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# Growth, photosynthesis and nitrogen metabolism in soybean varieties after exclusion of the UV-B and UV-A/B components of solar radiation



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## ABSTRACT

A field experiment was conducted to study the impact of the exclusion of the solar UV components on growth, photosynthesis and nitrogen metabolism in soybean (*Glycine max*) varieties PK-472, Pusa-24, JS 71-05, JS-335, NRC-7 and Kalitur. The plants were grown in specially designed UV exclusion chambers wrapped with filters to exclude UV-B or UV-A/B and transmitted all UV. Exclusion of UV significantly enhanced the growth of the aerial parts as well as the growth of the below ground parts in all of the six soybean varieties. Nitrate reductase activity (NRA) was significantly reduced, whereas leghemoglobin (Lb) content, total soluble protein, net photosynthesis ( $P_n$ ) and  $\alpha$ -tocopherol content were enhanced after UV exclusion. The exclusion of solar UV-A/B enhanced all parameters to a larger extent than the exclusion of solar UV-B in four of the six varieties of soybean except for NRC-7 and Kalitur. These two varieties responded more to UV-B exclusion compared to UV-A/B exclusion. A significant inverse correlation between the NRA and the number of nodules per plant was observed. The extent of response in all parameters was greater in PK-472 and JS71-05 than that in Kalitur and JS-335 after UV exclusion. The exclusion of UV augmented the growth of nodules, Lb content and  $\alpha$ -tocopherol levels and conferred higher rates of  $P_n$  to support better growth of nodules. Control plants (+UV-A/B) seemed to fulfill their N demand through the assimilation of  $\text{NO}_3^-$  resulting in lower symbiotic nitrogen fixation and higher NR activity.

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## 1. Introduction

The level of ambient UV-B (280–315 nm) radiation in sunlight varies with latitude and is relatively higher in tropical regions than in temperate regions. Due to the small solar zenith angle and thin stratospheric ozone layer in the tropics, terrestrial

plants encounter much higher levels of UV-B radiation than at higher latitudes [1]. India lies in a low ozone belt and receives more UV-B radiation than those of temperate regions with higher latitudes [2].

Data compiled from the last two decades suggest that nearly 50% of crop plants are affected by elevated levels of

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**Table 1 – Origin, pedigree and morphological characteristics of Indian soybean varieties.**

Variety	Origin	Hilum color	Growth habit	Days to maturity	Flower color	Pedigree	Year of release
NRC-7	Indore	Black	Determinate	90–99	Purple	Selection from S69-96	1997
JS-335	Jabalpur	Black	Determinate	95–100	Purple	JS-78-77XJS71-05	1994
Pusa-24	New Delhi	Black	Indeterminate	105–125	White	Shelby X Bragg	1987
JS71-05	Jabalpur	Black	Determinate	90–95	Purple	Selection from Leetype exotic material	1990
PK-472	Pantnagar	Brown	Determinate	100–105	White	Hardee X Punjab-1	1986
Kalitur	Wild variety	Black	Semi to indeterminate	110–120	Purple	Landrace	–

solar UV-B. Studies on a number of cultivated and native plant species have shown that ambient and enhanced levels of UV-B have detrimental effects on plant growth, development and morphology, photosynthesis, and biomass production [3–7]. Whether ambient UV-B has any effect on the underground organs such as roots or on the beneficial processes linked to nitrogen (N) fixation in leguminous plants is not clear.

Nitrogen requirement of legumes can be met by inorganic N assimilation and symbiotic N<sub>2</sub> fixation; in practice, they obtain N through both processes. A reduction of nitrate to nitrite (NO<sub>2</sub><sup>-</sup>) is catalyzed by nitrate reductase (NR; EC 1.6.6.4), an inducible enzyme whose activity depends on the availability of nitrate and light [8]. At high concentrations, nitrate inhibits both nodulation and N<sub>2</sub> fixation in almost all legume species [9]. Like most legumes, soybean has the potential to fix atmospheric nitrogen through symbiotic relationships with soil organisms [10].

Nitrogen fixation and assimilation are negatively affected by an elevated UV-B as confirmed by the reduced activities of nitrogenase, nitrate reductase, nitrite reductase and leghemoglobin (Lb) contents in the nodulated mung bean cultivars [11]. Supplemental UV-B also led to a reduced N<sub>2</sub> fixation in the tropical leguminous crops *Phaseolus mungo* and *Vigna radiata* [12]. Very little is known about the effects of ambient UV-B radiation on nitrogen metabolism. The number of nodules per plant in the bean grown in conditions of ambient UV radiation was higher than that on plants deprived of UV-B [13], and Chouhan et al. [14] found that ambient UV-B reduced the number and size of nodules, total protein and Lb content in the soybean. However, Shiozaki et al. [15] found that UV (300–400 nm) applied to leaves increased the amounts of nodulation and symbiotic N<sub>2</sub> fixation in pea plants. Baroniya et al. [7] reported that UV-excluded soybean plants had higher levels of  $\alpha$ -tocopherol, which plays an important role in translocating photoassimilates from the leaves to the roots [16].

Sensitivity to UV-B radiation varies considerably within and between plant species, but the factors underlying this diversity are not well understood [17]. Considerable intraspecific variation in response to enhanced UV-B radiation in terms of growth and yield has been observed in wheat [18], maize [19], rice [20], soybean [5,17,21] and tartary buckwheat [22]. Most of these studies examined the response of individual varieties to enhanced UV-B, but UV exclusion studies might provide more realistic assessments of the sensitivity of plants to current levels of UV radiation [5,7,23].

