage T4. Nature 227: 680-685.

Lindoo SJ, Caldwell MM (1978) Ultraviolet radiation induced inhibition of leaf expansion and promotion of anthocyanin production. Plant Physiol 61: 278-282

Lowry OH, Roseburg NJ, Farr AL, Randall RJ (1951) Protein Measurement with the folin phenol reagent. J Biol Chem 193: 265-275

Lyndon J, Teramura AH, Summers EG (1986) Effects of ultraviolet radiation on the growth and productivity of field-grown soybean. *In* RC Worrest and MM Caldwell, eds, Stratospheric Ozone Reduction, Solar Ultraviolet Radiation, and Plant Life. Springer-Verlag, Heidelberg, pp 313-325

Morikis D, Wright PE (1996) Hydrogen exchange in carbon monoxide complex of soybean leghemoglobin. Eur J Biochem 237: 212-220

Madronich S, McKenzie RL, Caldwell MM, Bjorn LO (1995)

Changes in ultraviolet radiation reaching the Earth's surface. Ambio 24: 143-152

Mazza CA, Batista D, Zima AM, Szwarcberg-Bracchitta M, Giordano CV, Acevedo A, Scopel AL, Ballare CL (1999) The effects of solar UV-B radiation on the growth and yield of barley are accompanied by increased DNA damage and antioxidant responses. Plant Cell and Environ 22: 61-70

Murali NS, Teramura AH (1986) Intraspecific differences in *Cucumis sativus* to ultraviolet B radiation. Physiol Plant 68: 673-677

Pinto ME, Casati P, Hsu TP, Ku MSB, Edwards GE (1999) Effect of UV-B radiation on growth, photosynthesis, UV-B absorbing compounds and NADP-malic enzyme in bean (*Phaseolus vulgaris* L.) grown under different Nitrogen conditions. Journal of Photochem and Photobiol B: Biology 48: 200-209

Pinto ME, Edwards GE, Riquelme, Ku MSB (2002) Enhancement of nodulation in bean (*Phaseolus vulgaris*) by UV-B irradiation. Funct Plant Biol 29: 1189-1196

Rinnan R, Keinanen MM, Kasurinen A, Asikainen J, Kekki TK, Holopainen T, Ro-Poulasen H, Mikkelsens TN, Michelsen A (2005) Ambient ultraviolet radiation in the Arctic reduces root biomass and alters microbial community composition but has no effects on microbial biomass. Global Change Biology 11: 564-574

Sanginga NB, Ibewiro P, Houngnandan B, Vanlauwe JA, Okogun IO, Akobundu, Versteeg M (1996) Evaluation of symbiotic properties and nitrogen contribution of *Mucuna* to maize grown in the derived savanna of West Africa. Plant and Soil 179:119-129.

Shiozaki N, Hattori I, Gojo R, Tezuka T (1999) Activation of growth and nodulation in a symbiotic system between pea plants and leguminous bacteria by near UV radiation. Journal of Photochem and Photobiol B: Biology 50: 33-37

Singh A (1997) Increased UV-B radiation reduces N_2 fixation in tropical leguminous crops. Environmental Pollution 3: 289-291

Sisson WB (1981) Photosynthesis, growth and ultraviolet irradiance absorbance of *Cucurbita pepo* L. leaves exposed to UV-B radiation (280-315 nm). Plant Physiol 67: 120-124

Solanki R, Lakshmi N, Athale R, Baroniya S, Guruprasad KN (2006) Growth and chlorophyll contents as affected by UV-A and UV-B components in cucumber and cotton. Physiol and Mol Biol of Plants 12(4): 321-323

Stefan H, Christian N (2002) Biomass production and N_2 fixation of five *Mucuna pruriens* varieties and their effect on maize yield in the forest zone of Cameroon. Journal of Plant Nutrition and Soil Sci 165: 101-109

Tezuka T, Hotta T, Watanabe I (1993) Growth promotion of tomato and radish plants by solar UV radiation reaching the Earth's surface. Journal of Photochem and Photobiol B: Biology 19: 61-66

Tezuka T, Yamaguchi F, Ando Y (1994) Physiological activation in radish plants by UV-A radiation. Journal of Photochem and Photobiol B: Biology 24: 33-40

Tezuka T, Shiozaki N, Yamaguchi F, Ando Y (1998) Responses of soybean plants to nodulation and N2 fixation by near UV radiation. Environ Sci 6: 107-112

Teramura AH (1983) Effects of ultraviolet radiation on the growth and yield of crop plants. Physiol Plant 58: 415-427

Teramura AH, Murali N (1986) Intraspecific differences in growth and yield of soybean exposed to ultraviolet–B radiation under green house and field conditions. Environmental and Experimental Botany 26: 89-95

Teramura AH, Sullivan JH (1994) Effects of UV-B radiation on photosynthesis and growth of terrestrial plants. Photosynth Res 39: 463-473

Tevini M, Braun J, Fiser G (1991) The protective function of the epidermal layer of rye seedlings against UV-B radiation. Photochem Photobiol 53: 529-533

Tevini M, Teramura AH (1989) UV-B effects on terrestrial plants. Photochem Photobiol 50: 479-487

Varalakshmi D, Lakshmi N, Guruprasad KN (2003) Physiological changes in soybean CV. JS 71-05 after the exclusion of UV-A and UV-B from the solar radiation. Indian Journal Plant Physiol (Special Issue): 602-606

Zaller JG, Caldwell MM, Flint SD, Scopel AL, Osvaldo SE, Ballare CL (2002) Solar UV-B radiation affects below-ground parameters in a fen ecosystem in Tierra del Fuego, Argentina: implications of stratospheric ozone depletion. Global Change Biology, 8: 867-871

Zavala JA, Botto JF (2002) Impact of solar UV-B radiation on seedling emergence, chlorophyll fluorescence, and growth and yield of radish (*Raphanus sativus*). Funct Plant Biol 29: 797-804