The aim of this paper was to evaluate the impact of ambient UV (280–400 nm) on the nodulation and nitrogen

metabolism, particularly nitrate reductase (NR) activity, net rate of photosynthesis, Lb content and total soluble protein (in nodules) in the six varieties of soybean (*Glycine max*) by the exclusion of UV-B and UV-A/B. We hypothesized that the exclusion of UV radiation would increase the growth and biomass of both the aerial and below ground parts of soybean, and also affect other physiological processes by an increased rate of photosynthesis and higher nitrogen fixation resulting from increased nitrate reductase activity (NRA), and Lb content. Such changes should ultimately lead to an increased yield.

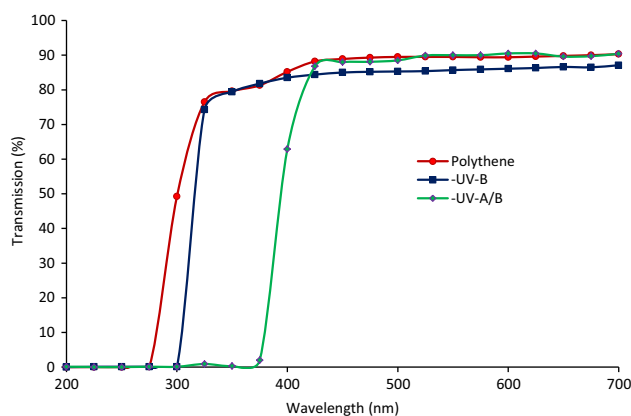
## 2. Material and methods

### 2.1. Plant materials

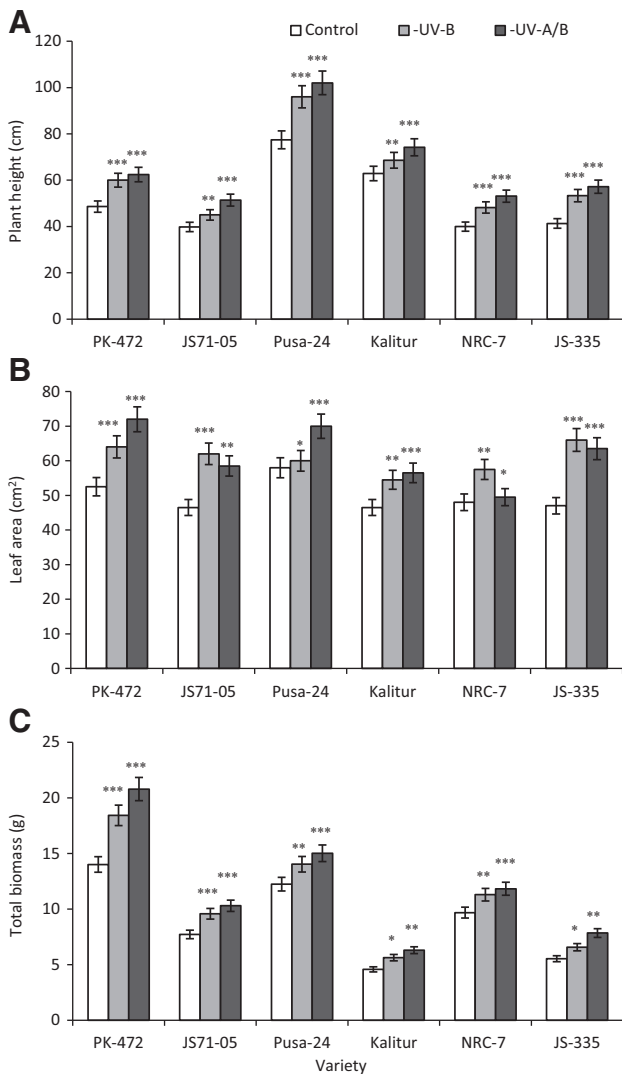
Seeds of soybean (*G. max*) varieties PK-472, Pusa-24, JS 71-05, JS-335, NRC-7 and Kalitur were obtained from the Directorate of Soybean Research, Indore. The origin and morphological characteristics of the varieties are listed in Table 1.

### 2.2. Experimental design of UV exclusion experiments

Field experiments under natural sunlight were conducted at the Botanical Garden, School of Life Sciences, Devi Ahilya University, Indore (22°43' N, 75°49'60" E). The experiments were carried out in January–April 2012, when the average daily solar UV is around 50% higher than the average daily level in temperate regions. The seeds were surface-sterilized with 0.1% HgCl<sub>2</sub> and then inoculated with a slurry of *Bradyrhizobium japonicum* before



**Fig. 1 – Transmission spectra for UV-B and UV-A/B exclusion filters on the metal cages under natural light conditions.**



**Fig. 2 – Effect of exclusion of solar UV-B and UV-A/B on plant height (A), leaf area (B) and total biomass (C) in soybean varieties. Vertical bars indicate  $\pm$ SE. Values are significantly different at ( $P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ ) from control (Newman–Keuls multiple comparison test).**

sowing in 1 m rows spaced 0.3 m apart and 6 cm between plants within rows. Each plot was placed under an iron mesh cage (120 cm L  $\times$  90 cm W  $\times$  120 cm H) wrapped with UV cut-off polyester filters (Garware Polyester Ltd., Mumbai) that selectively excluded UV-B ( $<315$  nm) and UV-A/B ( $<400$  nm) radiation. Control plants were grown under polythene filters transmissible to ambient solar UV-B and UV-A radiation. The transmission characteristics of the filters were measured by a Shimadzu (UV-1601) spectrophotometer (Fig. 1). The transmission characteristics of the filters did not change during the experimental period and the filters did not emit fluorescence in the visible region. The cages received full solar radiation during the day without shading. Seedlings were exposed to solar radiation from the time of emergence. There was no significant temperature difference between the control and the UV-excluded chambers

as the horizontal holes in the chambers allowed passive air ventilation. The experiments were conducted in a randomized block design with three replications of five plants for each treatment.

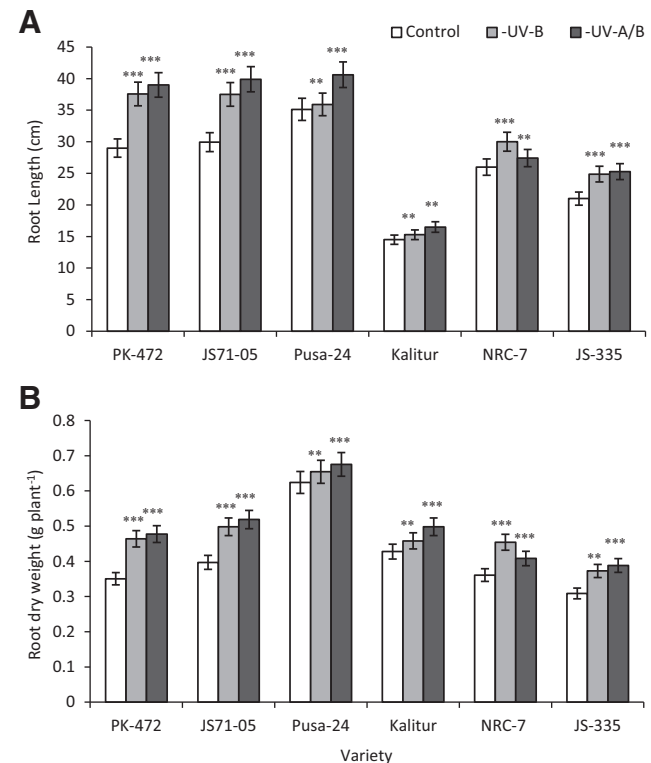
### 2.3. Radiation measurement

Absolute solar irradiance without UV-B or UV-A/UV-B was measured using a radiometer (Solar light-PMA 2100, Glenside, PA, USA). The midday photosynthetic active radiation (PAR) during the experimental period was at  $1378 \mu\text{E m}^{-2} \text{s}^{-1}$ , the loss in light intensity at midday using -UV-B filters was 10% ( $1240 \mu\text{E m}^{-2} \text{s}^{-1}$ ), and 14% ( $1180 \mu\text{E m}^{-2} \text{s}^{-1}$ ) under the UV-A/UV-B filter and 4.2% ( $1320 \mu\text{E m}^{-2} \text{s}^{-1}$ ) under the polythene filter transmissible to UV (filter control).

### 2.4. Above ground growth parameters

#### 2.4.1. Plant height and leaf area

Plant height was measured from the soil level to shoot tip in all the five plants in each replicate at crop maturity. The areas of the third trifoliolate leaves were estimated by tracing the outlines on gridded graph paper and weighing the cut paper outlines. A calibration curve was prepared by weighing 0–1500 mm<sup>2</sup> sections of the graph paper. The mean of the 15 leaves was taken as the measured value for each treatment.



**Fig. 3 – Effect of exclusion of solar UV-B and UV-A/B on root length (A) and root biomass (B) in soybean varieties. Vertical bars indicate  $\pm$ SE. Values are significantly different at ( $*P < 0.01$ ,  $***P < 0.001$ ) from control (Newman–Keuls multiple comparison test).**

#### 2.4.2. Total biomass accumulation

At maturity the 15 plants (five plants from each replicate) were randomly selected to measure the total dry weights after oven-drying at 60 °C for 72 h.

#### 2.5. Below ground growth parameters

Below ground growth parameters, including the root length, root biomass, number of root nodules and nodule fresh weight per plant, were measured for all the six varieties at 50 days after seedling emergence (DAE).

##### 2.5.1. Root length and root biomass

The roots were carefully removed and washed prior to measuring the lengths. The roots without the nodules were dried on a filter paper, then further dried at 60 °C for 72 h before weighing.

##### 2.5.2. Number of nodules/fresh weight of nodules

The number of nodules on the roots were counted and recorded on a plant basis, then weighed both individually (gm/nodule) and on a per plant basis for all treatments.

#### 2.6. Rate of photosynthesis

Net photosynthesis ( $P_n$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) was measured on intact plants grown in normal sunlight or UV excluded sunlight under field conditions using a portable system (Li-6200, LI-COR Inc., Lincoln, Nebraska, USA). Photosynthetic measurements were made on fully expanded leaves (3rd leaf from the apex) of the six plants in each treatment on clear days at noon; the photosynthetic photon flux density (PPFD) was 1200–1300  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ , air flow (500  $\text{mol s}^{-1}$ ), and  $\text{CO}_2$  concentration (380–400  $\mu\text{mol L}^{-1}$ ).

#### 2.7. Determination of NRA

NRA (EC 1.6.6.1) in leaves were determined by the intact tissue assay method of Jaworski [24]. Chopped leaf pieces (100 mg) were incubated for 1 h at 30 °C in a 10 mL reaction mixture containing 25  $\text{mmol L}^{-1}$  phosphate buffer, 100  $\text{mmol L}^{-1}$  potassium nitrate, and 1.25% isopropanol. The nitrite subsequently formed was measured at 540 nm after azo coupling with sulphanilamide and naphthylenediamine dihydrochloride. NRA was expressed as  $\text{nmol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$ .

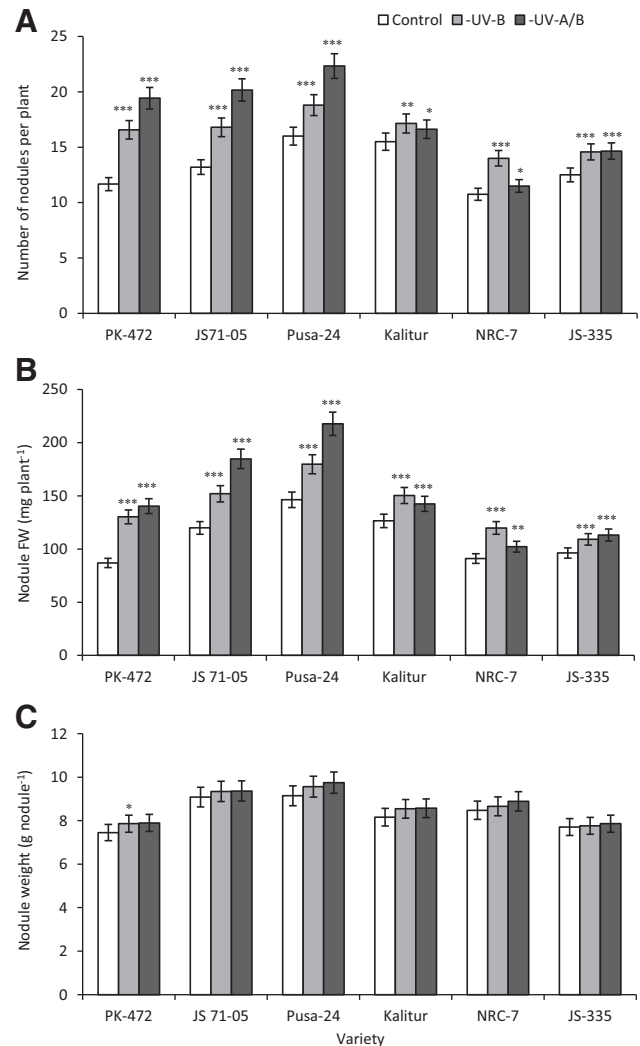
#### 2.8. Extraction and estimation of Lb content

Lb extracted from the root nodules at 50 DAE of the seedlings was measured by the method of Jun et al. [25]. The nodules (1.25 g) of the 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 a pestle. The resulting powder was suspended in 25 mL of 50  $\text{mmol L}^{-1}$  sodium phosphate buffer (pH 7.5) containing 1  $\text{mmol L}^{-1}$  EDTA, 1  $\text{mmol L}^{-1}$  PMSF, betamercaptoethanol and 10% polyvinyl pyrrolidone (PVPP), filtered through cheese cloth, and centrifuged at 20,000  $\times g$  for 20 min at 4 °C. The deep red supernatant was saturated to 50% with solid  $(\text{NH}_4)_2\text{SO}_4$  and centrifuged at 15,000  $\times g$  for 20 min at 4 °C. The pellet was discarded and the red supernatant

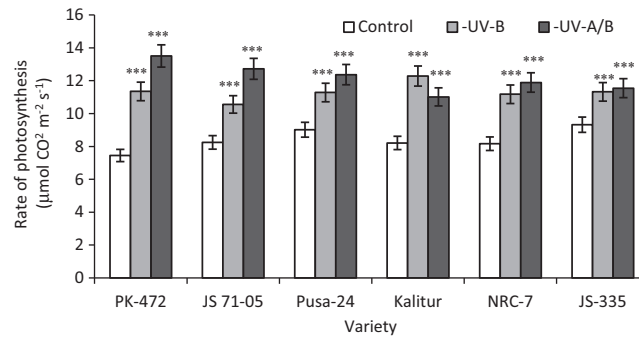
was saturated to 90% with  $(\text{NH}_4)_2\text{SO}_4$  and centrifuged at 15,000  $\times g$  for 20 min at 4 °C. The red pellet was resuspended in 15 mL of 20  $\text{mmol L}^{-1}$  Tris-HCl (pH 8.0) containing 1  $\text{mmol L}^{-1}$   $(\text{NH}_4)_2\text{SO}_4$ . The Lb-containing fractions (50% to 90% pellets) were detected at 410 nm using a UV-visible Shimadzu spectrophotometer.

#### 2.9. $\alpha$ -Tocopherol determination

$\alpha$ -Tocopherol was extracted from the leaves by the method of Walker and Slinger [26] and the concentrations were estimated by the method of Pearson et al. [27]. The amount of  $\alpha$ -tocopherol was calculated from a standard curve. The total soluble protein content was measured in Lb-containing fractions by the method of Lowry et al. [28].



**Fig. 4 – Effect of exclusion of solar UV-B and UV-A/B on number of nodules (A), nodule fresh weights (B) and individual nodule weights (C) in soybean varieties. Vertical bars indicate  $\pm$ SE. Values are significantly different at ( $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ) from control (Newman-Keuls multiple comparison test).**



**Fig. 5 – Effect of exclusion of solar UV-B and UV-A/B on rates of photosynthesis in soybean varieties. Vertical bars indicate  $\pm$ SE for the mean. All values are significantly different ( $P < 0.001$ ) from the controls (Newman–Keuls multiple comparison test).**

### 2.10. Statistical analyses

All the data are presented as means of the 15 plants, expressed as means  $\pm$ SE, and analyzed by the analysis of variance (ANOVA) followed by post hoc Newman–Keuls multiple comparison tests ( $^*P < 0.05$ ,  $^{**}P < 0.01$ ,  $^{***}P < 0.001$ ) using Prism 4 software for Windows, Graf Pad Software, Inc, La Jolla, CA, USA.

## 3. Results

### 3.1. Growth parameters of the aerial parts

The plant height, the leaf area (third trifoliolate leaves) and the total biomass accumulation were enhanced by the exclusion of UV-B as well as by the exclusion of UV-A/B from all of the six soybean varieties. A maximum enhancement of the plant height after the exclusion of UV-B was obtained in JS-335 (29%) and a minimum enhancement was recorded for Kalitur (9%). The plant height was further increased after the elimination of UV-A/B, with the maximum increase occurring in JS-335 (39%) and the minimum enhancement in Kalitur (18%) (Fig. 2-A).

The same trend was observed for the increases in the leaf area and the total plant biomass accumulation for all of the six varieties (Fig. 2-B and C).

### 3.2. Growth parameters of the below ground parts

The exposure of the plants to ambient UV-B and UV-A radiation resulted in less root growth/root biomass for all of the six varieties. The root length of the plants grown under UV-A/B at 50 DAE showed maximum increases in PK-472, JS 71-05 and JS-335 (34%, 33% and 20%, respectively) (Fig. 3-A). Similar trends were observed in the promotion of root biomass after the exclusion of UV-B and UV-A/B (Fig. 3-B).

The exclusion of UV-B and UV-A/B increased the number of nodules and fresh weights of nodules in all of the varieties (Fig. 4-A, B). Maximum increases were recorded for PK-472, NRC-7 and JS 71-05 (42%, 30% and 27%, respectively) relative to the respective controls. The exclusion of UV-A/B from solar radiation further increased the number of nodules on PK-472, JS 71-05 and Pusa-24 (67%, 53% and 40%, respectively) relative to the controls (Fig. 4-A). A similar trend was obtained for the increased fresh weights of nodules in all varieties by the

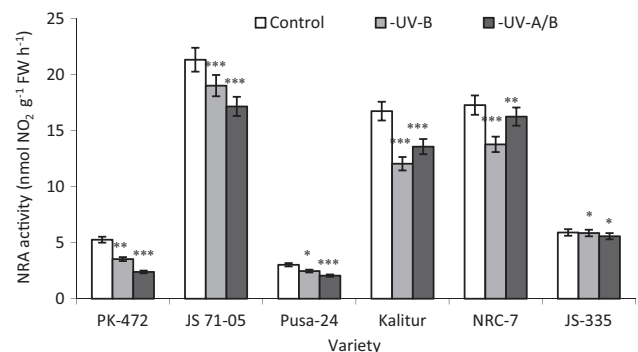
exclusion of UV-B and UV-A/B (Fig. 4-B), but individual nodule weights were not significantly altered after the exclusion of UV-B or UV-A/B (Fig. 4-C).

### 3.3. Rate of photosynthesis

Significant increases in the rates of photosynthesis were observed after the exclusion of solar UV-B and UV-A/B in all of the six varieties. However the extents of increase were greater in PK-472, Pusa-24 and JS 71-05 compared to the others (Fig. 5). The increased net rates of photosynthesis were higher after the exclusion of UV-A/B than UV-B relative to the respective controls (Fig. 5).

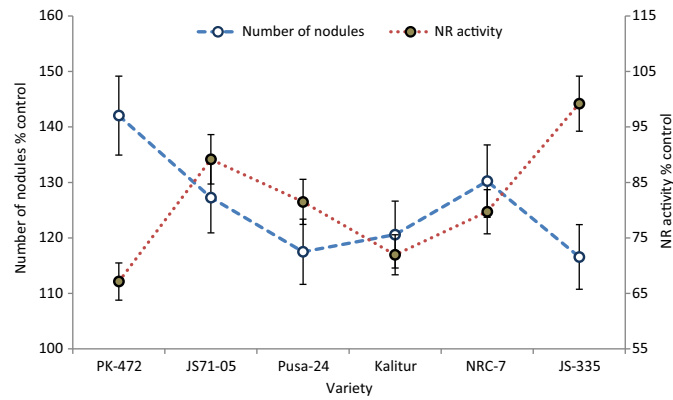
### 3.4. NRA

The mean NRA in the soybean leaves on the plants grown in ambient conditions ranged from 2.68 to 20.31 nmol  $\text{g}^{-1}$  FW  $\text{h}^{-1}$ . The UV-A/B exclusion significantly decreased the NRA in the leaves of PK-472, Pusa-24 and JS 71-05. A lower effect occurred in the plants subjected only to UV-B exclusion (Fig. 6). Maximum reductions in NRA after the exclusion of UV-B were shown by PK-472, Kalitur and NRC-7 (33%, 28% and 20%, respectively) compared to the respective controls.



**Fig. 6 – Effect of exclusion of solar UV-B and UV-A/B on nitrate reductase (NRA) activity in soybean varieties. Vertical bars indicate  $\pm$ SE for mean. Values are significantly different at ( $^*P < 0.05$ ,  $^{**}P < 0.01$ ,  $^{***}P < 0.001$ ) from control (Newman–Keuls multiple comparison test).**





**Fig. 7 – Negative correlations between number of nodules and NRA in soybean varieties after exclusion of solar UV-B. Vertical bars indicate  $\pm$ SE of means.**

There was a significant negative correlation between the number of nodules and NRA in the soybean varieties after the UV-B ( $-0.847$  at  $P = 0.008^{**}$ ) and UV-A/B ( $-0.763$  at  $P = 0.028^*$ ) exclusion. NRA was much lower in the varieties with the highest increases in the number of nodules after a UV exclusion (Figs. 7, 8).

### 3.5. Total soluble protein in the nodules and Lb

Biochemical analyses of the nodules from all six varieties indicated higher amounts of Lb and total soluble proteins. The amounts of the total soluble proteins in the nodules were increased by 25% (–UV-B) and 33% (–UV-A/B) in PK-472, and 9% (–UV-B) and 15% (–UV-A/B) in JS-335 compared to the controls (Fig. 9-A). Lb is a protein that plays an important role in the fixation of nitrogen in the nodules. The UV exclusion increased the Lb content in the root nodules by 36% (–UV-B), and 56% (–UV-A/B) (Fig. 9-B) in PK-472 with the corresponding values of 10% and 22% for JS-335 (Fig. 9-B).

### 3.6. $\alpha$ -Tocopherol

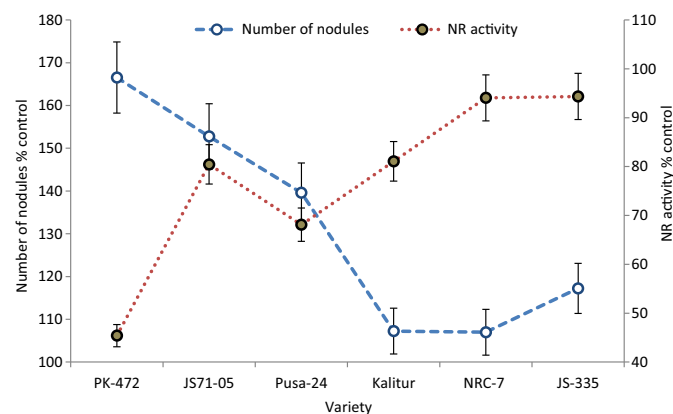
The UV-B and UV-A/B exclusion resulted in significant increases in the levels of  $\alpha$ -tocopherol in all varieties. Reductions in the

levels of  $\alpha$ -tocopherol caused by the exclusion of UV-B were further increased by the exclusion of UV-A/B (Fig. 10).

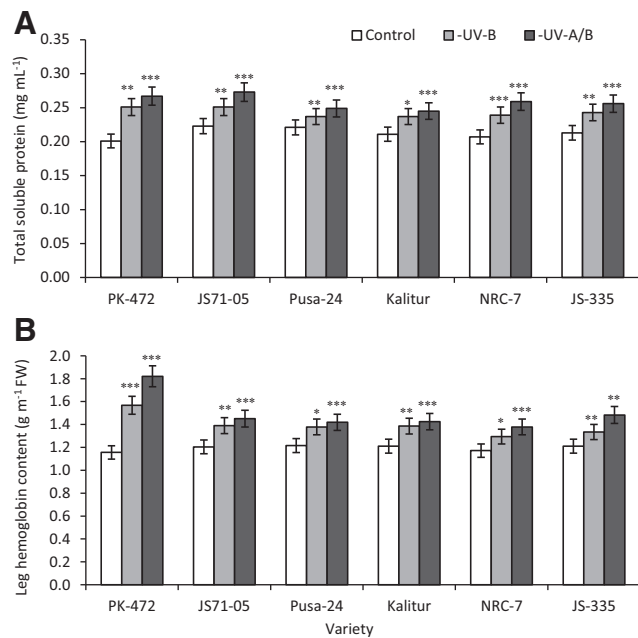
## 4. Discussion

The results of this study indicate the positive effects of UV exclusion on the aerial and the below ground parts of the six soybean varieties. Differences between the varieties were also apparent. The exclusion of solar UV-A/B led to larger effects than the exclusion of solar UV-B in the varieties PK-472, JS-7105, JS-335 and Pusa-24. The varieties NRC-7 and Kalitur responded more in response to the UV-B exclusion than in response to the UV-A/B exclusion for traits such as leaf area, root length, root fresh weight, nodule number per plant, nodule fresh weight and NRA.

Conflicting data have been published regarding the effects of UV-B on the underground parts or non-UV exposed parts, namely the roots, number of root nodules, weight of nodules and the amount of nitrogen fixation. Shiozaki et al. [15] found that the growth of pea plants was enhanced by the near UV (300–400 nm) radiation. Nodulation and symbiotic nitrogen fixation were also enhanced twofold and eightfold, respectively. Similarly, Tezuka et al. [29] found that UV-A (320–400 nm) sourced from UV lamps



**Fig. 8 – Negative correlations between number of nodules and NRA in soybean varieties after exclusion of solar UV-A/B. Vertical bars indicate  $\pm$ SE of means.**



**Fig. 9 – Effect of exclusion of solar UV-B and UV-A/B on total soluble protein (A) and leghemoglobin (B) contents in root nodules of soybean varieties. Values are significantly different at ( $P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ ) from controls (Newman-Keuls multiple comparison test).**

promoted nodulation on roots and increased symbiotic  $N_2$  fixation in soybean. Ambient UV-B affected the biomass partitioned to tubers and increased root diameter and root fresh weight of radish [30]. Pinto et al. [13] found that bean plants grown in a greenhouse, where UV-B levels are low compared to outside levels had almost 60% more nodules per plant and 2.5-fold increases in nodulation compared to the plants grown outside. However, moderate and elevated UV-B exposure had no effect on the number of nodules, nodule mass and nodule size, although nitrogen concentration was markedly reduced in the roots of soybean and common bean [31]. The responses in the root biomass as presented here are consistent with the report of increased root length in a *Carex* species subjected to a reduced UV radiation [32]. Similarly, our results indicated that the absence of solar UV components significantly enhanced the number of nodules and the fresh weight of nodules along with the increased root length and root mass; however, the weight per nodule was not significantly changed. Thus the greater N fixation was attributed to the increased number of nodules.

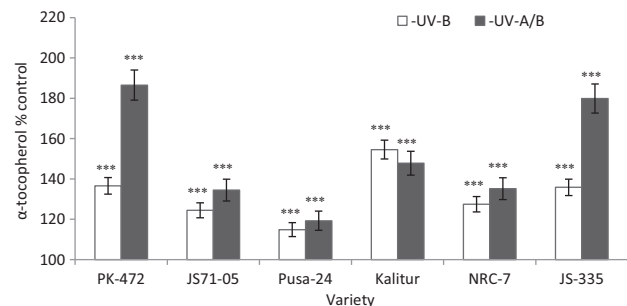
The number of studies on the effect of ambient UV irradiance on the N metabolism of plants is limited. Moreover, the enhancement of UV radiation with UV-B lamps has been more commonly used in experimental work than the exclusion of UV radiation with filters. In the present study, UV-A/B exclusion led to a significant reduction in the NRA in the leaves of soybean varieties when compared to control plants. Previous studies showed that UV-A/B exclusion in the field reduced NRA in the leaves of silver birch (*Betula pendula* Roth.) seedlings [33], and enhanced UV-B, reduced growth and decreased NRA

in dragon spruce (*Picea asperata*) needles [34] and in crop seedlings [35,36].

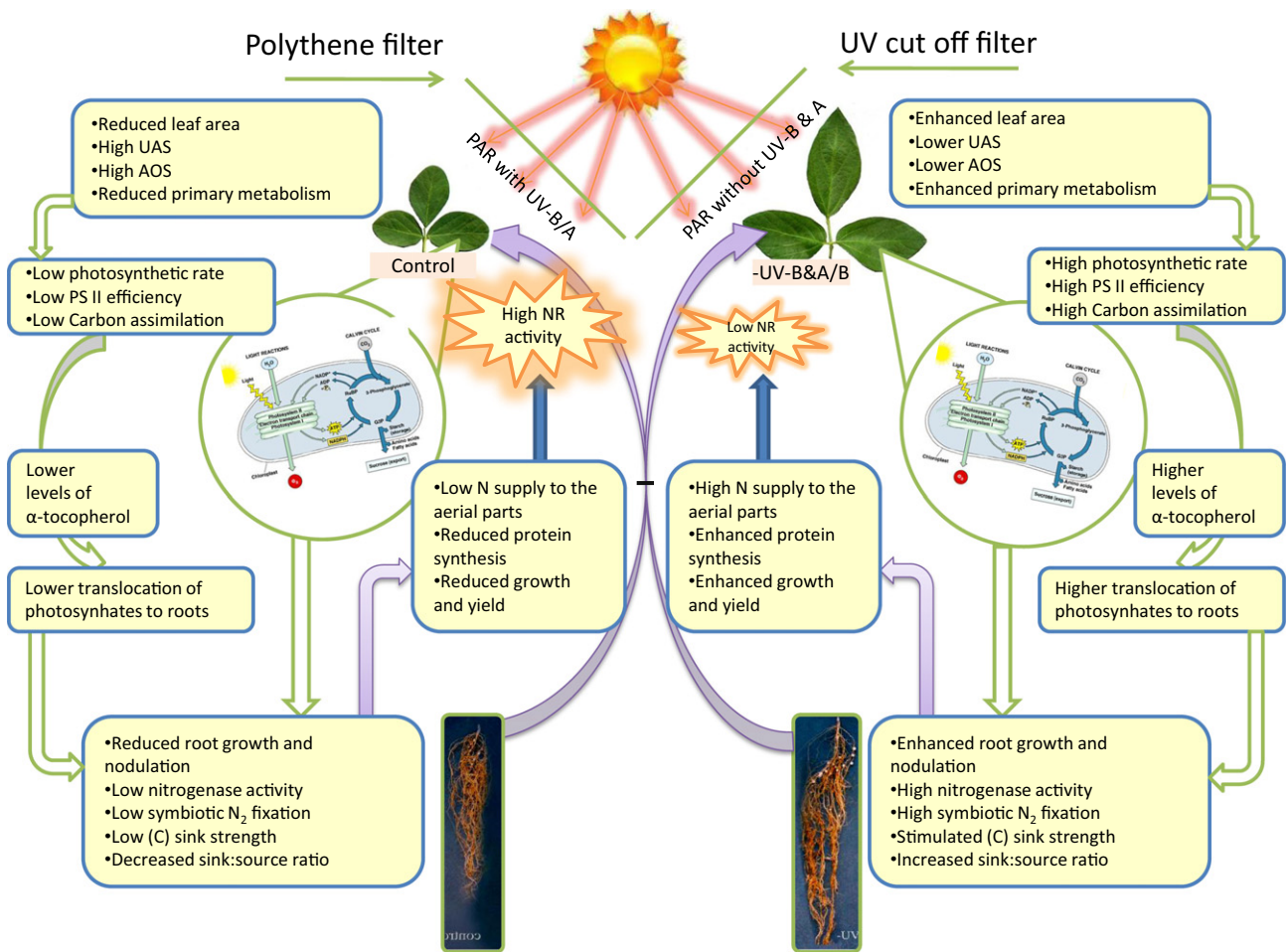
NR catalytic flux is controlled by substrate availability and level and activity of functional NR. Nitrate reduction capacity is regulated in relation to the overall plant metabolic level by metabolic sensors and signal transduction pathways [8]. Nitrate reductase that is located at the junction of two energy-consuming pathways, nitrate assimilation and carbon fixation, results in a controlled response to environmental changes that affect photosynthesis [37]. Biological  $N_2$  fixation can fulfill the N demand of leguminous crops such as soybean, resulting in significant increases in plant total N accumulation and higher N concentrations in the seeds [38] compared to N-fertilized plants. However, in terms of N acquisition, these benefits are accompanied by increased respiration costs of 14% or more of the current photosynthesis when compared to N-fertilized soybean [39]. Nitrate assimilation results in costs of up to  $2.5 \text{ g C g}^{-1} \text{ N}$  assimilated, whereas  $N_2$  fixation costs  $5.2\text{--}18.8 \text{ g C g}^{-1} \text{ N}$  [40]. Therefore,  $N_2$  fixation will be limited by the availability of photosynthate unless there is a simultaneous increase in the rate of photosynthesis [38,41].

The rates of photosynthesis also respond to factors other than leaf N concentration, such as environmental conditions and changes in the source:sink ratio of the plant [42,43]. There are reports showing that a decrease in the sink:source ratio caused by a removal of pods at the reproductive stage in soybean, decreases the rate of photosynthesis [42,44]. Moreover, changes in the sink:source ratio may lead to an absence of nodules and decrease photosynthetic response to elevated  $CO_2$  [43].

The higher contents of Lb increase the efficiency of the plants in terms of the capacity to fix atmospheric nitrogen [45]. The present data on the exclusion of solar UV radiation indicates an effect on the activity of nitrogen fixation by enhanced synthesis of Lb. Increased growth of the aerial parts seems to be deriving more nitrogen to support growth and enhanced protein synthesis. Increased number of nodules, Lb content and net rates of photosynthesis after the exclusion of UV-B and UV-A/B reflect the increase in sink:source ratio.  $\alpha$ -Tocopherol, plays an important role in translocating photoassimilates from the leaves to the roots [16]. In the present study  $\alpha$ -tocopherol levels were higher in UV excluded plants,



**Fig. 10 – Effect of exclusion of solar UV-B and UV-A/B on  $\alpha$ -tocopherol levels in third trifoliolate leaves of soybean varieties. Values are significantly different at ( $***P < 0.001$ ) from respective controls (Newman-Keuls multiple comparison test).**



**Fig. 11 – Impact of UV radiation on photosynthesis, UAS,  $\alpha$ -tocopherol, leghemoglobin, nodulation, NRA and growth in soybean plants.**

enabling them to translocate more photoassimilates from the leaves to the roots (Fig. 10). Recently, Kaschuk et al. [39] found N-independent effects of *Rhizobia* on photosynthesis, whereby the rates of photosynthesis were stimulated by the photosynthate (C) sink strength of symbiosis. Since the C costs of N<sub>2</sub> fixation are higher than those of the NO<sub>3</sub><sup>-</sup> uptake [40], our data support the assumption that higher C sink strength of N<sub>2</sub> fixation increases the rate of photosynthesis in UV excluded plants. Higher N<sub>2</sub> fixation by *Rhizobia* nitrogenase was reported in UV excluded soybean plants [46] suggesting that the plants with more nodules achieved higher rates of photosynthesis because they had a larger demand for photosynthate.

We found a significant negative correlation between the NRA and the number of nodules per plant after the exclusion of UV-B ( $r = -0.847$  at  $P = 0.008^{**}$ ) and UV-A/B ( $r = -0.763$  at  $P = 0.028^{*}$ ) (Figs. 7, 8).

The plants growing under ambient levels of UV-A/B radiation are constantly under stress, and a reduced photosynthesis (Fig. 5) may result in a reduced transport of photosynthates to the roots. The rate of N<sub>2</sub> fixation in a legume depends upon the amount of carbon product the host plant can provide and therefore any reduction in photosynthates decreases the nodule activity [12]. Abu-Shakra et al. [41] observed that nodulated soybeans with

higher nitrogenase activity had prolonged photosynthetic activity. They suggested that longer photosynthetic activity is related to higher N availability in the leaves as a consequence of higher N<sub>2</sub> fixation.

In the present study the varieties NRC-7 and Kalitur performed differently from the other four in showing lower increases in the number of nodules and larger increases in NRA following the exclusion of UV-A/B. On the other hand JS-7105, JS-335 and Pusa-24 showed lower number of nodules and larger increases in NRA by the exclusion of UV-B. These variations in the responses of the nodulated legumes to ambient UV-B and UV-A could be attributed to genotypic differences in their sensitivity.

In conclusion, the control plants (+UV-A/UV-B) with reduced photosynthesis, Lb content and reduced primary metabolism seem to fulfill their N demand through the assimilation of NO<sub>3</sub><sup>-</sup> by NR and thus NRA in the control plants is higher to compensate for the reduced symbiotic N<sub>2</sub> fixation. These results indicate that exclusion of UV increased the growth of nodules as well as primary metabolism, and the higher rate of photosynthesis associated with enhanced levels of  $\alpha$ -tocopherol permitted the translocation of photoassimilates from the leaves to the roots, to support better growth of nodules and Lb content for increased symbiotic nitrogen fixation (Fig. 11).



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